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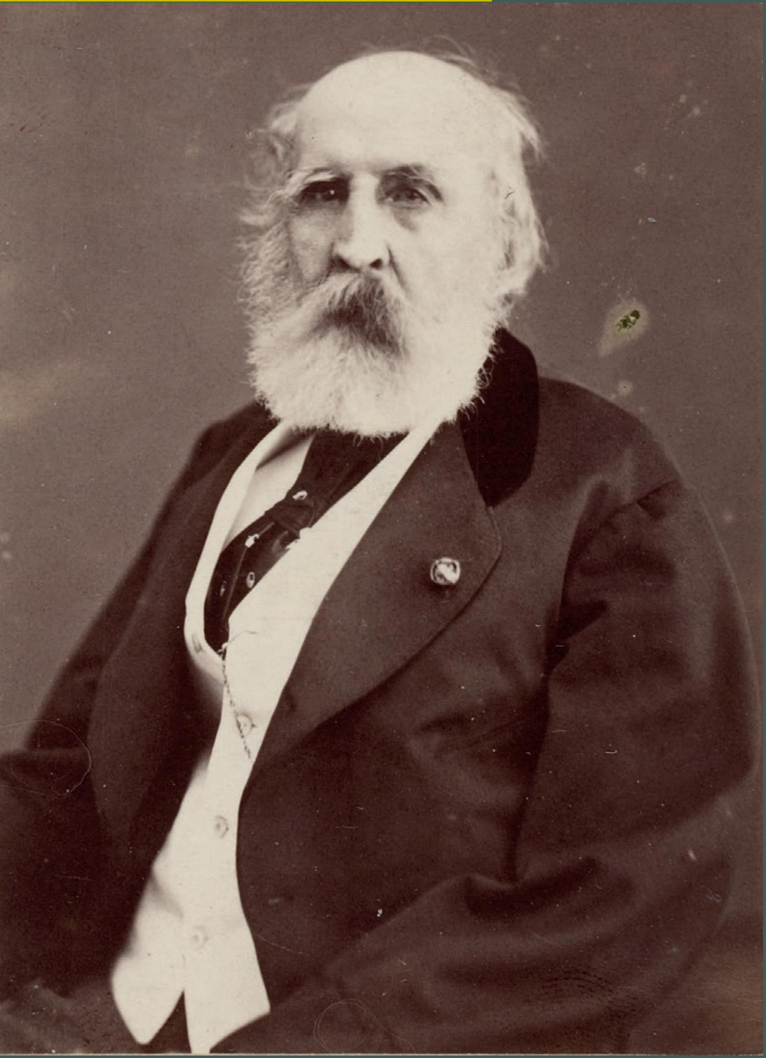

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Journal of Plant Taxonomy and Geography (*Webbia*) is a peer-reviewed journal on Plant Taxonomy, Nomenclature, Phylogeny, Phytogeography and Palaeobotany of the Vascular Plants.

The journal aims to allow research in botanical topics such as taxonomy, systematics, nomenclature, molecular phylogeny, conservation, biogeography, and history of botany, and botanical collections.

It was founded in **1905** in Florence by **Ugolino Martelli** (1860-1934), a botanist well known for his studies of and contributions to the systematics of the tropical genus *Pandanus* and on the Flora of Sardinia.

In the 19th century Florence represented one of the most important European centres in Plant Taxonomy and Phytogeography with several notable Italian botanists worth mentioning such as Filippo Parlatore, Teodoro Caruel, Eugenio Baroni, Stefano Sommier, Odoardo Beccari and Ugolino Martelli himself. In 1842 **Filippo Parlatore** (1817-1877) founded in Florence the *Herbarium Centrale Italicum (FI)*, which soon became one of the most important herbaria in the world. Most of the specimens described and/or cited in *Webbia* are still kept in it.

In 1905, and as a consequence of this multitude of activities in Plant Systematics and Phytogeography, Ugolino Martelli established the journal *Webbia-Raccolta di Scritti Botanici*, firstly published annually in a single issue, and later twice a year. Webbia also began to be a place of publication of contributions from Tropical Botany, especially after the Royal Colonial Herbarium founded in 1904 in Rome was moved to Florence in 1914, currently named Tropical Herbarium Study Center (Centro Studi Erbario Tropicale - Herbarium FT) belonging to the Department of Biology of the University of Florence.

Webbia had been created in honor of **Philip Barker Webb** (1793-1845), a close friend of Filippo Parlatore, who before passing away entrusted his personal herbarium and a library rich of old botanical books and publications to the then Botanical Museum in Florence.

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Cover images: Clockwise: Portrait of Sabin Berthelot in 1879 (courtesy of *Société de Géographie* and *Bibliothèque Nationale de France*, Paris). Flowering branch of *Berthelotia sericea* (Nutt.) Rydb. (accepted name *Pluchea sericea* (Nutt.) Coville), photo credit: Neal Kramer. Shop where Ayurveda medicinal plants are commonly sold, Kozhikode, Kerala, India, photo credit: Rahul R. Pathirickal. Capitula of *B. lanceolata* DC. (accepted name *P. lanceolata* (DC.) C.B. Clarke), photo credit: Nindhan Singh.

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Honoring Sabin Berthelot: Nomenclature and botanical history of *Berthelotia* DC. (Asteraceae, Inuleae)

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Abstract. The genus *Berthelotia* DC. (presently a synonym of the accepted name *Pluchea* Cass.), Asteraceae, Inuleae has been regarded as comprising two species, *B. lanceolata* DC. and *B. sericea* (Nutt.) Rydb. It honors the French naturalist Sabin Berthelot (1794–1880) for his contribution to the studies of the flora and fauna of the Canary Islands. Under the leadership of Philip B. Webb (1793–1854), Berthelot co-authored the most important work pertinent to the natural history of this archipelago: *Histoire Naturelle des Îles Canaries*. The nomenclature of *Berthelotia* and its associated names are revisited here. The holotype of *Polypappus sericeus* Nutt. (K000974653) was located, and lectotypes were assigned to *B. lanceolata* (G00450458) and *B. lanceolata* var. *senegalensis* DC. (G00452584). Phylogenetic analyses based on nucleotide sequences of the nrDNA ITS were conducted focusing on members of what is known as the Clade J of the Inuleae. A well-supported clade (100% P.P.) that we have labeled as "Clade I" was uncovered, and it is composed of three species that belong to the *Pluchea indica* Clade plus *P. dioscoridis* and *P. ovalis*; but this lineage did not support a previous hypothesis to include *P. lanceolata*. The two species of *Berthelotia* did not form a monophyletic group and seem to be distantly related; furthermore, none of these two taxa is sister to

Tessaria, a South American genus that has been suggested to be a close relative to *Berthelotia*. Ethnobotanical uses of *B. lanceolata* and *B. sericea* are reviewed.

Keywords: Asteraceae, *Berthelotia*, Canary Islands, India, Sonora Desert, Mexico, USA, Nomenclature, Phylogeny, Sabin Berthelot, Philip Barker Webb, Taxonomy.

INTRODUCTION

The botanical history of the Canary Islands has been highly influenced by the British botanist Philip B. Webb (1793–1854) and the French naturalist Sabin Berthelot (1794–1880; Figure 1). They were the editors and main authors of the *Histoire Naturelle des* Îles *Canaries* (thereafter "*Histoire Naturelle*"), a multivolume work published between 1835 and 1850, that includes the *Phytographia Canariensis* (thereafter "*Phytographia*"), the most important single work ever published on plant taxonomic diversity of the Canaries (Webb and Berthelot 1835–1850).

The journal *Webbia* honors Webb in recognition for establishing in his will that his extensive herbarium, botanical documents, and personal house located in Paris were to be bequeathed to the Grand Duke of Tuscany Leopold II of Lorraine (1797–1870). After the demise of Webb, his house in Paris was sold to establish an endowment for the curation of the archives and herbarium collection that he donated to Tuscany. Currently, Webb's specimens are a central part of the FI herbarium of the *Sezione Botanica "F. Parlatore"* of the Natural History Museum of the University of Florence, constituting the *Herbarium Webbianum*. His entire library and archives are kept in the *Biblioteca di Scienze – Fondi di Botanica* and *Fondi Archivistici*, also at the University of Florence.

In a recent work published in the journal *Webbia*, a botanical history and nomenclature study was presented, including a discussion of the description of the genus *Webbia* by three different botanists who published this name independently, referring to three taxa belonging to different families (Asteraceae, Hypericaceae) or tribes within Asteraceae (Astereae and Vernonieae). Francisco-Ortega et al. (2022) recognized the legacy of this outstanding British botanist, and the research that we are presenting in this issue of *Webbia* is in many aspects a follow up of the work that we previously published on the genus *Webbia*, as it provides nomenclature and historical insights into *Berthelotia* DC. (Asteraceae, Inuleae), a genus that the Swiss botanist Augustin Pyramus de Candolle (1778–1841, Figure 1) dedicated to Sabin Berthelot encompassing two species, *B. lanceolata* DC. and *B. sericea* (Nutt.) Rydb.

There have been a few extensive studies pertinent to the life of Sabin Berthelot (e.g., Zerolo 1881; Drouin 2007; Instituto de Estudios Canarios 1980; Santos-Guerra 2016; Le Brun 2016, 2020), and he also wrote autobiographical accounts (1838–1840, 1980) that help to reconstruct the most relevant aspects of his life. Therefore, a full biog-

Figure 1. Portraits of relevant botanists involved in the botanical history of *Berthelotia* DC. (Asteraceae, Inuleae). (A) French naturalist Sabin Berthelot to whom *Berthelotia* is dedicated. (B) Swiss botanist Augustin Pyramus de Candolle who described *Berthelotia* and *B. lanceolata* (accepted name *Pluchea lanceolata* (DC.) C.B. Clarke). (C) British botanist Thomas Nuttall who described *Polypappus sericeus* (accepted name *Pluchea sericea* (Nutt.) Coville). (D) Swedish-American botanist Per A. Rydberg author of the combination *Berthelotia sericea* (Nutt.) Rydb. (A) Courtesy of the *Museo Municipal de Bellas Artes*, Santa Cruz de Tenerife, image kindly provided by Carlos Gaviño de Franchy. (B) Courtesy of the Royal Botanic Gardens, Kew. (C) Courtesy of Harvard Art Museums. (D) Courtesy of Hunt Institute for Botanical Documentation.

raphy regarding his achievements and challenges are outside the scope of this contribution. However, information pertaining to his botanical contributions should be reviewed in order to fully understand why one of the most important plant taxonomists of the 19th century, Augustin P. de Candolle, described the genus *Berthelotia* to honor him. An overview of Berthelot's biography and botanical work is therefore presented below.

Sabin Berthelot: A biographical sketch

Unlike Webb, Berthelot did not have any formal academic training in biological disciplines. Born in Marseille (April 4, 1794), between 1804 and 1809 he only undertook secondary education studies in the Liceum of Marseille (also known as *Lycée Impérial*). Afterwards, he served in the French Navy from 1809 to 1814. Berthelot was uninterested in formal academic studies, and this could explain why he never pursued higher education (Le Brun 2016: 64–65). After this military service, he started to work as a civil sailor in 1816, and little is known about his professional activities between this year and 1819 (Le Brun 2016: 70–71). He arrived in the Canary Islands (Tenerife) in 1820. Berthelot (1838–1840: 11–14) indicated that he reached the Canaries by coincidence, as a trip he took to Senegal had several misfortunes that caused the vessel in which he travelled to change route to Tenerife. Once in the Canaries, in May 1824 he opened a high school in the city of La Orotava under the name of *Liceo de la Orotava*, but this initiative was halted in April 1825 by the Catholic clergy as they opposed the establishment of liberal education centers in Tenerife (Le Brun 2016: 120). During this time he was also connected with the Botanic Garden of La Orotava through its founder Alonso de Nava y Grimón (*VI Marqués de Villanueva del Prado*). During this time, in his publications, Berthelot (1827a, 1827b, 1828a, 1828b) cited his job title as the "Director" of the garden; however, he never had any official appointment for this or any position in this garden (Le Brun 2016: 121). Founded in 1788, it is the oldest botanic institution of Macaronesia and the second oldest of Spain. It was part of a network of *Jardines de Aclimatación* (acclimation gardens) that the Spanish Crown had in the Canaries and mainland Spain (Puerto Sarmiento 1988: 201; 2002: 34; Cioranescu 2010: 150–151). Berthelot (1844) had an interest in plant acclimation protocols to cultivate plants into temperate regions, and this could explain Alonso de Nava's interest in having him associated with this botanic garden.

In 1828, eight years after his arrival to the archipelago, he met Webb in one of the gorges located near the main harbor of the island in Santa Cruz de Tenerife. It seems that they met by chance when they were botanizing in this area (Santos Guerra 2007); importantly, this casual meeting led to a massive joint project that resulted in the afore-mentioned work entitled *Histoire Naturelle des* Îles *Canaries*. Webb's original plan was to continue his travel to Brazil to study its natural history; however, his plans were cancelled, and for two years Webb and Berthelot travelled through the archipelago, with Berthelot employed by Webb as his field assistant. Together they collected natural history specimens and gathered information pertinent to the Canarian flora, fauna, geography, and geology; with the exception of La Gomera and El Hierro, they visited all islands (including La Graciosa) (Relancio Menéndez and Breen 2006: 38, 56, 61). In 1830, Webb and Berthelot traveled back to Europe aiming to produce an extensive account on the natural history of the Canaries. After this long trip across the continent, they arrived in Paris in 1833.

Webb and Berthelot had several disagreements regarding the structure and focus of this work that was exacerbated by 1841 when their partnership collapsed (Suárez Martín 2018: 169–170). Webb's vision was to follow the approach done in other well-known natural history or flora projects that were published by naturalists such as Ruiz and Pavón (1794) in their *Florae Peruvianae, et Chilensis* or Augustin de Saint-Hilaire (1829–1833) in his *Flora Brasiliae Meridionalis*. These were based mostly on solid taxonomic treatments and were richly illustrated with color plates. In contrast, Berthelot preferred that an important component of the work to be written in French, mostly including aspects pertinent to the ethnography, society traditions, and history of the Canaries. These topics were rarely covered in other books that followed a scientific approach to the natural history of a particular area during this time. The volume-parts of the *Histoire Naturelle* that had Berthelot as the single author were written in French and did not have any formal plant taxonomic components (Berthelot 1835–1842, 1836–1839, 1838–1840, 1840–1842). Regarding Berthelot's botanical contributions to this project, he was the sole author of the *Geographie Botanique*, totaling 181 pages and represents the first part of Volume 3 of this work (Berthelot 1835–1842).

All taxa described in the *Phytographia* that involved Berthelot had him as the second author and Webb as the first author. Authorship of this extensive floristic and taxonomic treatment and of the *Géographie Botanique* was one of the reasons behind the intense arguments that they had in 1841 and 1842 (Suárez Martín 2018: 171, 213, 219), and it is likely that they also pertained to the author order of the new taxa included in the *Phytographia*. As far as we are aware, prior to his involvement in the *Histoire Naturelle*, Berthelot (1827b) validly published only a single new species by himself, the Tenerife endemic *Viola teydea* Berthel. (alternative name *V. canariensis* Berthel.); however, this is a heterotypic synonym of the previously validly published species *V. cheiranthifolia* Bonpl. in 1807[1]. Unlike Berthelot's background, Webb had a solid botanical training acquired in Oxford, with expertise in classical Greek and Latin languages, and importantly a good understanding of taxonomic methods and protocols. After their split in 1842, Berthelot followed other endeavors that included traveling across the Western Mediterranean to perform fisheries studies for the French government (Le Brun 2016: 285–291). Eventually, he returned to Tenerife in 1847 to start working in the French Consulate of the island, where in due course he was appointed First Class Consul in 1874 (Le Brun 2016: 360).

After 1842, several parts of the *Phytographia* were published until the project ended in 1850. Between 1842 and Webb's death (31 August 1854) they exchanged nine letters (Suárez Martín 2018: 38–39, 216–239, pers. comm.). However, only one of the letters (October 11, 1844) makes reference to galley proofs revised by Berthelot and discussions regarding the meaning of common plant names from the Canaries, suggesting that in spite of the intense arguments they had in 1842, Berthelot was still somehow associated with the *Histoire Naturelle* project.

Based on his life-time publication record, it is evident that the vast majority of Berthelot's single-author publications were devoted to the general public and had a wide range of topics. Appendix 1 lists the 29 botanical publications authored by Berthelot, 17 of them focused on the Canary Islands and the rest covered introductory works to Caribbean plants, ivies, larches, Orchilla lichens, palms, violets, and the importance of plant acclimation. These popular publications were important outreach initiatives aimed at generating interest in the general public, particularly those published in Spanish for Canary Islands magazines such as *Revista de Canarias*, *Eco de Comercio*, and *El Amigo del País* contributed to increase public awareness for the study and conservation of the botanical history heritage of these islands.

The importance of Berthelot's contributions to the botanical research history of the Canary Islands cannot be understated as he assisted Webb to make his floristic and taxonomic assessments. As Webb's project employee, Berthelot was actively involved in organizing the herbarium collections that were used for the extensive floristic component of this work, and there is no doubt that they had discussions pertinent to the identification of the plant material that was examined for the *Phytographia*. Indeed, when Candolle (1836: 375) described the sunflower genus *Berthelotia* as a homage to Berthelot's, he states "Fruticulum elegantem dicavi cl. [clarissimus] Berthelot florae faunaeque Canariensis strenuo ilustratori" [I have dedicated this elegant small shrub to honorable Berthelot, an active interpreter of the flora and fauna of Canary Islands], acknowledging the importance of Berthelot's input in the *Histoire Naturelle* to the study of the unique flora and fauna of these islands.

BOTANICAL HISTORY AND SYSTEMATICS

When Webb and Berthelot worked on the publication of the *Histoire Naturelle des Îles Canaries,* they were able to interact with some of the most prominent botanists from Europe. A total of 15 of the family treatments found in this work were written by some of these distinguished plant taxonomists (Relancio Menéndez and Breen 2006: 176; Santos-Guerra 2018). Among them was the Swiss botanist Alphonse Pyramus de Candolle (1806–1893), who prepared the revision of Myrsinaceae (currently merged within the Primulaceae). He was the son of Augustin Pyramus de Candolle who was the founder of the Candolle's botanical dynasty, a family based in Geneva which provided consequential botanical research contributions during four consecutive generations, with the last member of this lineage being Richard Émile Augustin de Candolle (1868–1920).

Augustin P. de Candolle did not write any text for the *Histoire Naturelle*, but he and Webb had extensive correspondence that included 38 letters housed in the *Conservatoire et Jardin botaniques de Genève– Archives* (22 letters) and in the University of Florence– Science Library (16 letters). Candolle exchanged with Berthelot only four letters, which are currently housed in the Archives of the Botanic Garden of Geneva. The content of these numerous letters includes many botanical inquires pertinent to the seminal work of Webb and Berthelot on the Canary Island flora, and it is unclear why this prominent Swiss botanist was not involved in preparing any of the plant taxonomy accounts of the *Histoire Naturelle*. Plausible reasons were that he already had a large commitment with the publication of his *Prodromus*, and that he suffered poor health during his last years of life and eventually passed away in 1841, when

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[1] Alexander von Humboldt (1769–1859) and Aimé Bonpland (1773–1858) have been usually considered as the coauthors of the species name *Viola cheiranthifolia*; however, Bonpland alone is credited for the text of the two volumes of *Plantae aequinoctiales* (title page: *"in Ordinem Digessit: Amatus Bonpland"*); Stafleu and Cowan (1979: 367, #3141) also mentioned that Bonpland is the author of the text. Therefore, the correct authorship for this species name is: *Viola cheiranthifolia* Bonpl. as indicated in the websites www.ipni.org and plantsoftheworldonline.org/

the *Phytographia* was still in progress. This section of the *Histoire Naturelle* was by far the largest (1403 pages and 287 plates) of this multivolume enterprise, and it was published in 106 independent *livraisons* (booklets) ranging from 1835 to 1850 (Stearn 1937).

Augustin de Candolle's autobiography has a few details regarding his interactions with Webb and Berthelot that confirm that they sought his help as they were preparing the *Phytographia* (Candolle 2004: 459). As already mentioned, in 1830 they moved from the Canaries to continental Europe to prepare the *Histoire Naturelle*. However, before reaching their final destination in Paris in 1833, they travelled extensively for three years in North Africa, the French Mediterranean region, Italy, and Switzerland. According to Candolle (2004: 459), during this journey Webb and Berthelot spent a few months in Geneva where they shared herbarium specimens with, and studied Canarian collections that were made by botanists who visited these islands, particularly Pierre M. Auguste Broussonet (1761–1807) from France, Antoine Courant from Switzerland, and Christen Smith (1785–1816) from Norway. Evidently, Berthelot had high respect for Candolle, and one year after he and Webb settled in Paris, he wrote a biographical account on him (Berthelot 1834) that was one of the few published biographies produced before Candolle passed away in Geneva in 1841 (Candolle 1862: 588). During his life Berthelot (1839a, 1839b) wrote only two other botanical biographies; they were for the French botanists Joseph P. Tournefort (1656–1708) and Valmont de Bomare (1731–1807).

It is worth mentioning that Augustin de Candolle's descriptions of *Berthelotia* and *Webbia* were published in volume 5 of his monumental *Prodromus* (Candolle 1836: 72, 375–376). Although, both genera belong to Asteraceae, *Berthelotia* is a member of the tribe Inuleae, whereas *Webbia* is placed within the tribe Vernonieae. The contents of two of the letters that Candolle exchanged with Webb and Berthelot reveal that he had other sunflower plants in mind to pay homage to his French and British friends. One of these letters is located in the University of Florence– Science Library (dated 13 April 1833, from Candolle to Webb, document #73.9.1–73.9.6), the second one is housed in the Archives of the library of Botanic Garden of Geneva (dated 12 July 1833, from Berthelot and Webb to Candolle, unnumbered document). The second of these letters has a more extensive account regarding Candolle's plans to describe *Berthelotia* than the other one. Therefore, an annotated translation of the latter is presented in Appendix 2, since it also deals with the taxonomic identity of Asteraceae specimens that Webb and Berthelot examined for their Canary Island flora work.

These two letters indicate that Candolle's original plan was to describe *Webbia* based on plants growing in Greece (Francisco-Ortega et al. 2022). However, Candolle (1836: 72) eventually described *Webbia* based on collections from Africa and not from the eastern Mediterranean. Nevertheless, *Webbia* DC. is illegitimate, as it is preceded by the earlier name *Webbia* Spach (Guttiferae = Hypericaceae). Therefore, those species that were placed in *Webbia* DC. are currently accommodated in the Vernonieae genera *Crystallopollen* Steetz or *Hilliardiella* H.Rob. (Francisco-Ortega et al. 2022; J.C. Manning pers. comm.; Manning and Govaerts in prep.).

The information found in these two letters is also relevant to the botanical history of *Berthelotia*. From these letters it appears that, at least initially, Candolle wanted to publish the new genus *Berthelotia* to accommodate both the Canarian-Salvagen endemic *Chrysocoma sericea* L.f., and a new species, restricted to Gran Canaria, that was going to be named "*B. glaberrima*." It seems that when Candolle exchanged these letters, he was unaware of *Schizogyne* Cass. (Asteraceae), which was published earlier by Cassini (Cuvier 1828). *Schizogyne*, endemic to the Canary and Savage islands, is the taxonomic choice to transfer *Chrysocoma sericea* to a different genus and to accommodate the new taxon to which Candolle assigned the species epithet of "*glaberrima*." Later, Candolle (1836: 75) was aware of *Schizogyne* as the taxonomic option for these rearrangements, because in the same work in which he described *Berthelotia* (Candolle 1836: 375–376), he made the new combination *S. sericea* (L.f.) DC. and validly published *S. glaberrima* DC. as a new species (Candolle 1836: 473). Candolle's plans to dedicate a genus name to Berthelot did not change, and he still described *Berthelotia*, but it was not based on Canary Islands plants but on a species that he named *B. lanceolata* DC. (Candolle 1836: 376, Figures 2-3), which has a relatively widespread distribution in India, Pakistan, Afghanistan, and Tropical Africa (Figure 4; King-Jones 2001: 82–84).

Following the well-known treatment of Bentham and Hooker (1873) on the classification of Compositae plant genera, taxonomists have not recognized *Berthelotia* as a distinct genus, and currently *Berthelotia* is treated as part of *Pluchea* Cass. (Compositae Working Group – CWG 2022). Bentham and Hooker's (1873) generic concepts for the Asteraceae have been greatly influential in the taxonomic history of this family (Cronquist 1977); however, from their work it is not readily evident whether Bentham and Hooker (1873: 291) recognized *Berthelotia* (printed in small cap font) as part of *Pluchea* but without any taxonomic rank, or they (1873: 550) proposed *Berthelotia* [printed in italics and regular font] to be a section

Figure 2. Selection of photos pertinent to *Berthelotia lanceolata* DC. (accepted name *Pluchea lanceolata* (DC.) C.B.Clarke). (A) Shop where Ayurveda medicinal plants are commonly sold, Kozhikode, Kerala, India. (B) Flowering branch. (C) Leaf fragments as sold in medicinal plant shop. (D) View of capitula in full flowering through stereomicroscope. Photo credits: Rahul R. Pathirickal (A, C, D), and D.S. Rawat (B).

Figure 3. Illustration of *Berthelotia lanceolata* DC. (accepted name *Pluchea lanceolata* (DC.) C.B.Clarke) published by Delessert and Candolle (1840). Drawn by Jean-Christophe Heyland (1791–1866) and engraved by Eugenia Taillant (?–?). Courtesy of the Linnean Society of London.

of *Pluchea*. Nevertheless, it was not Bentham and Hooker, but Clarke (1876: 94) who first made the legitimate transfer of *Berthelotia* to *Pluchea* when he published the combination *P. lanceolata* (DC.) C.B.Clarke. *Berthelotia* can be distinguished from other species of *Pluchea s. str.* by their lack of resin ducts, flattened filament collar cells, and divided styles (Anderberg 1991b).

Pluchea lanceolata has ample ethnobotanical use in India (Shanker and Srivastava 2017; Figure 2; see section on ethnobotany below), and it can also be a noxious weed (Inderjit et al. 1998). Because of its medicinal properties and widespread distribution, this species has been the subject of phytochemical (e.g., Srivastava and Shanker 2012; Pandey 2018), in vitro culture (e.g., Kumar et al. 2004), and ecological (e.g., Inderjit 1998, 2002) studies.

Pluchea sericea (Nutt.) Coville is a species restricted to arid parts of northeastern Mexico and western United States (Figures 5–7). This species is isolated among North American *Pluchea*, with its woody perennial habit and densely arranged waxy eglandular leaves, and Nesom (2006) stated that "*Pluchea sericea* is more similar to the segregates than to the herbaceous American groups [of the genus]." In 1848, it was originally described as *Polypappus sericeus* Nutt., and in 1906 this species was accommodated in *Berthelotia* (as *B. sericea* (Nutt.) Rydb.) by the Swedish-American botanist Per Axel Rydberg (1860–1931, Figure 1) who was the first curator of the New York Botanical Garden herbarium (NYBG 2005). The species is the type of Aven Nelson's genus *Eremohylema* A.Nelson.

Pluchea sericea has also ethnobotanical applications, and among Native Americans it was used both as material for building mud houses and to make arrows (Moerman 2022). Its roots have therapeutic properties to treat diarrhea, and indigestion as well as eye and dermatological inflammations. However, this species does not have any documented wide ethnobotanical use as compared to *P. lanceolata* in India.

Both *Pluchea sericea* and *P. lanceolata* are shrubs/subshrubs with white sericeous branches and sessile lanceolate leaves. Their floral parts are similar as well, with pink to purplish phyllaries, campanulate involucres, and

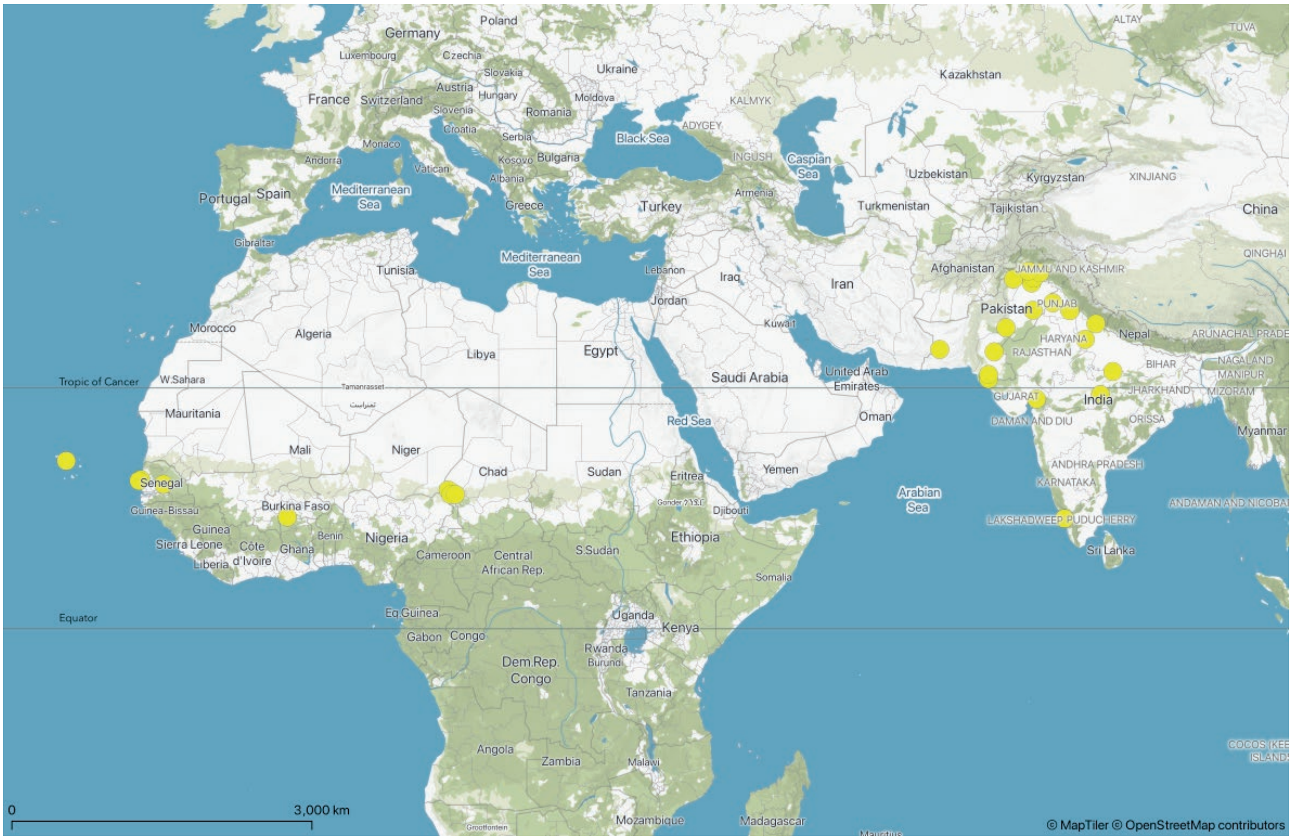

Figure 4. Distribution map of *Berthelotia lanceolata* DC. (accepted name *Pluchea lanceolata* (DC.) C.B. Clarke). Map is based on King-Jones (2001) and was created using QGIS 3.26 in conjunction with base maps from MapTiler.

pink to purplish corollas. These two species mainly differ in their growth habit, with *P. sericea* reaching up to 5 m in height (Barkley et al. 1993), while *P. lanceolata* usually reaches 1.5 m in height (King-Jones 2001). They also differ slightly in their leaves, with *P. sericea* characterized by narrower leaves (1–5 x 0.2–1 cm) than *P. lanceolata* (1–7.5 x 0.4–1.5 cm).

Molecular phylogenetics

Pluchea has been circumscribed to comprise taxa lacking the characteristic features of other smaller genera in the tribe Inuleae. Using floral micro-characters, Anderberg (1991b) found that *Pluchea* is not monophyletic. The genus as a whole lacks any diagnostic apomorphic characters; instead, it is defined by absence of characters found in related groups.

King-Jones's (2001) monograph for the Old World species of *Pluchea* included a phylogenetic analysis based on morphological data. This previous study suggested that *P. lanceolata* belongs to a monophyletic group with seven species that she informally named the "*Pluchea indica* Clade" and that was weakly supported with a low jackknife value of 63%. Additional taxonomic relationships pertinent to *Berthelotia* have been suggested by Nesom (1989, 2006), who indicated that the placement of *Tessaria* is relevant to understand the actual arrangement of *Berthelotia* in the Inuleae.

The polyphyletic nature of the genus was further reiterated by Nylinder and Anderberg's (2015) molecular phylogenetic studies based on nucleotide sequences of ITS and ETS (nuclear ribosomal DNA) and three chloroplast genome regions. In this work, *Pluchea* species are found in several different places in the recovered phylogeny, and many of the smaller genera of the tribe, such as the South American monotypic genus *Tessaria* Ruiz & Pav. (Anderberg 1994; Anderberg and Eldenäs 2007) became ingroups related to different *Pluchea* groups. Therefore, *Pluchea* as presently circumscribed is a diverse polyphyletic grade which is poorly understood and needs major taxonomic revisions.

Tessaria (one species; *Tessaria integrifolia* Ruiz & Pav.), *Pluchea indica* (L.) Less., and *P. sericea* belong

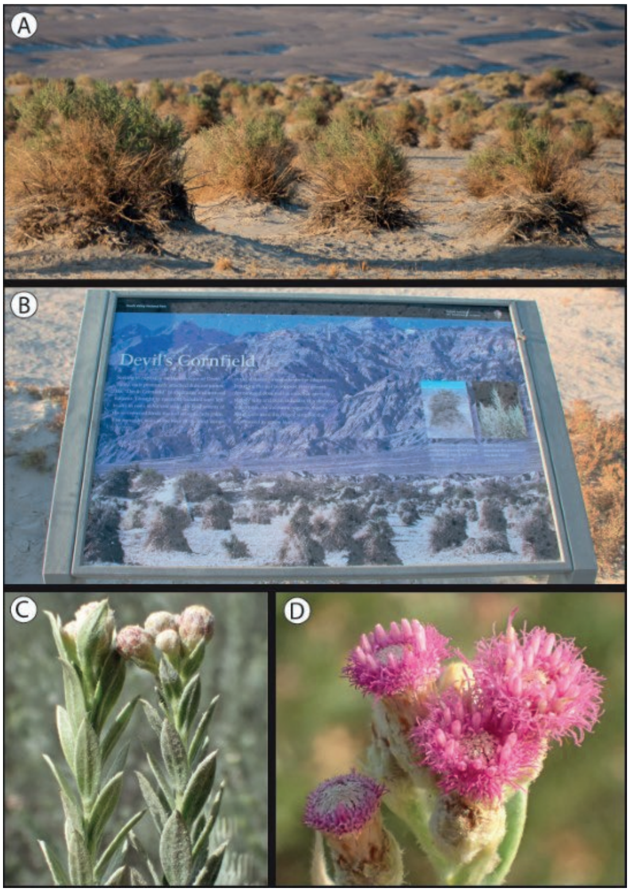

Figure 5. *Berthelotia sericea* (Nutt.) Rydb. (accepted name *Pluchea sericea* (Nutt.) Coville) in USA. (A) Plants in habitat in Dead Valley National Park, California. (B) Informative panel highlighting the ethnobotanical use of the species among Native Americans and its importance as a landmark species for the landscapes of Dead Valley National Park. (C) Young stems showing capitula in early developmental stages. (D) Capitula at different developmental stages. Photo credits: Mike Duran (A, B), Ron Vanderhoff (C, D).

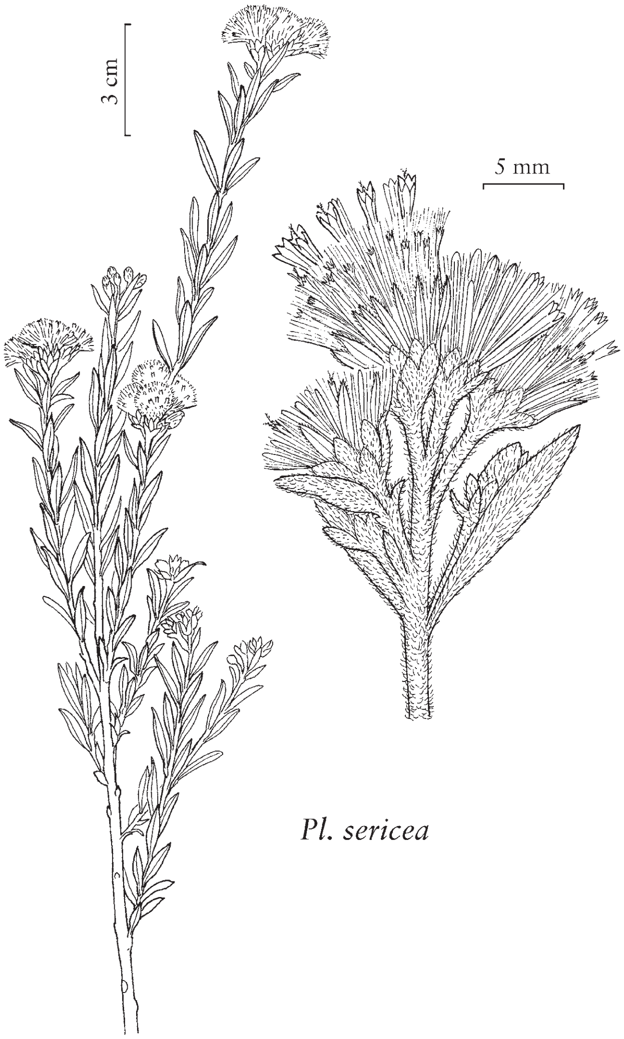

Figure 6. Illustration of *Berthelotia sericea* (Nutt.) Rydb. (accepted name *Pluchea sericea* (Nutt.) Coville). Courtesy Flora of North America Association, Volume 19; Barbara Alongi, illustrator; published by Nesom (2006).

to “Clade J” as recovered in Nylinder and Anderberg’s (2015) molecular phylogeny; however, this study did not include *P. lanceolata*. As a result, we aimed to analyze relationships across New and Old World members of Clade J of *Pluchea* to better understand relationships between the two *Berthelotia* taxa with *P. indica* and monotypic *Tessaria* as putative relatives of them.

MATERIAL AND METHODS

We chose ITS nucleotide sequences of 19 species representing two main clades of the Inuleae uncovered in recent literature, as part of Clade J in Nylinder and Anderberg (2015). They belong to the genera *Allopterigeron*, *Coleocoma*, *Epaltes*, *Pluchea*, *Streptoglossa*, *Tessaria*, and *Thespidium*, as our ingroups (including the two species described as *Berthelotia* as well as *P. indica*). Two species of *Epaltes,* which were found to be sister to Clade J (Nylinder and Anderberg 2015), are used as outgroup. For each species selected, ITS sequences were downloaded from GenBank (accessions in Table 1); all of them were previously published by Nylinder and Anderberg (2015) except that of *P. lanceolata* the unpublished one which was obtained by Rahul Pathirickal et al. in 2017. ETS data were not available for *Pluchea lanceolata*; therefore, we did not include this marker in our study.

Alignments were performed in MAFFT v.7 (Katoh and Standley 2013) for ITS. Matrices were visually

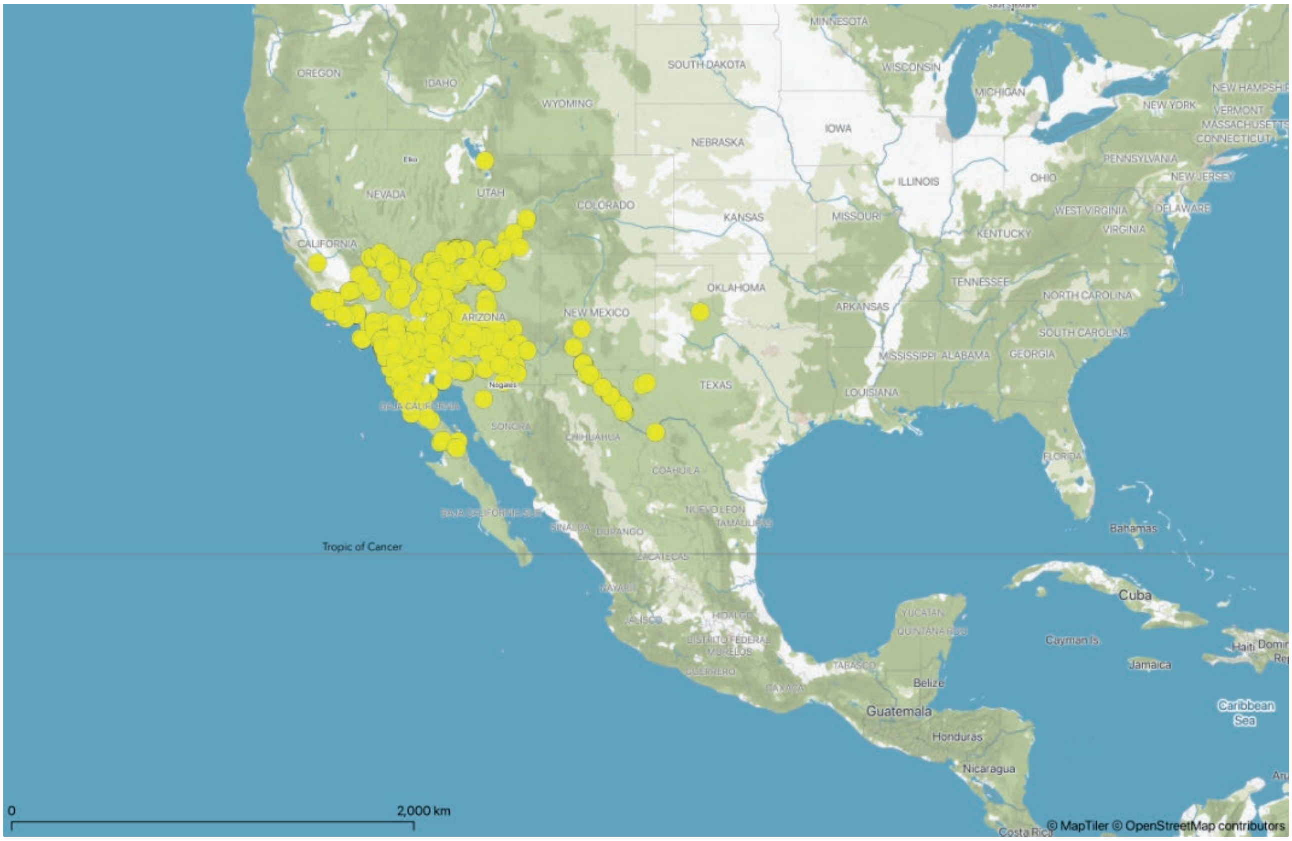

Figure 7. Distribution map of *Berthelotia sericea* (Nutt.) Rydb. (accepted name *Pluchea sericea* (Nutt.) Coville). Map was created using QGIS 3.26, in conjunction with base maps from MapTiler

inspected in order to check for false homologies. Phylogenetic analyses were performed across maximum likelihood and Bayesian methods. For the maximum likelihood analysis, the ITS gene tree was inferred for each of the individual gene alignments using RAxML v.8 (Stamatakis 2014), with 100 bootstraps (BS) using the GTRGAMMA model. For the Bayesian analysis, MrBayes was run for 1 million generations under the GTRGAMMA model, with 20% burn-in removed.

RESULTS AND DISCUSSION

The aligned length of the concatenated 21-sample, ITS matrix was 844 bp. For our Bayesian analysis, we recovered strong phylogenetic signal in the ancestral nodes of the ingroup, with low support for the clade including the members of *Tessaria* and *Pluchea* from both the New and Old World (68% posterior probability, P.P.). An additional clade with strong posterior probability support, identified here as "Clade I", was recovered. Clade I is composed of three species of the *Pluchea indica* Clade (i.e., *P. indica*, *P. bojeri*, and *P. lyciodes*) plus *P. dioscoridis* and *P. ovalis* (Figure 8). The latter two species were placed inside the "*Pluchea discoridis* clade" by King-Jones (2001). *Allopterigeron filifolius*, *P. caroliniensis* and *P. sericea*, formed a polytomy with the clade that has the rest of the species included in our analyses (Figure 8). Clade I exhibited equally high support (100% P.P., Figure 8) and the lineage composed of *P. sagittalis* and *Coleocoma centaurea* was also strongly supported (90% B.S.). Therefore, our analyses did not support the *Pluchea indica* Clade as a monophyletic group as defined by King-Jones's (2001) treatment. Maximum likelihood analysis of our ITS dataset showed similar results to our Bayesian analyses, with Clade I members resolved with high bootstrap support (99 B.S.). Almost all of the other shallower nodes in the ingroup had very low support.

In comparison to previous phylogenetic studies of the Inuleae and *Pluchea* (Anderberg 1991b; King-Jones 2001; Nylinder and Anderberg 2015), we found similarly low resolution at shallow nodes, however, our inclusion of *P. lanceolata* here reveals that: (1) the two species of *Berthelotia* are perhaps more distantly related than pre-

Table 1. Species included in the ITS phylogenetic analyses, along with their GenBank accession numbers, and their voucher specimen numbers (with herbaria acronyms). All sequences published by Nylinder and Anderberg (2015) except that of *Pluchea lanceolata* (MG273761) that is published by us in this contribution.

Species	GenBank	Vouchers
Allopterigeron filifolius (F.Muell.) Dunlop	LN607417	*Short & Dunlop 4758* (DNA)
Coleocoma centaurea F.Muell.	LN607432	*Albrecht 10563* (S)
Epaltes australis Less.	LN607444	*Anderberg & Anderberg 7938* (S)
Epaltes divaricata (L.) Cass.	LN607446	*Bremer et al. 43* (S)
Epaltes gariepina (DC.) Steetz	LN607447	*Wanntorp & Wanntorp 769* (S)
Pluchea bojeri (DC.) Humbert	LN607514	*Malcomber & Leeuwenberg 1137* (S)
Pluchea dentex Benth.	LN607516	*Crawford 1076* (CANB)
Pluchea dioscoridis (L.) DC.	LN607516	*Ryding & Ermias 1279* (UPS)
Pluchea dunlopii Hunger	LN607519	*Hunger & Kilian 3948* (B)
Pluchea fastigiata Griseb.	LN607520	*Beck & Lieberman 9613* (S)
Pluchea indica (L.) Less.	LN607524	*Kilian et al. NK 4601* (S)
Pluchea lanceolata (DC.) C.B.Clarke	MG273761	*S.P. Geetha 8769* (CMPR)
Pluchea lycioides (Hiern) Merxm.	LN607528	*Smook 765* (WIND)
Pluchea ovalis (Pers.) DC.	LN607534	*Gilbert & Thulin 328* (UPS)
Pluchea sagittalis (Lam.) Cabrera	LN607539	*Chung & Anderberg 1171* (S)
Pluchea sericea (Nutt.) Coville	LN607542	*Davis & Lightowlers 66328* (B)
Pluchea yucatanensisi G.L. Nesom	LN607546	*Jones & Jones 12656* (TEX)
Tessaria integrifolia Ruiz & Pav.	LN607592	*Daly et al. 6392* (S)
Thespidium basiflorum (F.Muell.) F.Muell.	LN607593	*Cowie & Dunlop 3923* (S)

viously thought, (2) *P. lanceolata* is not as closely related to *P. indica* as suggested by the morphological studies of King-Jones (2001), and (3) the *Pluchea indica* Clade is paraphyletic with *P. lanceolata* placed in a weakly supported polytomy (68% P.P.) made up of (1) eight additional species of *Pluchea*, *Epaltes*, *Thespidium*, and *Streptoglossa*; (2) Clade I; and (3) the assemblage composed of *P. sagittalis* and *Coleocoma centaurea*.

As indicated above, the monotypic genus *Tessaria* is placed in a weakly supported clade (68% P.P.) that has all the sampled species of *Pluchea*, except *P. carolinensis* (Jacq.) G. Don and *P. sericea* (Figure 8). If future studies confirm these relationships with greater phylogenetic resolution, this will have a profound impact on the taxonomy of the group, as the name *Tessaria* (described in 1794) holds precedent over the younger name *Pluchea*, which was only described in 1817. *Tessaria* also has priority over *Berthelotia* (described in 1836) and the other genera of Clade J (*Allopterigeron*, described in 1981; *Colecoma*, described in 1857; *Epaltes*, described in 1818, *Thespidium*, described in 1862; and *Streptoglossa*, described in 1863). Even if a large number of *Pluchea* species were renamed within a larger *Tessaria*, the latter genus would still likely be polyphyletic under current taxonomic definitions, and because of the phylogenetic placement of other genera that compose the Inuleae; therefore, this would require extensive nomenclatural modifications in order to have a species-rich monophyletic *Tessaria* (Nylinder and Anderberg 2015). Within the J Clade several ways of dealing with these generic mismatches can be proposed once nodes of these clade are better resolved; one of them would involve reinstating genera such as *Berthelotia* as described by Candolle (1836) and creating new monophyletic genera (likely most of them with few species). Such a solution could lead to consider *B. sericea* as a monotypic genus, in that case it will need to be treated as *Eremohylema sericea*. With *Tessaria* currently restricted to *T. integrifolia*, another solution would be for *Tessaria* to be expanded more broadly to include the two species listed within *Berthelotia* (*P. sericea* and *P. lanceolata*) and the other members of the J Clade.

ETHNOBOTANY AND MEDICINAL USE OF *PLUCHEA LANCEOLATA*

The ethnobotanical use of *Pluchea lanceolata* in India has its roots in the ancient Ayurveda, a natural system of medicine still widespread in the country (Figure 2), which originated more than 3,000 years ago and was part of the Vedic sacred literature of India (Padma 2005). The age of Ayurveda has been traced back

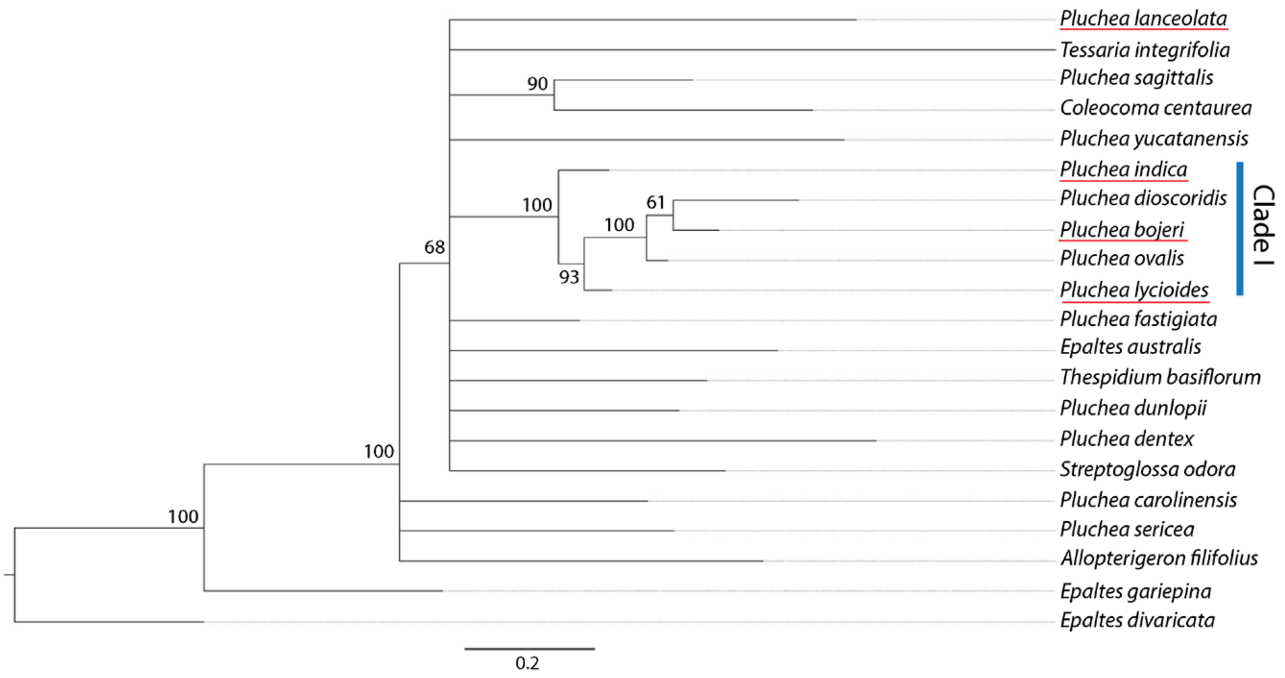

Figure 8. Bayesian topology for *Pluchea* species and related genera belonging to Clade J sensu Nylinder & Anderberg (2015) with *Epaltes divaricata* and *E. gariepina* as the outgroup. Species underlined in red were previously described as belonging to the "*Pluchea indica* Clade" (King-Jones, 2001). Node labels refer to Posterior Probability scores.

between 4500 and 600 BC (Kapoor 1990). The Ayurvedic plant naming structure does not have a binomial system of nomenclature as in Western botany, but it is based on polynomials, where one plant can be defined by a group of synonyms (one to fifty), each relating to its characters; such as morphology, habit, habitat, qualities, biological actions, therapeutic use, etc. Sometimes a synonym can be given to more than one plant resulting in a therapeutic "taxonomy" that helps an Ayurvedic physician in selecting a plant for its medicinal use (Viswanathan et al. 2003). This has also led to confusions, as technically precise descriptions of plants are not given. Therefore, this system of having numerous names for a single medicinal plant does not provide any morphological features sufficient for establishing its botanical identity. India is a place of diversity in all its forms including use of various languages/dialects in different regions, many tribal practices and folklore medicines contributing to use more than one name for the same plant, and sometimes different plants are having same names in different states of the country.

Only five species of *Pluchea* have widespread use as medicinal plants (Shanker and Srivastava 2017: 35–39). Three of them are from the New World (i.e., *P. carolinensis* (Jacq.) G.Don, and *P. odorata* (L.) Cass., *P. sagittalis* (Lam.) Cabrera), and at least one of them has been the subject of ethnobotanical field studies (Hodges and Bennett 2006). The aforementioned *P. indica* also has medicinal value in India and Southeast Asia (Shanker and Srivastava 2017: 35). Eleven additional species are also reported to have ethnobotanical interest, among them is *Pluchea sericea* (Shanker and Srivastava 2017: 35, 39).

Though plants of *Pluchea lanceolata* are commonly known as "rasna" in northern parts of India, they are also known by many other names in different regions of the country [i.e., rayasana, phaar (Hindi), rasnapat (Assam), chithramoolaka, dumme-rasna (Kannada), rasna, rashna, rayasana (Marathi), sanna rashtramu (Telugu), reshae, raasana, reshamee-sunnai, ra-sunna (Punjabi)]. The word "rasna" means "the process of tasting" or "perceptible by tongue". The species has been described in Sanskrit using many synonyms such as elaparni, mukta, rasna, rasya, sreyasi, sugandha, surabhi, surasa, suvaha, yukta. Consequently, the Ayurvedic text (Sanskrit) for rasna has been interpreted in different ways. The term "elaparni" could be interpreted for plants that have their leaf shape similar to the shape of Cardamom fruits (*Elettaria cardamomum* (L.) Maton), as considered by the northern Indian Ayurvedic physicians (Vaidyas). However, it can also be interpreted as plants bearing leaves whose shape is similar to the leaf shape of Cardamom, as it usually occurs in southern parts of India.

The words "sugandha" and "suvaha" can refer to tubers having fragrance, which can be found in different species of plants. Thus, while the leaves of *P. lanceolata* are mostly used in northern India, the rhizomes of *Alpinia galanga* (L.) Willd. (Zingiberaceae) are widely utilized in the Southern parts of India. They both share the Ayurvedic name of rasna and have similar therapeutic properties. Notwithstanding the controversy regarding the identity of the species, the Ayurvedic Pharmacopeia of India (API 2001) has recognized *P. lanceolata* as the plant "rasna" whose leaves (Figure 2) and roots are recommended for preparation of various herbal formulations, and *A. galanga* as the approved substitute in the Ayurvedic Formulary of India (AFI 2003).

In practice, the leaves of *Pluchea lanceolata* are usually traded as rasna, rasnapatti, vaaya-surai, or baisurai, while its roots are mostly traded as rasnamoola. However, at least 17 other plant species have their parts traded under the name rasna in various parts of the country (Viswanathan et al. 2003; Khare 2007; FRLHT Database 2016). A few examples of them include plants whose rhizomes or roots have medicinal use [e.g., *Alpinia galanga*, *A. calcarata* (Andrews) Roscoe, *Leucoblepharis subsessilis* Arn. (Asteraceae); and *Vanda tessellata* Roxb. (Orchidaceae)]; and others whose leaves exhibit similar therapeutic properties (e.g., *Dodonaea viscosa* (L.) Jacq., Sapindaceae). This medicinal plant classification system has made identification of the right material for Ayurvedic medicine preparation a daunting task. Some of the important Ayurvedic formulations containing rasna (*P. lanceolata*) are dasamularishta, devadarvarishta, karpasasthyadi taila, rasnadi kvatha churna, rasnairandadi kvatha churna. Some of these formulations are for ingestion (arishta/churna) and some are for external applications (taila/churna) (Shanker and Srivastava 2017). However, drug formulations containing rasna (*P. lanceolata*) are all indicated for treatment of inflammation, rheumatism, asthma, fever, dyspepsia, skin diseases, rheumatoid arthritis, gout, etc. Other of the widespread ethno-botanical uses associated with *P. lanceolata* in India include its action as a laxative, nervine tonic, uterine relaxant, and pain relief from scorpion stings. In the former Sind region of British India, the species was known as koura-sana or koura-sunna. Its leaves were used as aperient medicine, and the roots were regarded as an excellent purgative (Murray 1881; Dymock et al. 1891; Khare 2007).

In the traditional Ayurvedic treatments and literature there are references for the use of *Pluchea lanceolata* in poly-herbal formulations as it was never recommended as a single herb. Nevertheless, there has been a steadily increasing trend for naturopathic herbal companies to promote the use of single-herbs (such as rasna) that come from traditional Ayurvedic medicine. These companies are also making new combinations of Ayurvedic drug formulations, resulting in Ayurvedic medicinal patents that are associated with property rights (Patwardhan 2016). Products such as rasna churna (churna translates as powder), rasna capsules, and rasna tablets, which are not prescribed in the Ayurvedic classical medicine texts, fall in this category. They are marketed as herbal dietary supplements and advertised as having similar beneficial attributes as rasna formulations that have been used in India for millennia.

TAXONOMY & NOMENCLATURE

Berthelotia DC.[2] (Asteraceae, Inuleae).

Berthelotia DC., Prodr. 5: 375. 1836.

(≡) *Pluchea* Cass. subgen. *Berthelotia* (DC.) A. Gray, Syn. Fl. N. Amer 1(2): 225. 1884.

Type: *Berthelotia lanceolata* DC., Prodr. 5: 375–376. 1836.

(=) *Eremohylema* A.Nelson, Univ. Wyoming Publ. Sci., Bot. 1(3-4): 54. 1924, *nom. superfl. & illegit.* for *Berthelotia* DC.

Type: *Eremohylema sericea* (Nutt.) A.Nelson, Univ. Wyoming Publ. Sci., Bot. 1(3-4): 54. 1924 [Basionyn: *Polypappus sericeus* Nutt., Proc. Acad. Nat. Sci. Philadelphia 4: 22. 1848].

(=) ***Pluchea*** Cass., Bull. Sci. Soc. Philom. Paris 1817: 31. 1817.

Type: *Pluchea marilandica* (Michx.) Cass. in F.Cuvier, Dict. Sci. Nat., ed. 2 42: 2. 1826.
['*marylandica*'] [Basionym: *Conyza marilandica* Michx. Fl. Bor.-Amer. 2: 126. 1803].

Berthelotia lanceolata DC., Prodr. 5: 376. 1836.

(≡) *Berthelotia lanceolata* var. *indica* DC., Prodr. 5: 376. 1836.

(≡) ***Pluchea lanceolata*** (DC.) C.B.Clarke, Compos. Ind.: 94. 1876.

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[2] Accepted names indicated in bold font.

(≡) *Pluchea lanceolata* (DC.) Oliv. & Hiern in Oliv., Fl. Trop. Afr. 3: 329. 1877, *isonym*.

(≡) *Conyza lanceolata* Wall., Numer. List n. 2991. 1831, *nom. nud., inval.*

Type: "*Conyza lanceolata* Wall.! Cat. et herb. n. 2991. comp. n. 101". *N. Wallich 101*, *Conyza lanceolata* Wall. in herb. / 1830 [Lectotype designated here, G [G00450458 (photo!)]; isolectotypes, G [G00450438 (photo!)], K [K000974690 (photo!)], E [E00098190].

(=) *Conyza rubra* Buch.-Ham. in Wall., Numer. List n. 2991B. 1831, *nom. nud., inval.*

(=) *Saussurea mucronata* Spreng. ex DC., Prodr. 5: 376. 1836, *pro syn., inval.*

(=) *Berthelotia lanceolata* var. *senegalensis* DC., Prodr. 5: 376. 1836.

(=) *Conyza proteifolia* Perr. ex DC., Prodr. 5: 376. 1836 ["*proteiformis*"], *pro syn., inval.*

Type: [Senegal] "In arenosis circa Lamsar [Lampsar] et Gandiole mense junio legit cl. Perrottet. *Conyza proteifolia* ("*proteiformis*") Perr.! in litt. Folia infer. ex Perr. in litt. magis serrata. (v.s. comm. a cl. inv.)" (sic). Lectotype designated here: 15-June-1825, *G.S. Perrottett 2* /*Conyza proteifolia* nob.: G [G00452584 (photo!)].

Typification notes

Berthelotia DC. is typified by *B. lanceolata* DC.; Nelson (1924: 55) erroneously treated *Berthelotia* as a later homonym (non *Bertholletia* Bonpl. 1807) and included *Berthelotia* as a synonym of *Eremohylema* A.Nelson, and thus the latter genus name was superfluous and illegitimate when published. Nevertheless, Nelson cited *E. sericea* (Nutt.) A.Nelson as the type of *Eremohylema*. Therefore, *Eremohylema* was published as the name of a new genus, has its own type, and is not typified by the type of *Berthelotia* (see Shenzhen Code Art. 7.5).

Candolle (1836: 375–376) treated *Berthelotia* as a monospecific genus, and he included two varieties, viz., *B. lanceolata* DC. var. *indica* DC., and var. *senegalensis* DC. He did not include *B. lanceolata* DC. var. *lanceolata* or var. *typica*, or any such autonym. He did not cite any type collection for the species name *B. lanceolata*, but he did cite collections for vars. *indica* and *senegalensis*. His specific description for *B. lanceolata* was limited to a short text stating: ♄ *frutic. pedalis. An species forsan duae admittendae?*, which indicates Candolle's belief that the species was composed of two morphs (var. *indica* and var. *senegalensis*). Nevertheless, Candolle inadvertently created the autonym *B. lanceolata* DC. var. *lanceolata* (vide Art. 26.3).

In the above situation, one may question whether either var. *indica* or var. *senegalensis* is homotypic with var. *lanceolata*, and if so, whether such a varietal name is valid. In this regard, Art. 26.1 of the Code states that "The name of any infraspecific taxon that includes the type of the adopted, legitimate name of the species to which it is assigned is to repeat the specific epithet unaltered as its final epithet […]". It is ascertained here that since the species name *B. lanceolata* remained untypified at the time of its publication, neither var. *indica* nor var. *senegalensis* included the type of *B. lanceolata*, that Art. 26.1 does not apply here, and that the two varietal names were validly published. We add that theoretically, even if the names *B. lanceolata* and var. *indica* were typified with the same specimen and made homotypic, the validity of the varietal name *indica* would not be affected (cf., Art. 26.2 Ex. 4).

Prior to our study, King-Jones (2001: 82) provided nomenclatural assessments for these varietal names; however, we do not concur with two of her conclusions, as addressed below. King-Jones's treatment did not include any discussion on the nomenclature of *Berthelotia*; therefore, her reasoning behind a few of her nomenclatural conclusions is not clear.

Regarding the typification of the name *Berthelotia lanceolata* var. *senegalensis*, Candolle referred to a gathering collected in Senegal (in Lampsar and Gandiole) by George S. Perrottet (1793–1870), a Swiss-French botanist who performed field work in this country from 1824 to 1829 (Stafleu & Cowan 1983: 175). One specimen housed in G (G00452584) fits the protologue quite well and is designated here as the lectotype for the variety. The protologue of this variety includes a reference to the name "*Conyza proteiformis* Perr. in litt.!" (sic). The published epithet "*proteiformis*" may imply resemblance to the habit of the genus *Protea*. The designated lectotype specimen sheet, however, shows Perrottet's annotation as "*Conyza proteifolia* nob.", which suggests that he intended to allude resemblance to foliage of *Protea*. It is evident that there was a typographical error of the epithet spelling, which was overlooked by Candolle in the publication. In our assessment, we treat "*Conyza proteiformis*" as a typographical error.

Furthermore, King-Jones' (2001: 82) conclusion pertaining to the typification of *Berthelotia lanceolata* needs to be revisited. She mentioned that Clarke (1876: 94) typified this name ("-Lectotype (designated by Clarke, Compos. Ind.: 94. 1876): [India], Cawnpure [= Kanpur 26?28'N, 80?21'E], 1830, Wallich 2991/101 (E!, G!").

However, Clarke's treatment of this species shows "*Pluchea lanceolata* i.e., Berthelotia lanceolata DC. V. p. 376 Conyza lanceolata Wall. Cat. 2991 … Hab. Ad ripam Gangis prope Cawnpore in 1820 coll. Wallich. In omni Oude, India boreali-occidentali, Punjab et Afghanistan communis. Cl. Bth. in Gen. Pars. II. p. 291 Berthelotiam lanceolatam DC. cum Pluchea wallichiana DC. conjunxit. Berthelotia lanceolata est vera Pluchea sed Conyza lanceolata Wall. Cat. No. 2991 a Conyza sessilifolia Wall. Cat. No. 3029 longe discrepat, videl …" (sic). Clarke did not indicate the type element by direct citation of the term "type" (typus) or an equivalent and thus did not meet the mandatory requirement needed for a typification of a previously validly published name. (see Art. 7.11).

Although King-Jones (2001) cited a lectotype for the name *Pluchea lanceolata*, she did not inadvertently lectotypify the name because she did not use the phrase "designated here" (*hic designatus*) or an equivalent, which is required for typifications made from 1 January 2001 (Art. 7.11). Therefore, neither Clarke nor King-Jones typified the name, and we herewith designate the lectotype (G00450438; isolectotypes: K000974690, E00098190) of this taxon.

The protologue of *Berthelotia lanceolata* var. *indica* refers to three different specimens collected by the Danish physician and botanist Nathaniel Wallich (1786–1854) or the Scottish physician and botanist Francis Buchanan-Hamilton (1762–1829). The former lived in India during 1814–1846 (Stafleu and Cowan 1988: 37), and most of his career was associated with the Royal Botanic Garden, Calcutta. Buchanan-Hamilton also resided in India (between 1795 and 1815), and worked for the Botanic Garden of Calcutta, but for a shorter period (1814–1815; Stafleu and Cowan 1979: 35). According to the protologue, these three specimens were annotated as "*Conyza rubra* Ham." (G00452579), "*Conyza lanceolata* Wall." (G00450438, G00450458), and "*Saussurea mucronata* Spreng." (P01816057). Of the preceding three names, *S. mucronata* was proposed as a pro synonym of *B. lanceolata* var. *indica* by Candolle (1836: 376) and is invalid. The two other names are found in Wallich Catalogue (http://wallich.rbge.info/node/13421) without a description; they were treated by Candolle (1836: 376) as synonyms of *B. lanceolata* var. *indica*.

Since Candolle used the specific epithet *lanceolata* to coin for his new species name *B. lanceolata*, and since he did not cite any collection for his new species, it is construed here that his inclusion of the name "*Conyza lanceolata*" as a pro synonym of var. *indica* implies that he intended to treat this variety as the typical variety of the species. Thus, among the three specimens suitable for lectotypification we have designated G00450458 as the lectotype of *B. lanceolata* and of *B. lanceolata* var. *indica* (isolectotypes: G00450438, K000974690, E00098190).

Polypappus sericeus Nutt., Proc. Acad. Nat. Sci. Philadelphia 4: 22. 1848.

(≡) *Tessaria borealis* Torr. & A.Gray, Mem. Amer. Acad. Arts ser. 2, 4(1): 75, adnot. 1849, *nom. superfl. & illegit.* for *Polypappus sericeus* Nutt.

(≡) *Pluchea borealis* A. Gray, Proc. Amer. Acad. Arts and Sci. 17: 212. 1882, *nom. superfl. & illegit.* for *Polypappus sericeus* Nutt.

(≡) ***Pluchea sericea*** (Nutt.) Coville, Contr. U.S. Natl. Herb. 4: 128. 1893.

(≡) *Berthelotia sericea* (Nutt.) Rydb., Bull. Torrey Bot. Club 33: 154. 1906.

(≡) *Eremohylema sericea* (Nutt.) A.Nelson, Univ. Wyoming Publ. Sci., Bot. 1: 54. 1924.

(≡) *Tessaria sericea* (Nutt.) Shinners, Sida 3: 122. 1967.
Type: "In Upper California, towards the Rocky Mountains". *W. Gambel Polypappus sericeus* [/] Eastern California [/] I have only one bad [?] specimen [holotype, K: K000974653 (photo!)].

Typification notes

Polypappus sericeus was described by the British botanist and ornithologist Thomas Nuttall (1786–1859, Figure 1), who lived in USA during 1808–1841, and 1847–48 (Beidleman 1960; Graustein 1967), based on material collected in "Upper California, towards the Rocky Mountains" by botanist William Gambel (1823–1849), who actively collected especially in California and New Mexico (Graustein 1967: 342–344; Fischer, 2001: 26-27); Gambel also collected plant materials for Nuttall (Graustein 1967: 350).

Regarding Nuttall's herbarium and type specimens, Stafleu and Cowan (1981: 781) stated that "The Nuttall herbarium (5.750 species, acquired in 1860) is at BM. However, it would appear that up to the publication of the 'Genera [of North American plants]…' in 1818 he kept few or no specimens for himself, presenting a complete series of his plants to the Academy of Natural Sciences (PH) so that there repose his early types" (Pennell 1936). Further Nuttall specimens are found in CGE, DUKE, DWC, E, F, FI, G, GH, K, LIV, MANCH, MO, NY, OXF." Since Nuttall published the name *Polypappus* *sericeus* in 1848, it is reasonable to look for the Gambel's collection in the preceding herbaria. In our internet searches, we located only a single specimen, which is housed at the herbarium of the Royal Botanic Gardens, Kew (K000974653). Furthermore, in a personal communication, John Pruski (MO) informed us that he checked the PH microfiche for the name *P. sericeus* and did not find any specimen.

The Kew specimen, which we have identified as the original material, was examined and annotated by Nuttall. The specimen label shows the remark "I have only one bad[?] specimen" indicating that this was the only one specimen available for Nuttall to describe this species; the preceding remark also makes it clear that other herbaria do not have Gambel's collection for this species. Therefore, we recognize the K specimen as the holotype of *P. sericeus* Nutt. Two of the names (*Tessaria borealis* Torr. & A. Gray and *Pluchea borealis* A. Gray) that are relevant for our research were superfluous for *Polypappus sericeus* and illegitimate when published. Finally, "*Berthelotia borealis* Wooton", one of the names associated with the accepted *Pluchea sericea*, is not valid; Wooton (1913) provided a description for the genus *Berthelotia* but not for the listed species, *B. borealis*.

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APPENDIX 1.

Single-author botanical publications of Sabin Berthelot[3]

Berthelot S. 1826. Allegemeine benerkungen über die Canarische Inseln, besonders naturgeschichtlichen inhalts. Isis von Oken. 18–19: 960–975.

Berthelot S. 1827. Observations sur le *Dracaena draco* L. Nova Acta Academiae Caesareae Leopoldino-Carolinae Germanicae Naturae Curiosorum. 13: 775–788.

Berthelot S. 1827. Description d'une nouvelle espèce de *Viola*. Mémoires de la Société Linnéenne de Paris. 50: 418–420.

Berthelot S. 1827. Projet d'établissement d'un jardin de naturalisation aux Îles Canaries. Annales de la Société Linnéenne de Paris. 6: 491–496.

Berthelot S. 1828. Observations sur le *Dracaena draco* L. Annales des Sciences Naturelles. 14: 137–147.

Berthelot S. 1828. Observations sur le *Boehmeria arborea*. Nova acta Academiae Caesareae Leopoldino-Carolinae Germanicae Naturae Curiosorum. 14: 943–952.

Berthelot S. 1832. Observations sur l'accroissement et la longévité de plusieurs espèces d'arbres des environs de Nice. Bibliothèque Universelle des Sciences, Belles-Artes et Arts. 50: 280–293.

Berthelot S. 1832. Sur la longévité et l'accroissement des arbres. Bibliothèque Universelle des Sciences, Belles-Artes et Arts. 51: 365–390.

Berthelot S [as Anonymous author]. 1834. Tamarin. In: Cazeaux E, E Charton (eds.), Le Magazin Pittoresque. Year 2. Paris. Pp. 359–360.

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Berthelot S. 1837. Mancenillier. In: Dictionnaire de la Conversation et de la Lecture. Volume 36. Belin-Mandar, Paris. Pp. 489–490.

Berthelot S. 1837. Mélèse. In: Dictionnaire de la Conversation el de la Lecture. Volume 37. Belin-Mandar, Paris. Pp. 433–434.

Berthelot S. 1837. Orseille. In: Dictionnaire de la Conversation el de la Lecture. Volume 41. Belin-Mandar, Paris. Pp. 220–221.

Berthelot S. 1837. Palme. In: Dictionnaire de la Conversation el de la Lecture. Volume 42. Belin-Mandar, Paris. Pp. 26–29.

Berthelot S [as "S. B-t."]. 1837. Palme. In: Dictionnaire de la Conversation el de la Lecture. Volume 42. Belin-Mandar, Paris. Pp. 29–31.

Berthelot S [as "S. B."]. 1837. Palmiste. In: Dictionnarie de la Conversation el de la Lecture. Volume 42. Belin-Mandar, Paris. P. 33.

Berthelot S. 1839. Violette. In: Dictionnaire de la Conversation el de la Lecture. Volume 52. Belin-Mandar, Paris. Pp. 225–226.

Berthelot S. 1844. Considérations sur l'Acclimatement et la Domestication. Béthune et Plon, Paris.

Berthelot S. 1846. Note sur le mocan. Bulletin de la Société de Géographie. Série 3. 5: 334.

Berthelot S [as Anonymous author]. 1853. De algunos árboles célebres. Eco del Comercio. 100: 1–2

Berthelot S [as "Un Amigo del País"]. 1855. Montes de Canarias. Eco del Comercio. 297: 1–2; 298: 1–2; 299: 1; 302: 1–2.

Berthelot S. 1859. De l'acclimatation en Algérie des principales essences forestières des Îles Canaries. Bulletin de la Société de Géographie. Série 4. 18: 329–344.

Berthelot S. 1862. Sur les essences forestières des Canaries et la réoganisation du Jardin d´Acclimatation d´Orotava. Bulletin de la Société Impériale Zoologique d'Acclimatation. 9: 684–688; 778–786.

Berthelot S. 1866. Memoria dirigida a Gobernador Civil de las Islas Canarias en 1862 sobre la reorganización del Jardín de Aclimatación de La Orotava. El Amigo del País. Periódico de la Sociedad Económica de Santa Cruz de Tenerife. [1866]: 195–205.

Berthelot S. 1868. Montes. El Amigo del País. Periódico de la Sociedad Económica de Santa Cruz de Tenerife. [1868]: 102–105.

Berthelot S. 1879. Árboles y bosques (páginas de un libro inédito). Revista de Canarias. 15: 226–230; 16: 249–250; 17: 257–259;19: 290–293; 20: 310–313; 21: 329–330.

Berthelot S. 1880. Necesidad de la conservación y repoblación de los bosques y de la plantación de arbolados en la cuenca de Santa Cruz de Tenerife. Revista de Canarias. 29: 37–39; 30: 49–51.

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[3] Publications pertinent to agriculture or cultivated plants are not included. Likewise, contributions made to the *Histoire Naturelle* des *Îles Canaries* are not listed either.

APPENDIX 2.

Translation of the letter that Sabin Berthelot sent to Augustin Pyramus de Candolle on July 12, 1833. Notice that the last paragraph of this letter was written by Philip Barker Webb. Original letter is in French. Footnote bibliographies are listed within the Reference section of this contribution. Courtesy of *Conservatoire et Jardin botaniques de Genève–Archives*. Translation by Piero G. Delprete.

[Page 1]
Dear Sir,
In the hope of finding you in Paris, we are answering the interesting letter that you wrote at the eve of your departure from Geneva. When it arrived here, we learned with sadness that you left a few days before. We are sorry about this, which we are remembering as much unfortunate for us, and we are hasting to write to reply to your several questions and observations related to our Canarian Compositae.

The species about which you are asking some explanations and is with nº 3 bis in the collection of our radiate, is not cited in our catalogue and we have reasons to believe that this specimen cannot be other than a duplicate of nº 3, which following your announcement hereafter will be *Berthelotia glaberrima*[4]. We encountered this species only in Gran Canaria, in the volcanic peninsula called La Isleta[5] [and it] can be considered as the principal population, although in this locality is quite rare. This plant grows in this locality with *Berth. sericea*[6], which is found in abundance. We never observed intermediary stages between the two species; the first one was always with glabrous leaves, green, almost lucid and a little fleshy, characters which make it remarkable even from a distance; whereas the other is constantly conserved on hers [its leaves] with silky hairs that distinguish it and a grayish-green. Unfortunately, this brilliant color that gives to *Berth. glaberrima* a physiognomy so characteristic is lost during desiccation [when plants are dried] and despite all our cares we were unable to prevent our specimens from blackening.

Before passing to another subject, being the most interesting part, I should not finish this one without proper thanks. I accept with gratitude the dedication that you made for me for the new genus *Berthelotia*; it is unfortunate that the botanical laws oblige you in this case to maintain the old specific name *sericea*[7], because *villosa*, or even *pilosa* would be more in agreement, as both these adjectives would have alluded (more divi Linnée) to the more evident external character of my individual [plant] and that we could have been referred to that [the leaf indumentum character] of the species type. But I know too well that your decisions are final judgements, therefore leave to me these reflection on the silky hairs of my species and make the case that it deserves. Mr. Webb will also be very charmed of seeing his name dedicated to a genus from Greece[8]; you say that you have one [name] in reserve near *Berthelotia*; that one will establish a sort of confraternity between the two friends, they will be delighted of this friendship and they will feel glorified of being registered on the golden book under the auspices of such celebrated patron[9]. I can count on the gratitude of this new godson[10]. Regarding the other genus *Webbia*, which we have discussed in Geneva, it is true that Pavón[11] had the intention of publishing it and he also traced the characters in a manuscript; but certainly, the Spanish botanist published nothing on it[12].

My nº 6 is *Erigeron inuloides* Willd.[13] and the *Chrysoco-*

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[4] It refers to *Schizogyne glaberrima* DC. (Asteraceae, Inuleae). The species was described by Augustin Pyramus de Candolle in the same work in which he described the genera *Berthelotia* and *Webbia*. *Schizogyne* Cass. (two species) is endemic to the Canary and Salvage Islands and was described by the French botanist Alexandre de Cassini in 1828. Candolle never described "*B. glaberrima*." *Schizogyne glaberrima* is endemic to Gran Canaria.

[5] It refers to the small peninsula of La Isleta located in the main city of the island, Las Palmas de Gran Canaria.

[6] It refers to *Schizogyne sericea* (L.f.) DC. (Asteraceae, Inuleae). The species is found in the Savage Islands and in the Canaries (all islands except Lanzarote; https://www.biodiversidadcanarias.es/biota/). Candolle did not ever describe "*Berthelotia sericea*". See additional details in note 4.

[7] It refers to *Chrysocoma sericea* L.f. (Asteraceae, Inuleae), which is the basionym of *Schizogyne sericea* (L.f.) DC. See note 6.

[8] The genus *Webbia* DC. (Asteraceae, Vernonieae; *nom. illeg.*, non *Webbia* Spach, Hist. Nat. Veg. Phan. 5: 408 June 1836) does not occur in Greece and it is restricted to Africa. Therefore, Augustin Pyramus de Candolle did not refer to an Eastern Mediterranean species when he described *Webbia*. See further details on the botanical history of *Webbia* in Francisco-Ortega et al. (2022), as under this name three different genera were described by three plant taxonomists independently.

[9] It seems that in this sentence Berthelot is referring to a close association between *Berthelotia* (suggested in the letter as a Canary Island genus) and *Webbia* (suggested in the letter as a Greek genus) as both genera were described by Augustin Pyramus de Candolle. See additional details in note 8.

[10] Berthelot seems to metaphorically "treat" the genus *Berthelotia* as a close family relative of him.

[11] It refers to the Spanish botanist José Antonio Pavón Jiménez (1754–1840) who together with Hipólito Ruiz López led one of the most important botanical endeavors of the Spanish Crown: The *Expedición Botánica al Virreinato del Perú*. Some of the specimens of this expedition reached Webb's herbarium (Steinberg 1977).

[12] See Francisco-Ortega et al. (2022) regarding Pavón's plans to dedicate the genus *Webbia* to Webb.

[13] We did not find the name "*Erigeron inuloides* Willd." (Asteraceae, Inuleae) in the consulted taxonomic literature, and we initially interpreted that Berthelot referred to *Erigeron inuloides* Poir. (accepted name *Pulicaria inuloides* (Poir.) DC.). The protologue of *E. uniloides* Poir. is for a perennial herbaceous species that was collected by Pierre M. A. Broussonet [see additional details on Broussonet in note 16] in the Canaries (Poiret 1817: 464). Unfortunately, no original material collected by Broussonet has been

ma dichotoma[14], Canaries, according to our research in the herbarium of Mr. Bouchet[15]. The label was written by him and dictated by Broussonet[16].

[Page 2]
In the same herbarium we found only *Carlina sulphurea*[17] with the habitat of Tanger and with no species of this genus with the name of *simplex*.
We also did not see any *Carthamus rigidus*[18] from Gibraltar cited by Broussonet[19] or by Durand[20].
The *Carlina xeranthemoides*[21] in the Brouss. [Broussonet] herbarium is only a variety with large leaves of *Carlowizia salicifolia*[22]. This plant is found in the collection of Mr. Bouchet with two labels written by him and dictated by Broussonet. Mr. Bouchet confirmed that all the specimens of *Carlina xeranthemoides*[23] sent to other botanists by Broussonet, passed by his hands and they are all duplicates of the specimen that belongs to him. Therefore, our *Carlina xeranth*. [*xeranthemoides*] is not present in the Broussonet herbarium. In this case the herbaria of Linnaeus and Banks[24] could be useful in clarifying the doubts that we have about this species.
The *Centaurea calcitrapa*[25] is indigenous of to the Canaries; we can find it very frequently in the field surrounding the cities and in almost whole of the maritime region. We can say the same for *Galactites tomentosa*[26] [*tomentosus*] and *Silybum marianum*[27].
Regarding *Carthamus tinctorius*[28], this species is cultivated only in the Canaries [Canary Islands] and the *Carthamus teneriffae* Steud.[29], which we do not know, is probably just a variety of *C. tinctorius*[30] caused by the climate and that some good German could have considered as an indigenous plant.
During my visit at Montpellier, I made a drawing of your *Gonospermum elegans*[31] that probably I have in my herbarium as a variety, this drawing is the copy of that in the collection of the garden. If you wish to have it, I can send it to you, otherwise I will publish it with mine.
Here is, more or less, all the information that I can supply on the several species that you mentioned in your letter; if you wish that I do other searches on this subject or on other plants, you only have to indicate this to me, and with pleasure I will try to satisfy your desire. I will remain here another fifteen days and then I will leave for London where I will stay for about one month to check the conditions of my collections and give a glance at the Banks[32]

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found that allow us to properly interpret this name. *Pulicaria arabica* (L.) Cass. ssp. *hispanica* (Boiss.) Murb. is the only Canary Island native species of the genus to which this taxon could refer to. However, the text of this line of the letter can also be interpreted as Broussonet treating "*Erigeron inuloides*" as a synonym of *Chrysocoma dichotoma* L.f. We cannot rule this second possibility, because the account of *C. dichotoma* (as *Allagopappus dichotomus* (L.f.) Cass.) presented by Schultz Bipontinus (1844–1850: 224) in Webb and Berthelot's *Histoire Naturelle des Iles Canaries* lists *Conyza inuloides* Aiton as a synonym of *A. dichotomus*. There is a chance that Berthelot had an oversight and wrote "*Erigeron inuloides* Willd" instead of "*Conyza inuloides* Willd;" interestingly, Willdenow (1803: 1938) did list *C. inuloides* Aiton for the island of Tenerife.

[14] It refers to *Chrysocoma dichotoma* L.f. (accepted name *Allagopappus canariensis* (Willd.) Greuter, Asteraceae, Inuleae). *Allagopappus* Cass. (two species) is endemic to the Canaries, with *A. canariensis* occurring in Gran Canaria, La Gomera, and Tenerife. See note 13 for additional details on the interpretation of this line of the letter.

[15] It refers to the French botanist Dominique Bouchet (1770–1845) whose collections are housed at MPU (*Herbier de l'Université de Montpellier II*) and includes the herbarium of Pierre-Auguste-Marie Broussonett (Stafleu and Cowan 1976: 290; Le Brun 2018). See additional details on Broussonet in note 16.

[16] Pierre-Auguste-Marie Broussonett (1761–1807) was a French botanist and director of the Botanical Garden of Montpellier between 1803 and 1807. He lived in the Canary Islands between 1799 and 1803 where he was appointed French Commissioner for Trade. During this period, he actively botanized in this archipelago. Between 1794 and 1795, he exiled to the Iberian Peninsula where he was supported by botanists and friends from Spain, Britain, and Portugal (Motte 1970; Caillé 1972).

[17] *Carlina sulphurea* Desf. (accepted name *C. racemosa* L., Asteraceae, Cardueae) is restricted to the Western Mediterranean (including Morocco) and does not reach the Canary Islands (López Martínez and Devesa 2014; http://www.floraiberica.es/PHP/generos_lista.php).

[18] *Carthamus rigidus* Willd., nom. illeg. (Asteraceae, Cardueae) refers to *Carduncellus arborescens* (L.) Sweet a species restricted to the Western Mediterranean that does not reach the Canary Islands (López González 2014a; http://www.floraiberica.es/PHP/generos_lista.php).

[19] See note 16.

[20] It refers to the French priest and botanist Philippe Durand (1795–1815) who collected in southern Spain and Morocco. He worked in the Botanical Garden of Montpellier as Conservateur des Collections de Botanique (Veldkamp 2001; Michaud 2018).

[21] *Carlina xeranthemoides* L.f. (Asteraceae, Cardueae) is a species endemic to Tenerife.

[22] *Carlowizia salicifolia* (L.f.) Moench (accepted name *Carlina salicifolia* (L.f.) Cav., Asteraceae, Cardueae) is a species endemic to the Canary Islands and Madeira.

[23] See note 21.

[24] It refers to the herbarium of Sir Joseph Banks (1743–1820) who was the unofficial director of the Royal Botanic Gardens, Kew and one the most prominent figures in the history of science of his era. His herbarium is part of the collections of Natural History Museum of London (BM).

[25] *Centaurea calcitrapa* L. (Asteraceae, Cardueae) is a Mediterranean species that reaches the Canaries where it is found in all islands except La Palma (Devesa 2014a).

[26] *Galactites tomentosus* Moench (Asteraceae, Cardueae) is a Macaronesian-Mediterranean species, it is found in all Canaries except Fuerteventura and Lanzarote (Devesa 2014b).

[27] *Silybum marianum* (L.) Gaertn. (Asteraceae, Cardueae) is a species with a widespread distribution that reaches the Canary Islands (Devesa 2014c).

[28] *Carthamus tinctorius* L. (Asteraceae, Cardueae) has an unknown biogeographical origin and it is cultivated in the Canaries (López González 2014b).

[29] This is a *nomen nudum* (Steudel 1821: 164) and we are not certain of the taxonomic identity of this species. It could refer to *Carthamus lanatus* L. (Asteraceae, Cardueae) or *C. tinctorius* L.

[30] See notes 28 and 29.

[31] *Gonospermum elegans* DC. (accepted name *Gonospermum canariense* Less, Asteraceae, Anthemideae) is endemic to La Palma and El Hierro. The genus is restricted to the Canary Islands and it has seven species.

[32] See note 24.

herbarium for comparing the Canarian species collected by Masson[33]. I will then return to Paris to start working on my publications. I already finished the editing of several families and I hope that at my return to England Hayland[34] will have almost finished the drawings of the plants that I gave him in Lyon, as well as of our more important Compositae that you would give him for this purpose.

Here is the list:

Centaurea canariensis Brouss. Willd[35]
Carduus clavulatus[36]
Centaurea cynaroides[37]
Conyza canariensis Willd = *Jasonia dichotoma* DC. in Litt.[38]
Buphthalmum laevigatum Brouss. = *Jasonia laevigata* DC. in litt.[39]
Conyza gouanii Willd.[40]
Conyza sericea = *Berthelotia sericea* DC. in litt.[41]
Conyza glaberrima = *B. glaber*. DC. in litt.[42]
Phagnalon scariosum[43]
Conyza sordida n°1 bis in herb. Willd. B = *Phagnalon umbelliforme* DC. in litt.[44]
Gonospermum fruticosum[45]
[*Gonospernum*] *multiflorum*[46]

[Page 3]

and finally, the *Carlowizia xeranthemoides*[47].

I still did not have decided anything on my method of publication. I have great need of your advice on this, and your departure from Paris at the moment when I arrived to profit from your advice is for me a fatality. Is it better to deal with the same publisher for the different parts that I wish to publish on the natural history of the Canaries so that the parts are published successively with the same format that will be the same of our drawings? And which is, according to you, the publisher that I should prefer?

As I presume that you will not answer from Paris immediately, as you will be without doubt on the road towards Lugano at the reception of my letter, I am giving you the address of Mr. Webb in England.

Farewell, my dear Mister, please convey my compliments to all your family and rest assured of my sincere attachment.

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[48]I join my kinds regards to those of Berthelot, & should you have anything to make out in the herbaria of Linnaeus, Banks[49] or Lambert[50] beg leave to offer my humble services [illeg.] in London. My address is at my Brother's Milford House near Godalming, Surrey. I wish you a pleasant excursion to Italian Switzerland. Parolini[51] writes me that he shall send you hither our Greek Composite.

P. B.W.

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[33] It refers to the Scottish botanist Francis Masson (1741–1805), first official collector of the Royal Botanic Gardens, Kew who performed field work in the Azores, Canaries and Madeira between 1776 and 1779 (Francisco-Ortega et al. 2008).

[34] It refers to the German artist Jean-Christophe Heyland (1791–1866).

[35] It refers to *Centaurea canariensis* Brouss. ex Willd. (accepted name *Cheirolophus canariensis* (Brouss. ex Willd.) Holub, Asteraceae, Cardueae). This is a species endemic to Tenerife.

[36] *Carduus clavulatus* Link (Asteraceae, Cardueae) is a species endemic to the Canary Islands.

[37] It refers to *Centaurea cynaroides* Buch (accepted name *Rhaponticum canariense* DC., Asteraceae, Cardueae), a Tenerife endemic.

[38] These two names refer to *Conyza canariensis* Willd. and *Jasonia dichotoma* (L.f.) DC. (accepted name for both of them: *Allagopappus canariensis* (Willd.) Greuter, Asteraceae, Inuleae, see note 14).

[39] These two names refer to *Buphthalmum laevigatum* Brouss. ex Willd. and *Jasonia laevigata* (Brouss. ex Willd.) DC. (accepted name for both of them: *Vieraea laevigata* (Brouss. ex Willd.) Sch.Bip., Asteraceae, Inuleae). The genus *Vieraea* is monotypic and endemic to Tenerife.

[40] It refers to *Conyza gouanii* (L.) Willd. (accepted name *Erigeron gouanii* L., Asteraceae, Inuleae). This is primarily a continental African species (Morocco, Cameroon, eastern Africa, South Africa) that is also found in the Canaries (La Gomera, Gran Canaria, Tenerife, and La Palma). See https://africanplantdatabase.ch/en/nomen/97596.

[41] These two names refer to *Conyza sericea* (L.f.) W.T. Aiton and "*Berthelotia sericea* DC." (both with accepted name *Schizogyne sericea* (L.f.) DC., Asteraceae, Inuleae). See note 6 as the name "*B. sericea*" was never published by Candolle.

[42] The names "*Conyza glaberrima*" and "*Berthelotia glaberrima*" were never published; however, they both clearly refer to *Schizogyne glaberrima* DC. (Asteraceae, Inuleae). See note 4.

[43] It refers to *Phagnalon scariosum* DC. ex Lowe (Asteraceae, Gnaphalieae), species published by the British botanist Richard Thomas Lowe (1802–1874) using the name and brief description provided by Augustin Pyramus de Candolle. This is a poorly known species that seems to be morphologically similar to *Phagnalon rupestre* (L.) DC. (Lowe 1868: 443) The latter is a native (no-endemic) species in the Canary Islands.

[44] These two names refer to *Conyza sordida* L. (accepted name *Phagnalon sordidum* (L.) Rchb., Asteraceae, Gnaphaliea) and *P. umbelliforme* DC., respectively. The latter is a Canary Island endemic that occurs in all islands except Fuerteventura and Lanzarote. According to the protologue of *P. umbelliforme*, this specie is closely related to *P. sordidum* (Candolle 1836: 396), and this could explain why Berthelot listed *Conyza sordida* and *Phagnalum umbeliforme* together in this entry.

[45] It refers to *Gonospermum fruticosum* (Buch) Less. (Asteraceae, Anthemideae). This species is restricted to the islands of La Gomera, El Hierro, and Tenerife. See note 31 regarding biogeography of *Gonospermum*.

[46] It refers to *Gonospermum multiflorum* DC. (accepted name *G. fruticosum* (Buch) Less., Asteraceae, Anthemideae). See note 31 regarding biogeography of *Gonospermum* and note 45 on the distribution of this species in the Canary Islands.

[47] We have not found "*Carlowizia xeranthemoides*" in the taxonomic literature; however, this entry clearly refers to *Carlina xeranthemoides* L.f. (Asteraceae, Cardueae). See note 21.

[48] This part of the letter was written by Philip Barker Webb, and it is in English.

[49] See note 24.

[50] It refers to the British naturalist Aylmer Bourker Lambert (1761–1842) who was of the first fellows of the Linnean Society of London and owner of the largest private herbarium in Europe at that time (Miller 1970).

[51] It refers to the Italian botanist Alberto Parolini (1788–1867), a close friend of Webb to whom he dedicated the Canary Island endemic genus *Parolinia* (Brassicaceae), see further details in Francisco-Ortega et al. (2022).

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Wrong flowers? The evolutionary puzzle of *Jongkindia* (Passifloraceae s.l.), a new monotypic genus and tribe from Liberia, West Africa

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Abstract. *Jongkindia*, a small tree species endemic to a local area in southeast Liberia, is described as a new monotypic genus of Passifloraceae sens. lat. Its only species *Jongkindia mulbahii* combines floral characteristics of the Turneraceae and fruit characteristics of the Passifloraceae s.s. (or subfamily Passifloroideae in APG) and can therefore be regarded to occupy an isolated morphological position. This is confirmed here by DNA sequence-based phylogenetic analyses including most Passifloracean genera, which places it as sister to Passifloroideae. We delineate 16 morphological characters and their states and optimise them on our reconstructed phylogenetic tree. Based on these results we consider the Passifloraceae fruit characteristics (berries with arilled seeds) as synapomorphic for Passifloroideae. The monotypic *Pibiria* and *Jongkindia* are predominantly characterised by autapomorphies. On the other hand, the *Adenia*/*Passiflora* clade is characterised by polymorphisms. We place *Jongkindia* in a new tribe Jongkindieae Breteler & F.T.Bakker. A draft plastome sequence for *Jongkindia mulbahii* is presented and evidence for two mitome to plastome (mtpt) fragment transfers is discussed. Structurally the *Jongkindia* plastome appears similar to that of *Populus*, *Adenia*, *Mitostemma*, *Dilkea*, and *Passiflora pittieri*, but not to contain the previously-described major inversions within other, more derived, *Passiflora* plastomes.

Keywords: Passifloraceae, Passifloroideae, Turneroideae, plastome, floral evolution, West Africa.

INTRODUCTION

Ongoing botanical exploration of Liberia (Breteler 2020; Jongkind 2012, 2015a, b, c; 2016; 2017; 2019; Jongkind and Breteler 2020) revealed the presence of a thus far undescribed species of Passifloraceae s.l., near Sapo National park (Sinoe County, Liberia; see Figure 1) which contains "the second-largest area of primary tropical rainforest in West Africa." (Freeman et

Figure 1. Collection site of *Jongkindia mulbahii*, indicated by the red marker. Green area indicates boundaries of the Sapo National Park.

al. 2019). The undescribed species presented here appears somewhat of a conundrum: in spite of its 4- instead of 5-merous flowers it is best placed in Turneraceae (now Turneroideae sensu APG III (2009) and IV (2016)) because of its tubular calyx with the petals insterted on it and because of the absence of a corona (see Figure 10). However, Turneroideae have capsular fruits (Arbo 2007) whereas the new species presented here has an indehiscent fleshy fruit (see Figure 2), common in *Passiflora* (in Passifloroideae sensu APG).

Wurdack et al. (2009) maintained Turneraceae and Malesherbiaceae as separate under Passifloraceae s.l. but Takuoka (2012), based on comparison of rbcL, atpB, matK, and 18S rDNA sequences, considered a well-supported monophyletic Passifloraceae ss. and Turneraceae as (well-supported) sister groups. Both Xi et al. (2012), based on concatenated analysis of 82 plastid genes from 58 species, and Cai et al. (2021), based on multi-species coalescent analysis of 423 single-copy nuclear loci from 64 taxa, support a 'Parietal clade' in which Turneraceae and Passifloraceae are sister groups, with Malesherbiaceae sister to them. The two latter studies differ in the placement of Achariaceae, which is sister to the remainder of the Parietal clade (Xi et al. 2012) or in a derived position within it (Cai et al. 2021). Within the Parietal clade, the two studies differ with regards monophyly of the salicoids sensu Xi et al. (2012), which is supported by the concatenated analysis but not in any of the coalescent methods (Cai et al. 2021).

Within Passifloraceae s.s., Takuoka (2012) recognises monophyletic tribes Passifloreae and Paropsieae, wich is also adopted by APG. The first is distributed in the Old and New World and the second only in Old World (mainly Africa; Table 1). Maas et al. (2019) described the enigmatic 'unknown yellow' *Pibiria* as a lineage sister to Turneroidea and chose to place it at subfamily-level, which brings the number of recognised subfamilies in Passifloraceae to four, i.e. Passifloroideae, Turneroideae, Pibirioideae and Malesherbioideae

Given the remarkable combination of *Turnera*-like floral morphology and *Passiflora*-like fruits in the new species presented here it will be interesting to infer its phylogenetic position within this part of Malpighiales, known to present major challenges (such as incomplete lineage sorting, gene tree error and horizontal gene transfer) to phylogenetic reconstruction (Xi et al. 2012; Cai et al. 2020; APG IV). Therefore, we generated DNA sequences for this species, both Sanger and Illumina HiSeq, and compared it with publicly available sequence data for Passifloraceae s.l. We find our new species, which we refer to as *Jongkindia mulbahii* (see below), to be in an isolated position on a relatively short branch between, on the one hand, the Parietal clade (or Passifloroideae, to which it is sister) including a *Barteria*/*Paropsia* clade (both genera of small trees; Breteler 1999; 2003; de Vos and Breteler 2009), and the Turneroideae clade on the other. It is described and illustrated here as a new monotypic genus, and its draft plastome is compared with plastomes from *Adenia*, *Passiflora*, *Dilkea*, *Mitostemma* and allies.

Figure 2. *Jongkindia mulbahii* morphology. Abaxial surface glandular leaf tip (a), fruiting branch (b, c), with one fruit in longitudinal section (b) and sterile branch (d).

MATERIALS AND METHODS

Morphology

We delineated morphological characters and states in order to describe floral, fruit and vegetative morphology for our new species (see below under 'taxonomy') and its inferred allied genera (see below and Table 2). Characters were based on published taxonomic descriptions for the different genera (e.g. Feuillet and MacDougal 2007; Arbo 2007) plus our own observations (see the isotype specimen, Figure S1). We also included 'ecology' and habit in the descriptions in order to obtain a broad characterisation of all groups involved, within a phylogenetic framework.

DNA & Sanger sequencing

A few mgs of leaf tissue was used to extract DNA from, using standard CTAB protocols, including incubation with CTAB at 65°C, isopropanol precipitation at –20°C, followed by washing the DNA pellets with 70% EtOH. The DNA was dissolved in water and subjected to purification using the Promega clean-up system protocol. Cleaned DNA was then shipped to BGI HongKong, for library preparation and paired-end sequencing at the Illumina HiSeq2050 platform, using a read-length of 100bp. Part of the cleaned DNA sample was used for PCR amplification and Sanger sequencing of chloroplast rbcL, trnL-F as well as rDNA ITS regions, using standard protocols, and CodonCodeAligner (CodonCode Corporation, www.codoncode.com) for editing of sequence tracers.

Plastome assembly

Plastome contigs were assembled using GetOrganelle v20150226 (Jin et al., 2020) and IOGA (Bakker et al. 2016), the latter shown to be outperformed by the former (Freudenthal et al. 2019), but both having useful analytical aspects. For GetOrganelle we used default settings, for filtering plastid-like reads, conducting de novo assembly, purifying the assembly graph, and generating the plastome contigs. k-mer gradients were set as '-k 21,31,41,51,61,71,81,85,87,95,99'. For IOGA we used *Passiflora edulis* (NC_034285.1) as reference, and using only one instead of both Inverted Repeat (IR) regions in order to avoid possible artefacts related to assembling a linear sequence from a circular chloroplast genome. Typically, IOGA returns a full short single copy (SSC) region and full 'consensus IR', but the large single copy region (LSC) is usually recovered only in large parts, and compartments are rarely assembled together. Resulting contigs were assessed using nBLAST (www.ncbi.nlm.nih.gov, Altschul et al. 1990) in GenBank and then concatenated, along with the scaffolds from GetOrganelle, according to their position relative to the *Mitostemma brevilis* plastome. In GetOrganelle, contigs were automatically annotated as part of the post-assembly pipeline. Final assembly graphs, with connections between contigs, were visualized in Bandage (Wick et al. 2015). All data is available from the corresponding author on request, in addition all sequences will be available in GenBank.

Plastome structural analysis

Concatenated contigs were visualised using dotplots, generated at MAFFT on-line, in order to check plastome integrity and structure. Using the progressive Mauve algorithm, they were then aligned and visualised as Local Colinear Blocks (LCB's), relative to the *Populus trichocarpa*, *Adenia mannii*, *Mitostemma*, *Dilkea* and *Malesherbia* (partial) plastomes, in Mauve v2.3.1 (Darling et al. 2004) with default settings. One of the Inverted Repeats (B) was removed from all sequences (including the *Jongkindia* contigs) in order to not confound the alignment and allow for an optimal homology assessment (see Wicke et al. 2013). Strand orientation of the (numbered) LCBs was identified by progressiveMauve.

Phylogenetic analysis

Using *Populus alba* (Salicaceae) as outgroup, the Sanger sequences generated for our new species were used in nBLAST searches in GenBank and compiled into rDNA ITS, rbcL and trnL-F alignments using MAFFT (Katoh & Standley, 2013) with standard settings, apart from rDNA ITS where we used the 'Q-INS-I' iterative setting which considers secondary structure of RNA. In addition, an atpB alignment was compiled using one of the *Jongkindia* contigs as source and query for BLASTn searches. Alignments were then subjected to phylogenetic analysis using either IQ-TREE (Nguyen et al., 2015) for maximum likelihood based reconstruction (at the Vienna server), with the ultrafast bootstrap (Hoang et al. 2018) implemented in IQ-TREE, or MrBayes v3.2 (Ronquist et al., 2012) at a (local) laptop. For both, a partitioned analysis was set-up in which one partition contained codon positions 1+2 and the other partition contained codon position 3. A 10M generations Markov Chain was set-up using 'nst=mixed, rates=gamma and temp=0.05' for mcmc settings. The MrBayes consensus tree was visualised using FigTree, whereas the last two-third of trees sampled in the MCMCMC were converted into a Consensus Network with conflict threshold of 5% using SplitsTree. Resulting consensus trees were compared and, as taxonomic sampling for each gene sequence had been independent and non-overlapping, a majority consensus topology was inferred by visual inspection. That topology was then imported into TNT, after which the 'Optimise synapomorphies' and 'Optimise characters' commands were used to optimise (under parsimony as criterion) the non-DNA characters listed in Table 2. We used Mesquite to visualise the optimizations on the overall consensus tree topology.

RESULTS

For a general description of biogeographic distribution of Passifloraceae genera and species see Table 1. For morphological characters and their state delimitation in representative genera of Passifloraceae see Table 2. We distinguished 16 morphological characters, predominantly describing floral structures. Some of these we treated as (unordered) multi-state with, for instance, character 8 (stigma shape) having states 0, 1, 2 and 3 and no á priori assumed plesiomorphic state.

DNA extraction and purification of our leaf sample yielded <100 ng of DNA, most of which was used for library construction and sequencing at BGI HongKong, where seven million read pairs (forward and reversed) were generated. The rest of the DNA extract was used

Table 1. Passifloraceae, number of genera and species (according to Stevens, P.F. (2001 onwards).

Passifloraceae	genera/species	Distribution
Turneroideae Eaton	12/227	Tropical to warm temperate America and Africa (incl. Madagascar and Rodriguez Island)
Passifloroideae Burnett	16/775	Tropics to warm temperate, especially Africa and America
Paropsieae de Candolle	6/22	Tropical Africa, Madagascar, Malay Peninsula
Passifloreae de Candolle	10/705	Tropics to warm temperate, especially Africa and America
Malesherbioideae Burnett	1/24	South America from Peru southwards, esp. N. Chile
Pibirioideae Chase & Christenh.	1/1	South America, Guyana

Table 2. Passifloraceae s.l. selected floral morphological characters and states, as well as vegetative characters, habit and ecology; *or nearly so.

	Character	States			
		0	1	2	3
1	Flowers	pedicellate	sessile		
2	Flowers per inflorescence	one	several		
3	4/5-merous	four	five		
4	Anther shape	non-sagittate	sagittate		
5	Nr. of stamens	four	five	many	
6	Nr. of coronas	absent	one	two	
7	Extrafloral nectaries	absent	present		
8	Stigmas	punctate	disc-shaped	capitate	lobate-penicillate
9	Ovules	few	many		
10	Androgynophore	absent	present		
11	Calyx	tubular	sepals free*		
12	Indumentum flower buds	non-resinous	covered in resin		
13	Fruits	berry	capsule		
14	Number of arils	zero	one	two	
15	Stipules	absent	present		
16	Indumentum	absent	present		
17	Habit	herb	shrub	tree	climber
18	Ecology	forest	open, scrubby vegetation		

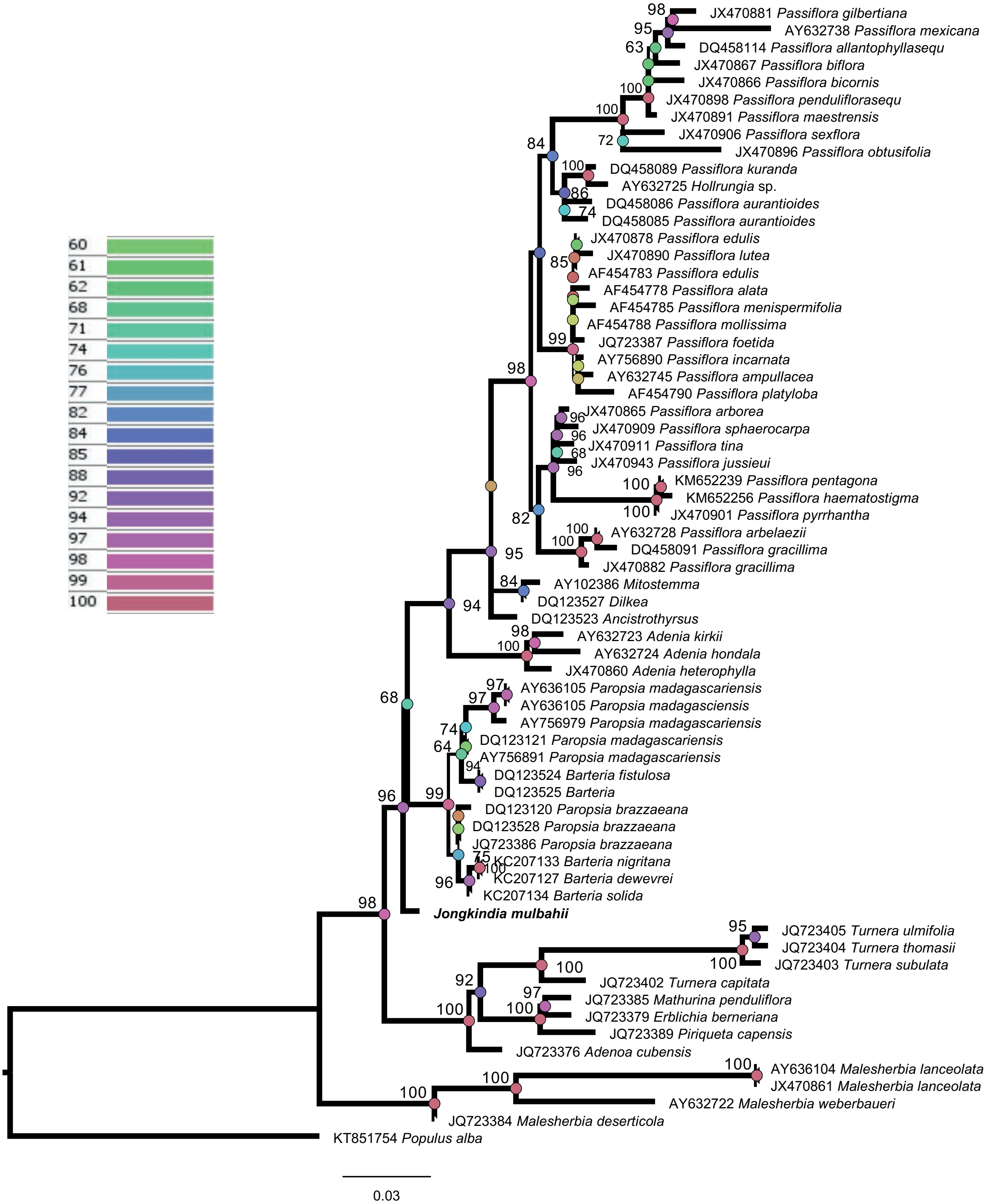

Figure 3. Maximum likelihood IQ-TREE analysis of trnL-F sequences, and rooted on *Populus alba*. *Jongkindia mulbahii* is shown in a well-supported position in between Turneroideae and Passifloroideae, but more closely related to the latter. Nodes without bootstrap frequencies indicate bootstrap values <60%. The colour coding indicates a range from green (no support) to red (100% support).

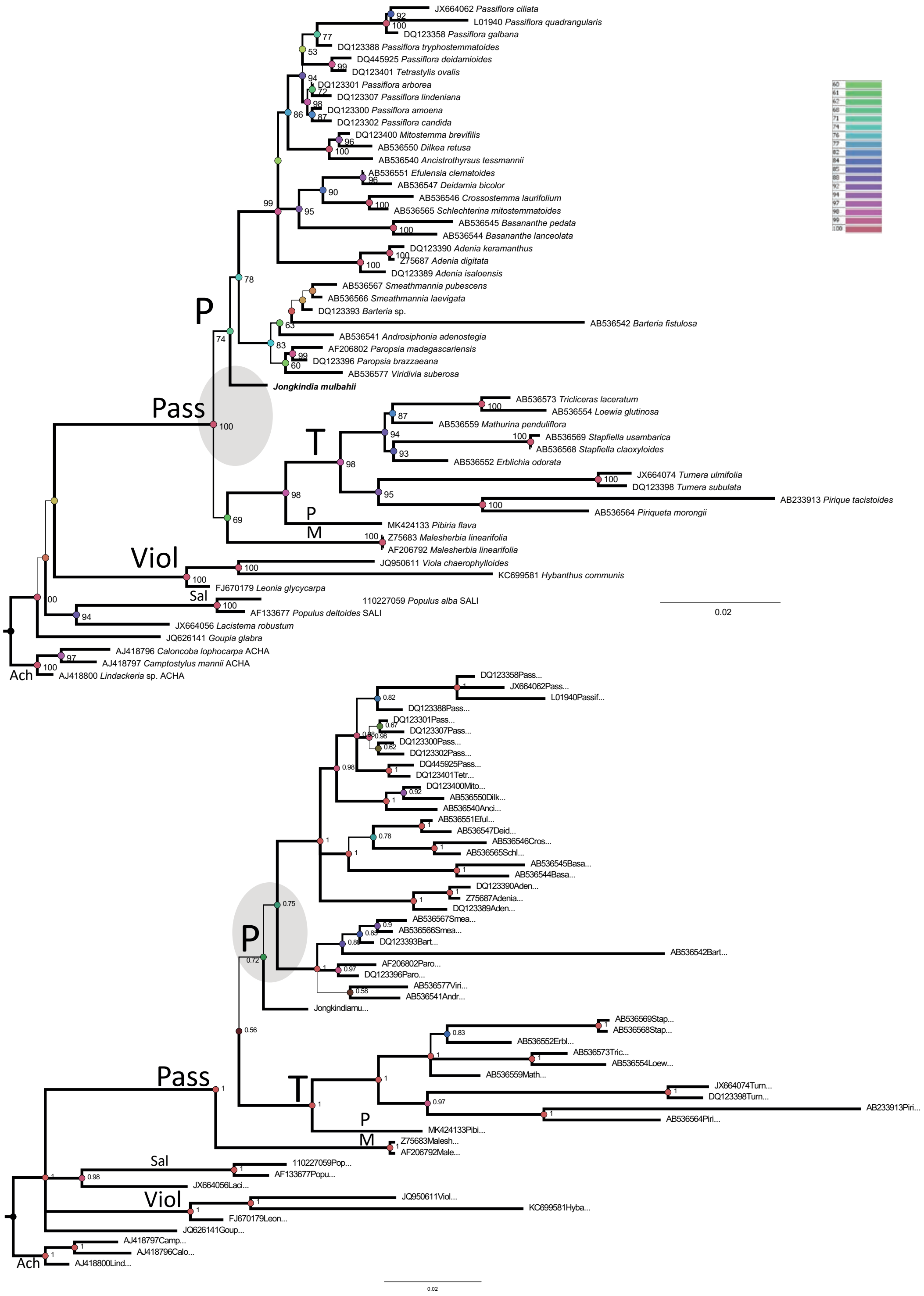

Figure 4. Passifloraceae phylogeny based on rbcL comparison, and rooted on Achariaceae; Maximum likelihood IQ-TREE analysis (top), using a partition according to codon position (1st +2nd versus 3rd); nodes without bootstrap frequencies indicate values <60%. And Bayesian Inference Markov Chain analysis (bottom) using the same data set and partitioning, with posterior probabilities indicated at the nodes. The colour coding indicates a range from green (no support) to red (100% support). Position of subfamilies Passifloroideae (P), Turneroideae (T), Pibirioideae (P), Malesherbioideae (M), as well as of Passifloraceae (Pass), Violaceae (Viol), Salicaceae (Sal) and Achariaceae (Ach) are indicated. In both analyses *Jongkindia mulbahii* is shown in a weakly-supported position, indicated with grey ellipses, in between Turneraceae and Passifloraceae, more closely related to the latter.

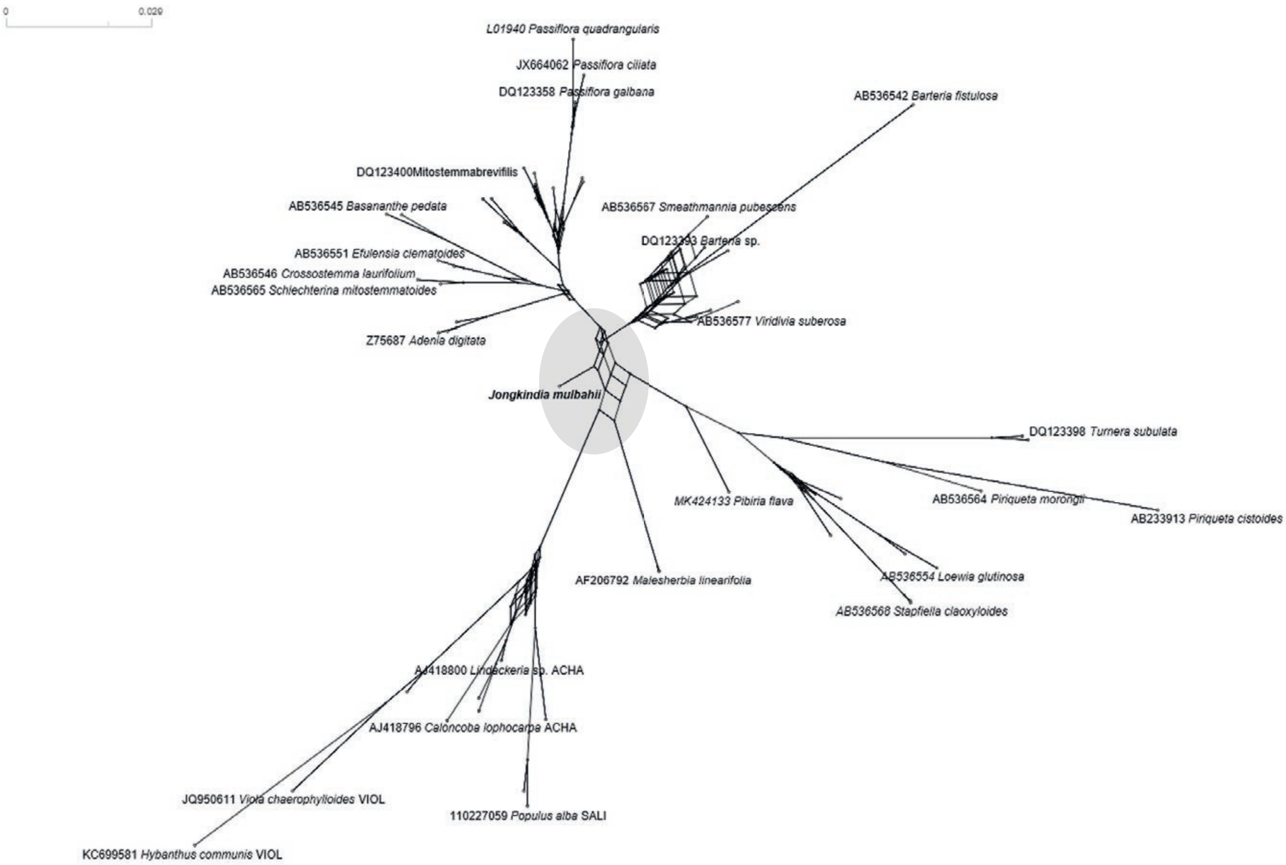

Figure 5. Passifloraceae phylogeny based on rbcL comparison, and rooted on Achariaceae; MrBayes analysis as in figure 5, summarised here as Consensus Network of the last 750 trees from one of the Markov Chains, using a splits-conflict threshold of 5% (NB in order to maintain readability not all terminal names are shown). The position of *Jongkindia mulbahii*, indicated with grey ellips, is shown in a relatively isolated position and connected with multiple splits to Turneraceae and *Barteria*/*Paropsia*.

for PCR and Sanger sequencing, aimed at phylogenetic reconstruction.

Phylogenetic analysis using chloroplast DNA as well as rDNA ITS sequences revealed that *Jongkindia mulbahii* has a relatively isolated position in between a Turneroideae clade and one containing Passifloroideae. Our new species is resolved as sister with low to medium support to a Passifloroideae clade containing *Barteria*, *Paropsia*, *Adenia*, *Mitostemma*, *Dilkea*, *Ancistrothyrsus* and *Passiflora*, supported by bootstrap values ranging between 68 and 99 (see Figures 3 and 4 for comparisons based on trnL-F and rbcL sequences) and 0.74 – 0.78 posterior probability (Bayesian rbcL, Figure 4 and 5). For rbcL comparisons we found a different tree topology and nodal support when comparing IQ-TREE ML and MrBayes tree inference (see Figure 4). Visualising among-tree conflict among the MrBayes Markov Chain tree sample of 751 trees with a Consensus Network (Figure 5) reveals that *Jongkindia mulbahii* is connected with multiple splits to (the outgroup) Achariaceae and Malesherbia and then to the other main clades. This could indicate that its rbcL sequence contains different, possibly conflicting, phylogenetic signals, whereas all other rbcL sequences in this analysis, apart from the *Barteria* clade and Achariaceae, show more 'tree-like' behaviour. For instance, *Pibiria flava* clearly groups with Turneroideae, without any extra splits separating its position. Phylogenetic relationships based on atpB were similar to those in the other trees with regards the placement of *Jongkindia mulbahii* (Fig S2) but indicated an incongruent placement of the Malesherbia clade, grouping as sister to Passifloroideae. This may be due to the fact that for atpB many more sequences were available in GenBank (stemming from Tokuoka 2012) and included in our alignment, as compared with the other marker regions. In any case is the (incongruent) position of Malesherbia clade not well-supported in our analyses, which is also apparent from the rbcL Consensus

Network (Figure 5). Phylogenetic relationships based on nrDNA ITS sequences (Figure S6) were congruent with those based on plastome sequences. Our four gene tree topologies then (trnL-F, rbcL, atpB and rDNA ITS) were overall congruent with regards the placement of *J. mulbahii*. A summary tree, placing *Malesherbia* as sister to

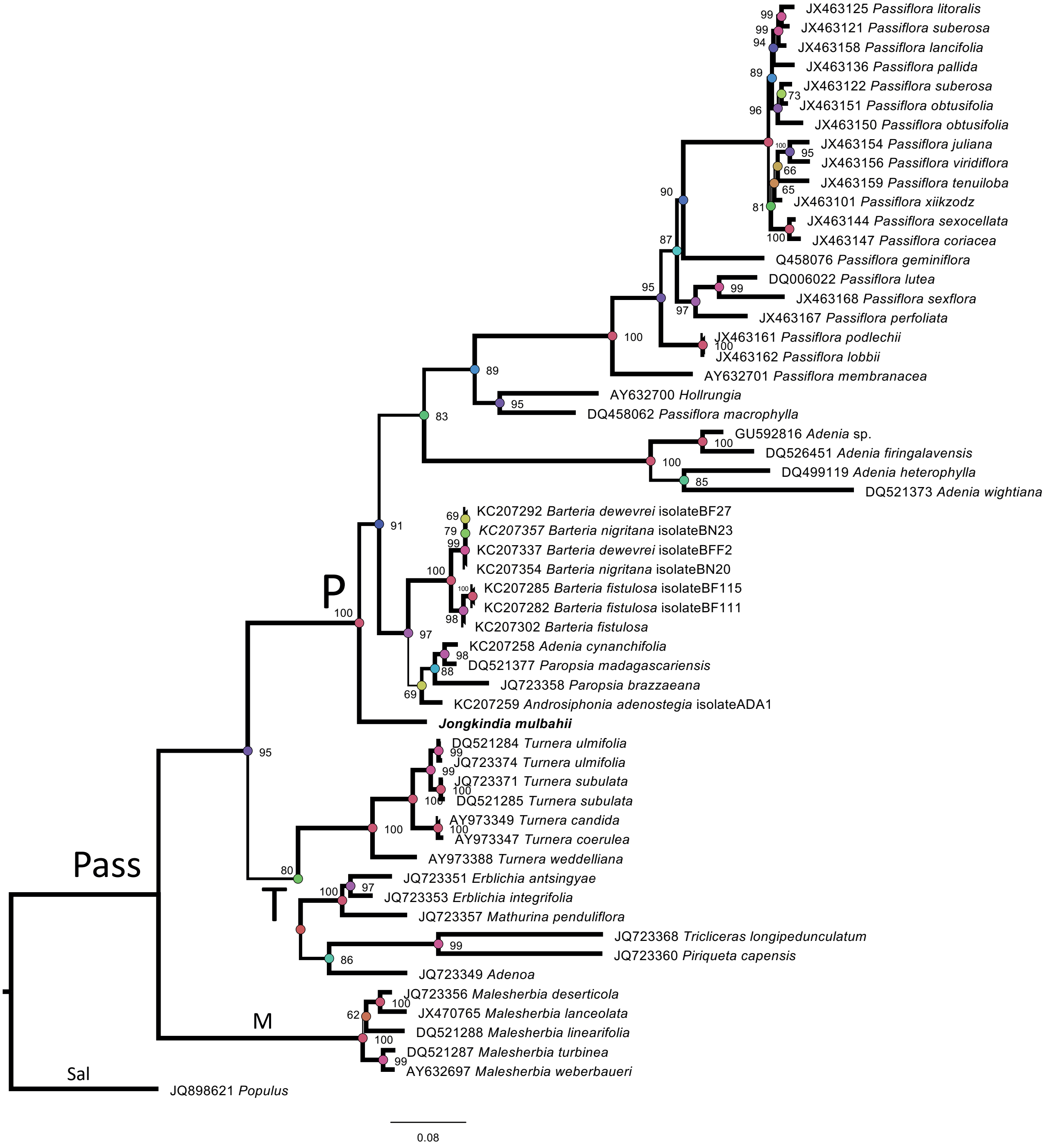

Figure 6. Passifloraceae phylogeny. Maximum likelihood IQ-TREE analysis of rDNA ITS sequences. *Jongkindia mulbahii* is shown in a well-supported position in between Turneroideae and Passifloroideae. Nodes without bootstrap frequencies indicate values <60%. The colour coding indicates a range from green (no support) to red (100% support). NB *Adenia* appears polyphyletic, however we suspect this could be due to mis-identification of the *Adenia cynanchifolia* KC207258 specimen.

Table 3. Scoring of characters in Table 2 for selected terminals in Passifloraceae. Question marks indicate unknown state.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.
Adenia	0	1	0/1	0	0/1	0/1	1	3	1	0	0/1	0	0/1	1	1	1	0/1/3	0/1
Barteria	1	1	1	0	2	2	1	2	1	0	0	0	0	1	0	1	2	0
Erblichia	0	0	1	0	1	1	1	3	0	0	1	0	1	1	1	1	1/2	1
Jongkindia	1	0	0	0	0	0	1	1	1	0	0	1	0	2	1	0	2	0
Malesherbia	0	0	1	0	1	1	0	0	1	0	1	0	1	0	0	1	0/1	1
Paropsia	0	1	1	0	1	1	1	1	0	1	1	0	0	1	1	1	1/2	0/ 1
Passiflora	0	1	1	0	1	1	1	1/2	1	1/2	1	0	0	2	1	0/1	3	0/ 1
Pibiria	0	1	1	1	1	0	0	0	0	0	0	0	?	?	0	1	1	0
Piriqueta	0	0/1	1	0	1	1	0/1	3	1	0	0	0	1	1	0/1	1	0/1/2	1
Turnera	0	0/1	1	0	1	0	1	3	1	0	0	0	1	1	1	1	0/1/2	0/1

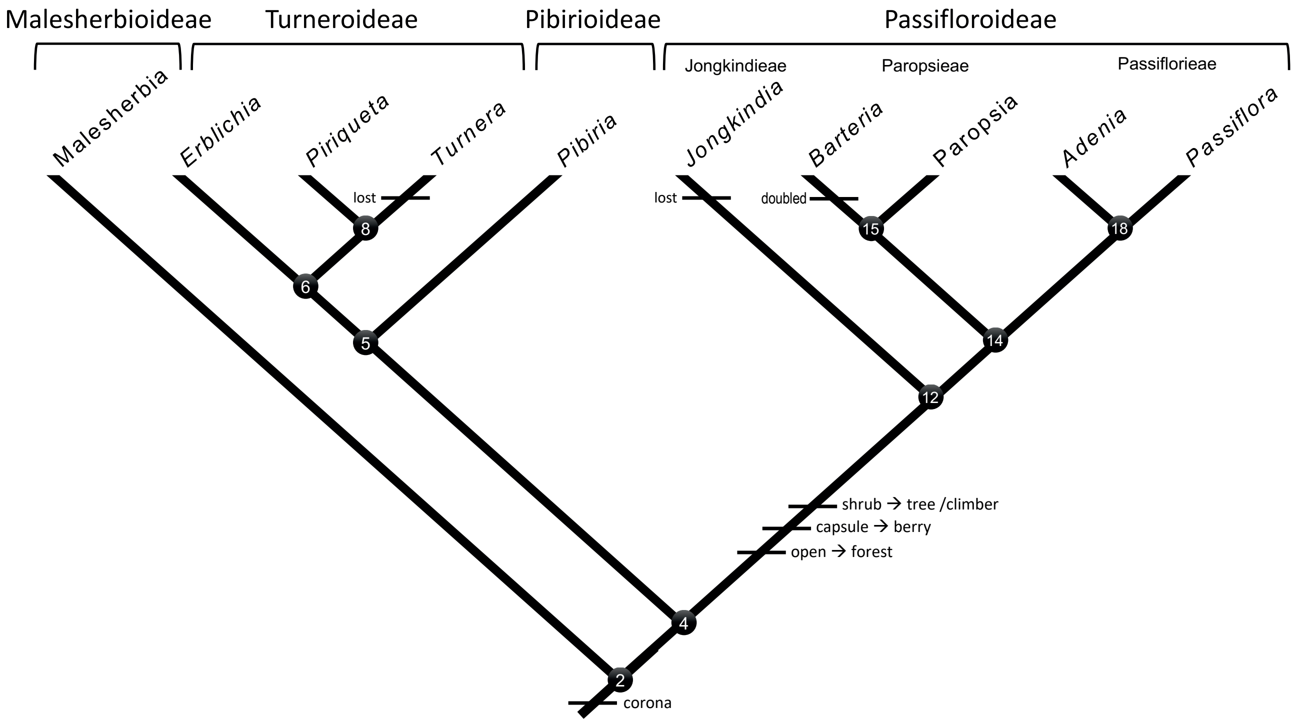

Figure 7. Summary tree depicting phylogenetic relationships in Passifloraceae s.l. based on chloroplast and rDNA sequence comparisons (see Figures 3-6), using exemplar genera to represent the clades. Note that Paropsieae also includes the African genera *Viridivia*, *Paropsiopsis* and *Smeathmannia* and that Passiflorieae also includes *Deidamia*, *Basananthe*, and *Ancistrothyrsus*. Node numbers and (selected) character state changes are indicated; 'lost' and 'double' refer to corona. For individual character optimizations see suppl. figure S4.

all other lineages, was inferred 'by eye' (Figure 7) and used to optimise the non-DNA characters inferred for the entire ingroup, using TNT. This topology was also found by Xi et al. 2012, based on plastome comparisons across Malpighiales.

Results from the morphological character optimisation (Figures 7, S3 and S4) indicate that i) Turneroideae and Passifloroideae are distinguished mainly by fruit type (capsular versus berry) and ii) that coronas have been lost in both *Turnera* and *Jongkindia*, but doubled in *Barteria*. Furthermore, we observe that whereas *Jongkindia* and *Pibiria* are characterised by significantly higher amounts of apomorphies than all other lineages, *Adenia* and *Passiflora* on the other hand show almost predominantly polymorphisms (see Fig. S3). No other lineage in our sampling exhibits such a level of polymorphism and could perhaps indicate their relatively recent origin or the occurrence of hybrids among these species. When looking at the ancestral states (Table S1) we see that for the most recent common ancestor of the Passifloroideae clade (node 12 in Figure 7) our reconstruction would imply a forest-dwelling tree with single, pedicellate, 5-merous flowers, a corona, extrafloral nectaries but no androgynophore. Its flowers would have many ovules and its fruits would be berries with single-arilled seeds; vegetatively, it would have stipules as well as indumentum on its leaves.

It took GetOrganelle only a few iterations to complete the plastome assembly process. This yielded 78 contigs (nodes) and 48 connections (edges) between them with a total length of 168.577 bp (163.172 without overlaps). This is slightly longer than the *Passiflora edulis* plastome length of 151,406bp (KX290855.1, Cauz-Santos et al. 2017). Nodes had an N50 of 3.985 bp and the longest node was 14,535 bp. The median read depth across all nodes was 18,7x and nodes were connected by Bandage into 33 'components' (see Fig. 8), the largest of which was 70,320 bp long. Total length of unconnected ('orphaned') nodes was 93,826 bp. It took IOGA >75 iterations to complete the assembly process which was stopped when no apparent N50 increase was observed. This yielded many contigs >1000bp with N50 =14,796. nBLAST analysis of the obtained contigs revealed that both plastome and mitome fragments had been assembled (with comparable read coverages each) and that *Jongkindia* IRs, SSC and LSC compartments had been partly assembled. Contigs from GetOrganelle and the IOGA analyses were mixed and mapped to the *Mitostemma brevilis* plastome (Fig. S6) which was covered by appr. 90%.

Given the fact that that several *Jongkindia* mitome sequences were assembled using a plastome reference,

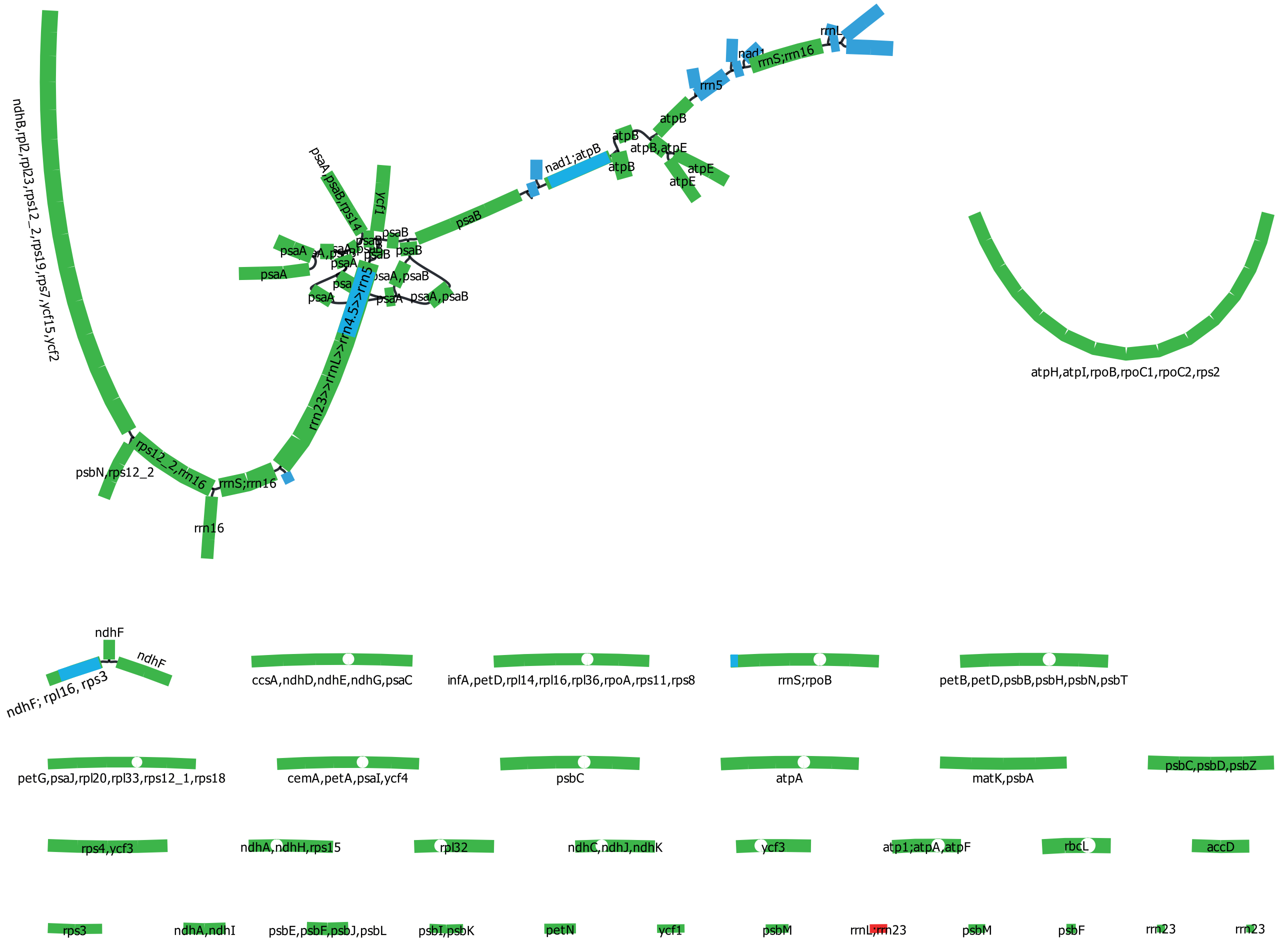

Figure 8. *Jongkindia mulbahii* mitome (blue) and plastome (green) assembly scaffolds sorted by size (kb) and resulting from GetOrganelle assembly using plastome reference sequences. Relative position and BLASTn hits of main contigs as reconstructed in GetOrganelle (see text) is indicated.

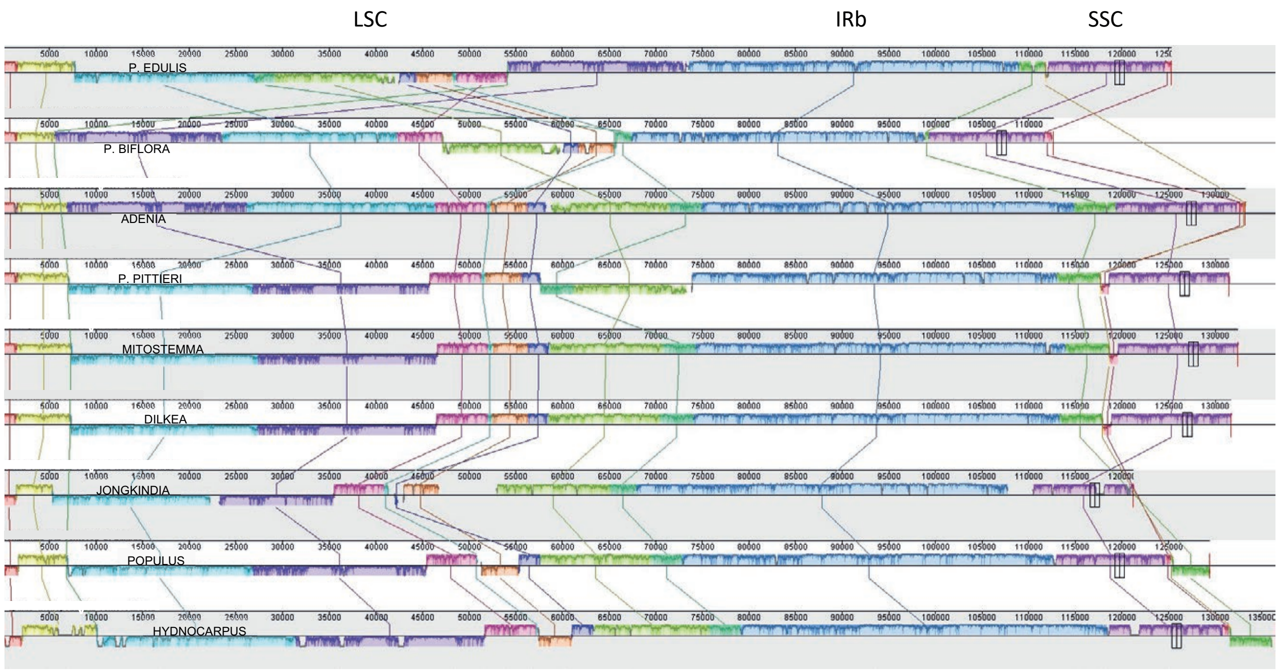

Figure 9. Mauve plastome alignments comprising the large single copy region (LSC), one of two inverted repeats (IRb) and the short single copy region (SSC). Shown are (from top to bottom, and approximately in phylogenetic arrangement) *Passiflora edulis* NC034285 (length 151,286), *P. biflora* NC038120, *Adenia mannii* NC043791 (length 165,364), *P. pittieri* NC 038125, *Mitostemma brevifilis* MT525867 (length 163,032), *P. edulis* MF807938, *Dilkea retusa* NC053302, *Jongkindia mulbahii*, *Populus trichocarpa* NC_009143.1 (length 157,033) and *Hydnocarpus hainanensis* (Achariaceae) NC_042720.1 (length 163,330). NB for *Malesherbia* only the IRb is available in GenBank and was therefore not included here. Colours indicate different Local Colinear Blocks and their relative position is indicated by coloured lines. Position of scaffolds indicates directionality relative to the reference, i.e. above the line is same orientation, below the line is in reverse orientation.

we interpret this as plastome fragments having, at some stage in the evolution of this lineage, been transferred to mitomes. This process, yielding so-called *mtpts*, has been documented as fairly frequently occurring among angiosperm organelle genomes (Jansen & Ruhlman 2012; Wang et al. 2007; 2017). Gandini and Sanchez (2018) estimate that "one out of five plant mtDNAs received plastid sequences by HGT". In case such a mtpt is still present in the reference plastome, it could prime the assembly process, extending the iterative assemblies into neighbouring mitome sequence. We found one scaffold consisting of both plastome and mitome associated contigs of 400-600bp, which means that read pairs had been found with one read being plastome-derived and the other stemming from the mitome, indicating the two sequences had been in linear order. We found the presumed *Jongkindia* mtpts to correspond to the consecutive atpB—atpE—ndhC intron region in *P. edulis*, which is part of the LSC. This region was found to comprise the smaller of two reversals found in *Passiflora* when compared across 12 Malpighialan plastomes (Cauz-Santos et al. 2017). The same region was also found to represent a mtpt in *Geranium brycei* (Adams et al. 2000), and for atpB in *Sapria himalayana* (Rafflesiaceae; Bock 2010).

The plastome structural analysis (Figure 9) indicated overall good alignability among the *Jongkindia* draft plastome with the other plastomes included. The dotplots (Figure S5) showed that *Jongkindia* contains, apart from the two Inverted Repeats, two small inversions which are present in all other plastomes too. It also clearly shows that with regards *Passiflora pittieri* there is a main inversion stretching from positions 20–65kb. This is also visible in the Mauve structural alignment in Figure 9, around 7–47kb. The implication is that *Jongkindia* has a *Paropsieae*/*Adenia* – like plastome structurally, and the elevated structural rearrangements known to have occurred in *Passiflora* plastomes (Cauz-Santos et al. 2017) occurred later within the *Passiflora* clade.

DISCUSSION

Jongkindia mulbahii as described here presents an interesting case evolutionarily as it combines a typical *Passiflora* (Passifloroideae) fruit syndrome with a Turneroideae floral syndrome. Assuming our summary tree reflects phylogenetic relationships accurately, *Jongkindia* *mulbahii* is in an isolated position phylogenetically, but not quite divergent from all other species groups in Passifloroideae, as judged from its moderate DNA-based branch lengths. This could indicate there having been other Jongkindia species now extinct, but probably no substitution rates increase in *J. mulbahii* after it split off from its common ancestor with the rest of Passifloroideae.

The fleshy fruits (berries) of *J. mulbahii* should be considered synapomorphic for Passifloroideae (Figure 7, S3 and S4) and this would imply that the 'genomic wiring' of *J. mulbahii* with regards to fruit formation and structure was already in place in the most recent common ancestor (MRCA) of this clade. In contrast, floral morphology for *J. mulbahii* appears to be less straightforward to interpret: its 4-merous flower seems to be, along with *Viridivia*, the only cases in Passifloraceae s.l. and can probably be considered independent autapomorphies. The absence of a corona in *J. mulbahii* should probably be interpreted as a secondary loss, as coronas are known from Turneroidea as well (but not *Turnera*, Figure 7, S3 and S4). For instance, the Neotropical *Piriqueta* is known to have coronas developed from calyx and a corolla not unlike that of *Passiflora* (Bernhard 1999). This could point to homology between the two clades with regards to their coronas, and possibly *Jongkindia* may have lost a corona 'secondarily' whereas in *Barteria* it got doubled (as well as in its sister group *Paropsiopsis*). Nevertheless, whether Turneroideae and Malesherbiodeae coronas are truly homologous with that what got lost in *Jongkindia* could be assessed based on, for instance, developmental studies, such as carried out by Bernhardt (1999). He concluded that the ancestral androecium for Passifloroidea would have been a single whorl. In our ancestral state analysis we reconstructed the MRCA of Passifloroidea (node 12 in Figure 7) to be a forest-dwelling tree with single, pedicellate, 4-merous flowers, a corona, extrafloral nectaries but no androgynophore, many ovules and berries with single-arilled seeds. It can be argued that this reconstruction might change upon the inclusion of additional Passofloracean lineages but nevertheless, we interpret our inferred pattern as indicative of conserved fruit morphology combined with unstable /labile floral morphology. *Jongkindia* would then have developed maintaining the Passifloracean fruit morphological syndrome but developing a new autapomorphic floral syndrome that could perhaps reflect adaptation to new environmental conditions in Miocene tropical Africa. To what extend pollinator-use may have played a role here is not known, but probably floral evolution was more 'adaptive' or under selective pressure (at least exerted by pollinators) then fruit morphology, for which the genomic wiring was probably already in place in the proto-*Jongkindia* lineage.

In the *Adenia/Passiflora* clade, we reconstructed predominantly polymorphic characters (see Figure S3) and interpret this as reflecting the possible young age of these clades, the occurrence of hybridization(?), biparental inheritance of plastids in *Passiflora* (Hansen et al. 2007) and possibly the occurrence of dioecy in *Adenia*. In such a scenario, with transitions in breeding system, evolutionary stasis or dead ends have been suggested but evidence is accumulating that this does not need to be the case (e.g. Muyle et al. 2020; Takahashi et al. 2022). Moreover, the genetic consequences of dioecy at the population level, for instance effective population size, are not yet fully understood, but may be relevant to the occurrence of (morphological) polymorphism.

Plastomics

For *Jongkindia mulbahii* we assembled appr. 90% of its plastome sequence as compared with *Passiflora edulis* and *Mitostemma*, currently the most closely-related plastomes available to date. Direction of our scaffolds relative to *Populus trichocarpa* and *Hydnocarpus hainanensis* (Achariaceae) was straightforward to determine and within the scaffolds itself we infer only a few rearrangements, especially within SSC and LSC. This leads us to conclude that the *Jongkindia* plastome looks rather similar in terms of genome structure to that in *Adenia*, *Mitostemma*, *Dilkea* and parts of *Passiflora*, especially the early-branching *P. pittieri* (see Cauz Santos et al. 2017). 'Downstream' in the Passiflora clade several inversions have occurred, sometimes overlapping but this has to be confirmed by *Malesherbia* plastome data (currently, only *Malesherbia* IRb plastome sequence is available in GenBank). In any case, plastome structural rearrangements in *Jongkindia* appear not to be present at the scale as seen in some other plants groups, e.g. in *Silene* (Sloan et al. 2014) and in *Pelargonium* (Weng et al. 2017). We argue that *Jongkindia* will be a suitable outgroup for future plastome evolution studies in Passifloraceae.

We found a possible *Jongkindia* mtpt to be in exactly the same atpB—atpE—ndhC region as where in Passiflora a reversion was reconstructed (and confirmed with PCR and Sanger sequencing) when compared across 12 Malpighialan plastomes by Cauz-Santos & al. (2017). It is interesting to find this same region to have probably moved to the *Jongkindia* mitome, and in addition in *Geranium brycei* (Adams et al. 2000), and for atpB in *Sapria himalayana* (Rafflesiaceae; Bock 2010). Whether this pattern is co-incidence or a structural factor or mechanism is involved remains to be investigated. In terms of mechanisms, whether inversions and transfers are related is not known yet. Rice et al. (2013) proposed a 'fusion-compatibility' model in which a foreign mitochondrion is captured, with subsequent fusion and genomic recombination of donor and recipient mitochondria. Gandini and Sanchez (2018) estimate that in most observed cases (i.e. 65%) mtpts are the result of mt—mt HGT following pt—mt transfer, rather than direct pt—mt transfer, although the amount of angiosperm mitome sequence data is still not sufficient in order to substantiate this further. It is not known to what extent *Jongkindia mulbahii*, like *Passiflora*, has biparental inheritance too and if so, whether it could explain the conspicuous mtpts found in our study.

In conclusion, we describe a new monotypic genus of Passifloraceae sens. lat., with the only species *Jongkindia mulbahii* combining floral characteristics of the Turneraceae and fruit characteristics of the Passifloraceae s.s. (or subfamily Passifloroideae). Phylogenetic analysis based on plastome-derived sequence comparisons places *Jongkindia* 'in between' the Passifloroidea and Turneroideae clades, but clearly more closely related to the former. We present evidence for two mitome-plastome fragment transfers in this species which correlate with transfers for the same region in other lineages. As a possible explanation for the 'chimaeric' morphological syndrome in *Jongkindia mulbahii* with its 'wrong flowers' we consider the following historical scenario: An ancestral polymorph proto-Passifloracean lineage in Africa, at the population-level and possibly driven by late Miocene/Pliocene climatic changes, as well as the earlier, Eocene Whole Genome Duplication events reconstructed in Malpighiales (Cai et al. 2019), would have undergone fixation, development and splitting into two lineages with either the Turneroideae or the Passifloroidean syndrome. Subsequent 'canalisation' and sorting of the genomic wiring of either syndromes would have fixed the capsule and berry syndromes that we still recognise today. Subsequently, and possibly by jump dispersal, *Passiflora* will have ended-up in Neotropics, and perhaps it was in Africa too but went extinct. In any case, the radiation of 600+ species of *Passiflora* in the Neotropics indicate that selection and pollinator pressures there must have been quite different from what was experienced in Africa, where *Jongkindia* did not undergo extensive floral morphological changes, keeping a more symplesiomorphic floral appearance. As such the Passifloroideae biogeographic pattern supports the more general pattern (e.g. Good 1964, Slik et al. 2015) of higher generic but lower species-diversity in Africa as compared with Neotropics.

TAXONOMY

Jongkindieae Breteler & F.T.Bakker, **trib. nov.**

Diagnosis

Small trees with *Turnera*-like, 4-merous flowers without corona and *Passiflora*-like, baccate fruits with arillate seeds. The phylogenetic position of this tribe as sister to Passifloreae plus Paropsieae is confirmed by chloroplast and rDNA sequence comparisons. Type: *Jongkindia* Breteler & F.T. Bakker

Jongkindia Breteler & F.T.Bakker, **gen. nov.**

Diagnosis

Glabrous treelet with alternate, glandular-dentate, simple, stipulate leaves and single, axillary, shortly pedicellate, bisexual flowers. Calyx tubular in lower half and with free petals inserted on rim of the calyx tube. Flowers *Turnera*-like, differing by being 4-merous. Fruits fleshy, many-seeded, indehiscent, typical *Passiflora*-like. Species: one. Type species: *Jongkindia mulbahii.*

Jongkindia mulbahii Breteler & F.T.Bakker, **sp. nov.** (Fig. 1, 10).

Type: LIBERIA. Sino, c. 50 km E of Greenville, riverbank forest, 5°05.09'N, 8°32.02'W, Alt. 56 m, 13 Mar. 2014 (fl., fr.), *Jongkind, Mulbah, Harris, Chaleson & Forkpah 12424* (holotype BR!, two sheets numbered BR 0000014915833 and BR 0000014915826); isotypes B!, COI!, G!, K!, LISC!, MA!, MO!, NY!, PRE!, WAG!).

Description

Treelet c.5 m tall and 5 cm dbh, glabrous in all its parts. Branchlets green, bark soon becoming brown and glossy. Stipules thick, deltoid to rim-like, up to 1 mm long, sometimes gland-tipped. Leaves distichous, subsessile; lamina coriaceous, ovate-elliptic to narrowly lanceolate, 2.5–4 times as long as wide, 4–10 x (1–)1.5–2.5(–4) cm, long-cuneate at base, gradually tapering to an acute to narrowly rounded apex or slightly, 0.5–1.5 cm acuminate; margin shallowly dentate, the teeth provided with a gland beneath; midrib and the 6–8 pairs of main lateral nerves ± equally prominent both sides. Flowers bisexual, 4–merous, yellow, solitary in the leaf axil; buds covered with resin, c.8 mm long in full grown state. Bracteoles small, rim-like ≤ 0.2 mm long. Pedicel articulate, the lower part c. 1 mm long, the upper part c. 2 mm long. Sepals united into a 3 mm long funnel-shaped base (hypanthium); lobes

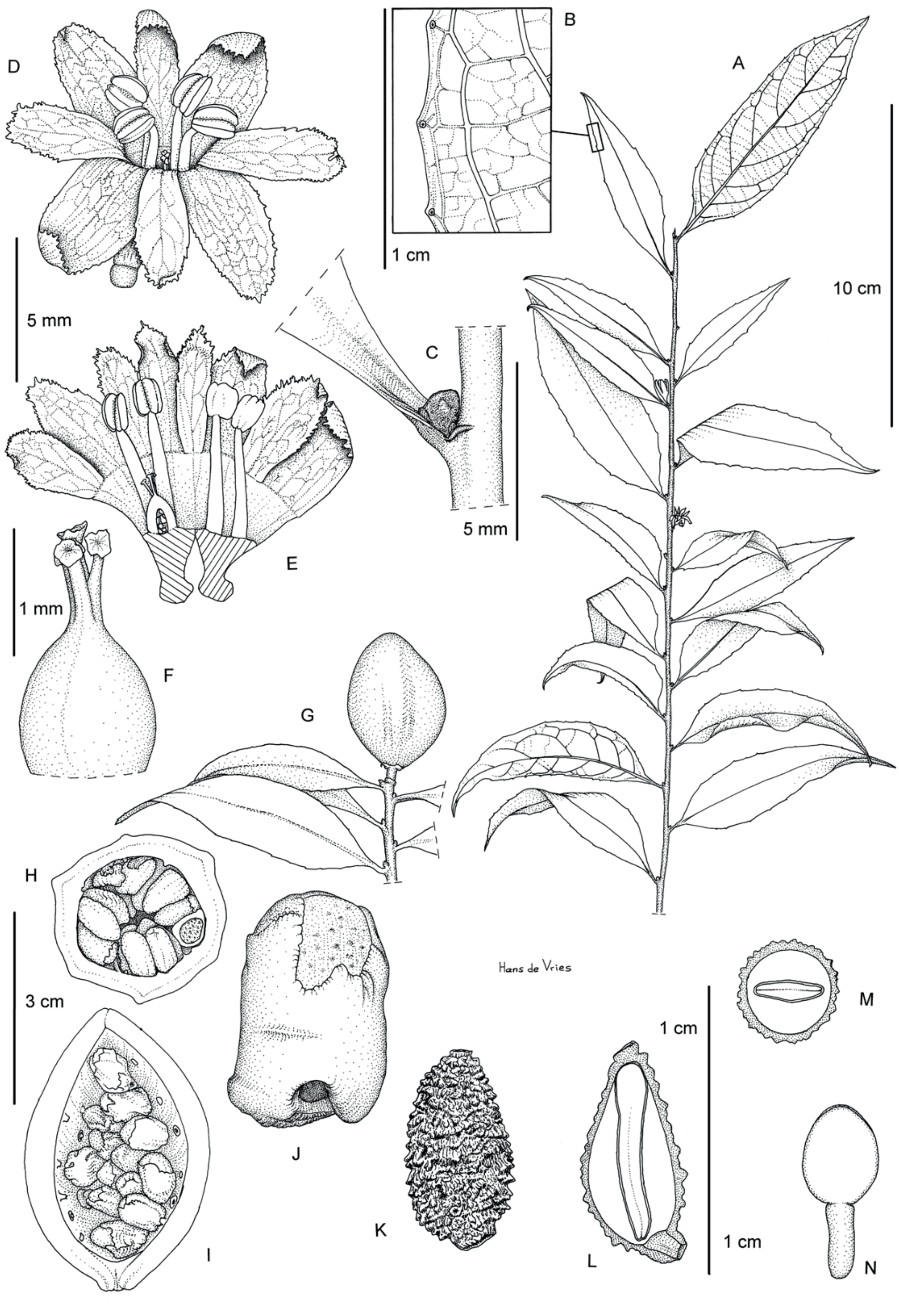

Figure 10. *Jongkindia mulbahii* morphology, with a) flowering branch zooming in b) on leaf margin at the abaxial leaf surface showing glands; c) leaf axil with resinous flower bud and stipule; d) flower; e) flower cut and laid open; f) pistil; g) branchlet with fruit; h) fruit in transverse section; i) fruit in longitudinal section; j) seed with arils; k) seed; l) seed in longitudinal section; m) seed in transverse section; n) embryo. After *Jongkind et al. 12424* (BR), see suppl. figure S1. Drawn by Hans de Vries.

imbricate, spreading at anthesis, oblong, c. 5 mm long, slightly hooded at apex, scarcely fimbriate in apical part. Petals spreading, free, valvate, inserted on the rim of the calyx tube and alternate with the calyx lobes, fimbriate in the upper half, slightly hooded at apex. Stamens 4, ± erect, alternate with the petals, free, inserted on the calyx tube near its base, c. 5 mm long; anthers exerted, c. 1.5 mm long, introrse, dorsifixed. Pistil c. 2 mm long, inserted on the bottom of the calyx tube; ovary ovoid, c. 1 mm long, with 3 multiovulate, parietal placentas; styles 3, c. 1 mm long, stigmas ± flat, ± circular in outline. Fruit indehiscent, orange, ellipsoid, (5–) 6–angled, slightly ribbed, 3–5 cm long, 2–3 cm in diameter, smooth, slightly glossy; wall 3–4 mm thick. Fruit stipe 3 mm long. Seeds numerous, black, ellipsoid, c. 7 mm long, tuberculate, with a ± thick, lobulate, free outer aril of 7–8 mm long, covering the seed for ⅔–¾ of its length, bulging over the hilum and an inner, thin, free aril, tightly covering the seed completely. Testa corrugate. Endosperm copious. Embryo straight, embedded in white, more or less soft endosperm.

Etymology

The generic name is derived from C.C.H. Jongkind, the first collector of the type specimen. He is best known as co-author of Woody Plants of Western African Forests (Hawthorn and Jongkind 2006) and continues his botanical exploration of western african forests, especially of Liberia. The specific epithet of the species is after D.M. Mulbah the second collector.

Distribution

SE Liberia (Figure 1).

Habitat and Ecology

Understorey treelet in primary riverine forest at low altitude of 56 m.

Conservation

The status of the new taxon cannot be calculated because of deficient data. Given the inconspicous habit of *Jongkindia mulbahii* it is likely that more individuals go unnoticed and therefore the area around its collecting locality needs to be further explored to establish *Jongkindia* distribution. Noticeably, the area is supposed to be rich in gold and may be exploited in the near future.

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A new species of *Pleroma* (Melastomataceae) from the Southern Espinhaço, Minas Gerais, Brazil

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Abstract. *Pleroma barbellatum* P.J.F.Guim., D.Nunes & I.M.Araújo a new species of Melastomataceae (Melastomateae) from the Espinhaço Range of Minas Gerais State, Brazil, is described and illustrated, and their affinities and diagnostic characters are here discussed. *Pleroma barbellatum* is closely related to *P. formosum*, *P. martiale*, and *P. riedelianum*. They share a similar habit and leaves that are alike in size and shape. However, they can be distinguished by differences in the trichomes of the branches, leaves and inflorescences, in addition to other characters related to the type of inflorescence and size of the bracteoles. We recommend a conservation status of Endangered for *P. barbellatum*.

Keywords: Campos rupestres, Diamantina Plateau, Espinhaço Meridional, Grão-Mogol, Melastomateae.

INTRODUCTION

Pleroma is a Neotropical genus of the tribe Melastomateae whose morphological characteristics are the extension of the connective beyond the thecae called pedoconnective, ovary apex with a persistent crown of erect trichomes surrounding the base of the style and cochleate seeds (Renner 1993; Guimarães et al. 2019; Veranso-Libalah et al. 2022). In Brazil there are 159 species of *Pleroma* distributed mainly in the Atlantic Forest with 105 species and Cerrado with 58 species, of which 4 are shared between these biomes (Guimarães 2022). The main features of *Pleroma* are the shrubby habit, rarely arboreal or herbaceous, flowers with 5 petals, purple to lilac or rarely white, anthers purple or pink, filaments with the frequent presence of trichomes

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capitate-glandular, and deciduous sepals that are absent on hypanthia that envelop mature capsules (Guimarães et al. 2019).

The Espinhaço Range is a major center of plant diversity in eastern Brazil (Giulietti and Pirani 1988; Giulietti et al. 1997; Colli-Silva et al. 2019) that extends for more than 1200 km along the states of Bahia and Minas Gerais (Almeida-Abreu and Renger 2002), with a predominance of *campo rupestre* vegetation (Vasconcelos 2011; Alves et al. 2014). This imposing mountainous expanse is composed mainly of herbaceous, subshrubby and shrubby life forms (Colli-Silva et al. 2019), originating from the Cerrado, Caatinga and Atlantic Forest (Conceição et al. 2016), and can be divided into two physiographic domains known as Chapada da Diamantina (comprising the northern portion, in the state of Bahia) and Serra do Espinhaço (southern portion, Minas Gerais) (Danderfer and Dardenne 2002; Gontijo 2008). These domains were recognized and characterized by Colli-Silva et al. (2019), respectively, as Chapada da Diamantina and Southern Espinhaço provinces. Following the classification by Colli-Silva et al. (2019), the Southern Espinhaço is subdivided into three districts: Grão-Mogol, Diamantina Plateau, and Iron Quadrangle.

In the Southern Espinhaço, Melastomataceae stand out as one of the vascular plant families with the most endemic species in the province (Colli-Silva et al. 2019). While in Grão-Mogol and Diamantina Plateau districts, the family is one of the richest [*e.g.*, Pirani et al. (2003, 2015); see also Paranhos (2020) for an updated list of Melastomataceae from the Diamantina Plateau]. However, the richness of the family in Grão-Mogol and Diamantina Plateau has increased significantly with 46 new species being described in the last 10 years (Supplementary file). Futhermore, floristic studies have contributed to the review of species occurrence and diversity within the provinces (*e.g.*, Candido 2005; Martins et al. 2009; Araújo 2013; Paranhos 2020).

During a floristic study focused on Melastomataceae from Biribiri State Park, in the state of Minas Gerais (Araújo 2013), an enigmatic species of *Pleroma* was found, but initially identified as *Pleroma formosum* (Cogn.) P.J.F.Guim. & Michelang. When we examined specimens more closely, we confirmed that it is a new species endemic to the Southern Espinhaço, occurring in the Diamantina Plateau and Grão-Mogol districts, Minas Gerais, Brazil. We here describe, illustrate and compare this new species to morphologically similar species. In addition, notes on its geographic distribution and conservation status, photos of living specimens, and scanning electron microscopy (SEM) images are provided.

MATERIALS & METHODS

Beentje (2016) was adopted for general morphological terminology. We emphasize that the term trichome barbellate is adopted here following Beentje (2016), but in Wurdack (1986) this same trichome is described as "elongated moderately roughened hairs". The stamen dimetrism index (SDI) was used to measure to the difference (or not) in the length between the antesepalous and antepetalous stamens (Melo et al. 2021). The morphological characteristics of the species that are compared to the new species were obtained from Guimarães (1997, 2022) and personal observations by the same author. The herbaria acronyms follow Index Herbariorum (Thiers 2022). The SEM images are based on herbarium specimens and taken with a EVO® 10 Zeiss microscope. The geographic distribution map was prepared using ArcGIS 10.5 (https://www.arcgis.com/features/index.html). The conservation status follows IUCN (2012, 2022) guidelines and criteria. The area of occupancy (AOO) and extent of occurrence (EOO) were calculated using the software GeoCAT (Bachman et al. 2011), with a user-defined cell width of 2 km.

TAXONOMIC TREATMENT

Pleroma barbellatum P.J.F.Guim., D.Nunes & I.M.Araújo, **sp. nov.** (Figures 1, 2, and 3).

Type: Brazil. Minas Gerais: Diamantina, Serra do Espinhaço, ca. 14 km SW of Diamantina on road to Gouveia, 1300–1360 m, 05 Feb. 1972 (bud, fl.), *W.R. Anderson, M. Stieber & J.H. Kirkbride, Jr. 35497* (holotype RB! barcode 01352109; isotypes MO herbarium number 3273401, NY! barcode 01404497, SP! barcode SP043387, UB! barcode UB0120844, US! barcode 01918515).

Diagnosis

A shrub or tree 1–2 m or 2–3 m tall, is readily recognized by its barbellate and eglandular trichomes (on the branches, petioles, leaf blade surfaces, pedicels, bracts and bracteoles, hypanthia, outer surface and margins of sepals, and base of style), oblong, elliptic or lanceolate leaf blades 15–44 × 6.5–13.5 mm, with strigose indumentum on the adaxial surface, and obtuse, rounded or cuneate base, terminal cyme inflorescences 3–5 cm long with up to 5 flowers or flowers solitary and terminal, campanulate hypanthia covered by a strigose bristly indumentum, and ensiform sepals 4–6 × 1.5–2.5 mm. *Pleroma barbellatum* is similar to *Pleroma formosum* (Cogn.) P.J.F.Guim.

& Michelang., but is readily distinguished by shorter leaf blades 15–44 mm long (*versus* 40–55 mm long in *P. formosum*), cyme inflorescences (*vs.* thyrsoids), 1–5 flowers per inflorescence (*vs.* 35–58 flowers per inflorescence), bracteoles 10–17.5 × 6.5–10 mm (*vs.* 4–6 × 1.4–2 mm), sepals 4–6 mm long (*vs.* 2.5–2 mm long).

Description

Shrub 1–2 m tall or treelet 2–3 m tall, with branching bi- or trichotomic; branches covered by a strigose indumentum of barbellate trichomes 0.3–0.8 mm long, eglandular, brownish yellow; internodes 5–13 mm long; distal branches quadrangular, light green, beige or brownish yellow; proximal branches circular, grey or dark greyish brown, sometimes with galls in the dichotomy (see Figure 3D). Leaves decussate, isomorphic in size per node, chartaceous, petiolate; hypostomatic; covered by a strigose indumentum of trichomes that are thick and stiff adaxially and pilose with trichomes elongated and fine abaxially; venation acrodromous, 3-nerved basal + one outer pair suprabasal, midrib and first pair of veins callous and second pair inconspicuous (on the abaxial surface); petiole canaliculate or flat, (1.2–)2–3 mm long, trichomes barbellate, (0.4–)1–1.2 mm long, brownish yellow (when dry); blades oblong, elliptic or lanceolate, 15–44 × 6.5–13.5 mm; abaxial surface light green, but the ribs turn clearer (when fresh) or brownish yellow (when dry), trichomes barbellate, 0.3–0.8(–1.5) mm long and adnate to the leaf surface for ½–⅔ of their length, eglandular; adaxial surface dark green (fresh material) or dark brown (dry material), trichomes barbellate, 0.1–0.8(–1) mm long, eglandular, and inconspicuous trichomes capitate-glandular; base obtuse, rounded or cuneate; margin entire and slightly revolute, trichomes 0.3–0.8 mm long; apex obtuse or acute. Inflorescences terminal cyme 3–5 cm long with 3–5 flowers or flowers terminal and solitary, erect; peduncle, rachis and pedicel quadrangular, covered by a strigose indumentum strigose of barbellate and eglandular trichomes; peduncles 4–5 mm long, trichomes 0.3–0.5 mm long; pedicels 1.5–2 mm long, trichomes 0.8–1.2 mm long; bracts and bracteoles caducous, sometimes persistent; bracts 2, oblong, ca. 12 × 2.5 mm, base and apex obtuse, margins entire and flat, 3-nerved, abaxial surface light green, covered by a strigose indumentum of barbellate trichomes 0.8–1.2 mm long, eglandular, adaxial surface dark green, covered by a strigose indumentum from the middle to the apex (⅔ of the blade), but the basal portion (⅓ of the blade) is glabrous or with sparse barbellate trichomes 0.3–0.8 mm long, eglandular; petiole flat, ca. 1 mm long; bracteoles 2, elliptic or ovate, 10–17.5 × 6.5–10 mm, concave, sessile, base rounded or obtuse, margin entire and revolute, apex obtuse, reddish or pinkish, inner surface glabrous, outer surface covered by a strigose indumentum entirely or only in the central portion from the base to the apex, white (fresh material) or brownish yellow (dry material), trichomes barbellate, 0.3–1 mm long, eglandular. Flowers 5-merous, diplostemonous; hypanthium campanulate, ca. 6 × 3–3.5 mm, outer surface light green, covered by a bristly strigose indumentum, white (when fresh) or brownish yellow (when dry), trichomes barbellate, 1.8–2 mm long, eglandular, mixed with inconspicuous gland-tipped trichomes, brownish yellow (when dry), the glands inconspicuous; sepals 5, ensiform, 4–6 × 1.5–2.5 mm, inner surface glabrous and reddish or pink, outer surface light green, covered by a strigose indumentum, white (when fresh) or brownish yellow (when dry), trichomes barbellate, 1–2.8 mm long, eglandular, margin flat with a hispid indumentum of barbellate trichomes 0.3–0.5(–1) mm long, eglandular; petals 5, obovoid, 11–17 × 6–8 mm, purple, the margins ciliate, white, trichomes capitate-glandular, 0.3–0.8 mm long, apex rounded, inner and outer surfaces glabrous; stamens 10, isometric or subdimetric in length (SDI ≅ 0.07–0.15), subisomorphic, filaments filiform, erect, lilac with a white base, indumentum pilose from base to ⅔ of the total length, white, trichomes capitate-glandular, appendages ventrally bituberculate, glabrous, lilac, dorsal appendages absent or present, when present calcarate, ca. 0.1 × 0.1 mm, glabrous, pedoconnectives slightly curved, lilac with a white base, anthers oblong, slightly curved, ventral surface rugulose, apex attenuate with a small ventral pore, purple or lilac with white apex; antesepalous stamens (larger) 5, 17–28 mm long, filaments 9–13 mm long, trichomes 0.3–2 mm long, ventral appendages 0.2–0.3 × 0.2–0.3 mm, pedoconnectives 1–3 mm long, anthers 7–12 mm long; antepetalous stamens (smaller) 5, 14.8–20.5 mm long, filaments 7.5–10 mm long, trichomes 0.3–0.5(–1) mm long, ventral appendages ca. 0.2 × 0.2 mm, pedoconnectives 0.3–0.5 mm long, anthers 7–10 mm long; ovary ovoid, 4–5.5 × 2.5–3 mm, light green, apical half with a puberulous indumentum of eglandular trichomes 0.5–1 mm long, 5-locular, pluriovulate, axile placentation; style filiform, ca. 15 mm long, straight or sigmoid, terete, glabrous or subglabrous with barbellate trichomes, erect and terete, purple or lilac with a white apex, stigma punctiform, white. Fruit a loculicidal capsule covered by the persistent globose hypanthium, capsule ovoid, ca. 6.2 × 4 mm, brownish, 5-locular; seeds elongate-cochleate, ca. 1 × 0.5 mm, brownish, testa tuberculate, cells of testa isodiametric; hilum terminal and elongate.

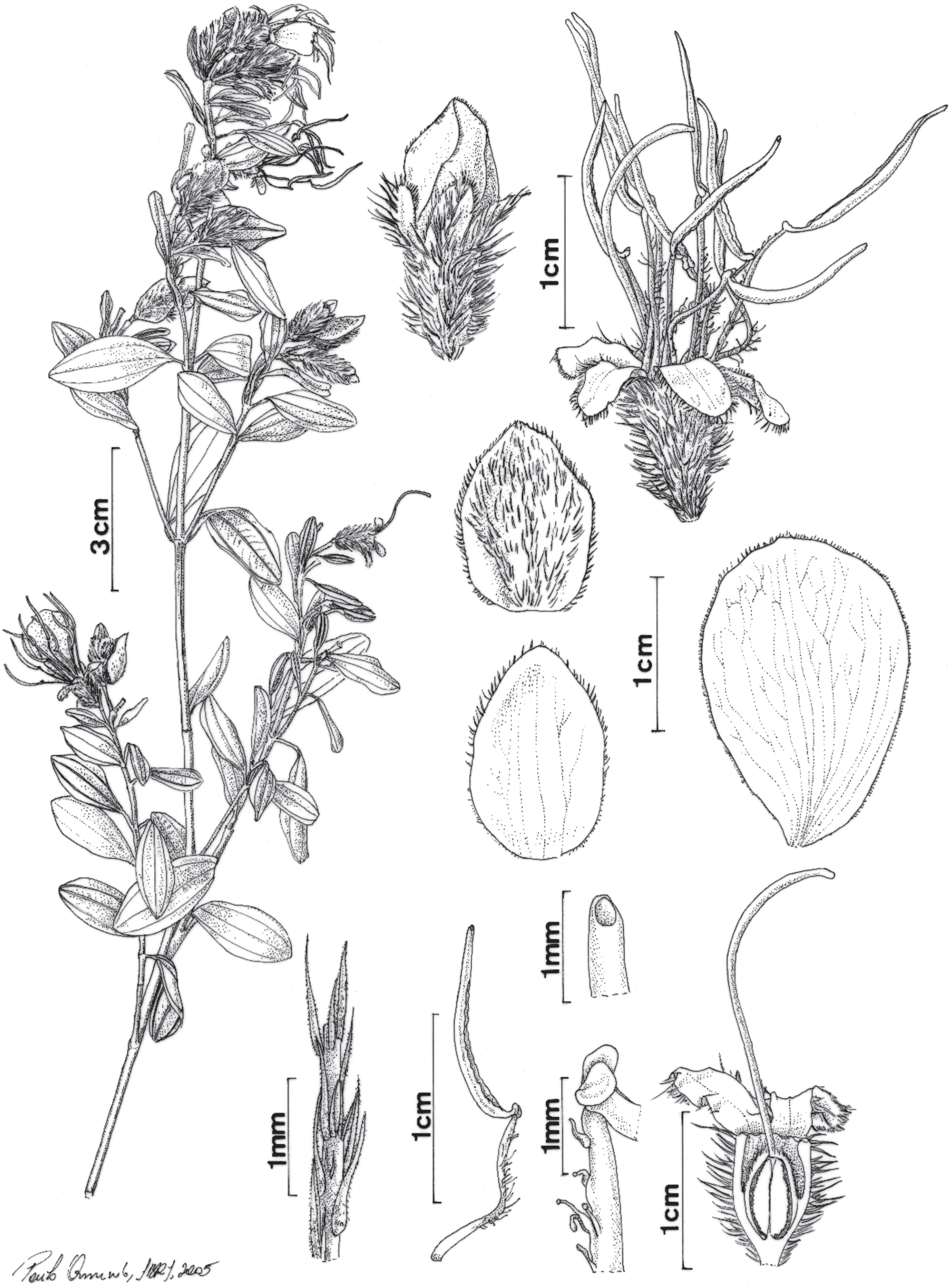

Figure 1. *Pleroma barbellatum* P.J.F.Guim., D.Nunes & I.M.Araújo. (a) Flowering branch. (b) Barbellate trichomes on the branch. (c) Floral bud. (d) Outer surface of bracteole. (e) Inner surface of bracteole. (f) Flower with petals removed. (g) Petal. (h) Antesepalous stamen. (i) Detail of the trichomes on the filament. (j) Anther apex. (k) Flower in longitudinal section. From *W.R. Anderson et al. 35497* (at RB).

Etymology

The specific epithet, *barbellatum*, refers to shortly barbed trichomes (see Beentje 2016) that are easily observed on branches, leaves, pedicels, bracts and bracteoles, hypanthia, outer surface and margins of sepals, and base of the style (see Figures 1B and 2).

Distribution and habitat

Pleroma barbellatum is endemic to the state of Minas Gerais, Brazil, where it occurs mainly in the Espinhaço Range, between 950–1400 m elevation around the city of Diamantina, including Datas, Gouveia and São Gonçalo do Rio Preto. It also occurs further north in Grão-Mogol, about 200 km away from Diamantina (Figure 4). Outside the Espinhaço Range towards southwest, *P. barbellatum* was also been collected in São Gonçalo do Abaeté, about 250 km away from Diamantina (Figure 4). This species has been found in the Cerrado, in *campos rupestres*, on steep rocky slopes with extensive outcrops (Figures 3A and 3B), as well as along the borders of gallery forests, preferably in sandy or fine gravel soils.

Conservation status

Based on distributional data of *Pleroma barbellatum,* the AOO is restricted to 68 km^2 and the AOO is equal to 34,283 km^2. To date, the species has only been collected in *campos rupestres*, especially along the southern portion of the Espinhaço Range (Figure 4), which is threatened by activities such as mining, fires, and agricultural activities (Verdi et al. 2015). *Pleroma barbellatum* occurs only in one Conservation Unit (UC), the Biribiri State Park. Even within the limits of this UC, its long-term survival is directly affected by the degradation of natural resources, areas of swidden and pasture, mineral extraction, damming of streams, accumulation of garbage, bushfires, transmission lines, exotic vegetation, excessive trails and disorderly tourism (STCP Engenharia de Projetos 2004). In view of this panorama, we recommend a conservation status of Endangered [EN B2b(iv)].

Taxonomic notes

In nature, *Pleroma barbellatum* attracts attention by its showy reddish bracts and bracteoles that subtend a characteristic hypanthium covered with a strigose bristly indumentum and leaves which range from oblong, elliptic or lanceolate reaching 15–44 mm long. The leaf blades are covered above with a strigose indumentum composed of barbellate trichomes 0.3–0.8(–1.5) mm long, which are adnate to the blade for ½–⅔ of their length. The abaxial surface of the leaf is covered with barbellate trichomes 0.1–0.8(–1) mm long and inconspicuous capitate-glandular trichomes.

This new species is most closely related to *Pleroma formosum* which has a similar size of about 1 m tall, in having lanceolate leaves 40–45 mm long with five nerves. Both species have leaf blades covered with barbellate trichomes (see the figures 2I and 3I in Guimarães et al. 1999). On the contrary, *P. formosum* has smaller bracteoles (4–6 mm long *vs.* 10–17.5 mm long) and the trichomes on the hypanthium are not bristled. In addition, *P. formosum* has two elongated ventral appendages on the connectives (*vs.* bituberculate ventral appendages), that are narrower and longer than those observed in *P. barbellatum*. Finally, *P. formosum* has a thyrsoid inflorescence with 35–58 flowers (*vs.* cyme with up to 5 flowers or flowers solitary) (Table 1).

Another related species is *P. martiale* (Cham.) Triana due to the shrubby habit with 0.7–1.5(–3) m tall and leaves with 25–43 mm long. On the other hand, *P. martiale* has shorter bracteoles that are 3–5 mm long (*vs.* 10–17 mm long) subtending a strigose hypanthium with smooth non-bristly trichomes and smooth trichomes on branches, surfaces of leaf blades, and hypanthia (*vs.* barbellate) (Table 1). *Pleroma riedelianum* is also a shrub 1.2–3 m tall with leaves that are 25–30 mm long. Bracteoles are also evident but only 6–8 mm long (*vs.* 10–17 mm long), and the hypanthia indumentum is equally strigose but the trichomes are smooth and not bristly (Table 1). The absence of barbellate trichomes on the leaves on both surfaces and on the hypanthia rules out the possibility of considering them the same species. The closest species to *P. riedelianum* is *P. martiale* due to the morphology of the leaves and hypanthia, being distinguished from it mainly by the size of the bracteoles and the indumentum on the filament (Table 1).

Additional specimens examined (Paratypes)

BRAZIL: Minas Gerais: [Datas], Serra do Espinhaço, ca. 18 km by road SW of Diamantina on road to Curvelo, 1400 m, 10 Apr. 1973 (bud, fl.), *W.R. Anderson et al. 8513* (MO herbarium number 3274512, UB! barcode UB0120843, US! barcode 01918607). Diamantina, Parque Estadual do Biribiri, Alto da Jacuba, 18°11’46”S, 43°35’14”W, 28 Feb. 2016 (fr.), *J.E.Q. Faria 5398* (HDJF! herbarium number 3382, HUFU! barcode HUFU00072698, RB! barcode 01306989, UB! barcode UB0120845); Parque Estadual do Biribiri, “Alto da Jacuba”, próximo à “Casa dos ventos”, 15 Jan. 2013 (bud, fl.), *I.M. Franco 1194* (HUFU! barcode HUFU00068485); P.E. Biribiri, “Alto da Jacuba”, 18°08’32.2”S, 43°36’32.2”W, 1382 m, 14 Mar. 2012 (bud, fl., fr.), *I.M. Araújo et al. 261* (HUFU! barcode HUFU00067348);

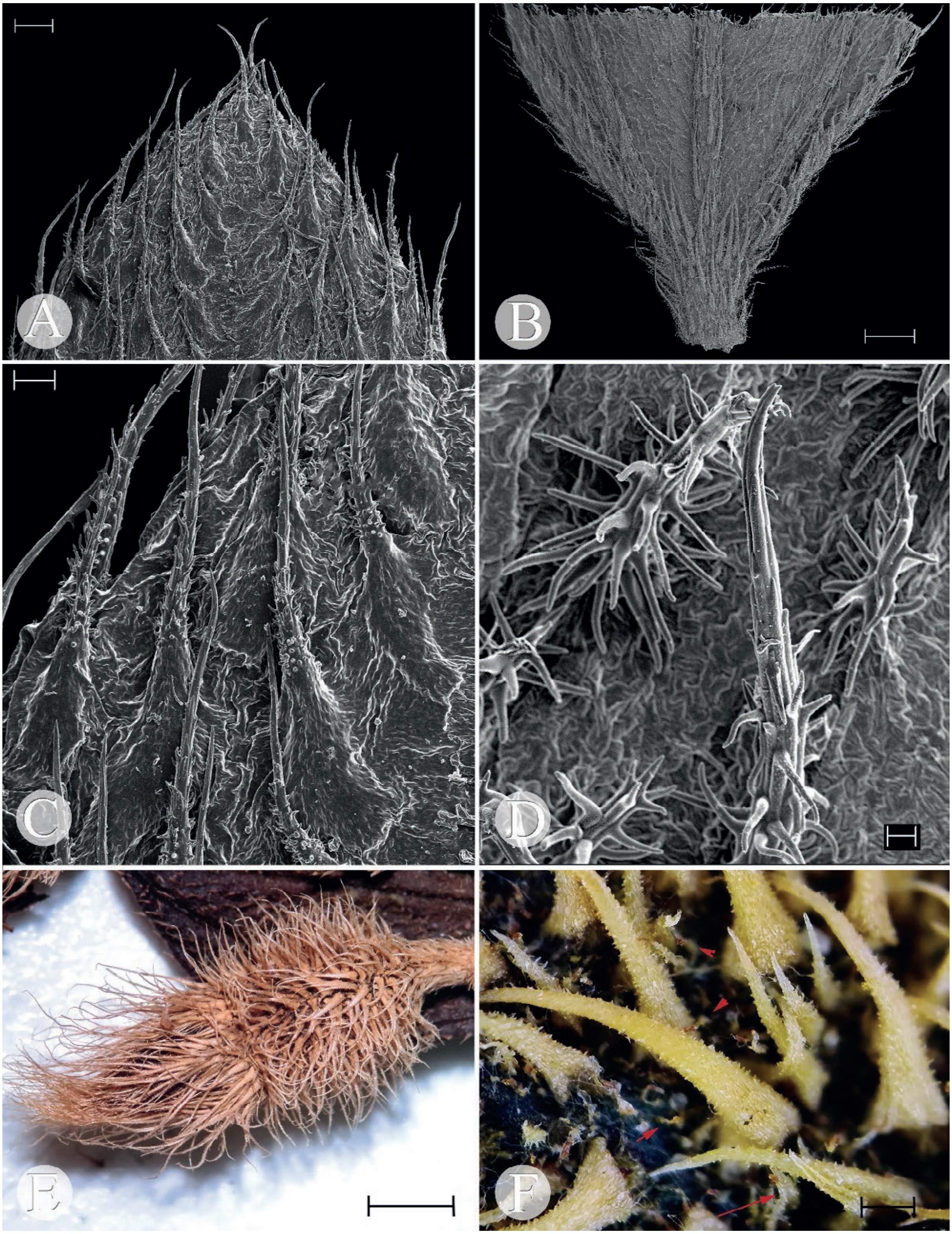

Figure 2. Images of *Pleroma barbellatum* P.J.F.Guim., D.Nunes & I.M.Araújo. SEM: (a) Adaxial leaf surface [bar = 300 μm]. (b) Adaxial leaf surface [bar = 1 mm]. (c) Adaxial leaf surface [bar = 100 μm]. (d) Abaxial leaf surface [bar = 20 μm]. Macro photography: (e) Floral bud [bar = 3.5 mm]. (f). Hypanthium with barbellate trichomes and capitate-glandular trichomes at red arrows [bar = 0.25 mm]. A–D: From *W.R. Anderson et al. 35497* (at RB); E–F: From *A.C. Brade 13756* (at RB).

P.E. Biribiri, Salto do Mocotó, próximo ao Rio do Biribi, 18°08'38.8"S, 43°36'44.5"W, 1065 m, 13 Mar. 2012 (fl., fr.), *I.M. Araújo et al. 239* (HUFU! barcode HUFU00067346, RB! barcode 01460890); Parque Estadual do Biribiri, 18°11'24"S, 43°37'36"W, 1108 m, 18 May 2011 (fl.), *I.M. Araújo et al. 91* (HUFU! barcode HUFU00067344, RB! barcode 01460881); Parque Estadual do Biribiri, 18°12'59"S, 43°37'24.4"W, 1172 m, 21 Sep. 2010 (fr.), *A.R. Rezende et al. 45* (HUFU! barcode HUFU00067349, RB! barcode 01460893); estrada

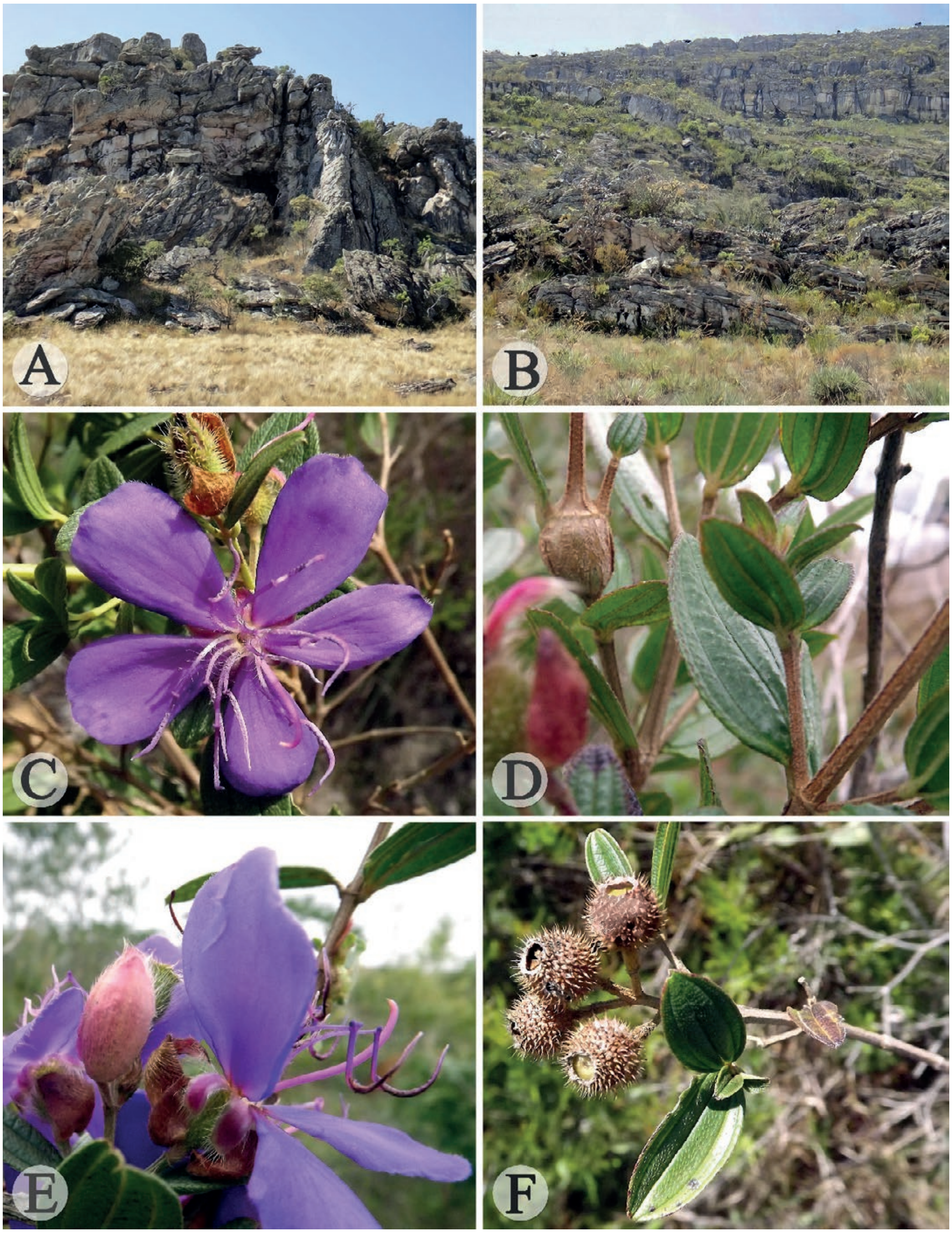

Figure 3. Environments and living specimens of *Pleroma barbellatum* P.J.F.Guim., D.Nunes & I.M.Araújo. (a) Rocky outcrop of *campo rupestre* in Biribiri State Park. (b) Steep rocky slopes with extensive outcrops in Biribiri State Park. (c) Flower. (d) Leaf blades and gall. (e) Detail of bracts. (f) Fruits. Living specimens from *I.M. Araújo et al. 261*. Photos: I.M. Araújo.

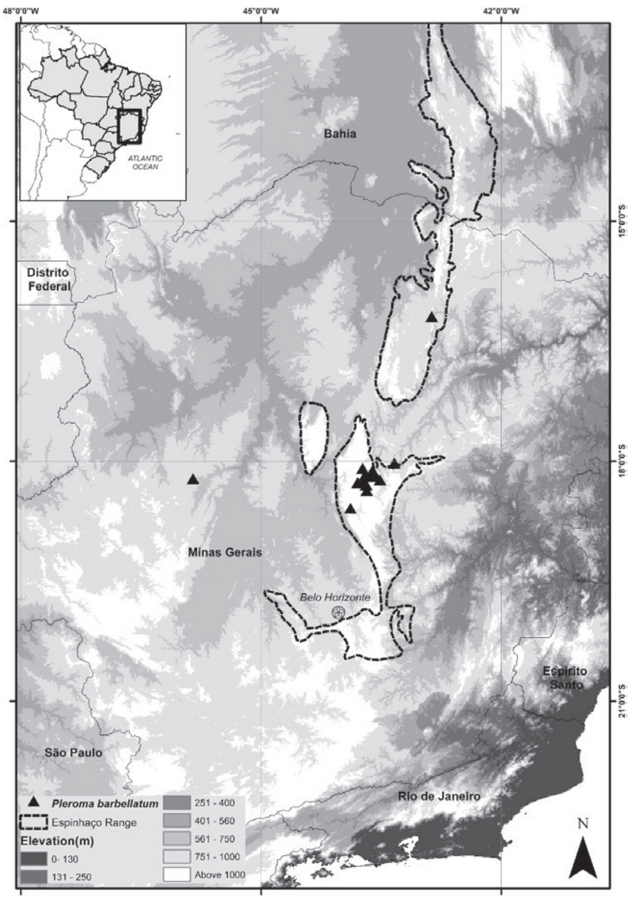

Figure 4. Map showing the geographical distribution of *Pleroma barbellatum* P.J.F.Guim., D.Nunes & I.M.Araújo (black triangles).

para Biribiri, ca. 4 km aquém de Biribiri, 18°10'13.3"S, 43°36'53.8"W, 950 m, 23 Jan. 2007 (bud, fl.), *J.R. Pirani et al. 5690* (K! barcode K001072465, HUFU! barcode HUFU00067347, SPF! barcode SPF00179063); estrada vicinal entre Sopa e São João da Chapada, sentido Diamantina - São João da Chapada, 18°11'57"S, 43°42'11"W, 1304 m, 07 Feb. 2015 (bud, fl., fr.), *C.N. Fraga et al. 3633* (MBML! barcode MBML00038817, NY, RB! barcode 01202829, UPCB herbarium number 88378); estrada Sopa - São João da Chapada, 02 Feb. 2017 (bud, fl., fr.), *R. Pacifico 334* (HUEM! barcode 000015512, RB! barcode 01394204, SPF barcode SPF00231834, UPCB herbarium number 98036); estrada Sopa - São João da Chapada, 18°06'47.1"S, 43°44'03.8"W, 10 Feb. 2017 (fl., fr.), *R. Pacifico 350* (HUEM! barcode 000015566, SPF! barcode SPF00231857, UPCB herbarium number 98035); estrada para Extração, ca. 9 km S de Diamantina, 18°15'27"S 43°30'59"W, 1241 m, 11 Jan. 2003 (bud, fl.), *L.P. de Queiroz et al. 7623* (HUEFS! barcode HUEFS000144643); Serra do Espinhaço, ca. 12 km S.W. of Diamantina, 1350 m, 23 Jan. 1969 (bud, fl.), *H.S. Irwin et al. 22471* (NY! barcode 00941884, UB! barcode UB0120842, US! barcode 01906485); Serra do Espinhaço, ca. 15 km E of Diamantina, 1100 m, 20 Mar. 1970 (fr.), *H.S. Irwin et al. 27984* (NY! barcode 00941892, UB! barcode UB0120839, US! barcode 01906484); Serra do Espinhaço, ca. 20 km S.W. of Diamantina, 1200 m, 20 Jan. 1969 (bud, fl.), *H.S. Irwin et al. 22307* (CAS herbarium number 573142, NY! barcode 00941882, UB! barcode UB0120840, US! barcode 01906487); Serra do Espinhaço, ca. 20 km S.W. of Diamantina, 1300 m, 21 Jan. 1969 (bud, fl.), *H.S. Irwin et al. 22397* (NY! barcode 00941883, UB! barcode UB0120841, US! barcode 01906486); Serra do Mendanha, 28 Nov. 1937, (bud, fl.), *Mello Barreto 10058* (HB! herbarium number 24879 [2 sheets], Jardim Botânico de Belo Horizonte [not found at BHCB] herbarium number 23098, RB! barcode 00231478); s.l., 1400 m, Jun. 1934 (bud, fl.), *A.C. Brade 13756* (RB! barcode 00231052); s.l., 17 Jan. 1947 (bud, fl.), *D. Romariz 0101* (RB! barcode 00231862); s.l., 21 Jan. 1947 (bud, fl., fr.), *D. Romariz 0118* (RB! barcode 00231869); Gouveia, Contagem, vale do Ribeirão da Contagem ao longo da estrada para Prata, a ca 4 km E da BR-259 (Rod. Gouveia-Curvelo), 18°36'51"S, 43°53'07"W, 1108 m, 22 Jan. 2004 (bud, fl.), *J.R. Pirani et al. 5235* (K! barcode K001072464, SPF! barcode SPF00168490); Grão-Mogol, Torre Telemig, 1000 m, 12 Jun. 1990 (fl., fr.), *G. Hatschbach et al. 54175* (MBM! herbarium number 137956, US! barcode 01906482); São

Table 1. Main characters that distinguish *Pleroma barbellatum* P.J.F.Guim., D.Nunes & I.M.Araújo and relative species.

	P. barbellatum	*P. formosum*	*P. martiale*	*P. riedelianum*
Leaf blade (mm)	15–44 × 6.5–13.5	40–55 × 13–20	25–43 × 9–16	25–30 × 9–13
Trichome surface on branches, both sides of leaf blade and inflorescence	Barbellate	Barbellate	Smooth	Smooth
Inflorescence	Cyme	Thyrsoid	Dichasium or Thyrsoid	Cyme
Number of flowers per inflorescence	Solitary or up to 5	35–58	3 or 7–19	Solitary or up to 5
Bracteoles (mm)	10–17.5 × 6.5–10	4–6 × 1.4–2	3–5 × 1.5–2	6–8 × 3–5
Sepal length (mm)	4–6	2.5–2	ca. 4	ca. 4

Gonçalo do Abaeté, Rod. BR-365, 30 km S of the intersection with BR-040, 3 Apr. 1992, *G. Hatschbach et al. 56457* (MBM! herbarium number 151290); S. Gonzalo do Abaete [São Gonçalo do Abaeté], Rod. BR 365, km 261, 20 Mar. 1980 (bud, fl.), *G. Hatschbach 42815* (MBM! herbarium number 65479, US! barcode 01918512); Felisberto Caldeira [São Gonçalo do Rio Preto], Curtidor, 16 Feb. 1973 (bud, fl.), *G. Hatschbach & Z. Ahumada 31654* (MBM, NY! barcode 00941955, US! barcode 01906483); [without informed municipality], between Beribiry [Biribiri] and Diamantina 02 Apr. 1892 (bud, fl.), *A.F.M. Glaziou 19302* (BR!, C!, K! barcode K001072463, P!, R! barcode R000009290).

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Lychnophora pseudovillosissima (Asteraceae: Vernonieae: Lychnophorinae), a new species restricted to Minas Gerais, Brazil

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Abstract. *Lychnophora pseudovillosissima*, a new species from the State of Minas Gerais, Brazil, is here described and illustrated. The new species is unique because of the combination of petiolate linear leaves with revolute margins, reticulodromous venation, and 3–5 florets per capitulum. The new species is compared to a morphologically similar species, *L. villosissima*, resembling in habit, leaves, venation, and number of florets per capitulum, but differing by the shape and size of the leaf and petiole. Both species may occur sympatrically, but are uniform in their morphology with diagnostic features that differentiate them. Accompanying the description and the illustration, we provide a photographic plate, a first assessment of the species' conservation status, as well as comments on the geographic distribution, ecology, and identification of the new species.

Keywords: Campo rupestre, Compositae, Espinhaço Range, neotropical flora, taxonomy.

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INTRODUCTION

The Neotropical *campos rupestres* are a type of open vegetation, mostly shrubby and herbaceous, found in nutrient-poor quartzite and ironstone soils and outcrops, in elevations above 900 m a.s.l., rarely lower (Silveira et al. 2016; Miola et al. 2021). This physiognomy in Brazil occurs in Central and Eastern portions, in Bolivia and in the Guyana Shield, with the core area concentrated in the uplands of the Espinhaço Range, a mountain range that extends over 1000 km along the central parts of Minas Gerais and Bahia states, in a North-South axis (Miola et al. 2021). *Campos rupestres* are well-known for having a

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high diversity of plants, including several endemic species, among which some families are quite diverse and stand out as characteristics of this vegetation, such as Eriocaulaceae, Velloziaceae and Asteraceae (BFG 2015; Colli-Silva et al. 2019). Within Asteraceae, the subtribe Lychnophorinae (tribe Vernonieae) stands out for the high number of species endemic to *campos rupestres*, particularly of the genus *Lychnophora* Mart. (Loeuille et al. 2019).

Lychnophora is restricted to Brazil, with ca. 30 species distributed almost exclusively in the *campos rupestres* of the Brazilian Central Plateau, especially in the Espinhaço Range, which makes the genus a distinct taxon of this physiognomy (Loeuille et al. 2019; Marques et al. 2020, 2021). Recently, the genus delimitation has been subject to several changes to recognize only monophyletic genera, including the synonymization of *Lychnophoriopsis* Schultz-Bip. under *Lychnophora*, the description of *Lychnophorella* Loeuille, Semir & Pirani, and the reestablishment of *Lychnocephalus* Mart. ex DC. (Loeuille et al. 2015, 2019).

Species of *Lychnophora* have a distinctive morphology, with a candelabriform habit, thick indumentum covering leaves and stems, and a large syncephalium (secondary capitulum) that can protect against herbivory and enhance reproductive success (Loeuille et al. 2019). Additionally, some species are used in folk medicine (known as "arnica") due to their diversified secondary compounds (Keles et al. 2010; Semir et al. 2011). These unique features aroused the curiosity of the 19th century naturalists that collected in Brazil, resulting in the description of the genus and several new species (e.g., Martius 1822; Gardner 1846). Nonetheless, systematic evaluations of the genus have only been carried out much later by Coile and Jones (1981) and Semir (1991), Semir et al. (2011), and with a recent synopsis (Loeuille et al. 2019). Among these studies, the work by Semir (Semir 1991; Semir et al. 2011) stand out as the most complete taxonomic assessment ever made of *Lychnophora*.

Based on morphological evidence, we describe and illustrate a new species of *Lychnophora* from the *campos rupestres* of Minas Gerais State, Brazil. The new species was previously recognized by João Semir (1937–2018) in his unpublished thesis (Semir 1991). In addition, we characterize the new species, discuss its morphological affinities, and provide a first assessment of the species conservation status.

MATERIAL AND METHODS

Morphological descriptions were based on specimens analyzed in the following herbaria: BHCB, DIAM, HUFU, K, MBM, SAMES, SPF, UEC and UFP (acronyms according to Thiers, continuously updated). In addition, the online databases Reflora Virtual Herbarium (Reflora, 2022) and SpeciesLink (2022) were consulted. A 10–60 × magnification stereomicroscope was used to examine morphological features of the specimens. Terminology follows Harris and Harris (2001) for general morphology, Hickey (1973) for leaf shape, and Roque et al. (2009) and Loeuille et al. (2019) for specific terms.

A first assessment of the species' conservation status was made based on the IUCN criterion B, following the IUCN categories and criteria (2012) and guidelines (2022). This analysis was made in the Geospatial Conservation Assessment (GeoCAT) tool (Bachman et al. 2011) using the IUCN default values for Extent of Occurrence (EOO) and Area of Occupancy (AOO). A geographic distribution map was produced in QGIS version 2.18.15 (QGIS Development Team 2018). For all non-georeferenced herbarium specimens, geographic coordinates were approximated using the locality described on the specimen label.

TAXONOMIC TREATMENT

Lychnophora pseudovillosissima Semir ex Antar, M.Monge & Loeuille, **sp. nov.**

Type: Brazil. Minas Gerais: Diamantina, km 184 da MG220 na direção de Conselheiro Mata, 18°17'30"S, 43°44'15"W, elev. 1300 m, 7 February 2009, *J.R. Pirani et al. 5834* (holotype SPF [barcode SPF 203228], isotypes CTES, HUFU, NY, UEC, UFP).

Diagnosis

Species *Lychnophorae villosissimae* habitu, foliis breviter petiolatis, venation reticulodroma et floribus 3-5 similis, sed petiolo 1–2.4 mm longi (non usque 6 mm), basi expansa (non angustata), foliis linearibus (non angustissime lanceolatis vel angustissime ellipticis), plerumque glaucis, in sicco brunneolis ad cinerascentia (non nunquam glaucis, in sicco viridulis ad cinerascentia), lamina apice acuto (non apice acuto vel obstusato), basi atenuata vel truncata (non atenuata vel rotundata), longitudine foliorum maiore pro ratione ad latitudinem 1: (8.7–)14–57 (non 1:5–12.2(–17)) differt.

Description

Treelet, candelabriform, 1–3 m tall. Stems highly branched at apex, densely lanate, glabrescent, whitish, greyish, or ochraceous, surface tesselate, mamillated; leaf-scars circular, punctiform. Leaves alternate, simple, patent and reflexed at base of stem, densely imbricate, and ascendant near apex; subsessile, petiole 1–2.4 mm long, concealed by lanose indumentum, base expanded; lamina linear, (2.2–)4.5–12.7(–15) × 0.15–0.4 cm, discolorous, coriaceous, base attenuate or truncate, margins entire, revolute, apex acute, frequently with a short, blunt mucron, ca. 0.5 mm long, abaxial surface whitish to greyish in sicco, whitish in vivo, lanate, denser near base, peeling off, midrib slightly prominent, venation obscured by indumentum, adaxial surface greyish in sicco, green in vivo, glabrescent, midrib impressed or slightly canaliculate, pubescent, sometimes restricted to base, secondary veins slightly prominent, venation obscurely reticulodromous. Inflorescence a syncephalium with capitula fused, solitary, terminal or lateral; syncephalium 15–21 × 20–31 mm diam., hemispherical, surrounded by secondary leaf-like bracts. Capitula 30–40, homogamous, discoid, sessile, slightly appressed at base, interspersed by leaf-like subinvolucral bracts; involucre 10–11.2 × 2.9–3.5 mm in diam., cylindrical; phyllaries imbricate, 4–6-seriate, light stramineous, apex acute, outer phyllaries 6.1–6.6 × 0.8–1 mm, lanceolate, glabrous or glabrescent, except for apex with a tuff of long white trichomes sometimes extending to base, inner phyllaries 10.2–11 × 0.7–1 mm, narrow oblanceolate, lanceolate, linear or very narrow elliptic, glabrescent; receptacle scrobiculate, glabrous, epaleaceous. Florets 3–5 per capitulum, bisexual, fertile; corollas actinomorphic, deeply 5-lobed, corolla tube (5.5–)7.1–10.2 × 0.9–1.2 mm, glabrous, corolla lobes (3.5–)4.5–5.6 × 0.6–0.9 mm, glabrous, apex acute, purple or magenta; anther calcarate, 3.7–4.1 × 0.2–0.4 mm, whitish, or magenta, apical appendages lanceolate, apex acute, anther base sagittate; style white or pale lilac, shaft 12–12.6 mm long, glabrous throughout except for pubescent upper 2.5–3.5 mm beneath style-arms, style-base glabrous, nectariferous disc present, style-arms 1.6–2.0 mm long, apex acute, pubescent outside throughout, hairs acute. Cypsela cylindrical or narrow obconical, 3.2–3.6 × 1.2–1.7 mm, 10-ribbed, castaneous, glandular-punctate; carpopodium inconspicuous; pappus setae biseriate, paleaceous, whitish to stramineous, apex acute, outer series, 0.9–2.0 mm long, persistent, free, margins erose, inner series 7.5–9.6 mm long, caducous, barbellate, twisted. Figures. 1–3.

Etymology

The specific epithet refers to its morphological similarity with *Lychnophora villosissima*.

Distribution and habitat

Lychnophora pseudovillosissima is restricted to Minas Gerais State, Brazil (Figure 4). Its occurrence is known from two localities alongside the Meridional portion of the Espinhaço Range, corresponding to its southern distribution limit in *Quadrilátero Ferrífero*, near Belo Horizonte, and its northern distribution limit in Diamantina plateau (Colli-Silva et al. 2019). The species inhabits *campo rupestre* physiognomies, alongside rocks or in *campo rupestre/cerrado* ecotone zones, at elevations between 1,010 and 1,400 m.

Phenology

Flowering and fruiting between November and August.

Preliminary assessment of conservation status

Lychnophora pseudovillosissima has an Extent of Occurrence (EOO) of 7,505 km² and an Area of Occupancy (AOO) of 80 km² (Figure 3). Most of the known populations are located along the road that connects the cities of Diamantina to Conselheiro Mata. This is an area very rich in plant species, many of which are endemic and little known (e.g., Antar et al. 2019; Cavallari et al. 2006; Konno et al. 2006; Semir et al. 2011). According to the national action plan for conservation of biodiversity in southern Espinhaço, this is an area of conservation priority, yet still unprotected as it does not currently possess any conservation units (Pougy *et al.* 2015). Current threats to the plant diversity in the area are agriculture, cattle raising, and quartzite mining (Pougy et al. 2015). The southern populations from the *Quadrilátero Ferrífero*, where the first known collection record of the new species came from (specimen *Roth 1660*, from Belo Horizonte), are located in or near areas subject to significant habitat destruction from increasing urbanization and iron mining. Additional collection efforts in these areas are necessary to evaluate better the conservation status of the southern populations. *Lychnophora pseudovillosissima* has populations protected only in the Parque Estadual do Biribiri, in Diamantina, and in the Parque Nacional das Sempre Vivas, in Buenópolis. Despite having the threshold for being accessed as Vulnerable due to its limited EOO or Endangered due to its AOO, the species occurs in more than 10 locations and does not seem to present extreme fluctuations, what preclude the assessment in categories of higher risk of extinction. Therefore, according with these data, *Lychnophora pseudovillosissima* should be assessed as Near Threatened (NT).

Comments and affinities

Lychnophora pseudovillosissima disjunction (*Quadrilátero Ferrífero* and Diamantina plateau–Figure 4) is noteworthy as the range break is considerable in its extension and there are several cases of endemic species

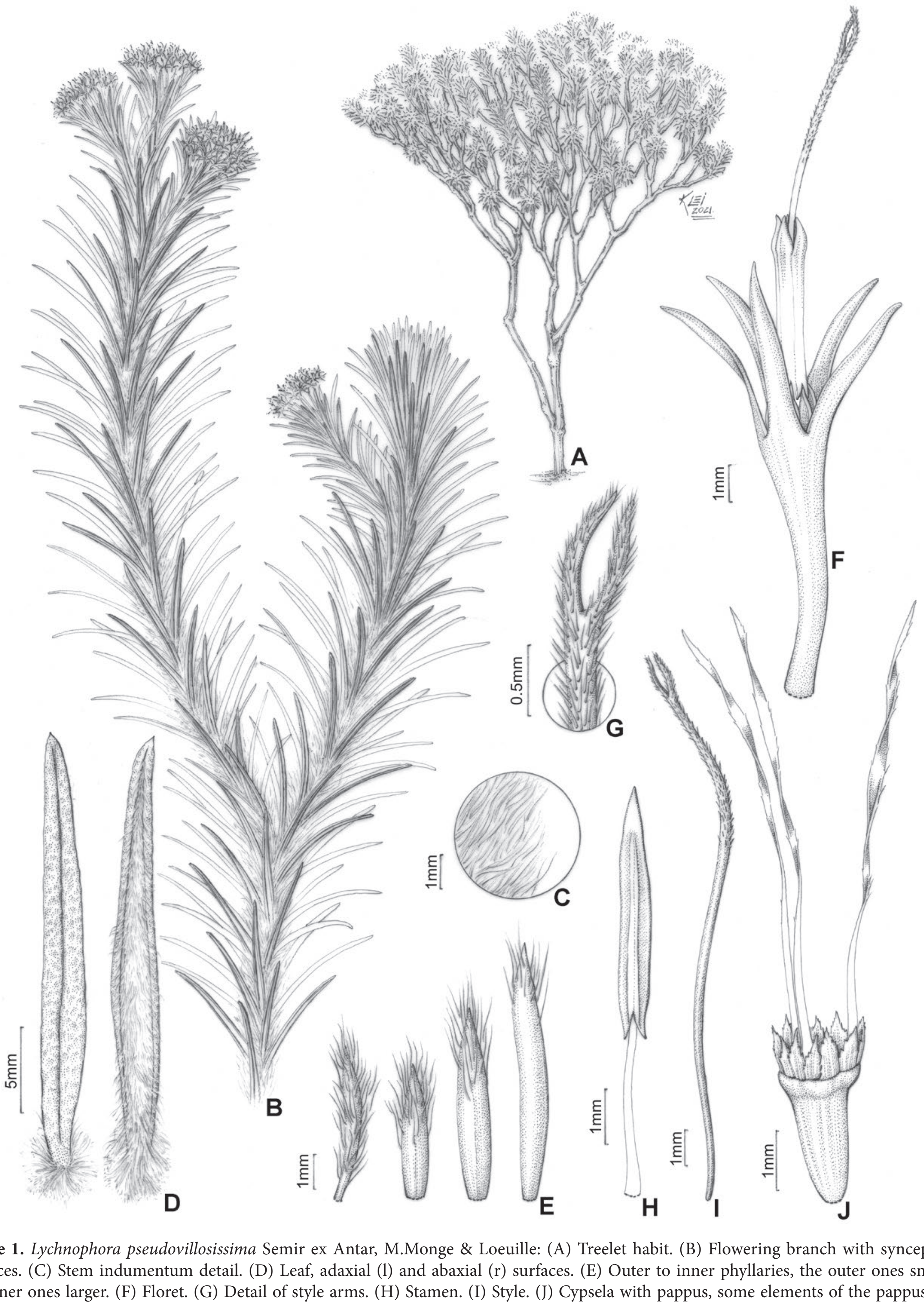

Figure 1. *Lychnophora pseudovillosissima* Semir ex Antar, M.Monge & Loeuille: (A) Treelet habit. (B) Flowering branch with syncephalia at apices. (C) Stem indumentum detail. (D) Leaf, adaxial (l) and abaxial (r) surfaces. (E) Outer to inner phyllaries, the outer ones smaller, the inner ones larger. (F) Floret. (G) Detail of style arms. (H) Stamen. (I) Style. (J) Cypsela with pappus, some elements of the pappus have been removed for clearer view. Illustration by Klei Souza based on *J.R. Pirani et al.* 5834 (SPF).

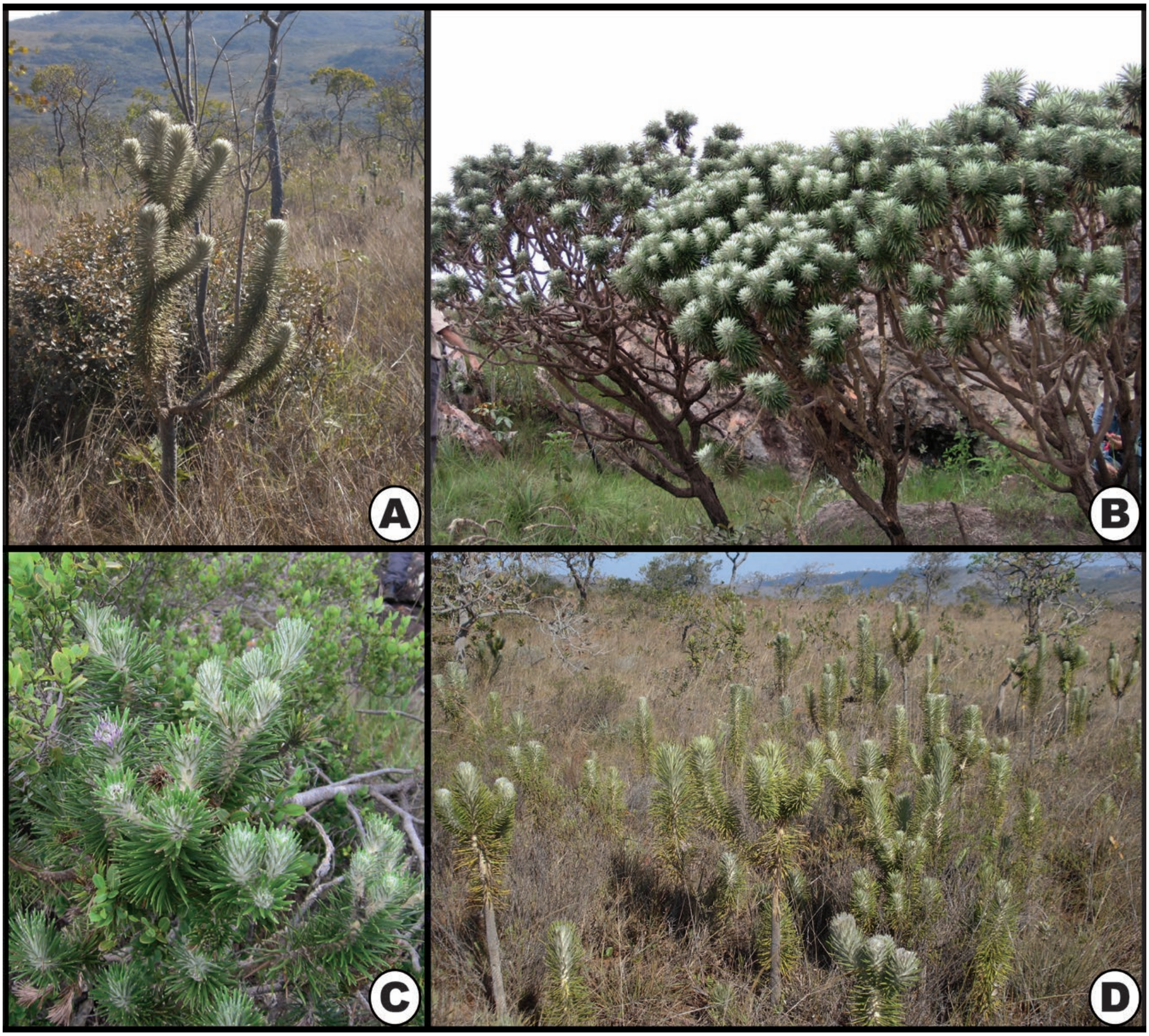

Figure 2. *Lychnophora pseudovillosissima* Semir ex Antar, M.Monge & Loeuille: (A) Treelet habit. (B) Treelet habit. (C) Branches bearing immature inflorescences (capitulescences). (D) Dense population growing in campo rupestre. A–D. Photos by B. Loeuille.

from both localities (e.g. Carmo et al. 2018; Cota et al. 2020). There are very few examples of plants presenting the same disjunctive pattern, e.g. *Chamaecrista itabiritoana* (H.S.Irwin & Barneby) H.S. Irwin & Barneby (Cota *et al*. 2020). This disjunction is basically due to the absence of records of these species in the *campos rupestres* of Serra do Cipó, a well-studied area (Zappi et al. 2013; Pirani et al. 2015). Nevertheless, several species of Lychnophorinae frequently recorded in the *Quadrilátero Ferrífero* and Diamantina plateau are known from very few recent collections in the Serra do Cipó, e.g. *Chronopappus bifrons* (DC. ex Pers.) Pers., *Heterocoma albida* (DC. ex Pers.) DC. and *Piptolepis ericoides* Sch.Bip. (Loeuille et al. 2019). The evolutionary history of several plant lineages from Serra do Cipó is strongly marked by environmental filters (e.g., edaphic factors, elevation and microenvironmental aspects) (Mattos *et al*. 2021), this may explain the difficulty for these cited species (incl. *L. pseudovillosissima*) to establish permanent population in the Serra do Cipó.

Although both localities (*Quadrilátero Ferrífero* and Diamantina plateau) share a similar *campo rupestre* physiognomy, the ones from *Quadrilátero Ferrífero* are usually composed of ferruginous soils, which par-

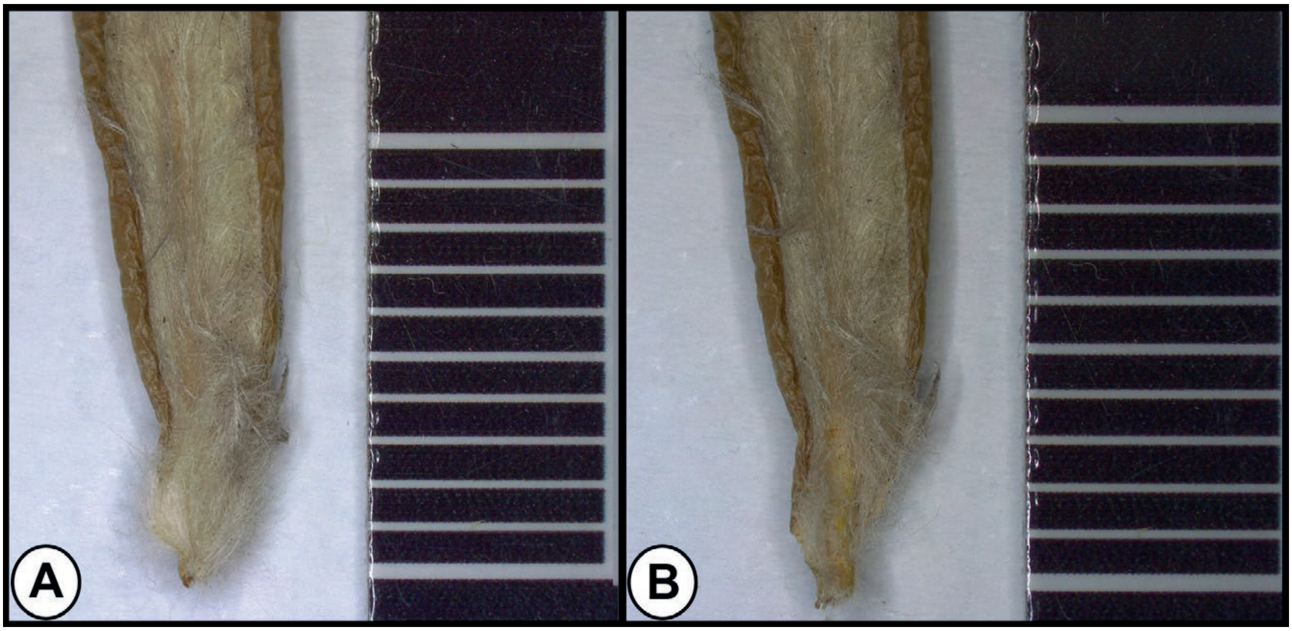

Figure 3. *Lychnophora pseudovillosissima* Semir ex Antar, M.Monge & Loeuille: (A) Detail of the petiole covered by dense indumentum. (B). Detail of the petiole, with indumentum removed for clearer view. Each rectangle corresponds to 1 cm in scale bar. Photos of the specimen *Loeuille et al.* 81 (K).

tially explain its unique flora (Carmo et al. 2018). For *Lychonophora pseudovillosissima*, we found just some differences between gatherings from both localities. The specimens from Diamantina Plateau usually dry greyish and have a higher blade length/wide ratio and the specimens from *Quadrilátero Ferrífero* dry brownish and have a slightly smaller blade length/wide ratio. Despite of that, gatherings from both localities are clearly representatives of the same taxon, having solid differences from other species. Future phylogeographic studies with the species are desired in order to better understand its genetic diversity as well as further expeditions to uncover other localities for the species and better understand its distribution.

The new species is distinguished from other *Lychnophora* species by petiolate leaves (Figure 3), with the petiole obscured by lanose trichomes, long linear lamina usually drying glaucous, leaves with acute apex, frequently with a short blunt mucron, ca. 0.5 mm long, reticulodromous venation, and 3–5 florets per capitulum.

As stated in the diagnosis, the morphologically closest species is *Lychnophora villosissima*. The new species shares with it a similar habit, leaves shortly petiolate, reticulodromous venation and 3–5 florets per capitulum, but differs in the combination of mature leaves linear (vs. mature leaves narrow lanceolate or very narrow elliptic in *L. villosissima*), usually glaucous, with leaves drying greyish or brownish (vs. leaves drying brownish or greenish), apex acute (vs. apex acute or obtuse), base attenuate or truncate (vs. attenuate or rounded), blade length/wide ratio 1:(8.7–)14–57 (vs. 1:5–12,2(–17)), and petiole 1–2.4 mm long with expanded base (vs. petiole up to 6 mm long, with narrowed base). Furthermore, individuals of *L. pseudovillosissima* seem to possess less robust stems (Figure 2) when compared to *L. villosissima*; it should be noted, however, that due to variation in collection procedures, sometimes only the apical part of the stems was sampled, and no measurements were taken.

In addition, *Lychnophora pseudovillosissima* is morphologically similar to *L. ericoides* Mart. and *L. pinaster* Mart. which also possess linear leaves but it differs from both species by having shortly petiolate leaves (vs. sessile in *L. ericoides* and *L. pinaster*), reticulodromous venation (vs. broquidodromous), and lanose or villose trichomes fully covering the midrid abaxially (vs. midrid glabrous or tomentose partially covering the midrib). The main morphological differences among *L. pseudovillosissima* and related species are summarized in Table 1.

Semir (1991) in his unpublished thesis recognized 68 species in *Lychnophora* (with a different circumscription), 27 of which were proposed as new to science. Some of Semir's proposed new species have already been published (Semir et al. 2014; Loeuille et al. 2019; Gomes and Loeuille 2021), but other clearly recognizable taxa

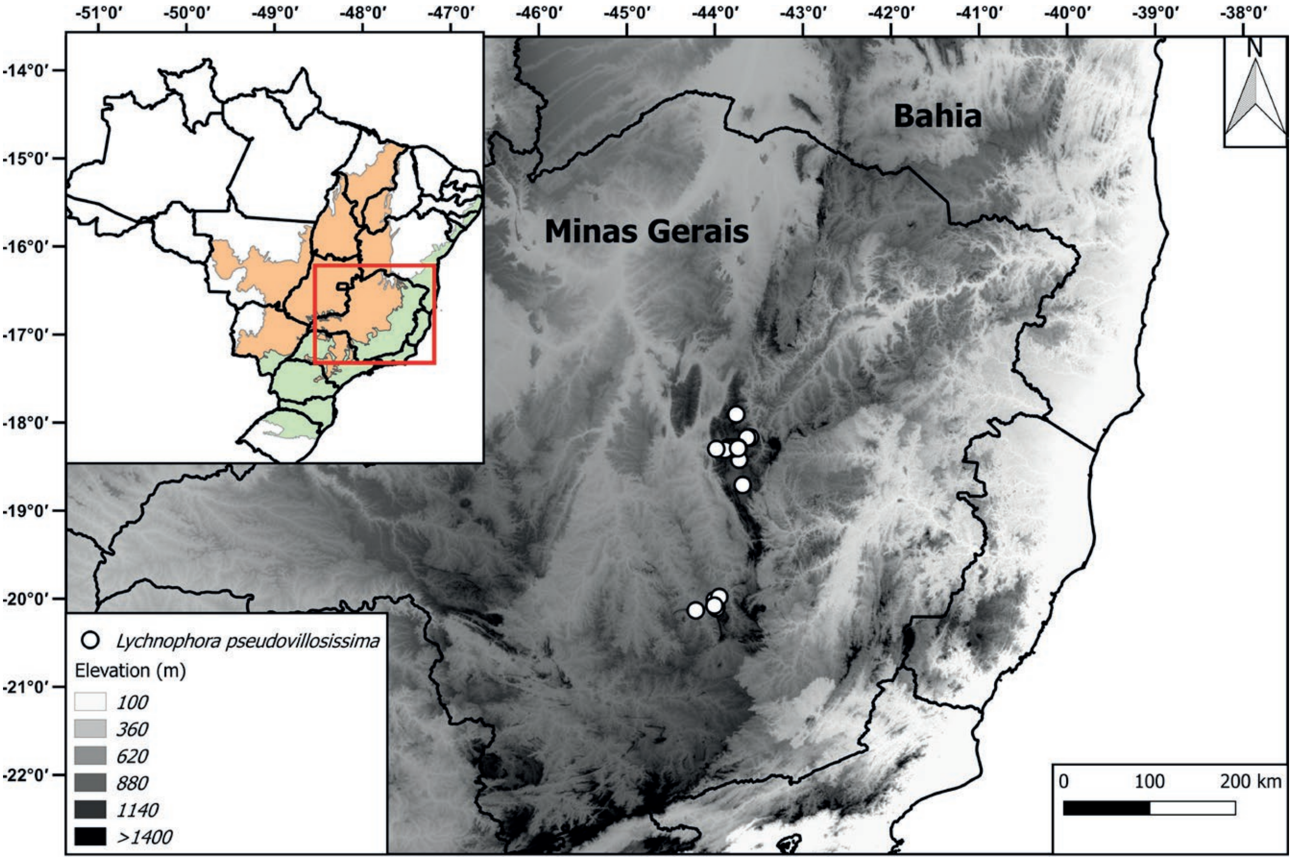

Figure 4. Geographic distribution of *Lychnophora pseudovillosissima* Semir ex Antar, M.Monge & Loeuille (white circles). In the smaller map, the green shaded area represents the Atlantic Forest domain and the orange shaded area represents the Cerrado phytogeographic domain.

remained unpublished. Here, we described and illustrated another new species previously recognized in João Semir's (1937–2018) thesis (Semir 1991), then named *L. pseudovillosissima* and also treated in Semir et al. (2011) as *Lycnhophora* sp. 5. Although *L. pseudovilossisima* has never been formally described before, the name is present in the IPNI (IPNI 2022) and in The Plant List (The Plant List 2013), which makes the formal publication of the species name urgent. This is especially important in view of the recent and diverse threats of *campo rupestre* areas, including open pit mining, wood extraction, agriculture, altered fire regimes, and invasive species (Silveira *et al.* 2016).

We understand that the value of the *campo rupestre* vegetation can be better understood from the description and recognition of its floristic diversity. This recognition can subsidize conservationist actions by the regional and national governments and lead to advances in specific legislation for the protection of the vegetation of Brazilian *campos rupestres*.

Additional specimens examined (Paratypes)

BRAZIL: Minas Gerais: Belo Horizonte, Serra do Curral, BR3, a 15 km de Belo Horizonte, 1300 m, 16, July 1956, *L. Roth 1660* (HUFU, MBM, RB). Buenópolis, Parque nacional das Sempre Vivas, ao lado da Serra do Landi, 1306 m, 01 May 2007, 17°54'27.7"S, 43°45'24.2"W, *T.E. Almeida et al. 974* (BHCB). Brumadinho, Serra da Calçada (Serra da Moeda), Retiro das Pedras, caminho para o Forte de Brumadinho, depois da descida da escada de pedras, à direita e terrenos na divisa da EXPLO perto da mina de cristal, 20°08'S, 44°13'W, February 1989, *L.A. Martens 93* (SPF, UEC); ibid, nas proximidades da mina de cristal, 20°08'S, 44°13'W, February 1990, *L.A. Martens 372* (K, SPF, UEC); arredores do condomínio Retiro das Pedras, 14 September 1999, *J.R. Stehmann & M. Gonçalves 2543* (BHCB); Serra da Moeda, Retiro das Pedras, campo de quartzito, 20°05'35"S, 43°59'01"W, 20 August 2001, *P.L. Viana 162* (BHCB); Serra da Moeda, 20°06'28"S, 43°59'02"W, 17 February 2012, *C.V. Vidal & J. Paula-Souza 915* (BHCB). Con-

Table 1. Diagnostic morphological characters of *Lychnophora pseudovillosissima* and related species.

Character	*L. pseudovillosissima*	*L. villosissima*	*L. ericoides*	*L. pinaster*
Leaf petiole	shortly petiolate	shortly petiolate	sessile	sessile
Leaf arrangement	dense	dense	dense	lax, rarely dense
Blade shape	linear	narrow lanceolate or very narrow elliptic	linear	linear
Blade length	up to 15 cm	rarely exceeding 10 cm	up to 15 cm	rarely exceeding 6 cm
Venation	reticulodromous	reticulodromous	broquidodromous	broquidodromous
Leaf color (when dry)	greyish or brownish, rarely greenish	brownish or greenish	greyish	brownish or greenish
Blade apex	acute	acute to obtuse	acute	obtuse to rounded
Mucron	short blunt, ca. 0.5 mm long, rarely absent	inconspicuous, less than 0.2 mm, commonly absent	short blunt, ca. 0.5 mm long, rarely absent	inconspicuous, less than 0.2 mm, commonly absent
Blade base	attenuate or truncate	attenuate or rounded	rounded, rarely truncate	rounded to auriculate, rarely attenuate
Indumentum midrib abaxially	lanose or villose	lanose or villose	glabrous or tomentose	glabrous or tomentose

gonhas do Norte, estrada para Costa Sena, ca. 15 km de Congonhas do Norte, 18°42'33.8"S, 43°41'04.8"W, 1010 m, 21 January 2007, *B. Loeuille et al. 81* (K, SPF). Diamantina, 15 km S. de Diamantina, rodovia para Conselheiro Mata, 17 May 1977, *P.E. Gibbs et al. 5264* (UEC); 18°18'S, 43°59'W, *J.E.M. Brazão 239* (RB); estrada Guinda-Conselheiro Mata, km 178, 4 June 1985, *H.F. Leitão-Filho 17340* (UEC); ibid, *H.F. Leitão-Filho 17350* (UEC); estrada Diamantina-Conselheiro Mata, km 185 próximo à grande inselberg, 23 February 1986, *J. Semir et al.* CFCR *9492* (UEC); 20-26 km WSW de Diamantina, camino a Conselheiro Mata, MG-220, 18°17'S, 43°49'W, 1270-1300 m alt., 18, May 1990, *M.M. Arbo et al. 4355* (CTES, SPF, UEC); km 183-184 estrada de terra para Conselheiro Mata, margem esquerda, 11 September 1999, *N.P. Lopes 213* (UEC); estrada para Conselheiro Mata, 10 km do asfalto, 18°17'28"S, 43°45'27"W, 29 November 2000, *Mansanares et al. 00/27* (UEC); estrada para Conselheiro Mata, 17.2 km do asfalto, afloramento lado D, 18°18'00"S, 43°48'48"W, 29 November 2000, *M.E. Mansanares et al. 00/28* (UEC); estrada para Conselheiro Mata, 18 km, 20 October 2007, *J.N. Nakajima et al. 4673* (HUFU); estrada Diamantina-Conselheiro Mata (km 176), 10 km do trevo, 31 March 2001, *J.N. Nakajima & R. Romero 3096* (DIAM, HUFU, MBM); ibid, *J.N. Nakajima & R. Romero 3098* (HUFU); estrada Diamantina-Conselheiro Mata, km 164, 18°18'36.5"S, 43°53'11.9"W, 20 June 2001, *J. Semir et al. 01/108* (UEC); ibid, *J. Semir et al.* 01/112 (UEC); estrada pra Conselheiro Mata, km 176.5, ao lado direito da estrada, 18°17'S, 43°47'W, 14 November 2002, *F. Feres et al. 74* (UEC); estrada para Conselheiro Mata, 8 July 2004, *M.E. Mansanares et al. 412* (UEC); estrada para Conselheiro Mata, km 172, 8 July 2004, *Mansanares et al. 414* (UEC); estrada Diamantina-Conselheiro-Mata, km 184, 2.5 km da estrada Diamantina-Gouveia (BR 259), 18°17'30"S, 43°44'10"W, 22 January 2007, *B. Loeuille et al. 87* (K, MBM, MO, SPF, UFP, US); ibid., 1280 m, *B. Loeuille et al.* 89 (K, SPF); Parque Estadual do Biribiri, estrada para Pinheiro, mirante da Guinda, 10 August 2010, *B. Loeuille et al. 532* (SPF); ibid, 18°10'20.5"S, 43°38'2.1"W, 10 August 2010, *I.M. Franco et al. 584* (DIAM, HUFU); Conselheiro Mata, Estrada Diamantina-Conselheiro Mata, ca. 5 km da BR, 22 January 2012, *D. Gonçalves et al. 381* (UEC); Parque Estadual do Biribiri, Alto do Guinda, 18°10'9.5"S, 43°35'54.6"W, 15 March 2012, *D. Marques et al. 444* (BHCB, DIAM, HUFU); Serra do Pasmar, 18°17'53"S, 43°45'16"W, 24 February 2010, *I.M. Franco et al. 48* (DIAM, HUFU). Gouveia, morro da torre de televisão, alto do morro, entrada a oeste da rodovia Gouveia-Diamantina (BR 259), a 3,3 km norte de Gouveia, 18°25'24"S, 43°43'24"W, 1280 m elev., 22 January 2007, *B. Loeuille et al. 85* (SPF, UFP). Nova Lima, campo rupestre perto da BR040 próximo ao BH Shopping e Copasa, 22 February 1990, *A.M.G. Anjos 125* (BHCB, UEC).

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A new species of *Schismatoglottis* (Araceae) from Sabah, Malaysian Borneo

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Abstract. *Schismatoglottis mons* Kartini is described as taxonomically novel species assigned to the Asperata Complex. *Schismatoglottis mons* is a highland mesophytic species found at c. 718 m a.s.l. most like lowland *Schismatoglottis gillianiae* P.C. Boyce and *Schismatoglottis shaleicola* P.C.Boyce & S.Y.Wong.

Keywords: Araceae, Malaysian Borneo, Schismatoglottideae, Schismatoglottis.

INTRODUCTION

The first informal Asperata Group was proposed by Hay in Hay and Yuzammi (2000). Boyce and Wong (2014) proposed a modified informal taxon, the Asperata Complex, defined within the Asperata Group and defined by asperate to coarsely granulate petioles, a petiolar sheath with the wing extended into a free ligular portion, erect inflorescences in which the spathe limb irregularly crumbles and breaks away at or after staminate anthesis, and erect infructescences with a persistent lower spathe with an unconstructed terminal orifice and with thickened walls remaining white beyond fruit maturity. Low et al. (2018) demonstrated, at broadest sense (i.e., Hay, 2000), that the Asperata Group is very diverse and heterogeneous, but nested within are several well-supported clades delineated both molecularly and by consistent characteristics.

Currently the Asperata Complex (sensu Low et al. (2018) comprises 11 species which are six mesophytes (*Schismatoglottis asperata* Engl., *S. scortechini* Hook.f., *S. sejuncta* A.Hay, *S. shaleicola* P.C.Boyce & S.Y.Wong, *S. tahubangensis* A.Hay & Herscovitch, *S. zainuddinii* Kartini, P.C.Boyce & S.Y.Wong, and four are rheophytes (*Schismatoglottis crypta* P.C.Boyce & S.Y.Wong, *S. gillianiae* P.C.Boyce, *S. jelandii* P.C.Boyce & S.Y.Wong, and *S. tegorae* P.C.Boyce & S.Y.Wong) and here a new, mesophytic species, *S. mons* Kartini.

Until now three species of the Asperata Complex has been recorded in Sabah. *Schismatoglottis tahubangensis* recorded from Tahubang River along route to Marak-Parai (Hay and Herscovitch, 2003) and *S. zainuddinii* found in Tawau Hills Park, Tawau (Kartini et al. 2017)

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Key to Sabahan species of the *Schismatoglottis* Asperata Complex

1a. Petiolar sheath broader at the base extended into fleshy ligule. Tawau Hills Park, Tawau………………………………………………… ………………………………………………***Schismatoglottis zainuddinii***

1b. Petiolar sheath only at the extreme base extended into a narrowly triangular ligule ………………………………………………2

2a. Papery ligule, peduncle short c. 3 cm long, creeping or decumbent. Tahubang River, Ranau……………………………… ………………………………………***Schismatoglottis tahubangensis***

2b. Fleshy ligule, peduncle long c. 9 cm long, suberect. Mensalong Forest Reserve, Kota Marudu……………………………… ……………………………………………………***Schismatoglottis mons***

Schismatoglottis mons Kartini, **sp. nov.**

Type: Malaysia, Sabah, Kota Marudu, Mensalong Forest Reserve 06°13.915'N 116°46.261'E, 30 July 2019 *Kartini Saibeh 2708* (holotype BORH!).

Diagnosis

The inflorescence of *Schismatoglottis mons* is most similar to that of rheophytic *S. gillianiae*. However, *S. gillianiae* has a very short peduncle (c. 2-3.5 cm long-, fleshy persistent ligular petiolar sheaths and narrowly oblanceolate leaves whereas *S. mons* have long peduncle c.10 cm, a marcescent free ligular petiolar sheath and ovate to oblong-ovate leaves. *Schismatoglottis mons* differs from mesophytic *S. shaleicola* by the inflorescence peduncle up to 10 cm long, the yellow spadix, a slender cylindrical appendix about twice as long as remainder of spadix, pink sterile interstice.

Description

Moderately robust mesophytic herb to c. 30 cm tall. *Root* densely covered with short soft minute hairs, c. 0.3 cm diam. *Stem* condensed, suberect, becoming epigeal, to c. 2 cm diam., obscured by leaf bases, usually red; pleionanthic. *Leaves* several together (6-10) with roots emerging from their bases; petiole 9–15 cm long, D-shaped in cross-section, usually shorter than the length of the blade, verruculose-pubescent, erose on each side on the adaxial face, dark green, dark red sometimes more than half length, sheathing only at the extreme base with the sheath extended into a narrowly triangular, fleshy ligular portion 2-4 cm long, marcescent, emerging leaves pink; blade ovate to oblong-ovate, rather leathery, 14–26 cm long x 5–7 cm wide, glossy medium green adaxially, paler abaxially, the base retuse to narrowly truncate or less narrowly cordate, the tip acute to obtuse and shortly apiculate; midrib abaxially prominent; abaxial primary lateral veins conspicuous, crowded, c. 15 on each side, alternating with lesser interprimaries, diverging at c. 45-60°; secondary venation almost all arising from the midrib; tertiary venation indistinct. *Inflorescence* solitary to two or more together, with a sweet floral scent at anthesis, strongest during pistillate anthesis; peduncle 9-10 cm long, subtended by two or more broad membranous cataphylls,; Spathe c. 12 cm long, sub-cylindrical, tapering; lower spathe c. 2 cm long, slightly creamy pinkish and greenish, spathe limb differentiated from the limb by colour but no obvious constricting separating them; limb c. 10 cm long, apiculate for c. 4 mm, pale pink to creamy, opening wide than crumbling and breaking away at late staminate anthesis. Spadix sessile, shorter than the spathe, c. 10 cm long; pistillate flower zone 1–2 cm long, oblique insertion, 0.5–1 cm diam. at base, faintly conoid; pistils crowded, creamy, squat mushroom-shaped, c. 1 mm diam.; stigma sessile, discoid to irregularly polygonal, centrally impressed, papillate, wider than ovary; interpistillar staminodes few, white, scattered among the pistils, slightly taller than the pistils, c. 0.8 mm diam., the tops depressed in the middle; sterile interstice c. 2-3 mm long, pinkish, isodiametric with top of female and base of male zones, 2–3 whorls of irregularly polygonal sterile stamens c. 1 mm diam., interspersed with a few interpistillar staminodes; staminate flower zone 0.8–1 cm long, cylindric; stamens pinkish, crowded, truncate with the thick connective slightly elevated above the thecae, more or less rectangular to triangular from above; appendix yellow, about twice the length of the rest of the spadix, widest shortly above the base and there somewhat wider than male zone, then gradually tapering to a narrow blunt tip; staminodes of appendix more or less flat-topped, irregularly polygonal, c. 1 mm diam., crowded.

Etymology

Mons, Latin meaning mountain, and referring to the occurrence of the species at altitude.

Distribution

Schismatoglottis mons occurs in the Mensalong Forest Reserve, Kota Marudu at c. 718 m a.s.l. Populations are small and scattered.

Ecology

Terrestrial in wet hill forest on steep slopes on soils derived from Middle Eocene sandstone sediments [Crocker Formation], c. 720 m a.s.l.

Figure 1. *Schismatoglottis mons* – A: flowering plant in habitat; B: inflorescence at onset of pistillate anthesis (spathe artificially removed); C: pistillate zone (spathe artificially removed); D: inflorescence at late of pistillate anthesis (spathe artificially removed); E: spathe limb irregularly crumbling and breaking away after staminate anthesis; F: marcescent ligular petiolar leaf sheath – All from *Kartini BORH* 2708. – All photographs by Kartini Saibeh.

Notes

The Mensalong Forest Reserve was formerly part of Lingkabau Forest Researve located in the Kota Marudu, Sabah. The reserve shares approximately 5 km of its boundary in the west with the Kinabalu National Park. *Schismatoglottis mons* is the second recorded species of S. asperata Complex occurring above 700 m. *Schismatoglottis tahubangensis* known only from the type was recorded at c. 1,100 m.

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Studies on the genus *Capparis* L. (Capparaceae) in Lao PDR. VI: a new species from the Bolikhamxai Province

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Abstract. A new species of *Capparis*, *C. phatadke*, is described and illustrated from the Bolikhamxai Province, central Lao PDR. The new species is characterized by lianous habit, brown-reddish indumentum, straight stipular thorns, laminar bracts, large flowers arranged in terminal corymbs or subumbels, long filaments and large ovary. It is so far known from a single locality, where has been observed in mixed deciduous forest and secondary forest in a limestone area. Its ecology and phenology are discussed, and its conservation status is assessed.

Keywords: Bolikhamxai Province, *Capparis* sect. *Monostichocalyx*, endemism, Indo-Pacific area, plant taxonomy.

INTRODUCTION

The genus *Capparis* L. comprises about 150 species (POWO 2022), widespread in the tropical and subtropical regions of the Old World and occurring in a wide range of habitats from sea level to c. 3600 m (Souvannakhoummane et al. 2020). Jacobs (1965) recorded in the Indo-Pacific area 82 species, but recently several new taxa were described from various areas of southern Asia and New Caledonia (Viswanathan 2000; Srisanga and Chayamarit 2004; Sy et al. 2013, 2015, 2018, 2020; Fici 2012, 2016a, 2016b, 2017a, 2017b, 2021; Murugan et al. 2020; Julius 2022). With regard to Lao PDR, floristic surveys carried out during the last years in poorly investigated areas allowed the description of a few new species (Souvannakhoummane et al. 2018, 2020; Fici et al. 2018, 2020; Fici and Souvannakhoummane 2020), while two species were recorded for the first time from the country (Fici 2016a; Tagane et al. 2020). Based on the available data, the genus *Capparis* includes in Lao PDR 22 species, all belonging to *Capparis* sect. *Monostichocalyx* Radlk.

During recent fieldwork in the Bolikhamxai Province, central Lao PDR, a population of *Capparis* characterized by lianous habit, brown-reddish indumentum, large flowers in terminal corymbs or subumbels and large ovary, was observed in forest habitats. Material collected from this population turned out to belong to an undescribed species, to be referred to the Trinervia-Group (Jacobs 1965) within *C.* sect. *Monostichocalyx*. The new species is here described and illustrated, and data on its distribution, ecology, conservation status and affinities are given.

MATERIALS AND METHODS

Field investigations were undertaken in the Bolikhamxai Province in 2021 and 2022. The new species was firstly observed in April 2021 in the Khamkeut District, and specimens were collected in the same locality in April 2022, and kept at the National University of Laos (FOF) and Herbier National du Laos (HNL).

The species concept adopted, as well as the terminology of the vegetative and reproductive structures, follow Jacobs (1965). The description and illustration are based on herbarium material. The herbarium acronyms follow Thiers (continuously updated), while authors and plant names are based on the IPNI (2020). The examination of the type specimens of related taxa was carried out through electronic images available at JSTOR Global Plants (n.d.). The conservation status was assessed according to *IUCN Red List Categories and Criteria* (IUCN 2012).

TAXONOMIC TREATMENT

Capparis phatadke Fici, Lanors., Lamxay & Souvann., **sp. nov**.

Type: Lao PDR, Bolikhamxai Province, Khamkeut District, Pongpatao village, 7 April 2022, *Lanorsavanh et al.* LP123 (holotype FOF!; isotype HNL!). (Figure 1).

Diagnosis

Differs from *Capparis trinervia* Hook. f. & Thomson var. *trinervia* in the longer, foliaceous bracts, sepals outside glabrous, pubescent at the base, longer filaments and larger ovary.

Description

Climber up to c. 8 m long. Twigs brown-reddish pubescent. Stipular thorns straight, c. 2 mm long. Petiole 5–8 mm long, densely pubescent. Leaf blade elliptic, (2.2–) 2.9–3.7 (–4) times as long as wide, widest at or above the middle, (7.7–) 8.5–9.6 (–10) × (2–) 2.3–3 (–3.6) cm; base cuneate, apex acuminate with tip up to 1.2 cm long; surfaces glabrous; nerves 3–4 pairs. Flowers c. 10–12 in terminal corymbs or subumbels; pedicels (1.2–) 1.7–2.5 (–3) cm long, pubescent; bracts foliaceous, persistent, up to 1.2 cm long; sepals (0.8–) 0.9–1.2 × 0.6–0.8 (–1) cm, outside glabrous, pubescent near the base, inside glabrous, the inner pair with margins membranous, ciliate; petals white, obovate, (1–) 1.2–1.5 (–1.6) × 0.9–1.2 (–1.3) cm, crisp at the top, outside glabrous or pubescent at the base, inside pubescent at the base, ciliate at margins; stamens (48–) 60–70 (–90), filaments (3–) 3.2–4 (–4.4) cm long; gynophore (2–) 2.5–3 (–3.5) cm long, glabrous; ovary ellipsoid or ovoid, 3.5–4 × 3–3.2 mm, shortly beaked, glabrous. Fruit unknown.

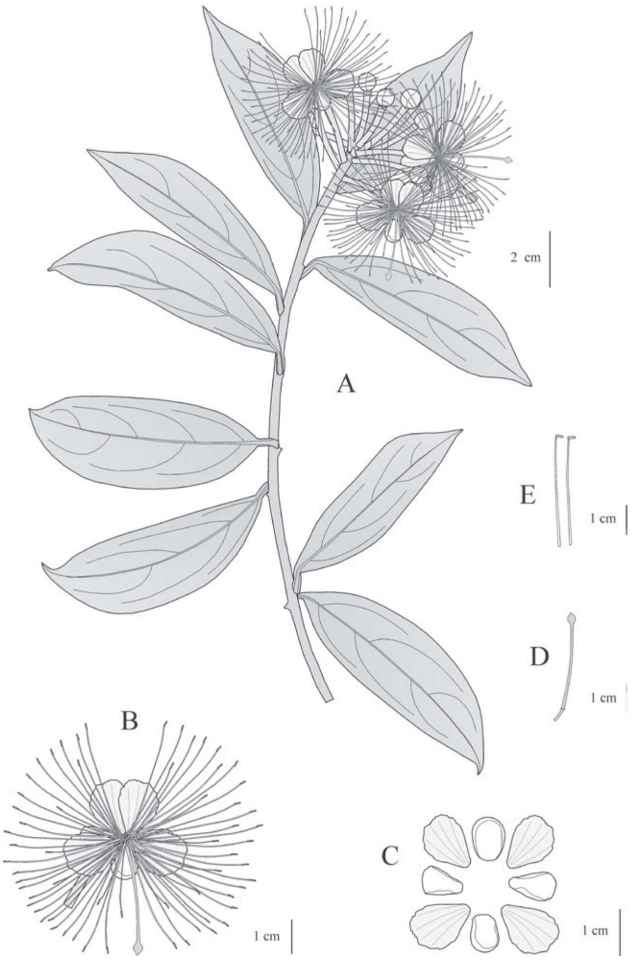

Figure 1. *Capparis phatadke.* (A) Flowering branch. (B) Flower. (C) Dissected flower showing sepals and petals. (D) Gynophore and ovary. (E) Stamens. All from *Lanorsavanh et al.* LP123 (holotype). Drawn by S. Fici.

Figure 2. *Capparis phatadke.* Flowering branch. Photo by S. Lanorsavahn.

Etymology

The new species is named after Pha Tad Ke, the first botanical garden in Laos, involved in plant conservation and in educational programs on the flora of the country and its ethnobotanical uses.

Distribution

The new species is known only from the type locality, at 18°12’43.7” N 104°46’51.8” E, where few individuals were observed.

Ecology

Mixed primary deciduous forest and older secondary forest with *Neonauclea purpurea* (Roxb.) Merr., *Nauclea orientalis* (L.) L., *Barringtonia* sp. and *Syzygium* sp., in a limestone area at elevation of 730 m a.s.l.

Conservation status

Capparis phatadke is here assessed as Vulnerable (VU D1) following the *IUCN Red List Categories and Criteria* (IUCN 2012), due to its restricted area of occupancy in a single location so far known, and low number of individuals observed.

Phenology

Flowering in April.

Notes

The new species, characterized by brown-reddish indumentum, straight thorns, laminar bracts and large flowers arranged in terminal corymbs or subumbels (Figures 2, 3), belongs to the Trinervia-Group (Jacobs 1965), which includes a few species from south-eastern Asia. It is related to *Capparis trinervia* var. *trinervia*, a taxon recorded from Myanmar, Laos, Vietnam, Malaysia, Indonesia and doubtfully Thailand (Jacobs 1965), differing mainly in the bracts laminar, persistent, up to c. 1.2 cm long (vs linear, caducous, c. 4 mm long), sepals outside glabrous, pubescent only at the base (vs outside densely puberulous), filaments (3–) 3.2–4 (–4.4) cm long (vs c. 2.7 cm) and ovary 3.5–4 × 3–3.2 mm (vs 2–2.5 × 1.5 mm) (Jacobs 1960, 1965). The recently described *C. trinervia* var. *chungiana* Julius from Peninsular Malaysia differs in the larger leaf blade, 13–16 × 5.5–8.5 cm (vs (7.7–) 8.5–9.6 (–10) × (2–) 2.3–3 (–3.6) cm), nerves 5–7 (–8) pairs (vs 3–4 pairs), sepals 1.2–1.5 × 1.2 cm, the outer pair velvety ferruginous outside (vs (0.8–) 0.9–1.2 × 0.6–0.8 (–1) cm, the outer pair glabrous, pubescent only at the base) and stamens in number of 30–40 (vs (48–) 60–70 (–90)) (Julius 2022).

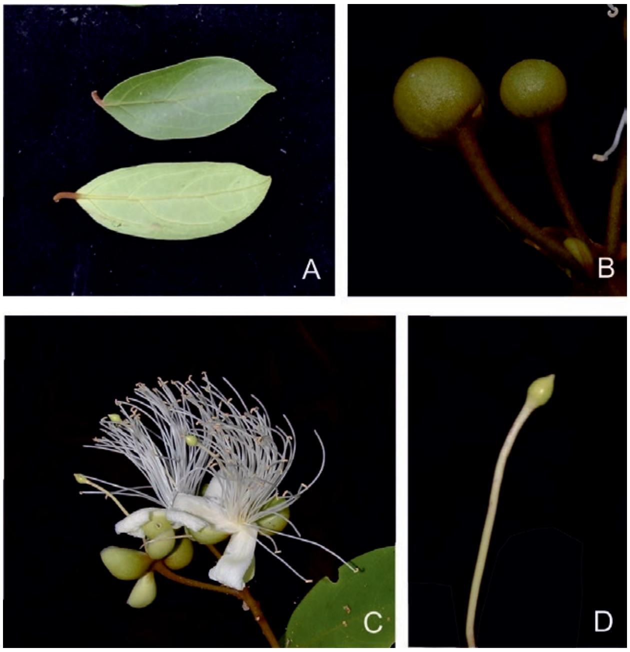

Figure 3. *Capparis phatadke.* (A) Leaves, adaxial side (above) and abaxial side (below). (B) Flower buds. (C) Flowers. (D) Gynophore and ovary. Photos by S. Lanorsavahn.

The large ovary of *C. phatadke* is a remarkable character if compared with the other species of the Trinervia-Group, with the exception of *C. klossii* Ridl., a species endemic to peninsular Thailand, in the Isthmus of Kra, which differs from the new species in the longer petiole, c. 12–15 mm long (vs 5–8 mm), larger leaves, c. 16.5–22.5 × 8–13 cm (vs (7.7–) 8.5–9.6 (–10) × (2–)

2.3–3 (–3.6) cm), nerves 6–7 pairs (vs 3–4 pairs), flowers in racemes (vs corymbs or subumbels), bracts c. 1.7 cm long (vs up to c. 1.2 cm), and gynophore 3.5–5.5 cm long (vs (2–) 2.5–3 (–3.5) cm) (Jacobs 1965; Chayamarit 1991). Large bracts, in some cases resembling small leaves, are recorded for *C. scortechinii* King var. *scortechinii*, a taxon widespread in Malaysia, differing from *C. phatadke* in the recurved thorns (vs straight), nerves 5–6 pairs (vs 3–4 pairs), flowers in racemes (vs in corymbs or subumbels), pedicels 0.5–1 cm long (vs (1.2–) 1.7–2.5 (–3) cm), petals 0.8–0.9 × 0.45–0.6 mm (vs (1–) 1.2–1.5 (–1.6) × 0.9–1.2 (–1.3) cm), filaments c. 1.5 cm long (vs (3–) 3.2–4 (–4.4) cm), gynophore c. 5–6.5 cm long (vs (2–) 2.5–3 (–3.5) cm), and ovary 1.7 × 0.7 mm (vs 3.5–4 × 3–3.2 mm) (Jacobs 1960, 1965). The recently described *C. scortechinii* var. *ruthiae* Julius from Peninsular Malaysia differs from *C. phatadke* in the petiole 10–15 mm long (vs 5–8 mm), pedicels 0.4–0.5 cm long (vs (1.2–) 1.7–2.5 (–3) cm), bracts (13–)16–25 mm long (vs up to 12 mm long), sepals 0.4–0.6 × 0.4–0.6 cm, densely hairy outside (vs (0.8–) 0.9–1.2 × 0.6–0.8 (–1) cm, pubescent only at base), petals 0.65–0.7 × 0.45–0.5.5 cm (vs (1–) 1.2–1.5 (–1.6) × 0.9–1.2 (–1.3) cm), and ovary c. 2.5 × 1.5 mm (vs 3.5– 4 × 3–3.2 mm) (Julius 2002).

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Wurfbainia rubrofasciata (Zingiberaceae), a new species from Palawan, Philippines

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Abstract. A ginger species collected in Palawan, Philippines was initially thought to be *Wurfbainia palawanensis* but differs by the dark brown fertile bracts and red spinose fruits. This species is also morphologically similar to the continental Asian *Wurfbainia microcarpa* but can be distinguished from this by the entire ligule, petiolate lamina, congested rachis, and presence of red stripes at the base of the labellum. Therefore, we here describe *Wurfbainia rubrofasciata* providing colour plates, data on phenology, distribution, habitat, and a provisional conservation assessment. A key to five species of *Wurfbainia* in the Philippines is provided and we discuss the likely closest relatives of *Wurfbainia palawanensis*.

Keywords: *Amomum* s.l., endemic, endangered, taxonomy, *Wurfbainia*.

INTRODUCTION

In 2018, the ginger genus *Wurfbainia* Giseke was reinstated by De Boer et al. as a monophyletic genus within the polyphyletic *Amomum* Roxb. sensu lato. The spoon-shaped labellum, the trilobed anther crest in which the side lobes usually pointing upwards and the mid lobe positioned behind the stigma distinguishes *Wurfbainia* from its closely related genera, especially from its sister, *Meistera* Giseke. *Wurfbainia* is distributed with 26 species in North East India, Bangladesh, China, and South East Asia with a centre of diversity in Thailand that harbours 18 species (Kaewsri and Sangvirotjanapat 2022).

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The taxonomic revision by De Boer et al. (2018) divided the Philippine species hirtho places in *Amomum* into three genera namely *Amomum* (1 species), *Meistera* (3 species), and *Wurfbainia* (4 species). Two species, *Amomum luzonensis* Elmer and *A. warburgii* (K.Schum.) K.Schum., however, were listed as *incertae sedis* since both have very limited protologues and their respective type is either lost or destroyed. Thus, there is not sufficient evidence to conclude their generic placement.

The four species of *Wurfbainia* in the Philippines are all endemic (Pelser et al. 2011 onwards). Ridley (1905) described *Amomum elegans* Ridl. and *A. trilobum* Ridl. Turner (2001), however, made a new name, *A. hedyosmum* I.M.Turner, for *A. trilobum* since the name was already used for a different species in Vietnam. Furthermore, Elmer (1915) described *A. mindanaense* Elmer and *A. palawanense*, both named after the island where they were discovered. The combinations of *A. elegans, A. hedyosmum, A. mindanaense,* and *A. palawanense* in *Wurfbainia* was based entirely on morphology since none of them had been included in a molecular-based phylogeny.

The present paper focuses on a species of *Wurfbainia* in Palawan which was at first misidentified as *W. palawanensis* (Elmer) Škorničk. & A.D.Poulsen (Figure 1). The resulting new species is described in detail and illustrated below and an updated key to all species of the genus in the Philippines is provided.

MATERIAL AND METHODS

On the website of Co's Digital Flora of the Philippines (CDFP), a photograph of a *Wurfbainia* taxon in Palawan was identified by M.A.K. Naive as *W. palawanensis* (see http://www.phytoimages.siu.edu/imgs/pelserpb/r/Zingiberaceae_Wurfbainia_palawanensis_122428.html). In 2018, this taxon was collected during fieldwork in Palawan.

Herbarium specimens, including types and specimens relevant to *Wurfbainia* deposited in several herbaria (BM, BO, E, FI, FEUH, G, GH, K, L, NY, PNH, U, US, USTH, and Z), protologues, and published morphological descriptions of relevant species were examined. Specimens that were examined using only digital images are indicated with an apostrophe (*). Geospatial Conservation Assessment Tool (GeoCAT) (Bachman et al. 2011: http://geocat.kew.org/) was used to calculate the area of occupancy (AOO) and was used to assess the conservation status of the new species using the International Union for Conservation of Nature (IUCN) criteria (IUCN Standards and Petitions Committee 2019). The distribution map presented was generated using SimpleMappr (Shorthouse 2010).

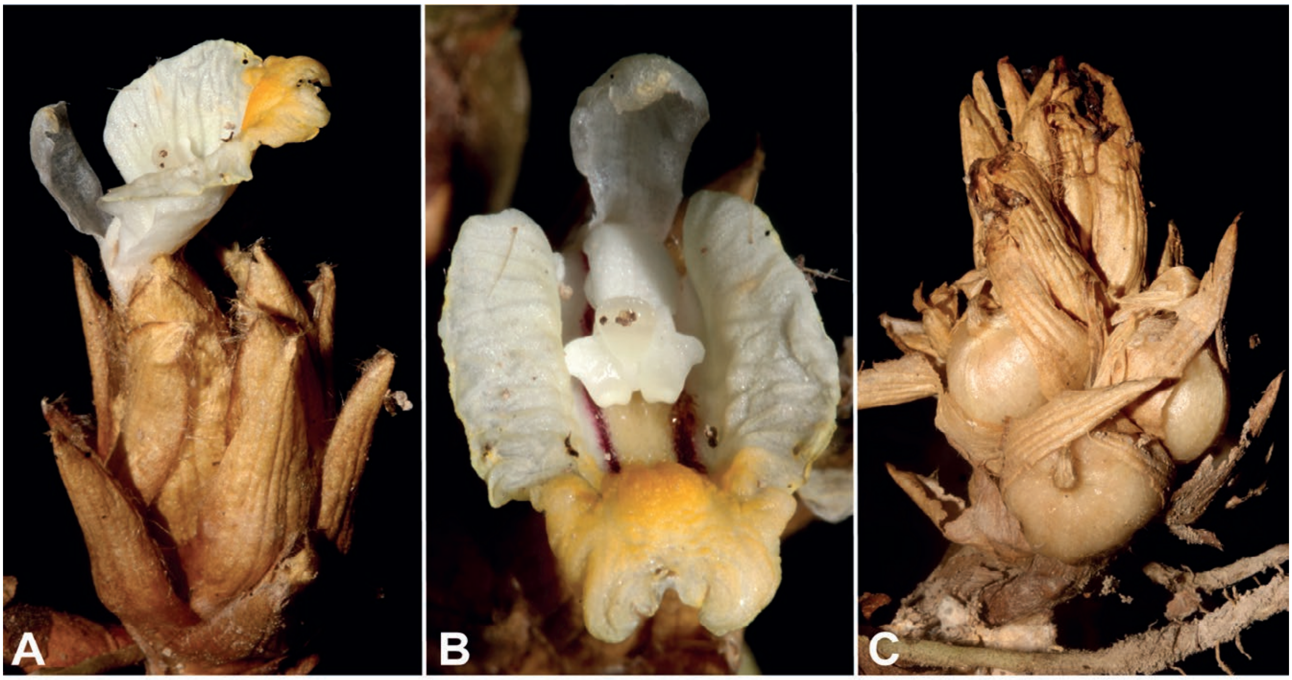

Figure 1. *Wurfbainia palawanensis*: **A** Inflorescence **B** Flower **C** Infructescence. Based from *R.V.A. Docot et al. 299* (Photographs by R.V.A. Docot).

TAXONOMIC TREATMENT

Considering the proximity of the type locality of *Wurfbainia palawanensis* it is not surprising that one might jump to the conclusion that photographs taken of this genus in this area of Palawan are of this species (Figure 2). An examination of our recent material, however, demonstrated that it clearly did not match *W. palawanensis* nor any other known species of Philippine *Wurfbainia*. Of these, *W. elegans* (Ridl.) Škorničk. & A.D.Poulsen shares some characters but the continental Asian, *W. microcarpa* (C.F.Liang & D.Fang) Škorničk. & A.D.Poulsen, is most similar (Table 1). The material is at the same time distinguished from all this and a novel species is therefore described below.

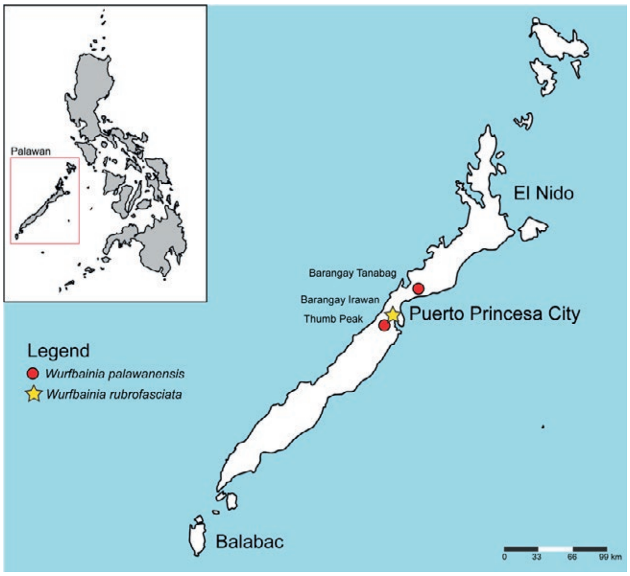

Figure 2. Distribution map of *Wurfbainia palawanensis* and *W. rubrofasciata* sp. nov.

Wurfbainia rubrofasciata Docot & Domingo, **sp. nov.** (Figure 3).

Type: Philippines, Palawan Puerto Princesa City, Barangay Irawan, 1 July 2018, *C.B.M. Domingo & R.V.A. Docot PL18–006* (holotype PNH; isotypes FEUH + spirit, L, PPC, USTH).

Diagnosis

Wurfbainia rubrofasciata is morphologically similar to *W. microcarpa* (C.F.Liang & D.Fang) Skornick. & A.D.Poulsen in having white flowers with a clawed and saccate labellum but differs in the entire ligule (vs emarginate to bilobed), 7–11 mm long petiole (vs < 2 mm); 2–4 flowers open at a time (vs 1–2); congested rachis (vs elongated); white calyx tube and corolla lobes (vs white to red), presence of 10–12 red stripes at the base of the labellum (vs minute red dots) and ovate and petaloid lateral lobes of the anther crest (vs oblong and fleshy).

Description

Terrestrial herb in loose clump. Rhizome 1–2 cm wide, axis yellowish-green, scales 10–16 mm long, dark brown, glabrous, stilt roots absent. Leafy shoots 1–2 m long, arching at various degrees, with ca. 30 leaves per shoot, 6–7 cm apart, base 20–34 mm wide, white; sheath reticulate, mid-green, pubescent including the margin; ligule ovate, 2–4 × 3–4 mm, brownish-green, sericeous, apex rounded to obtuse; petiole 7–11 mm long, mid-green, pubescent; lamina narrowly ovate, 29–35 × 5–7 cm, mid-green and glabrous above, light green and pubescent beneath, veins slightly plicate, base obtuse, apex acuminate, margin entire and pubescent. Flowering shoot 4–7 cm long, arising near base of the leafy shoot or from rhizome; peduncle nodding at various degrees, pubescent, 4–7 cm long at flowering state, elongates up to 9 cm long at fruiting state; peduncular bract tubular, 5–7 mm long, dark brown, glabrous; spike bouquet-like, with 4–8 flowers, 2–4 open at a time, 3–4 cm long; rachis congested even at fruiting state; fertile bracts each subtending one flower only, ovate, 13–21 × 8–11 mm, dark brown, glabrous, apex mucronate; bracteole spathaceous, 9–16 mm long, dark brown, sparsely pubescent, apex acute; pedicel ca. 2 mm long, pubescent, elongates up to 4 mm at fruiting state; flowers 3–4 cm long; calyx spathaceous, 17–22 mm long, semi-transparent white, pubescent, apex 2–3-dentate; corolla tube 18–23 cm long, white, glabrous outside, ciliate inside; corolla lobes narrowly ovate, white, margin transparent, glabrous, apex cucullate; dorsal corolla lobe 10–14 × 4–5 mm; lateral corolla lobes 8–13 × 3–4 mm; labellum saccate, trilobed, glabrous, basal part with 10-12 red stripes adaxially, lateral lobes white, central lobe yellow with minute red dots, emarginate; lateral staminodes oblong or tooth-like, ca. 3 mm long, red and white; stamen 8–12 mm long; filament flattened, 5–7 × 1–2 mm, white, glabrous; anther 4–6 × 3–4 mm, connective white and glabrous; anther crest trilobed, the lobes ovate, lateral lobes 3–4 mm long, central lobe 2–3 mm long and sometimes emarginate, white, glabrous; thecae 4–6 × 1–2 mm, white, margin facing each thecae ciliate; style 27–35 mm long, white, glabrous; stigma ca. 2 mm wide, white, glabrous; epigynous glands linear, 3–4 mm long, yellow, glabrous; ovary subglobose, 3–5 × 2–3 mm, light brown, pubescent. Infructescence 5–10 cm long, with

Figure 3. *Wurfbainia rubrofasciata* Docot & Domingo (A) Habit. (B) Ligule. (C) Inflorescence and infructescence. (D) Inflorescence showing different angle of the flowers. (E) Infructescence. (F) Floral dissection. (G) Stamen at different views. (notice the white arrow pointing a lateral staminode). Based from *C.B.M. Domingo & R.V.A. Docot PL18-006* (type). *Abbreviations*: br: bracteole; ca: calyx tube; dc: dorsal corolla lobe; fb: fertile bract; fl: flower; inf: inflorescence; la: labellum; lc: lateral corolla lobes; ov: ovary with epigynous glands on the top. Scale bars = 1 cm. (Photographs by R.V.A. Docot).

3–4 fruits; fruits globose, 8–11 × 7–10 mm, dark red, sparsely pubescent, spinose; seed irregular to subglobose, ca. 2 mm long, black, aril white.

Etymology

The specific epithet refers to the red stripes at the base of the labellum.

Distribution and habitat

Wurfbainia rubrofasciata is endemic to Palawan, Philippines, where it inhabits semi-shaded areas of secondary forest near and along streams.

Phenology

Flowering and fruiting from March to August.

Provisional IUCN conservation assessment

Based on the IUCN red list categories and criteria (IUCN Standards and Petitions Committee, 2019), *Wurfbainia rubrofasciata* is assessed as Endangered (EN B2ab(iii), D). The area of occupancy (AOO) is estimated to be less than 10 km^2 (total AOO is c. 4 km^2) as the species is only known from the type locality. *Wurfbainia rubrofasciata* is abundant within Barangay Irawan with more than 200 individuals observed. There are reports that the new species is likely present in Northern Palawan (e.g., El Nido) but specimens are needed in order to support this.

Notes

In the Philippines, *Wurfbainia elegans* resemble the new species by having dark brown fertile bracts and red spinose fruits. *Wurfbainia rubrofasciata,* however, can be easily distinguished by the presence of red stripes at the base of the labellum (vs minute red spots) and ovate and petaloid side lobes of the anther crest (vs linear and fleshy).

Based on overall morphology (Table 1), the most similar species is *Wurfbainia microcarpa* of continental Asia. Although both *W. microcarpa* and *W. rubrofasciata* can reach 2 m long, *W. rubrofasciata* can be readily distinguished in its vegetative state by the sericeous and entire ligule (vs tomentose and emarginate to bilobed), 7–11 mm long petiole (vs subsessile), and obtuse lamina base (vs attenuate). In the floral morphology, both has white spikes. In *Wurfbainia rubrofasciata,* the rachis of the spike is congested while it is elongated in *W. microcarpa*. During anthesis, there are 2–4 flowers open at a time in *W. rubrofasciata* whereas in *W. microcarpa,* 1–2 flowers are open. In terms of flower colour, the calyx and corolla of *W. macrocarpa* vary from white, light pink to red. In *W. rubrofasciata,* however, it is white only. The most observable difference between the two species is the labellum colour and pattern in which there are 10–12 red stripes at the base in *W. rubrofasciata* while only minute red dots are present in *W. microcarpa*. There are no reported local name or use of the new species.

Table 1. Morphological comparison between *Wurfbainia rubrofasciata, W. microcarpa, W. elegans,* and *W. palawanensis*

Morphological characters	*Wurfbainia rubrofasciata*	*Wurfbainia microcarpa*	*Wurfbainia elegans*	*Wurfbainia palawanensis*
Ligule indumentum and apex	sericeous, entire	tomentose, emarginate to bilobed	pubescent, entire	glabrous, bilobed
Petiole length	7–11 mm	1–2 mm (subsessile)	4-8 mm	5-9 mm
Lamina base	Obtuse	attenuate	obtuse	attenuate
Lamina apex	acuminate	caudate	acuminate	attenuate
Flower anthesis	2–4 open at a time	1–2 open at a time	1–2 open at a time	1-2 open at a time
Rachis	congested	elongated	congested	congested
Bracteole apex	1-dentate	2-dentate	1-dentate	2-dentate
Calyx color	white only	white, light pink or red	reddish at the base, becoming white towards the apex	white only
Corolla lobes color	white only	white or red	white only	white only
Labellum color and pattern	white with 10–12 red stripes at the base of the labellum	white with minute red dots at the base of the labellum	white with yellow and minute red dots at the middle	white with yellow band and two parallel purplish-red lines at the middle
Filament color	white only	white or red	white only	white only
Lateral lobes of the anther crest	ovate, 3-4 mm long, petaloid	oblong, 2-3 mm long, fleshy	linear, 4-5 mm long, fleshy	linear, 1-2 mm long, fleshy
Fruit	red, spinose	red, spinose	red, spinose	light brown, smooth

Closest relatives of Wurfbainia palawanensis

Elmer (1915) described *Amomum palawanense* using a set of specimens collected in Mount Pulgar (= now known as Thumb Peak), Palawan and noted that *Amomum loheri* K.Schum. (now placed in *Meistera*) as the most related species. So far, *W. palawanensis* is restricted in Palawan (Figure 2). Examination of the type, protologue, and relevant materials revealed that *W. palawanensis* is instead, due to the light brown fertile bracts and light brown smooth fruits (Figure 1C), more closely related to *W. mindanaensis* (Elmer) Škorničk. & M.F.Newman of the Philippines and *W. compacta* (Sol. ex Maton) Škorničk. & A.D.Poulsen in Indonesia. Future molecular-based phylogenetic work will be able to test this prediction.

Specimens examined

Wurfbainia elegans. Philippines. Luzon, Bataan, Mount Mariveles, Lamao River, May 1905, *T.E. Borden 3033* (K, US*); Orani, Barangay Tala, Mount Natib, 12 June 2019, R.V.A Docot et al. 274 (FEUH); Ilocos Norte, Pagudpod, Barangay Balaoi, Kalbario-Patapat Natural Park, 18° 34.012’N, 120° 53.789’E, 360 m. a.s.l., 14 June 2022, *R.V.A. Docot et al. 321* (FEUH); 18°33.831’N, 120°53.574’E, 385 m. a.s.l., 14 June 2022, *R.V.A. Docot et al. 324* (FEUH).

Wurfbainia microcarpa. Laos. Oudomxia, Ban Nam Peng, 21°01’05 N, 101°39’32 E, 810 m.a.s.l., 22 June 1999, *M.F. Newman 870* (E); Vientiane, Feuang, Bane Ne Xeng, 18°48’27’N, 102°6’31’E, 264 m.a.s.l., 1, May 2008, *V. Lamxay et al. VL1214* (E + spirit).

Wurfbainia mindanaensis. Philippines. Mindanao, Davao del Sur, Todaya, Mount Apo, June 1909, A.D.E. Elmer 10822 (BO, C, FI, G, GH, HBG, K, L, U, Z); Surigao del Norte, Tubod, Barangay Sapa, 9°31’43.1”N 125°43’52.4”E, 11 July 2019, *C.B.M. Domingo et al. SDN19-001* (FEUH + spirit, L, PNH, USTH).

Wurfbainia palawanensis. Philippines. Palawan, Puerto Princesa City, Mount Pulgar, March 1911, *A.D.E. Elmer 12795* (BM, BO, FI, G, GH, K, L, U, US*, Z); Beside trail to first camp to San Rafael to Cleopatras Needle, 09°48’N, 118°56’E, 100 m.a.s.l., 15 January 1998, *Q. Cronk et al., 25351* (E + spirit); Barangay Tanabag, 10 March 2020, *R.V.A Docot et al. 299* (FEUH + spirit, PNH, PPC, USTH).

Key to the Philippine *Wurfbainia* species

1a. Fertile bract light brown, persistent; fruit smooth, light brown **2**

1b. Fertile bract dark brown, non-persistent; fruit spinose, red. **3**

2a. Margin of sheet and ligule with brown hairs; flowering shoot elongates with age; lateral lobes of anther crest ovate and petaloid .... ***W. mindanaensis***

2b. Margin of sheet and ligule glabrous; flowering shoot does not elongate with age; lateral lobes of anther horn-like and fleshy .... ***W. palawanensis***

3a. Base of the labellum with red stripes; lateral lobes of the anther crest ovate and petaloid .... ***W. rubrofasciata***

3b. Base of the labellum with minute red spots; lateral lobes of the anther crest linear and fleshy **4**

4a. Lamina narrowly ovate to linear, velvety beneath .... ***W. elegans***

4b. Lamina narrowly ovate only, glabrous beneath .... ***W. hedyosma***

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Micro-morphological characters in Polypodiaceae and its taxonomic significance

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Abstract. The present study insights into the interrelationships and taxonomic segregation of some Polypodiaceous fern taxa based on leaf architecture and foliar micro-morphology. Twenty-seven fern species were examined and valuable qualitative and quantitative data were obtained to generate UPGMA dendrogram. A dichotomous key differentiating the taxa was prepared. Results reveal that even though species have overlapping characters, certain specific traits prove taxonomically valuable. The results proved that traits like leaf shape, higher order leaf venation, stomatal and epidermal features are indeed important diagnostic characters and hence can be used for the identification of fern species in their immature stage or even in absence of sori. These data sets often combined with other morphological as well molecular data would contribute to fern phylogenetic study particularly of the large and complex family Polypodiaceae.

Keywords: Epidermis, ferns, leaf architecture, stomata.

INTRODUCTION

Polypodiaceae *s.l.* is an extant, monophyletic family of ferns that includes Polypodiaceae and previously segregated families Grammitidaceae and Platyceriaceae (Smith et al. 2008). As per PPGI (2016), Polypodiaceae comprise 6 sub-families, 65 genera, and 1,652 species and it is the second largest family of ferns (Hori et al. 2022). However, earlier reports estimate approximately 50 genera under the family worldwide (Tryon and Tryon 1982; Hennipman et al. 1990; Parris 1990; Smith et al. 2008). The family is a sub-cosmopolitan group mainly characterized by creeping stems covered with varying scales, fronds attached to phyllopodia, venation free or sometimes areolate with free or included veins, round to globose exindusiate, sori on abaxial lamina surface with yellowish to greenish monolete spores (Tryon and Tryon 1982; Hennipman et al. 1990; Parris 1990; Smith et al. 2008).

Kubitzki (1990) initially coined the term “polygrammoid ferns” until a phylogenetic study established the name Polypodiaceae (Schneider et al. 2004). Phylogenetic studies of major derived fern groups, such as aspleniod, dryopteroid, and polygrammoid ferns, have been of great importance since the ferns are an integral component of the tropical vegetation (Schneider et al. 2004). Major generic-level recircumscriptions have been suggested for Polypodiaceae with a redefinition of *Polypodium* L., *Pleopeltis* Humb. & Bonpl. ex Willd. and allied genera (Smith et al. 2008). Christenhusz et al. (2011), suggested the need for more studies at the generic level, especially on relationships among genera in some of the large families including Polypodiaceae. The order Polypodiales have been segregated into three groups with Polypodiaceae included within eupolypods I (PPG I 2016). The group eupolypods I showed diverse morphological variations with species richness and limited data sets, hence the classification or identification up to generic and specific levels is often associated with difficulty (Rothfels et al. 2012; Tan and Buot 2020).

The correct identification and classification of fern is associated with complexities since the time of Linnaeus and even in the modern era. Several genera like *Loxogramme* (Blume) C.Presl lacked published generic revision and comprehensive analyses of the genus *Arthromeris* (T.Moore) J.Sm. are unavailable. *Microsorum* Link is apparently paraphyletic relative to some other species but requires further study (PPG I 2016). The concept of family is not well established due to the existence of shared morphological features between the families (Christenhusz and Chase 2014). Even with the availability of molecular data for classification and phylogeny, Takhtajan (1996), Christenhusz and Chase, (2014), and Christenhusz et al. (2015) pointed out several difficulties such as random changes in DNA sequences, convergent evolution, parallelism, splitting and lumping of huge data set.

Morphological references of medicinal fern species are of utmost importance so that the samples can be correctly identified especially when there are chances leading to confusion and improper use of those particular taxa (Oliveira et al. 2017).It seems that Pteridaceae and Polypodiaceae contribute the highest number of medicinal fern species with worldwide distribution (Muhammad et al. 2020). Some of the species investigated in our study have important medicinal aspects. The roots of *Arthromeris wallichiana* (Spreng.) Ching antiseptic properties (Manandhar 2002), and anti-dysentery (Gaur and Bhatt 1994; Nwosu 2002). Similarly, *Drynaria quercifolia* L. has wound healing properties, lumbago treatment, rhizome astringent, and against hectic fever and cough (May 1978; Gaur and Bhatt 1994). *Microsorum membranaceum* (D.Don) Ching provides relief for chest pain, cough and cold of infant, diarrhea, and dysentery (Gaur and Bhatt 1994). Leaf juice of *Microsorum punctatum* (L.) Copel. serves as a purgative, diuretic, and wound healer (May 1978). *Phymatosorus scolopendria* (Burm.f.) Pic.Serm. is used against chronic diarrhea, anti-inflammatory, pulmonary and liver disease treatment (Mannan et al. 2008; Hoet al. 2011). *Pyrrosia lanceolata* (L.) Farw. has been used to treat skin disorders, colds, and sore throats (Benjamin and Manickam 2007).

Leaves have significance in evolutionary and developmental studies, because they are the most conspicuous organs of the plants. The leaves or fronds of ferns display great morphological diversity (Creese et al. 2010; Vasco et al. 2013). Systematists overlook the importance of vegetative characters such as leaf venations because of the perception that they are phenotypically plastic characters (Larano and Buot 2010).

However, over the years numerous species of angiosperms have been identified, described and delineated, generating phylogenetic relationships using leaf architecture Cervantes et al. 2009; Pacheco-Trejo et al. 2009; Sarala and Vijay 2014; Sharma et al. 2016; Fayed et al. 2020). Foliar micro-morphological traits like epidermal cell size, stomatal features, and laminar indument have great taxonomic implications in distinguishing species (Baronova 1992; Chukwuma et al. 2017; Chukwuma et al. 2022).

Most studies reveal that leaf venation was genetically fixed and closely related to the development and their evolution (Roth-Nebelsick et al. 2001). Therefore, it can be utilised by systematics, especially for plants without reproductive parts (Carlquist 1961; Dilcher 1974). Recent studies have further associated the lamina shape and petiole structure with plant carbon budget (Takenaka 1994; Semchenko and Zobel 2007; Niinemets et al. 2007). Description and characterization of the families under eupolypods I have included leaf dissection and venation (Pray 1960; Wagner 1979; Pryer et al. 1995; Ding et al. 2014). Some of the earlier works on leaf architecture of ferns included *Diplazium* Sw. species (Conda et al. 2017), *Lygodium* Sw. (Shinta et al. 2012), *Ophioglossum* L. (Magrini and Scoppola, 2010), and some terrestrial and epiphytic eupolypod ferns (Tan and Buot 2019) from Araucaria forest (Larcher et al. 2013).

Exploration of leaf architectural characters in selected eupolypods I group exhibited higher degree of venations until areoles only in some species under Polypodiaceae and Tectariaceae (Tan and Buot 2020). More studies for other species through leaf architecture are highly recommended to strengthen its affectivity and usefulness (Conda et al. 2017).

Over the years, significant information on certain species within the family Polypodiaceae has been generated by several studies worldwide on morphological, molecular, ecological, and distributional aspects. However, a comparative study on the species with respect to leaf architecture and venation pattern is limited. The

Polypodiaceous ferns are exceptionally diverse and thus an ideal system for investigating taxonomic and systematics of leaf form venation, epidermal features, and their variations.

This study explores the use of leaf architecture and other details in some members of Polypodiaceae and helps in taxonomic delineation which would complement the already established diagnostic characters for identification of the taxa.

MATERIALS AND METHODS

Plant material

Fresh specimens of Polypodiaceae were used for the present study. They were collected mainly from the forests of Darjeeling Himalaya which is a part of the eastern Himalaya hotspot, extending between 27°13'10''N to 26°27'05''N and 88°53'E to 87°59'30''E and lies on the northern part of the Indian state of West Bengal. The specimens were carefully identified with the help of available literature (Mehra and Bir 1964; Ghosh et al. 2004; Fraser-Jenkins 2008; Kholia 2010; Frazer-Jenkins et al. 2021). Lloyd Botanic Garden Herbarium, Darjeeling and Calcutta University Herbarium (CUH) were also consulted for proper identification. Correct nomenclature was maintained following Smith et al. (2006), Pteridophyte Phylogeny Group (PPG I 2016), and databases like Global Biodiversity Information Facility (GBIF 2022) and World Flora Online (WFO 2022). The species were assigned with a code having three lettersto the generic and specific names (see Table 1).

Leaf morphometric and venation study

Mature leaves of the target species were collected from 3 representative plants and the samples were then washed properly. For the venation study, the method of Yu and Chen (1986) was followed with some modifications. Leaves were boiled in water for 10-20 minutes, then placed in 1-5% NaOH, the strength depending on the thickness of the material. NaOH solution was changed every 1-2 days during the clearing process, which generally took 2-10 days. For some species with thick lamina, the leaves were boiled in water before being macerated in 35% NaOCl solution. Cleared leaves were then rinsed in running water thoroughly, dried, stained in 1% safranin, and mounted on slides with glycerine.

The minor venation patterns were studied by cutting a small bit from the mid portion of the leaf skeleton. Leaves were examined and photographed under Wild M3 Heerbrugg and binocular microscope Leitz Laborlux D. The terminology of Hickey (1973), Ash et al. (1999), Ellis et al. (2009), Conda et al. (2017) and Conda and Buot (2018) have been followed for the description of the leaf architecture and venation patterns.

Leaf epidermal study

The epidermal characters were analysed using different quantitative measures *viz.*,the number of epidermal cells, epidermal cell size (L x W), stomata size (L x W), stomatal pore size (L x W), and stomatal index (SI). Epidermal cell measurements from adaxial and abaxial surfaces were determined under 40x magnification with a fitted ocular scale. The stomatal index was measured following Salisbury (1928, 1932), as SI = S/E+S x100.

Statistical analysis

All the quantitative data were subjected to descriptive statistics and analysed using PAST version 4.03 (Hammer et al. 2001) to obtain a UPGMA (Unweighted Pair Group Method with Arithmetic Mean) based dendrogram.

RESULTS

The leaf architectural characters of 27 fern species were examined based on three aspects. First, the morphological characters like lamina division, shape, apex shape, blade class, base shape, base angle, base symmetry, and margin were studied. Secondly, the leaf venation details from primary to tertiary and higher order vein categories and areoles were focused upon. The leaf epidermal cells and stomata were later analysed with the aid of microscopy.

Leaf morphological trait and venation

The species under the Polypodiaceae family exhibited variable morphological characteristics that were taxonomically significant such as the leaf organisation varying from simple to pinnate, pinnatifid, imparipinnate, etc. The leaf shape ranges from lanceolate, ovate-lanceolate, oblong, linear-lanceolate to elliptic. The blade class ranged from nanophyll to macrophyll while the margins observed were mostly entire to serrate, serrulate, crenate etc. (Table 1).

Table 1. Summary of foliar micro-characters of the studied taxa

Species	Species code	Lamina division	Shape	Apex shape	Blade class	Base shape	Base angle	Base symmetry	Margin
Arthromeris himalovata Fraser-Jenk. & Kandel	*Art him*	1-pinnate	ovate-lanceolate	caudate	notophyll	rounded	obtuse	symmetrical	entire
A. lehmannii (Mett.) Ching	*Art leh*	1-pinnate	lanceolate	acuminate	microphyll	rounded	acute	symmetrical	minutely toothed
A. wallichiana (Spreng.) Ching	*Art wal*	1-pinnate	ovate-lanceolate	acuminate	mesophyll	obliquely cordate	acute	symmetrical	entire
Drynaria propinqua (Wall. ex Mett.) J.Sm. ex Bedd.	*Dry pro*	pinnatifid	ovate-lanceolate	acute	mesophyll	adnate	obtuse	symmetrical	slightly crenate
D. quercifolia L.	*Dry que*	pinnatifid	broadly lanceolate	acute	mesophyll	adnate	obtuse	symmetrical	entire
Goniophlebium argutum (Wall. ex Hook.) J.Sm.	*Gon arg*	1-pinnate	linear lanceolate	acuminate	mesophyll	broad	obtuse	symmetrical	mucronate
Lepisorus contortus (Christ) Ching	*Lep con*	simple	linear to elliptic lanceolate	acute	microphyll	attenuate	acute	symmetrical	entire
L. loriformis (Wall. ex Mett.) Ching	*Lep lor*	simple	linear	acuminate	notophyll	decurrent	acute	symmetrical	entire
L. mehrae Fraser-Jenk.	*Lep meh*	simple	linear-lanceolate	acuminate	mesophyll	attenuate	acute	symmetrical	entire
L. normalis (D.Don) C.F.Zhao, R.Wei & X.C.Zhang	*Lep nor*	simple	lanceolate	acuminate	notophyll	attenuate	acute	symmetrical	entire
L. nudus (Hook.) Ching	*Lep nud*	simple	lanceolate	acuminate	notophyll	attenuate	acute	symmetrical	entire
L. rostratus (Bedd.) C.F.Zhao, R.Wei & X.C.Zhang	*Lep ros*	simple	elliptic	acuminate	nanophyll	attenuate	acute	symmetrical	entire
L. sublinearis (Baker ex Takeda)	*Lep sub*	simple	broadly lanceolate	acuminate	microphyll	attenuate	acute	symmetrical	entire
Loxogramme involuta (D.Don) C.Presl	*Lox inv*	simple	lanceolate	acuminate	mesophyll	attenuate	acute	symmetrical	entire
Microsorum membranaceum (D.Don) Ching	*Mic mem*	simple	lanceolate	acute	mesophyll	decurrent	obtuse	symmetrical	entire
M. punctatum (L.) Copel.	*Mic pun*	simple	linear-lanceolate	acute	mesophyll	decurrent	acute	symmetrical	entire
Phymatosorus cuspidatus (D.Don) Pic.Serm.	*Phy cus*	imparipinnate	linear-lanceolate	acuminate	microphyll	attenuate	acute	symmetrical	entire
P. scolopendria (Burm.f.) Pic.Serm.	*Phy sco*	pinnatifid	oblong	acuminate	microphyll	cuneate	obtuse	symmetrical	entire
Pichisermollodes ebenipes (Hook.) Fraser-Jenk.	*Pic ebe*	palmatifid	lanceolate	acuminate	microphyll	adnate/ deflexed	obtuse	symmetrical	slightly serrulate
P. stewartii (Bedd.) Fraser-Jenk.	*Pic ste*	pinnately parted	lanceolate	acuminate	microphyll	adnate	obtuse	symmetrical	serrulate
Polypodiodes amoena (Wall. ex Mett.) Ching	*Pol amo*	deeply pinnatifid	oblong-lanceolate	acute	microphyll	adnate	obtuse	symmetrical	serrate
Pyrrosia costata (C.Presl ex Bedd.) Tagawa & K.Iwats.	*Pyr cos*	simple	oblong-lanceolate	caudate	mesophyll	decurrent	acute	symmetrical	entire
P. heteractis (Mett. ex Kuhn) Ching	*Pyr het*	simple	ovate-lanceolate	caudate-acuminate	microphyll	round	obtuse	symmetrical	entire
P. lanceolata (L.) Farw.	*Pyr lan*	simple	narrow-lanceolate	acuminate	nanophyll	attenuate	acute	symmetrical	entire
P. mannii (Giesenh.) Ching	*Pyr man*	simple	lanceolate	acute	notophyll	attenuate	acute	symmetrical	entire
Selliguea griffithiana (Hook.) Fraser-Jenk.	*Sel gri*	simple	lanceolate	acuminate	microphyll	cuneate	acute	symmetrical	entire
S. oxyloba (Wall. ex Kunze) Fraser-Jenk.	*Sel oxy*	deeply pinnatifid	ovate	acute	microphyll	adnate	obtuse	symmetrical	entire

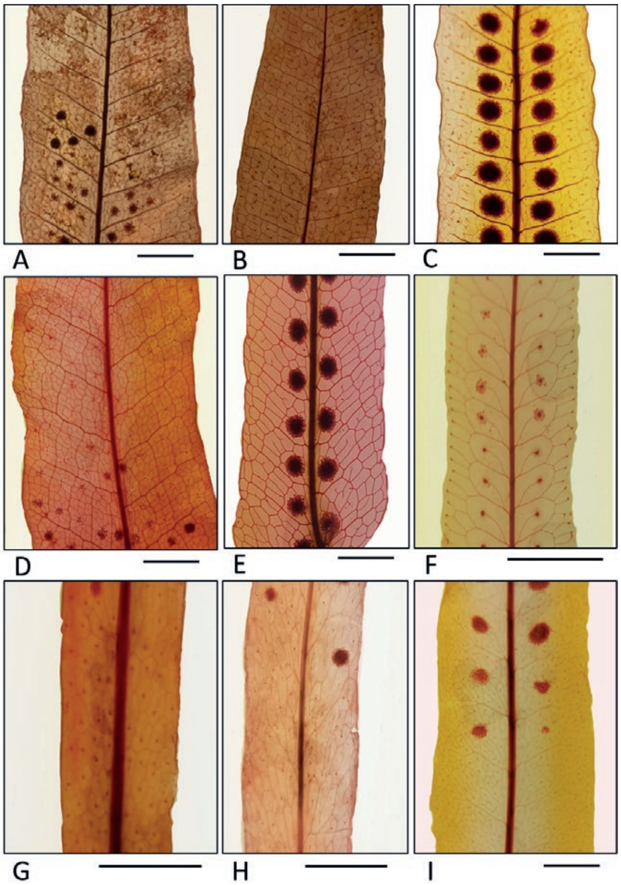

Figure 1. Photomicrographs of leaf venation in Polypodiaceae: **A**-*Arthromeris himalovata*; **B**-*Arthromeris lehmanii*; **C**-*Arthromeris wallichiana*; **D**-*Drynaria quercifolia*; **E**-*Drynaria propinqua*; **F**-*Goniophlebium argutum*; **G**-*Lepisorus contortus*; **H**-*Lepisorus loriformis*; **I**-*Lepisorus mehrae* (Scale bar-5mm).

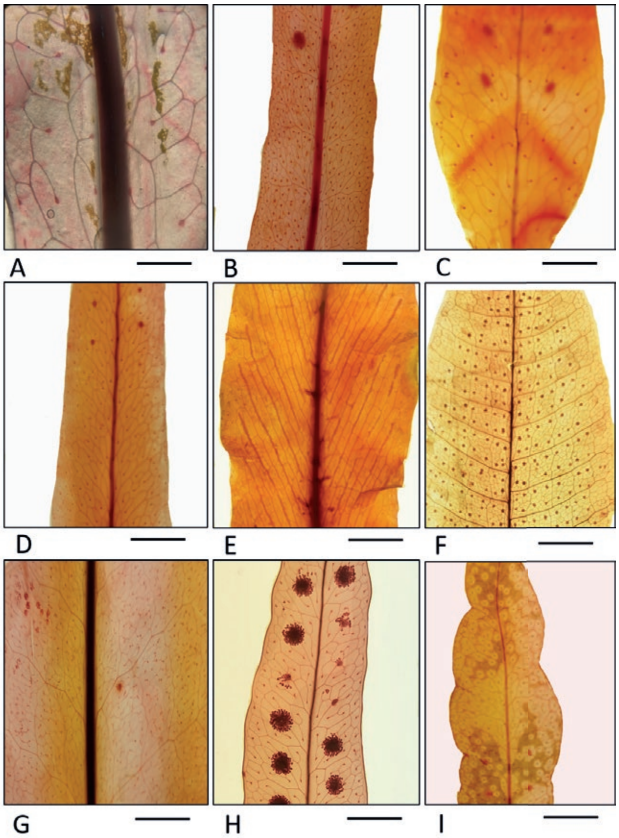

Figure 2. Photomicrographs of leaf venation in Polypodiaceae: **A**-*Lepisorus normalis;* **B**-*Lepisorus nudus*; **C**-*Lepisorus rostratus*; **D**-*Lepisorus sublinearis*; **E**-*Loxogramme involuta*; **F**-*Microsorum membranaceum*; **G**-*Microsorum punctatum*; **H**- *Phymatosorus cuspidatus*; **I**-*Phymatosorus scolopendria* (Scale bar-5mm).

The venation patterns of the species were complex which mostly ended up to 4° vein with areoles. All the species exhibited pinnate 1° vein with moderate to stout primary vein. The higher and finer secondary (2°) and tertiary (3°) venations up to quaternary (4°) vein, and areoles were also observed in most of the species (Table 2, Figures 1-3).

The marginal venation of the leaves was observed and their variation were noted which aids in the taxonomic delimitation of the species. Prominent marginal secondary veins were observed in *Arthromeris wallichiana*, *Drynaria quercifolia*, and *Drynaria propinqua*. In the species of *Lepisorus, Pyrrosia heteractis*, *Microsorum membranaceum, Phymatosorus cuspidatus* and *Phymatosorus scolopendria* the marginal veins are looped and in some taxa like *Microsorum punctatum*, *Pyrrosia costata*, *Pyrrosia mannii*, and *Pyrrosia lanceolata,* incompletely looped margins have been observed (Figures 4-6).

The areoles were mostly formed by the tertiary and quaternary veins in all the studied taxa except in *Goniophlebium argutum* and *Polypodoides amoena* where secondary veins anastomoses to form large costal areole and free forked marginal veins (Figures 1 & 3). The size of the areoles and other quantitative details of the studied taxa have been tabulated (Table 3).

Epidermal cells and stomata

Our study is focused on the mature epidermis. The epidermal cells of most of the species were irregular, with the anticlinal walls sinuous, slightly lobed in *Pyrrosia heteractis* and *Pyrrosia costata* to straight in *Pyrrosia lanceolata* and *Pyrrosia mannii*. A significant variation was observed in epidermal cell length and width among species. Mean epidermal cell length on the abaxial surface was least (26.8±0.4µm) in *Arthromeris himalovata* to (85.3±0.7µm) in *Pyrrosia costata* while mean

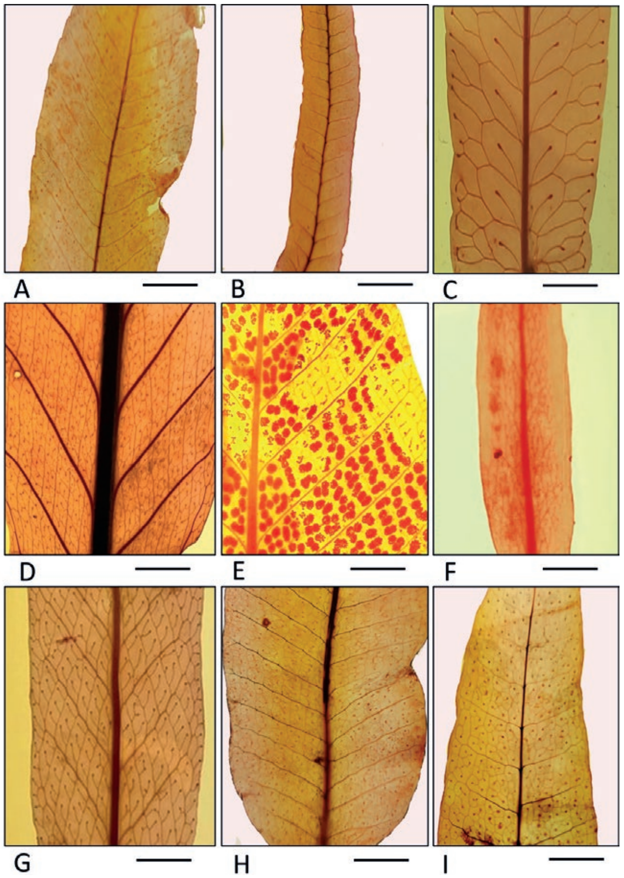

Figure 3. Photomicrographs of leaf venation in Polypodiaceae: **A**-*Pichisermollodes ebenipes;* **B**-*Pichisermollodes stewartii*; **C**-*Polypodoides amoena*; **D**-*Pyrrosia costata*; **E**-*Pyrrosia heteractis*; **F**-*Pyrrosia lanceolata*; **G**-*Pyrrosia mannii*; **H**-*Selliguea griffithiana*; **I**-*Selliguea oxyloba* (Scale bar-5mm).

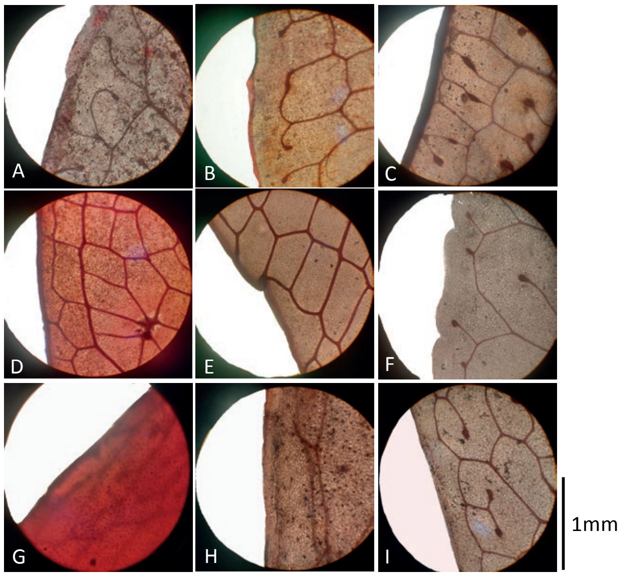

Figure 4. Photomicrographs of marginal leaf venation in Polypodiaceae: **A**-*Arthromeris himalovata*; **B**-*Arthromeris lehmanii*; **C**-*Arthromeris wallichiana*; **D**-*Drynaria quercifolia*; **E**-*Drynaria propinqua*; **F**-*Goniophlebium argutum*; **G**-*Lepisorus contortus*; **H**-*Lepisorus loriformis*; **I**-*Lepisorus mehrae.*

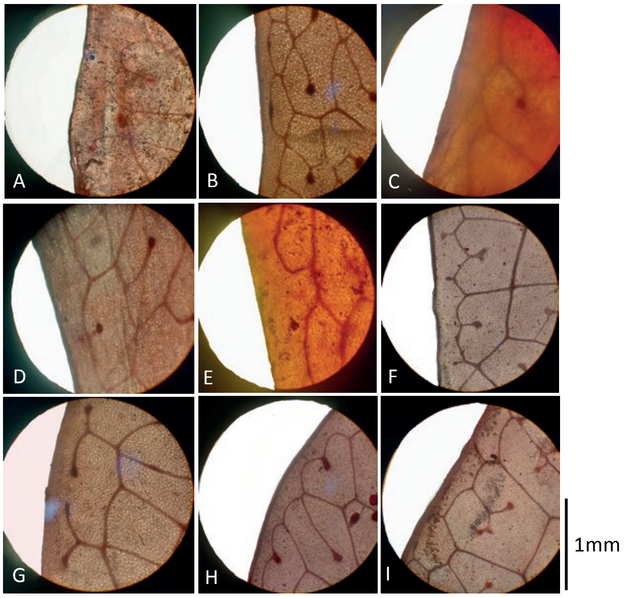

Figure 5. Photomicrographs of marginal leaf venation in Polypodiaceae: **A**-*Lepisorus normalis;* **B**-*Lepisorus nudus*; **C**-*Lepisorus rostratus*; **D**-*Lepisorus sublinearis*; **E**-*Loxogramme involuta*; **F**-*Microsorum membranaceum*; **G**-*Microsorum punctatum*; **H**-*Phymatosorus cuspidatus*; **I**-*Phymatosorus scolopendria.*

width ranged from16±0.5µm in *Arthromeris himalovata* to 55.2±0.4µm in *Lepisorus nudus*. On the abaxial side, the mean epidermal cell length was lowest (88.6±0.5µm) in *Pyrrosia costata* to highest (27.5±0.3µm) in *Arthromeris himalovata* (Table 4). Likewise, the number of lobes per cell varied from 3 to 16 among studied taxa. The minimum number of lobes per cell differed from 3 to 4 on the abaxial surface of *Lepisorus loriformis* whereas 8 to 16 on the adaxial surface of *Selliguea griffithiana*. Stomata in Polypodiaceous species under investigation are restricted to the abaxial surface of the leaf, hence they are hypostomatic. Stomatal cells have been observed all over the lamina except the vein on the abaxial surface. The stomata on a single leaf can be categorized into two or more types in all the species. The average length of stomata varied from 18.5±0.6µm in *Arthromeris himalovata* to 48.3±0.1µm in *Loxogramme involuta*, while mean stomatal width ranged from11.5±0.4µm in *Selliguea griffithiana* to 33.8±0.3µm in *Loxogramme involuta*.

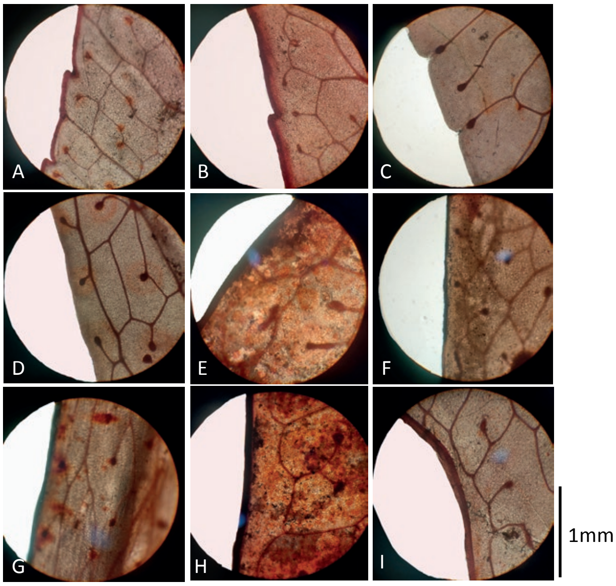

Figure 6. Photomicrographs of marginal leaf venation in Polypodiaceae: **A**-*Pichisermollodes ebenipes*; **B**-*Pichisermollodes stewartii*; **C**-*Polypodoides amoena*; **D**-*Pyrrosia costata*; **E**-*Pyrrosia heteractis*; **F**-*Pyrrosia lanceolata*; **G**-*Pyrrosia mannii*; **H**- *Selliguea griffithiana*; **I**-*Selliguea oxyloba.*

The stomatal index value ranged from 9.04±0.1 to 46.15±0.3 with the lowest in *Loxogramme involuta* followed by *Pyrrosia lanceolata* and the highest in *Arthromeris lehmanni*. The size of the epidermal cells of both abaxial and adaxial surfaces has been tabulated for all the species. The position of the stomata varied, in some species, it was clearly visible along with the epidermal cells whereas deeply sunken in species like *Arthromeris wallichiana*, *Lepisorus contortus*, *Lepisorus loriformis*, *Microsorum punctatum*, *Pyrrosia lanceolata* and *Pyrrosia mannii*. However, it was possible in all cases to distinguish subsidiary cells from other epidermal cells. In the studied taxa, mostly 6 different types of stomata namely, polocytic, copolocytic, seppolocytic (*Loxogramme involuta*), anisocytic (*Lepisorus rostratus*), pericytic, and copericytic (*Pyrrosia heteractis*) were observed (Figure 7-9). The epidermal cell types were mostly sinuous and broadly lobed or angular. The qualitative and quantitative epidermal characters of all the examined species have been presented in Table 4.

Key to the studies species based on leaf architecture, venation pattern and epidermal features

1a. Leaves simple, margin entire.. 2

b. Leaves compound, margin entire or toothed...................... 16

2a. Secondary venation reticulodromous................................. 3

b. Secondary venation brochidodromous, weak brochidodromous or festooned brochidodromous 7

3a. Tertiary venation free forked 4

b. Tertiary venation opposite percurrent .... ***Loxogramme involuta***

4a. Primary vein size stout, secondary vein spacing uniform, quaternary venation random reticulate..... ***Pyrrosia costata***

b. Primary vein moderate, secondary vein spacing uniform or irregular, quaternary venation absent................................. 5

5a. Secondary vein spacing irregular or uniform, copericytic or pericytic stomata 6

b. Secondary vein spacing uniform, polocytic stomata .... ***Pyrrosia mannii***

6a. Secondary vein spacing uniform, looped marginal ultimate venation ....***Pyrrosia heteractis***

b. Secondary vein spacing irregular, incompletely looped marginal ultimate venation ....***Pyrrosia lanceolata***

7a. Primary vein size stout, tertiary venation opposite per current venation 8

b. Primary vein size moderate, tertiary venation random reticulate or dichotomising 9

8a. Variation in angle of divergence of secondary veins is regular, freely ending veinlets 1-branched, looped marginal ultimate venation................... ***Microsorum membranaceum***

b. Variation in angle of divergence of secondary veins of upper veins slightly acute than lower, freely ending veinlets 2 or more branched, incomplete looped marginal ultimate venation .... ***Microsorum punctatum***

9a. Secondary venation brochidodromous, weak brochidodromous or festooned brochidodromous or indistinct, presence of looped marginal ultimate venation 10

b. Secondary venation weak brochidodromous, presence of a marginal secondary vein as marginal ultimate venation ....***Selliguea griffithiana***

10a. Leaf blade class nanophyll, leaf shape elliptic, cyclocytic stomata .... ***Lepisorus rostratus***

b. Leaf blade class microphyll to mesophyll, leaf shape linear lanceolate to broadly lanceolate, polocytic or copolocytic stomata 11

11a. Secondary venation weak brochidodromous,tertiary venation random reticulate.. 12

b. Secondary venation indistinct or brochidodromous or festooned brochidodromous, tertiary veins random reticulate or dichotomizing... 13

Table 2. Qualitative venation details of the studied taxa.

Species	Primary Veins		Secondary Veins				Tertiary Veins Category	Quaternary Veins Category	F.E.V.S.	Marginal ultimate venation
	Category	Size	Category	Spacing	AD	VAD				
Art him	pinnate	moderate	weak brochidodromous	uniform	wide	Regular	random reticulate	dichotomizing	1-branched	marginal secondary vein
Art leh	pinnate	moderate	weak brochidodromous	uniform	wide	regular	random reticulate	dichotomizing	1 -branched	marginal secondary vein
Art wal	pinnate	moderate	weak brochidodromous	uniform	right angle	regular	random reticulate	dichotomizing	unbranched	marginal secondary vein
Dry pro	pinnate	stout	reticulodromous	uniform	wide	regular	random reticulate	opposite percurrent	unbranched	marginal secondary vein
Dry que	pinnate	moderate	festooned brochidodromous	uniform	wide	regular	random reticulate	alternate percurrent	unbranched	marginal secondary vein
Gon arg	pinnate	moderate	semicraspedodromous	uniform	wide	regular	absent	absent	unbranched	free forked
Lep con	pinnate	moderate	indistinct	random	acute	irregular	random reticulate	dichotomizing	unbranched	looped
Lep lor	pinnate	moderate	weak brochidodromous	irregular	acute	upper vein slightly acute than lower	random reticulate	dichotomizing	unbranched	looped
Lep meh	pinnate	moderate	festooned brochidodromous	uniform	wide	regular	random reticulate	dichotomizing	1-branched	looped
Lep nor	pinnate	moderate	weak brochidodromous	irregular	acute	irregular	random reticulate	free	unbranched	looped
Lep nud	pinnate	moderate	festooned brochidodromous	uniform	wide	regular	dichotomising	dichotomizing	unbranched	looped
Lep ros	pinnate	moderate	weak brochidodromous	uniform	wide	upper vein slightly acute than lower	random reticulate	dichotomizing	unbranched	looped
Lep sub	pinnate	moderate	brochidodromous	uniform	acute	upper vein slightly acute than lower	random reticulate	free	unbranched	looped
Lox inv	pinnate	moderate	reticulodromous	uniform	acute	regular	opposite percurrent	dichotomizing	absent	looped
Mic mem	pinnate	stout	weak brochidodromous	uniform	wide	regular	opposite percurrent	dichotomizing	1-branched	looped
Mic pun	pinnate	stout	weak brochidodromous	uniform	wide	upper veins slightly acute than lower	opposite percurrent	dichotomizing	2 or more branched	incomplete loops
Phy cus	pinnate	moderate	brochidodromous	uniform	wide	upper vein slightly acute than lower	dichotomising	absent	1-branched	looped
Phy sco	pinnate	stout	weak brochidodromous	irregular	wide	upper veins more acute than lower	random reticulate	dichotomizing	1-branched	looped
Pic ebe	pinnate	moderate	weak brochidodromous	uniform	wide	upper veins slightly acute than lower	random reticulate	absent	1-branched	marginal secondary vein

(Continued)

Species	Primary Veins		Secondary Veins				Tertiary Veins	Quaternary Veins	F.E.V.S.	Marginal ultimate venation
	Category	Size	Category	Spacing	AD	VAD	Category	Category		
Pic ste	pinnate	moderate	weak brochidodromous	uniform	wide	regular	random reticulate	absent	1-branched	marginal secondary vein
Pol amo	pinnate	moderate	semicraspedodromous	uniform	wide	regular	absent	absent	1-branched	free forked
Pyr cos	pinnate	stout	reticulodromous	uniform	wide	upper vein slightly acute than lowe	free forked	random reticulate	1-branched	incomplete loops
Pyr het	pinnate	moderate	reticulodromous	uniform	wide	upper vein slightly acute than lower	free forked	absent	unbranched	looped
Pyr lan	pinnate	moderate	reticulodromous	irregular	wide	upper vein slightly acute than lower	free forked	absent	unbranched	incomplete loops
Pyr man	pinnate	moderate	reticulodromous	uniform	wide	upper vein slightly acute than lower	free forked	absent	unbranched	incomplete loops
Sel gri	pinnate	moderate	weak brochidodromous	uniform	wide	regular	random reticulate	dichotomizing	1-branched	marginal secondary vein
Sel oxy	pinnate	moderate	weak brochidodromous	uniform	wide	regular	random reticulate	dichotomizing	1-branched	marginal secondary vein

Key: AD-Angle of Divergence; VAD-Variation in Angle of Divergence; F.E.V.S-Freely Ending Veinlet(s).

Table 3. Quantitative characters of areoles and veins of the studied taxa.

Species	Mean areole size (mm^2)	Veinlet entering areole/mm^2	Vein termination/ mm^2
Art him	3.04±0.2	2-3	1-2
Art leh	3.69±0.7	3-5	1-2
Art wal	5.98±0.4	3-5	1-2
Dry pro	3.22±0.2	0-1	1
Dry que	1.20±0.1	0-2	1-3
Gon arg	12.23±0.3	1	1
Lep con	2.9±0.09	1-2	1
Lep lor	16.01±0.5	1-3	1
Lep meh	8.75±0.4	3-4	1-2
Lep nor	5.24±1.0	1-2	1
Lep nud	2.34±0.1	1-3	1-2
Lep ros	4.75±0.2	3-4	1
Lep sub	5.63±0.2	1-2	1
Lox inv	13.3±1.4	1-3	1-2
Mic mem	12.6±1.0	3-4	1-2
Mic pun	2.52±0.2	1-2	1-2
Phy cus	3.5±0.6	1-3	1
Phy sco	0.5±0.04	1-4	1
Pic ebe	3.9±0.2	1-2	1
Pic ste	2.42±0.1	1-2	1-2
Pol amo	9.06±2.2	1	1
Pyr cos	4.16±0.3	1-2	1-2
Pyr het	3.95±0.4	3-5	1-2
Pyr lan	2.38±0.18	2-3	1-2
Pyr man	10.61±0.9	3-4	1
Sel gri	2.33±0.1	2-6	1
Sel oxy	6.31±0.4	2-4	1-2

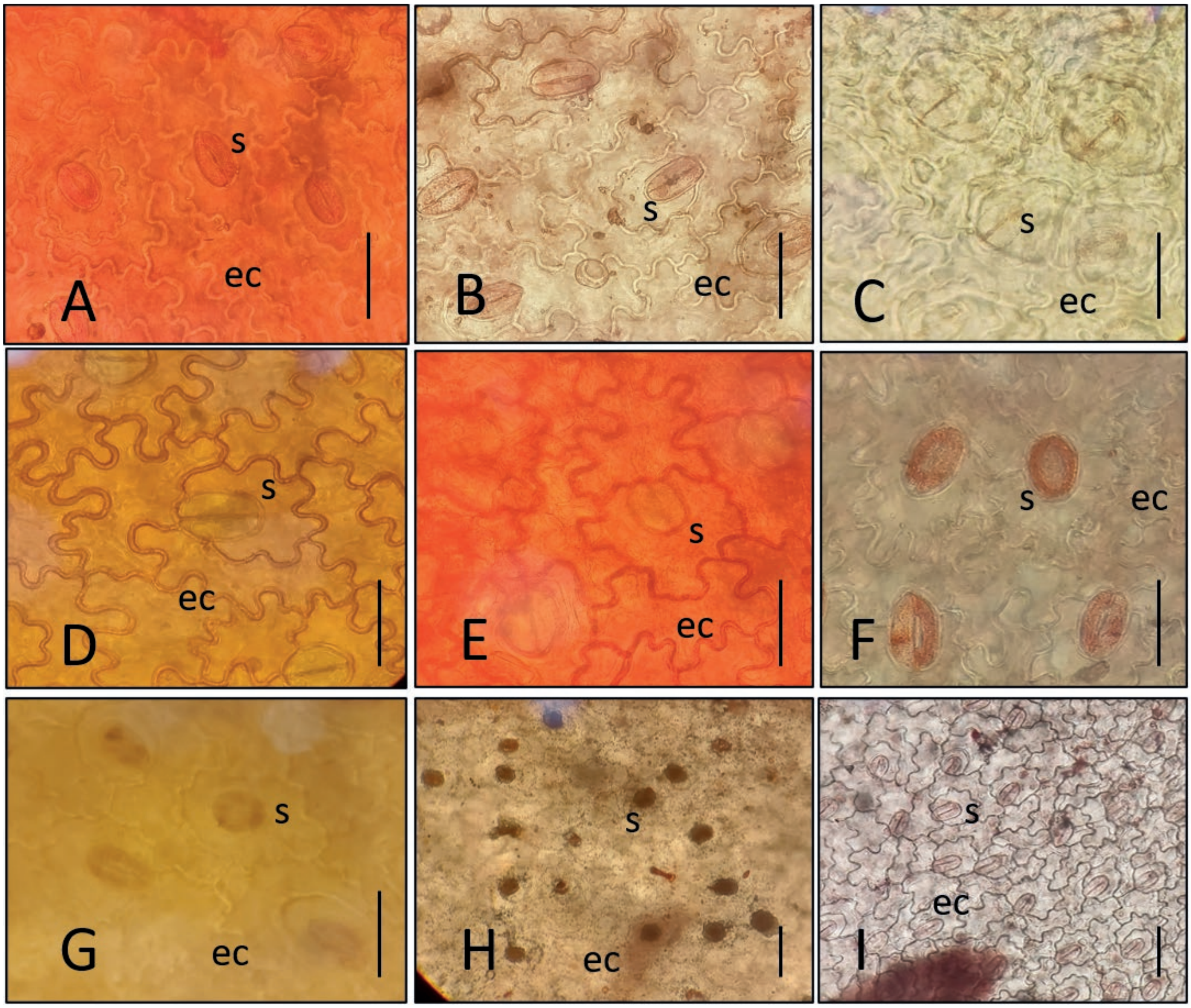

Figure 7. Leaf epidermis with stomata in Polypodiaceae: **A**-*Arthromeris himalovata*; **B**-*Arthromeris lehmanii*; **C**-*Arthromeris wallichiana*; **D**-*Drynaria quercifolia*; **E**-*Drynaria propinqua*; **F**-*Goniophlebium argutum*; **G**-*Lepisorus contortus*; **H**-*Lepisorus loriformis*; **I**-*Lepisorus mehrae* (Key: s-stomata; ec-epidermal cell; Scale bar-25μm).

12a. Secondary vein angle of divergence irregular, quaternary veins free .... ***Lepisorus normalis***

b. Secondary vein angle of divergence of upper veins slightly acute than lower, quaternary veins dichotomizing .... ***Lepisorus loriformis***

13a. Secondary venation indistinct, secondary vein spacing random, tertiary venation random reticulate .... ***Lepisorus contortus***

b. Secondary venation brochidodromous or festooned brochidodromous, secondary vein spacing uniform 14

14a. Brochidodromous secondary venation, secondary venation angle of divergence of upper vein slightly acute than lower, tertiary venation random reticulate, quaternary veins free .... ***Lepisorus sublinearis***

b. Festooned brochidodromous secondary venation, secondary venation angle of divergence regular, tertiary venation random reticulate or dichotomizing, quaternary veins dichotomising 15

15a. Tertiary veins random reticulate, freely ending veinlets 1-branched .... ***Lepisorus mehrae***

b. Tertiary veins dichotomizing, freely ending veinlets unbranched .... ***Lepisorus nudus***

16a. Secondary venation semicraspedodromous, tertiary venation absent 17

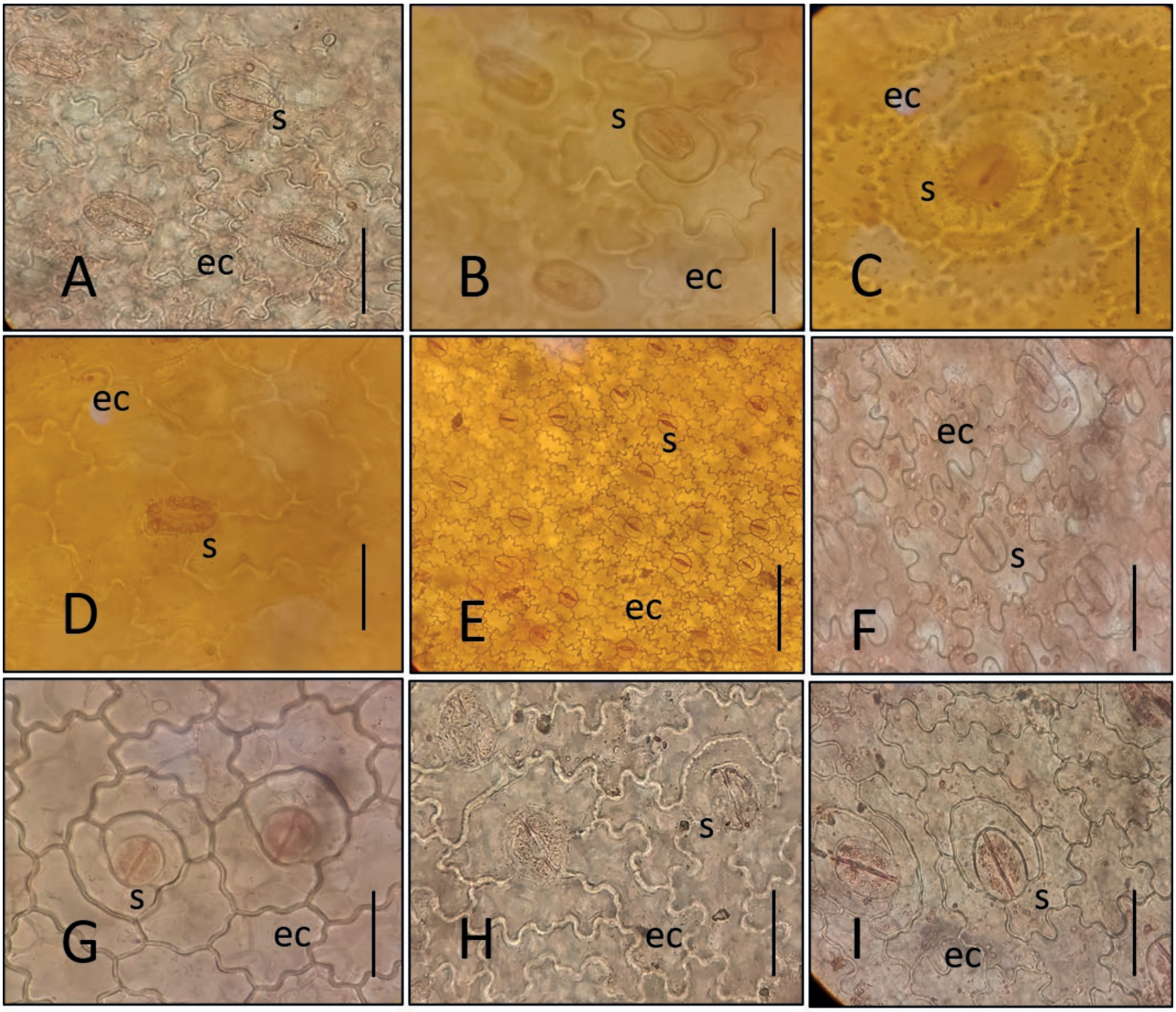

Figure 8. Leaf epidermis with stomata in Polypodiaceae: **A**-*Lepisorus normalis;* **B**-*Lepisorus nudus*; **C**-*Lepisorus rostratus*; **D**-*Lepisorus sublinearis*; **E**-*Loxogramme involuta*; **F**-*Microsorum membranaceum*; **G**-*Microsorum punctatum*; **H**-*Phymatosorus cuspidatus*; **I**-*Phymatosorus scolopendria* (Key: s-stomata; ec-epidermal cell; Scale bar-25μm)

b. Secondary venation reticulodromous, brochidromous, festooned brochidodromous or weak brochidodromous, tertiary venation present 18

17a. Lamina division 1-pinnate, leaf margin mucronate............ .... ***Goniophlebium argutum***

b. Lamina division deeply pinnatifid, leaf margin serrate ....***Polypodiodes amoena***

18a. Primary vein size stout, lamina division pinnatifid, secondary venation reticulodromous.......***Drynaria propinqua***

b. Primary vein size moderate, lamina division pinnafid, deeply pinnatifid, palmafid, 1-pinnate or imparripinate, secondary venation brochidodromous, weak or festooned brochidodromous 19

19a. Secondary venation brochidromous, lamina division imparipinnate...............................***Phymatosorus cuspidatus***

b. Secondary venation weak brochidodromous or festooned brochidodromous, lamina division 1-pinnate or pinnatifid...... 20

20a. Secondary venation festooned brochidodromous,tertiary venation random reticulate, quaternary vein alternate percurrent, lamina division pinnatifid .... ***Drynaria quercifolia***

b. Secondary venation weak brochidodromous, tertiary venation present or absent 21

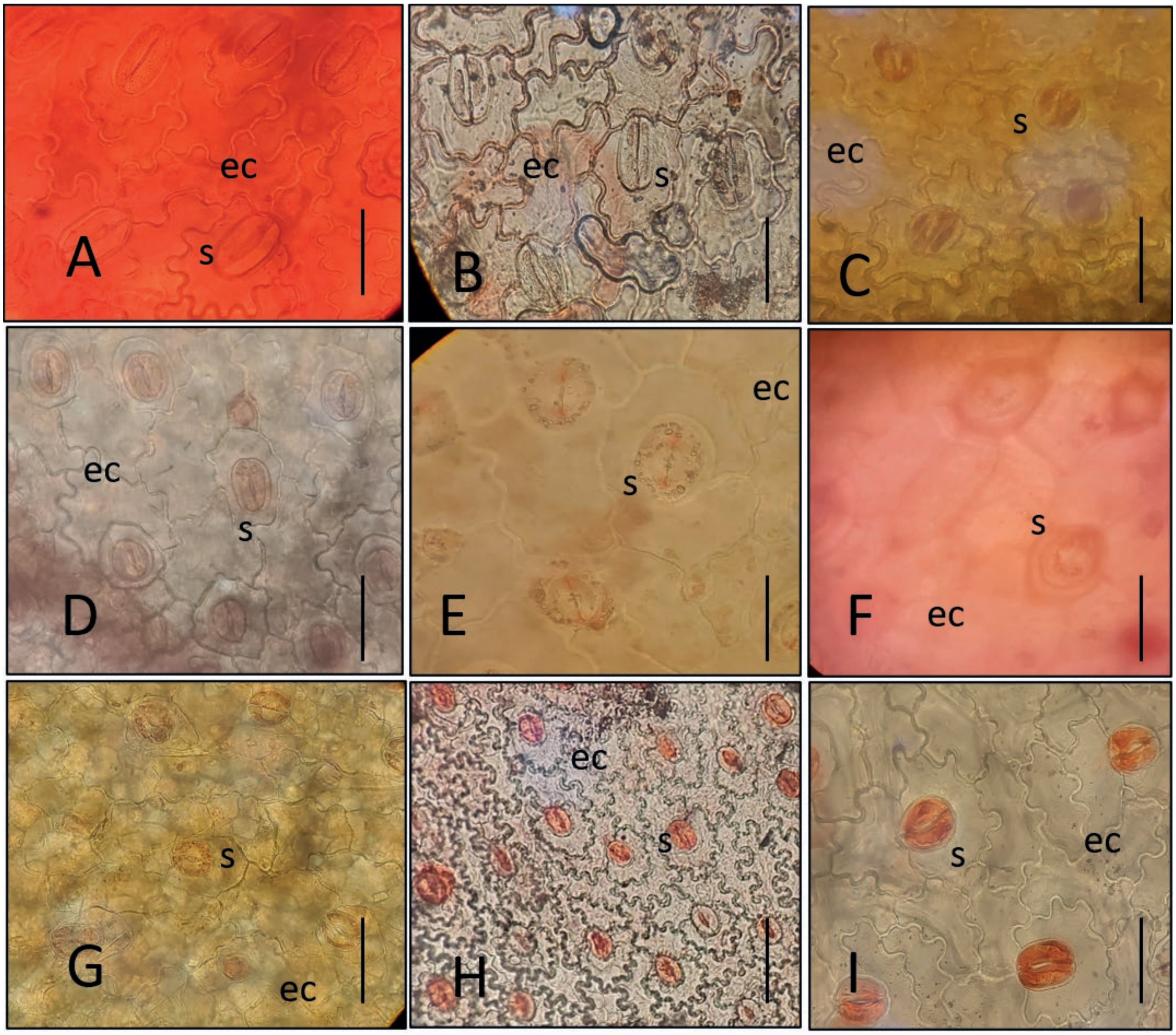

Figure 9. Leaf epidermis with stomata in Polypodiaceae: **A**-*Pichisermollodes ebenipes;* **B**-*Pichisermollodes stewartii*; **C**-*Polypodoides amoena*; **D**-*Pyrrosia costata*; **E**-*Pyrrosia heteractis*; **F**-*Pyrrosia lanceolata*; **G**-*Pyrrosia mannii*; **H**-*Selliguea griffithiana*; **I**-*Selliguea oxyloba* (Key: s-stomata; ec-epidermal cell; Scale bar-25µm).

21a. Tertiary venation present 23

b. Tertiary venation absent 22

22a. Lamina division pinnately parted, secondary venation angle of divergence regular......***Pichisermollodes stewartii***

b. Lamina division palmatifid, secondary venation angle of divergence upper veins slightly acute than lower .... ***Pichisermollodes ebenipes***

23a. Secondary vein spacing irregular, tertiary venation random reticulate, quaternary venation absent .... ***Phymatosorus scolopendria***

b. Secondary vein spacing uniform,tertiary venation random reticulate, quaternary venation present 24

24a. Lamina division deeply pinnatifid, leaf margin entire, mean areole size greater than ±6mm^2 .... ***Selliguea oxyloba***

b. Lamina division 1-pinnate, leaf margin entire or minutely toothed, mean areole size lesser than ±2-5 mm^2.............25

25a. Leaf base rounded, Angle of divergence in secondary veins are wider angle (≥90°) 26

b. Leaf base obliquely cordate, Angle of divergence in secondary veins are at right angle (90°)..***Arthromeris wallichiana***

Table 4. Detailed epidermal and stomatal cell characteristics of the studied taxa.

Species	Surface	EC wall type	ECL (μm)	ECW (μm)	Lobes per cell	ST type(s)	STL (μm)	STW (μm)	STS (μm^2)	SI
Art him	AB	sinuous	26.8±0.4	16±0.5	5-8	copolocytic, polocytic	18.5±0.6	14.2±0.2	267±0.8	30.75±0.1
	AD	sinuous	27.5±0.3	16.2±0.4	6-10					
Art leh	AB	sinuous	35.6±0.9	20±1.1	6-9	polocytic, copolocytic	20±0.4	18.5±0.3	370±0.6	46.15±0.3
	AD	sinuous	37±0.5	22.3±0.7	7-10					
Art wal	AB	sinuous	29±0.8	17.5±0.9	4-10	polocytic	19±0.3	15.5±0.0	294.5±0.1	29.51±0.2
	AD	sinuous	30±0.3	18.6±0.6	4-11					
Dry pro	AB	sinuous	52.5±0.4	32.5±0.5	6-10	polocytic	32±2.1	17±0.9	544±0.8	26.66±0.1
	AD	sinuous	55±0.4	33.5±0.4	5-10					
Dry que	AB	sinuous	75±0.9	40±0.3	6-10	polocytic, copolocytic	25±1.3	22.5±1.2	562.5±0.9	25.78±0.3
	AD	sinuous	77.3±0.6	42±0.7	6-10					
Gon arg	AB	sinuous	57.5±1.1	40±0.9	8-12	polocytic, copolocytic	33±0.6	29±0.3	957±1.0	22.22±0.1
	AD	sinuous	57±0.2	43±0.1	10-12					
Lep con	AB	sinuous	110.9±0.8	45±0.6	6-7	copolocytic, polocytic	37±0.5	23.5±0.4	869.5±0.3	20.5±0.3
	AD	sinuous	115±0.6	38.33±0.2	6-9					
Lep lor	AB	slightly lobed	75.26±2.9	24±2.4	3-4	copolocytic, polocytic	28±0.3	21±0.2	588±0.5	15.9±0.4
	AD	sinuous	78±1.8	26.8±0.3	5-7					
Lep meh	AB	sinuous	55±1.7	34±1.1	4-5	polocytic, copolocytic	23.5±0.5	19±0.5	446.5±0.5	40±0.1
	AD	sinuous	53±1.9	35±1.3	4-7					
Lep nor	AB	sinuous	65.4±0.3	38.11±0.0	5-6	copolocytic, polocytic	32.7±0.1	25±0.4	817.5±0.3	30.5±0.2
	AD	sinuous	66±0.4	40±0.5	5-6					
Lep nud	AB	sinuous	60±3.2	55.2±0.4	4-8	copolocytic, polocytic	44±0.2	27±0.3	1188±0.2	20.5±0.1
	AD	sinuous	61±2.1	53.6±0.8	4-9					
Lep ros	AB	sinuous	48.83±0.4	33±4.3	8-10	cyclocytic	27.3±2.8	19±2.1	518.7±0.9	24±0.1
	AD	straight	48±0.5	31±1.8	-					
Lep sub	AB	sinuous	51±0.5	35±2.3	4-6	polocytic	25.4±0.7	12.3±1.7	304±0.4	18.18±0.2
	AD	slightly lobed	53±0.7	35.5±0.1	5-7					
Lox inv	AB	sinuous	45±0.3	33.3±0.2	6-10	seppolocytic	48.3±0.1	33.8±0.3	1584±0.6	9.04±0.1
	AD	sinuous	44±0.2	32±0.6	6-12					
Mic mem	AB	sinuous	75±1.1	35±0.3	4-6	polocytic	31±0.2	19±0.3	589±0.2	14.76±0.3
	AD	sinuous	77±0.5	34.4±0.2	4-6					
Mic pun	AB	sinuous	63.2±0.4	33.2±0.6	4-8	polocytic	33±1.9	20±0.7	660±1.1	16.66±0.1
	AD	sinuous	65.4±0.4	31.7±0.4	5-10					
Phy cus	AB	sinuous	53±0.3	34±0.2	5-8	copolocytic, polocytic	32±0.7	23.7±0.5	752±0.3	19.14±0.2
	AD	sinuous	50.2±0.6	35±0.7	5-10					
Phy sco	AB	sinuous	61±1.7	30.64±0.3	8-12	copolocytic, polocytic	30.33±0.2	21.02±1	637.53±0.3	24.22±0.1
	AD	sinuous	58.9±1.9	30.1±0.5	8-14					
Pic ebe	AB	sinuous	84.5±1.1	43±3.6	8-10	copolocytic, polocytic	28±0.5	21.5±0.8	602±0.4	33.33±0.1
	AD	sinuous	82.3±0.4	40±4.3	8-12					
Pic ste	AB	sinuous	65.9±2.3	22±1.7	5-8	polocytic	31±0.8	21±0.4	651±1.1	21.67±0.2
	AD	sinuous	66.2±2.1	21.5±0.7	6-10					
Pol amo	AB	sinuous	55.7±0.2	43.5±0.1	5-8	polocytic	35±0.4	23.5±1.3	822.5±0.7	25.49±0.1
	AD	sinuous	56±0.2	43±0.5	5-9					
Pyr cos	AB	slightly lobed	85.3±0.7	25.6±0.2	-	pericytic	29±0.2	17±0.5	493±0.1	25.8±0.1
	AD	slightly lobed	88.6±0.5	24±0.3	-					
Pyr het	AB	slightly lobed	66.2±0.7	28±0.5	-	copericytic, pericytic	23.5±0.1	19±0.8	446.5±0.2	20.45±0.3
	AD	slightly lobed	65.4±1.2	26.8±0.5	-					
Pyr lan	AB	straight	77.5±0.5	26.2±0.9	-	pericytic	28.5±0.6	19±1.7	541.5±0.4	13.51±0.1
	AD	straight	79.2±0.4	28±1.1	-					
Pyr man	AB	straight	29±0.8	21.5±1.7	-	polocytic	19.5±0.4	13.5±1,1	263.25±0.4	18.6±0.2

(Continued)

Species	Surface	EC wall type	ECL (μm)	ECW (μm)	Lobes per cell	ST type(s)	STL (μm)	STW (μm)	STS (μm²)	SI
	AD	straight	30.12±0.7	21±0.7	-					
Sel gri	AB	sinuous	71±2.3	48±0.6	8-14	polocytic	29±3.6	11.5±0.4	333.5±0.3	16.67±0.1
	AD	sinuous	73±2.1	52.7±0.9	8-16					
Sel oxy	AB	sinuous	75±0.6	30±0.2	7-12	polocytic	20±2.9	16±0.3	320±0.2	16±0.4
	AD	sinuous	78±0.8	33.3±3.6	8-12					

Key: AB-Abaxial; AD-Adaxial; ECL-epidermal cell length; ECW-epidermal cell width; STL-Stomatal length; STW-Stomatal width; STS-Stomatal size; SI-Stomatal index. All measurements expressed as mean ± standard error.

26a. Leaf shape ovate-lanceolate, leaf apex shape caudate, leaf margin entire, 2-3 veinlet entering areole per .... ***Arthromeris himalovata***

b. Leaf shape lanceolate, leaf apex shape acuminate, leaf margin minutely toothed, 3-5 veinlet entering areole per mm² .... ***Arthromeris lehmannii***

A UPGMA dendrogram based on similarity was obtained from the quantitative and qualitative data analysed during the study (Figure 10). The inter relationship between the taxon can be understood. Higher degree of similarity (≥0.95) was observed between *Drynaria propinqua* and *Drynaria quercifolia*, *Lepisorus contortus* and *Lepisorus sublinearis* and *Microsorum membranaceum, Microsorum punctatum* and *Pyrrosia costata.* Over 90% similarity has been observed between *Goniophlebium argutum* and *Polypodoides amoena* while *Lepisorus rostratus* and *Lepisorus loriformis* show ≤ 0.85 similarity with other *Lepisorus* species. *Loxogramme involuta* shares ≤ 0.70 of similarity with the rest of the Polypodiaceous species.

DISCUSSION

Fern leaf or frond shares a common character which is the presence of a stalk and a lamina. However, the leaves exhibit a wide diversity, especially in size and shape (Vasco et al. 2013). Christenhusz and Chase (2014) suggested that families under eupolypods I clade have enormous morphological diversity thus leading to the difficulty to visualise the group as a single clade. From our study, it can be observed that such diverse characteristics exists even within the species of Polypodiaceae which is a part of the eupolypods I. Polypodiaceous ferns exhibited variable morphological characters such as lamina division, shape, leaf blade class, and margin. The morphological traits prove to be more effective in taxonomic delineation if supported by other stable characters such as leaf venation (Magrini and Scoppola 2010; Sundue and Rothfels 2014; Tan and Buot 2020).

On examination of the leaf venation traits, it has been observed that the 27 representative species of family Polypodiaceae possess pinnate type of primary venation and the variations mostly occur in the higher degree vein order. The higher venation character offers great taxonomic value (Sack and Scoffoni 2013; Tan and Buot 2020).

The overall species in our study exhibited weak brochidodromous (11 species), festooned brochidodromous (three species), brochidodromous (two species), reticulodromous (seven species), and two species with semi craspedodromous secondary venation pattern. Species showed nearly uniform in terms of variation in angle of divergence. Those species having weak brochidodromous and reticulodromous secondary vein usually had upper veins more acute than lower veins in terms of variation in angle of divergence.

Similar results were observed from earlier works (Conda and Buot 2018; Tan and Buot 2019; Tan and Buot 2020), in which ferns differed in 2° vein, 3° vein, 4° vein, angle of divergences of the secondary veins, and areolation. In works of Tan and Buot (2019), semi-craspedodromous secondary veins were observed in *Goniophlebium subauriculatum*. In our study, *Goniophlebium argutum* and *Polypodoides amoena* exhibited semi-craspedodromous 2° venation. They also have similar polocytic stomatal type. The dendrogram obtained also reveals more than 90% similarity between the two species. Therefore, it supports the fact that the *Polypodoides amoena* and *Goniophlebium amoenum* are homotypic synonyms.

The epidermal cells generally vary in size and shape among the studied taxa. The shape of epidermal cells is mostly irregular. The anticlinal walls are slightly lobed and sinuous. The shape of anticlinal wall of epidermal cells is a result of environmental adaptation, mesophytic species generally have sinuous walls while xerophytes have straight walls (Gifford 1989). Majority of *Pyrrosia* species are extremely drought tolerant with xerophytic adaptations (Wei et al. 2017). In our study, we observed epidermal cells with straight walls in *Pyrrosia lanceolata*

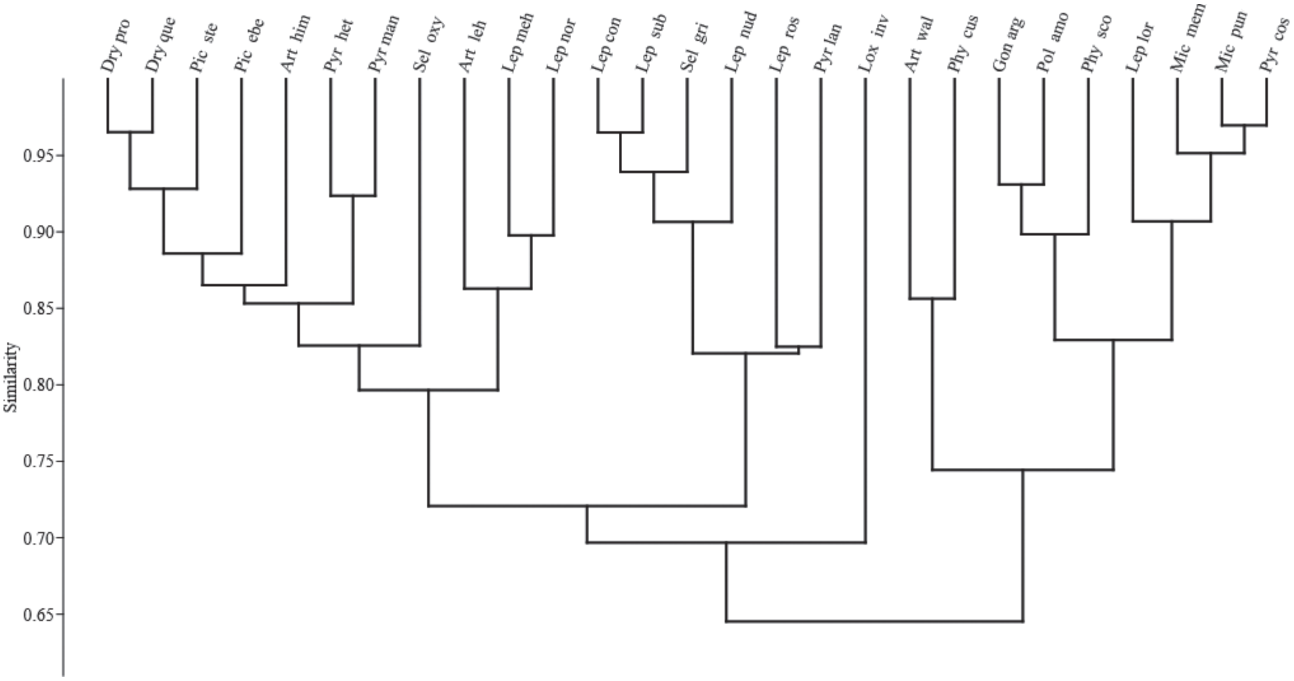

Figure 10. UPGMA dendrogram based on similarity among the studied taxa.

and *Pyrrosia mannii*. Fern leaves are mostly hypostomatic (Wang et al. 2009; Deng and Wang 2010; Shah et al. 2018). All the species possess stomata only on abaxial surface. From the variations observed, it is seen that most of the Polypodiaceae ferns have Polocytic, copolocytic, pericytic type of stomata. *Loxogramme involuta,* mostly exhibit seppolocytic type of stomata with lower stomatal index compared to the rest of the studied taxa. In the works of Van Cotthem (1970) and Pichi-Sermolli (1970), stomatal features have been used to differ Grammitidaceae and Loxogrammaceae from Polypodiaceae, however, it was not confirmed by Sen and Hennipman (1981). Molecular data consistently indicate that *Loxogramme* is sister to the rest of the Polypodiaceae (Schneider et al. 2004; Kreier and Schneider 2006; Wei et al. 2021). In our study, it is can be visualised from the dendrogram that *Loxogramme involuta* shares lesser than 0.70 similarity from rest of the Polypodiaceous taxa. Molecular studies consistently suggests that Loxogrammoid ferns are sister to the rest of the Polypodiaceae (Schneider et al.2004; Wei and Zhang 2022).

According to the new classification of *Lepisorus* proposed by Zhao et al. (2020), *Tricholepidium normale* (D.Don) Ching and *Lemmaphyllum rostratum* (Bedd.) Tagawa are now considered homotypic synonyms of *Lepisorus normalis* (D.Don) C.F.Zhao, R.Wei & X.C. Zhang and *Lepisorus rostratus* (Bedd.) C.F.Zhao, R.Wei & X.C.Zhang (Wei and Zhao 2019). Combined molecular studies showed that *Lepisorus* were recovered to be monophyletic when *Tricholepidium*, *Lemmaphyllum*, *Neolepisorus* and some other related genera were included (Zhao et al. 2020). The stomatal type of *Lepisorus normalis* varies from copolocytic to polocytic as in all other *Lepisorus* species in our study. However, cyclocytic stomatal type has been observed in *Lepisorus rostratus* which is quite different from the rest. In recent studies with some *Lepisorus* species by Mondal and Moktan (2022), it was observed that *Lepisorus rostratus* was out-grouped from the rest of the studied taxa based on significant morpho-anatomical features. Although the secondary and higher venation orders, the epidermal cell sizes, the stomatal index is closer to the other species of these genera. Though the mature stomata in the polypodioid ferns show different forms, all of them go through the polocytic conditions during their development. One such lines of development lead to the formation of cyclocytic and cocyclocytic stomata. The other line is characterised by the formation of stomata types like desmocodesmocytic, peri-, and copericytic forms (Sen and Hennipman 1981).

The reticulodromous venation with frees forked tertiary veins having pericytic and co pericytic stomatal type (*P. mannii* being exception in having polocytic stomata) makes *Pyrrosia* genus distinct from other members of Polypodiaceae. The origin and relationship of the genus has been debated among pteridologists. Christensen (1938) and Copeland (1947), associated *Pyrrosia* with Pleopeltoid group of ferns of the Polypodiaceae, whereas Copeland considered it closer to the microsorioid group. Based on phylogenetic analyses the infrageneric classification of *Pyrrosia s.l.* is controversial. Reticulate evolution was suggested among the species (Wei et al. 2017). The predominant pericytic stomata type in *Pyrrosia s.l.* is a recent adaptive feature in Polypodiaceae. Some species of *Pyrrosia* still have polocytic stomata, which are observed in other genera of Polypodiaceae. The occurrence of polocytic stomata in *Pyrrosia* may be due to the reversion or secondary development (Wei et al. 2017).

Ching (1978), established sub-family Lepisoroideae replacing Pleopeltidoideae and Pyrrosioideae. Raised several subfamilies like Gymnogrammitis (Gymnogrammitidaceae), *Drynaria* (Drynariaceae), and *Platycerium* (Platyceriaceae) to the family level. Later, phylogenetic studies based on multiple parameters provided better comprehension of the familial and generic circumscription of Polypodiaceae (Schneider et al. 2004; Schuettpelz and Pryer, 2007; Kreier et al. 2008; Testo et al. 2019; Zhao et al. 2020).

Major classifications by Smith et al. (2006), Christenhusz et al. (2011) and PPG I (2016), settled with a broader definition of Polypodiaceae by considering several closely associated groups like Loxogrammoids, Grammitids, Drynariaceae, and Platyceriaceae. Similar inter relationship between the taxon can be understood from the UPGMA obtained in this investigation. In our present study based on the leaf venation patterns reveals that higher degree of similarity (≥ 0.95) was observed between *Microsorum membranaceum* and *Microsorum punctatum* as species belonging to the same genera usually possess the same pattern of stomata, leaf venation, lamina division etc. A phylogenetic and morphological analyses conducted by Testo et al. 2019 proposed two new genera *Bosmania* and *Zealandia* under subfamily Microsoroideae of Polypodiaceae family. *Microsorum membranaceum* was shifted to the genera *Bosmania* therefore *Bosmania membranacea* (D.Don) Testo and *Microsorum membranaceum* (D.Don) Ching are homotypic synonyms (Testo et al. 2019).

Over 90% similarity has been observed between *Goniophlebium argutum* and *Polypodoides amoena* which is quite evident in their secondary venation pattern being semicraspedodromous type which is distinct from rest of the studied taxa. *Lepisorus rostratus* and *Lepisorus loriformis* shows lesser than 85% similarity with other *Lepisorus* species. *Loxogramme involuta* shares around 70 % similarity with rest of the Polypodiaceae species. The reticulodromous secondary venation pattern as well as stomatal type being seppolocytic is less observed in Polypodiaceae.

Therefore, it is evident from the study that leaf micro-morphological details and venation patterns can serve as an additional set of data in line with molecular and morphological characters which could help in decoding the existing problems up to generic and specific levels. The combination of diagnostic morphological characters like rhizome scales, leaf shapes, venation patterns, and features of the paraphyses proved crucial in untangling the clades and sub-clades of genus *Lepisorus, Pleopeltis*, and the grammatid ferns (Ranker et al. 2004; Otto et al. 2009; Wang et al. 2010; Zhao et al. 2020).

CONCLUSION

The present investigation reveals that leaf architectural traits and venation patterns especially higher degree veins are useful characters in delineating species. It can be concluded that features like leaf venation and stomata type is genetically stable and related to ontogeny and phylogeny. The results can serve as an additional and complementary data for the ferns under Polypodiaceae family. It is a reliable and economical tool in identification and classification of fern taxa. It is suggested that other fern species can be explored and classified precisely through leaf architectural approaches.

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Supplementary file. Voucher specimens used for the foliar micro-morphological study.

Species	Voucher number	Collector(s)	Locality	Habitat	Date
Arthromeris himalovata	SM-0358	S Mondal, S Moktan	Third mile	Epiphyte	15/10/2020
Arthromeris lehmannii	SM-0360	S Mondal, S Moktan	Sixth mile	Lithophyte or epiphyte	16/10/2020
Arthromeris wallichiana	SM-0363	S Mondal	Third mile	Lithophyte or epiphyte	16/10/2020
Drynaria propinqua	SM-0501	S Mondal	Kurseong	Epiphyte or lithophyte	22/09/2021
Drynaria quercifolia	SM-0550	S Mondal	Sukna	Lithophyte or epiphyte	27/09/2021
Goniophlebium argutum	SM-0582	S Mondal	Jorebunglow	Epiphyte or lithophyte	29/09/2021
Lepisorus contortus	SM-0344	S Mondal	Third mile	Epiphyte	15/10/2020
Lepisorus loriformis	SM-0493	S Mondal, S Moktan	Kaiyakatta	Epiphyte	21/09/2020
Lepisorus mehrae	SM-0365	S Mondal	Mungpoo	Lithophyte or epiphyte	24/09/2020
Lepisorus normalis	SM-0599	S Mondal	Lebong	Epiphyte or lithophyte	29/09/2021
Lepisorus nudus	SM-0239	S Mondal	Lebong	Epiphyte or lithophyte	29/09/2021
Lepisorus rostratus	SM-0554	S Mondal, S Moktan	Rajahatta	Epiphyte or lithophyte	28/09/2021
Lepisorus sublinearis	SM-0324	S Mondal, S Moktan	Third mile	Epiphyte or lithophyte	14/10/2020
Loxogramme involuta	SM-0223	S Mondal, S Moktan	Mahanadi	Lithophyte or epiphyte	24/09/2020
Microsorum membranaceum	SM-0464	S Mondal, S Moktan	Lebong	Lithophyte	18/09/2021
Microsorum punctatum	SM-0512	S Mondal, S Moktan	Pankhabari	Epiphyte or lithophyte	23/09/2021
Phymatosorus cuspidatus	SM-0211	S Mondal	Rohini	Lithophyte	21/09/2020
Phymatosorus scolopendria	SM-0552	S Mondal	Sukna	Lithophyte or terrestrial	27/09/2021
Pichisermollodes ebenipes	SM-0315	S Mondal, S Moktan	Third mile	Epiphyte or lithophyte	14/10/2020
Pichisermollodes stewartii	SM-0614	S Mondal, S Moktan	Ghoom	Epiphyte or lithophyte	17/10/2021
Polypodiodes amoena	SM-0498	S Mondal	Kurseong	Epiphyte or lithophyte	22/09/2021
Pyrrosia costata	SM-0533	Mondal,S	Pankhabari	Lithophyte or epiphyte	23/09/2021
Pyrrosia heteractis	SM-0602	S Mondal	Bagora	Lithophyte or epiphyte	02/10/2021
Pyrrosia lanceolata	SM-0386	S Mondal	Rongtong	Lithophyte or epiphyte	18/09/2021
Pyrrosia mannii	SM-0546	S Mondal, S Moktan	Panighatta	Lithophyte or epiphyte	25/09/2021
Selliguea griffithiana	SM-0355	S Mondal	Third mile	Lithophyte or epiphyte	15/10/2020
Selliguea oxyloba	SM-0371	S Mondal	Senchal	Lithophyte or epiphyte	03/09/2021

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Evaluating the species distribution patterns of the genus *Saurauia* Willd. in the Philippines using geospatial analysis

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Abstract. Understanding the species distribution patterns of endemic and threatened species is very useful for conservation planning and actions. However, research on the distribution of many important and often overlooked species in the country, such as the *Saurauia*, is extremely limited. This paper provided an overview of the distribution patterns of *Saurauia* species across the Philippine archipelago based solely on the existing digital checklist of vascular plants. Specifically, the study produced distribution maps of the 56 endemic *Saurauia* species and determined their climatic distribution ranges. The methods involved geospatial and geoprocessing analysis in a geographic information system (GIS) environment. The findings showed that 29 *Saurauia* species are currently distributed in a single province, whereas seven (7) species have only been recorded in a certain region. Furthermore, 35 species was found to have a single type of climate. The *Saurauia* species identified in this paper that have only a single provincial or regional distribution record and are restricted to a single type of climate are recommended for conservation because they are mostly likely to be affected by environmental and climatic changes. Finally, the information and data derived in this paper could serve as a baseline for assessing conservation status, habitat modelling studies, species-level conservation planning, and understanding climate change impacts.

Keywords: Climate change, conservation, geographic information system, *Saurauia*, species distribution, systematics, taxonomy.

INTRODUCTION

Saurauia Willd. is one of the three (3) genera included in the family Actinidiaceae Gilg & Werderm. (WFO, 2022), the other two being the *Actinidia* Lindl. and *Clematoclethra* (Franch.) Maxim. Based on the Plants of the World Online (POWO) (2022), there are currently about 391 accepted

Saurauia species worldwide, with the majority of them found in Asia and the Americas' tropical and subtropical countries. In the Philippines, the Co's Digital Flora of the Philippines (CDFP) website, a digital checklist of Philippine vascular plants, currently lists 56 species under this genus (Pelser et al. 2011), and all of these are endemic to the Philippines (POWO, 2022) or found nowhere else on the planet.

Many species of *Saurauia* have long been used to treat a wide range of ailments worldwide by the indigenous communities (Pasaribu et al. 2020), which includes digestive problems (Silalahi et al. 2015), asthma, boils, bronchitis, depression of the central nervous system, fever, hepatitis B, piles, rheumatism, skin problems, seizure disorders, and ulcers (Uddin and Rahman 2006; Ahmed et al. 2013). In the Philippines, the fruit of *S. bontocensis* is edible and used as raw materials for the food processing center that was built for enhancing livelihood opportunities for the Ikalahan Indigenous Peoples (Rice 2002). Other products that can be derived from these species include raisins from dried fruits, juice, jelly, spread, vinegar, and wine (Subilla and Baoanan 2020; Tacloy et al. 2021). Despite their significant economic potential and therapeutic properties, the *Saurauia* species in the country, if not in the world, have been understudied (Fabelico 2020), not only in terms of their medicinal properties but also in terms of their distribution, taxonomy, and conservation status.

At present, however, no studies have been conducted or published yet concerning the species distribution of all the *Saurauia* in the Philippines, which is also true for other frequently overlooked endemic and threatened floral species in the country. Typically, the most convenient way to determine the distribution of *Saurauia* or other plant species in the country is to use online plant databases and checklists like the CDFP. Although this digital checklist includes the general distribution data of different floral species from other countries down to the provincial level and some specific mountains in the country where the species occurred, one limitation of this digital database is that it does not show any spatially explicit distribution maps of these species unlike the other websites such as the Global Biodiversity Information Facility (GBIF), wherein the species distribution or occurrence was projected in a worldwide map. This lack of information and studies regarding the distribution of many plant species in the country, like the *Saurauia,* limits their conservation and protection strategies. Moreover, prior to engaging in any type of biological research that has the potential to bring limitless advantages and/or benefits to human development, one must first comprehend the taxonomy and systematics as well as distribution of the organism with which one would be working (Narendran 2000).

With the advent of Geographic Information System (GIS) technology in the early 80s', numerous studies relative to the species distribution have already been carried out (Corsi et al. 2000) worldwide. The GIS technology is a powerful tool that can handle, manipulate, and analyze huge amount of spatial and temporal data (Burrough et al. 2015), which is very relevant in species distribution studies. In the Philippines, numerous studies have examined the distribution of other floral species using GIS-assisted methodologies and related technologies such as the *Musa balbisiana* (Rabara et al. 2020), sago palm (Santillan and Makinano-Santillan 2016), *Shorea guiso* (Blanco) Blume and *Parashorea malaanonan* (Blanco) Merr. (Tumaneng et al. 2019), among others.

Mapping the distribution of the different species using the GIS, based on reliable dataset, could provide better understanding of species diversity and richness, which is a vital component in the forest functioning (Paquette and Messier 2011). In addition, a species diversity map database at different levels ranging from individual trees to entire communities has the potential to understand functional diversity, which can aid in determining forest ecosystem productivity and stability (Schneider et al. 2017). This may offer basic information needed in conservation planning as well as forecasting the impacts of global environmental changes on species and ecosystem level (Franklin 2010). Species distribution maps could serve as a baseline information in evaluating the impacts of climate change on this taxon as plants can only thrive in a specified temperature and moisture condition. In other words, climate factors influence species distributions in general, and thus climate change can result in changes in species distributions (Lawler et al. 2013).

The general objective of this paper is to evaluate the distribution patterns of *Saurauia* species across the Philippine archipelago. This paper specifically aimed at generating species distribution maps using geospatial analysis for all the accepted species at the provincial and regional scale. Also, this paper will analyze the ranges of climatic types optimal for *Saurauia* species as an aid to species conservation.

MATERIALS AND METHODS

Data gathering

In this study, the list of *Saurauia* species and their corresponding geographical distribution data were extracted from the CDFP webpage, particularly the names of provinces where these are mostly found. The

CDFP is a digital checklist of the Philippines' native, naturalized, and invasive vascular plant species. The basic occurrence information, conservation status information, and references to scientific literature are all included in this checklist (Pelser et al. 2011). Here, only the accepted *Saurauia* species were included and the species synonyms were excluded in the analysis. Since there are very limited information on the confusing or unresolved taxonomy of *Saurauia* species in the country, this paper solely relied on the checklist provided in the CDFP website.

Species distribution mapping

A GIS software was used in this paper, specifically the Quantum GIS (QGIS) version 3.16 (Hannover long term released), in creating a distribution map of all the *Saurauia* species in the Philippines. The provincial boundary layer of the Philippines downloaded from IGISMAP website was used to reflect the occurrence information of the species. The extracted information from the CDFP (species list and their distribution) were processed and entered in the provincial boundary layer through the attribute table. The flowchart showing the data extraction and species distribution mapping using the GIS is presented in Figure 1. All the map layers were projected to Universal Transverse Mercator (UTM) with the coordinate system World Geodetic System (WGS) 84 Zone 51 North.

Species distribution of Saurauia species based on climatic types

This paper also determined the climatic types, based on the Modified Corona's Climatic Classification, where the *Saurauia* species are currently distributed. Knowing the type of climate in which the *Saurauia* species are found may provide relevant information in the species conservation efforts such as reforestation and plantation establishment. Proper species-level conservation planning takes into account the climatic distribution range of species because growing it outside of its natural climatic conditions may directly affect its growth and survival after outplanting. To determine the climatic classification of the *Saurauia* species, the climatic type layer and the species distribution layer were intersected in the QGIS environment using the intersection algorithm under the vector geoprocessing options (Figure 1). The species distribution map of the *Saurauia* was used as an input layer, whereas the climatic type map was used as the overlay layer.

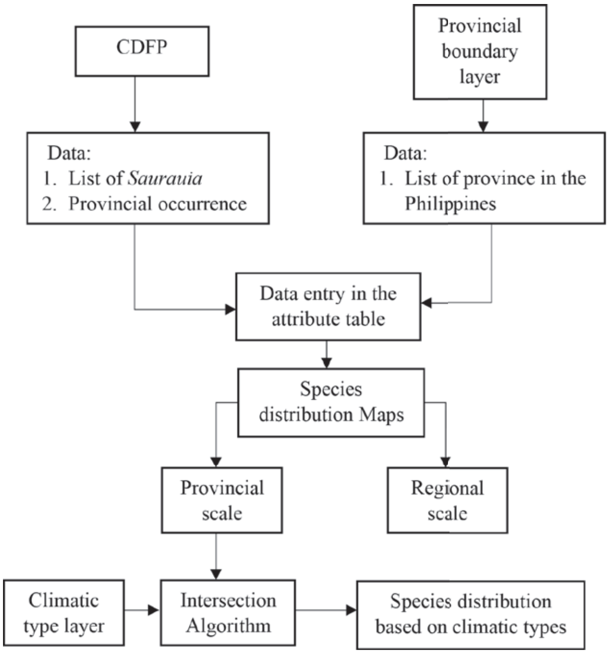


Figure 1. The flowchart of species distribution mapping of *Saurauia* species using Geographic Information System (GIS) platform.

RESULT AND DISCUSSION

Current distribution of Saurauia species at the provincial scale based on the CDFP

The current species distribution map of the *Saurauia* species based on existing data provided by CDFP website showed that *S. elegans* is the most widely distributed throughout the country, which can be found in 12 provinces mostly in Luzon (Figure 2a). This is followed by *S. clementis* with a total of nine (9) provincial occurrences mostly in Mindanao (Figure 2b). Both *S. latibractea* and *S. polysperma* had six (6) provincial distribution. In terms of the number of *Saurauia* species occurred per province (Table 1), the provinces of Agusan Del Norte, Quezon, and Zamboanga del Norte recorded the highest number of different *Saurauia* species, with a total of eight (8) different *Saurauia* species in each of these provinces. The species found in Quezon province include the *S. bakeri, S. bicolor, S. elmeri, S. luzoniensis, S. panduriformis, S. sparsiflora, S. whitfordii, and S. cinnamomea.* On the other hand, the *Saurauia* species found in Zamboanga del Norte include *S. clementis, S. confusa, S. denticulate, S. philippinensis, S. trunciflora, S. zamboangensis, S. longipedicellata*, and the new to science species, the *S. abbreviate*. Other provinces with more than five *Saurauia* species include Agusan del Norte (7 species), Lanao del Sur (6 species), Bukidnon, Benguet, and Cat-

Table 1. The provincial distribution of each *Saurauia* species in the Philippines based on the CDFP website.

No.	Saurauia Species	Provincial Distribution
1	*Saurauia abbreviata* K.R.Mazo	Zamboanga del Norte
2	*Saurauia alvarezii* Merr.	Lanao del Sur
3	*Saurauia ampla* Merr.	Samar, Agusan del Norte
4	*Saurauia avellana* Elmer	Negros Oriental, Lanao del Sur, Bukidnon
5	*Saurauia bakeri* Merr.	Quezon, Catanduanes
6	*Saurauia bicolor* Merr.	Quezon
7	*Saurauia bontocensis* Merr.	Mountain Province, Abra, Kalinga, Ifugao
8	*Saurauia cinnamomea* Merr.	Rizal, Quezon
9	*Saurauia clementis* Merr.	Lanao del Sur, Panay, Leyte, Camiguin, Zamboanga, Bukidnon, Davao, Agusan, Surigao del Norte
10	*Saurauia confusa* Merr.	Laguna, Camarines, Sorsogon, Catanduanes, Zamboanga
11	*Saurauia copelandii* Elmer	Davao, Camiguin, Bukidnon,
12	*Saurauia cordata* Quisumb.	Catanduanes
13	*Saurauia denticulata* C.B.Rob.	Zamboanga
14	*Saurauia elegans* (Choisy) Fern.	Benguet, Ilocos Norte, Mountain Province, Cagayan, Isabela, Nieva Viscaya, Nueva Ecija, Aurora, Bataan, Camarines, Albay, Oriental Mindoro,
15	*Saurauia elmeri* Merr.	Quezon,
16	*Saurauia erythrotricha* Elmer	Agusan Del Norte,
17	*Saurauia fasciculiflora* Merr.	Palawan
18	*Saurauia gigantifolia* Quisumb.	Agusan
19	*Saurauia glabrifolia* Merr.	Surigao Del Norte
20	*Saurauia gracilipes* Merr.	Lanao Del Sur, Agusan Del Norte, Surigao Del Norte,
21	*Saurauia involucrata* Merr.	Davao
22	*Saurauia klemmei* Merr.	Isabela, Cagayan, Apayao
23	*Saurauia knemifolia* Quisumb.	Palawan
24	*Saurauia lanaensis* Merr.	Lanao Del Sur, Misamis Occidental,
25	*Saurauia latibractea* Choisy in Zoll.	Albay, Catanduanes, Sorsogon, Mindoro, Cebu, Cagayan
26	*Saurauia leytensis* Merr.	Leyte
27	*Saurauia loheri* Merr.	Rizal
28	*Saurauia longipedicellata* Merr.	Zamboanga Del Sur, Zamboanga Del Norte,
29	*Saurauia longistyla* Merr.	Palawan
30	*Saurauia luzoniensis* Merr.	Rizal, Zambales, Laguna, Quezon,
31	*Saurauia macgregorii* Merr.	Nueva Viscaya
32	*Saurauia merrillii* Elmer	Leyte, Samar
33	*Saurauia mindorensis* Merr.	Oriental Mindoro,
34	*Saurauia negrosensis* Elmer	Panay, Negros
35	*Saurauia oblancilimba* Quisumb.	Zambales
36	*Saurauia oligantha* Merr.	Sorsogon,
37	*Saurauia oligophlebia* Merr.	Catanduanes
38	*Saurauia palawanensis* Merr.	Palawan
39	*Saurauia panayensis* Merr.	Capiz
40	*Saurauia panduriformis* Elmer	Quezon, Kalinga, Benguet, Laguna, Batangas
41	*Saurauia papillulosa* Merr.	Ifugao, Mountain Province
42	*Saurauia philippinensis* Merr.	Oriental Mindoro, Basilan, Zamboanga, Misamis Occidental,
43	*Saurauia polysperma* (Blanco) Merr.	Bataan, Ilocos Norte, Benguet, La Union, Nueva Viscaya, Pampanga, Bataan
44	*Saurauia samarensis* Merr.	Samar
45	*Saurauia sampad* Elmer	Agusan Del Norte
46	*Saurauia sibuyanensis* Elmer	Sibuyan
47	*Saurauia sorsogonensis* Merr.	Sorsogon
48	*Saurauia sparsiflora* Elmer	Benguet, Ilocos Norte, Nueva Ecija, Bulacan, Quezon
49	*Saurauia tayabensis* Quisumb.	Aurora

No.	Saurauia Species	Provincial Distribution
50	*Saurauia trichophora* Quisumb.	Aurora, Quirino
51	*Saurauia trunciflora* Merr.	Zamboanga, Lanao Del Sur, Bukidnon,
52	*Saurauia urdanetensis* Elmer	Agusan Del Norte, Bukidnon
53	*Saurauia vanoverberghii* Merr.	Mountain Province, Benguet
54	*Saurauia wenzelii* Merr.	Leyte, Samar
55	*Saurauia whitfordii* Merr.	Quezon
56	*Saurauia zamboangensis* Merr.	Zamboanga

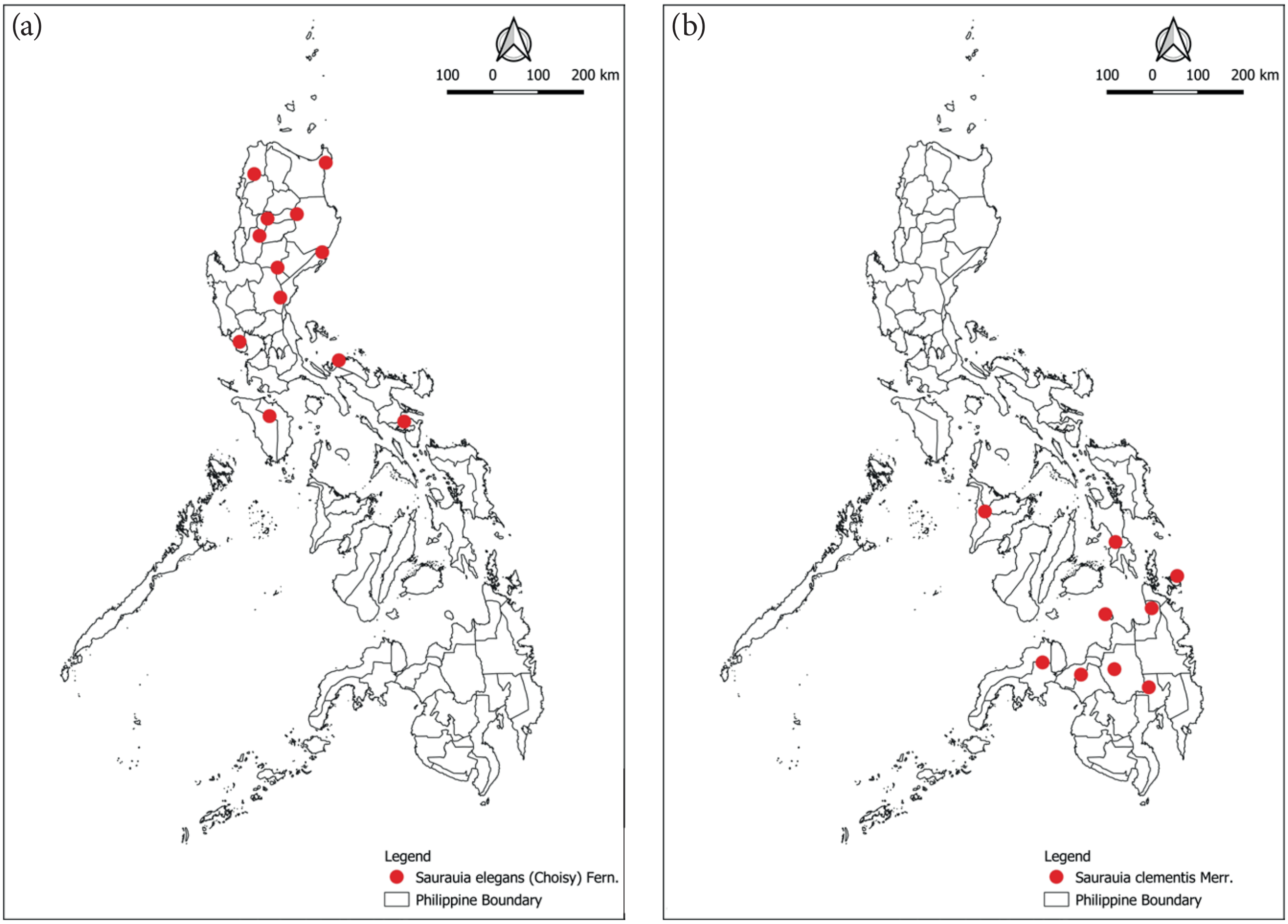


Figure 2. Species distribution maps of (a) *Saurauia elegans* and (b) *Saurauia clementis* based on the CDFP data.

anduanes, all of which have five (5) *Saurauia* species. In terms of species conservation prioritization on a provincial scale, the aforementioned provinces, as shown in the density map (Figure 3), should be given preference since they are home to numerous *Saurauia* species.

Saurauia species with single provincial distribution range

Out of the 56 *Saurauia* species, 29 of these have single provincial distribution at present or found only on a certain province as reported in the CDFP (Fig. 4). These are *S. abbreviate, S. alvarezii, S. bicolor, S. cordata, S. denticulate, S. elmeri, S. erythrotricha, S. fasciculiflora, S. gigantifolia, S. glabrifolia, S. involucrate, S. knemifolia, S. leytensis, S. loheri, S. longistyla, S. macgregorii, S. mindorensis, S. oblancilimba, S. oligantha, S. oligophlebia, S. palawanensis, S. panayensis, S. samarensis, S. sampad, S. sibuyanensis, S. sorsogonensis, S. tayabensis, S. whitfordii, and S. zamboangensis*. This may infer that these 29 species are more vulnerable to threats because they are

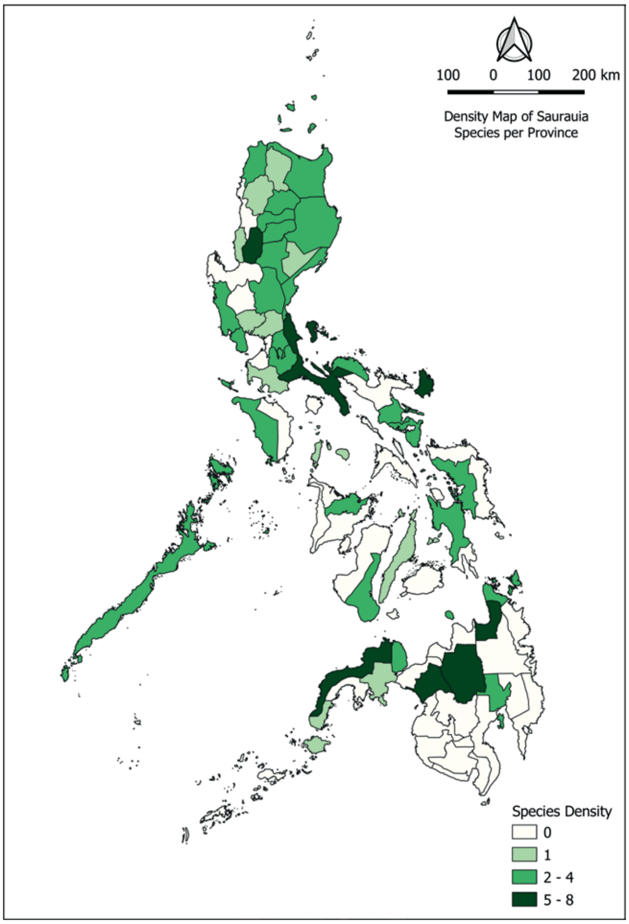


Figure 3. The generated density map of the occurrence of different *Saurauia* species in the Philippines based on the CDFP.

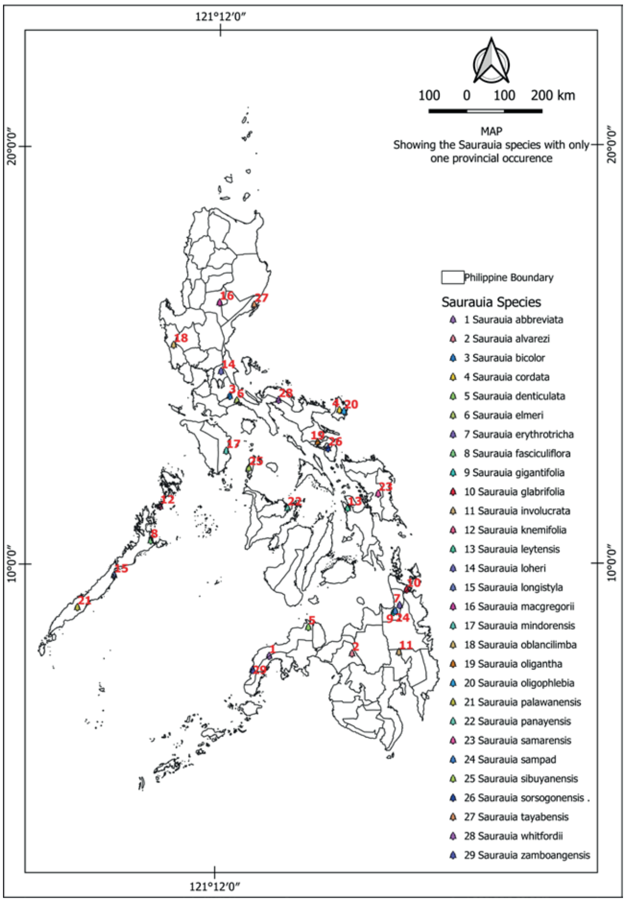


Figure 4. The *Saurauia* species with single provincial record based on the CDFP.

currently found nowhere else in the country (local endemism) other than their present occurrence. As pointed out by Howard et al. (2015), endemic taxa (at local or national level) are at greater risk than non-endemics and they are more vulnerable to extinction. This may be attributed to their narrow and sometimes single geographical range as presented in this paper. Hence, there is an urgent need to assess the conservation status of these 29 species, which may result in their inclusion on the national list of threatened floral species or even in the IUCN Red List. At present, only *S. longistyla*, one (1) of the 29 species, and *S. bontocensis* are categorized as Vulnerable under the DAO 2017-11. It should be noted, however, that *S. bontocensis* has a wider distribution compared to the aforementioned *Saurauia* species.

Saurauia species with regional geographic distribution

There are also seven (7) *Saurauia* species that have regional distribution or found only on a specific region. These are *S. bontocensis, S. papillulosa, S. vanoverberghii* (Figure 5a) found only in the Cordillera Administrative Region (CAR); the *S. cinnamomea* found only in the CALABARZON region (IV-A) (Figure 5b); *S. longipedicellata* distributed only in the Zamboanga Peninsula region (IX) (Figure 5c); and the *S. merrillii* and *S. wenzelii* found only in the Eastern Visayas Region (VIII) (Figure 5d). These *Saurauia* species should be considered for conservation as well because they are only found in specific range and well-defined habitats. According to Engler et al. (2011), plant taxa adapted to colder conditions such as in the mountainous regions, particularly the endemics, are at the highest risk of species extinction due to climate change. According to Rebollido-Jacob (2015), *S. bontocensis* are pioneer trees and often found in stream banks of mountains as well as in open areas. On the other hand, Pelser et al. (2011) stated in the CDFP webpage that this species can be found in thickets and forests. This was supported by the findings of Lumbres et al. (2014); Taguiling (2014); and Malabrigo

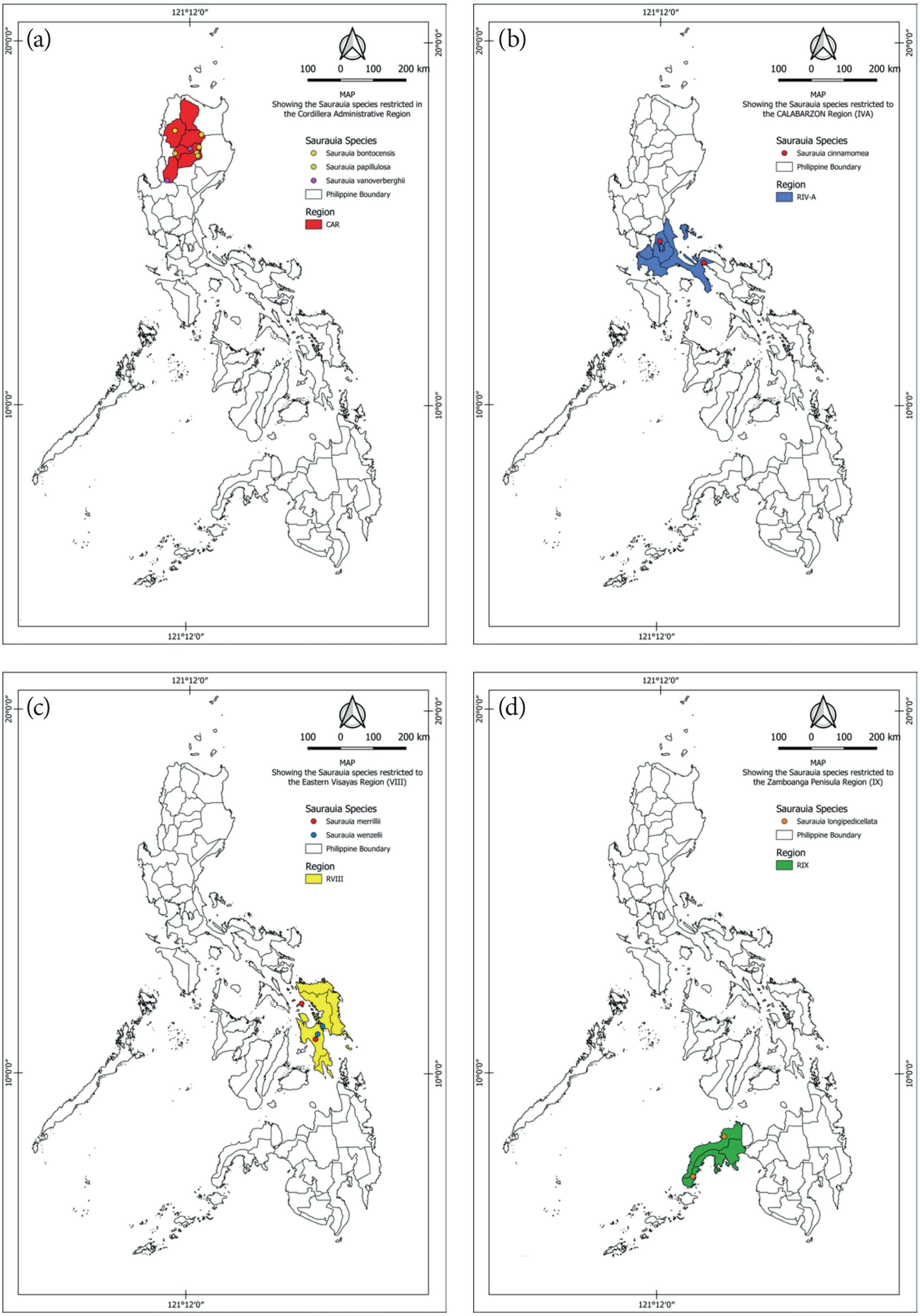


Figure 5. The Saurauia species destributed to a specific region based on the CDFP. (a) Cordillera Region, (b) CALABARZON (IV-A), (c) Eastern Visayas (VIII), (d) Zamboanga Peninsula (IX)

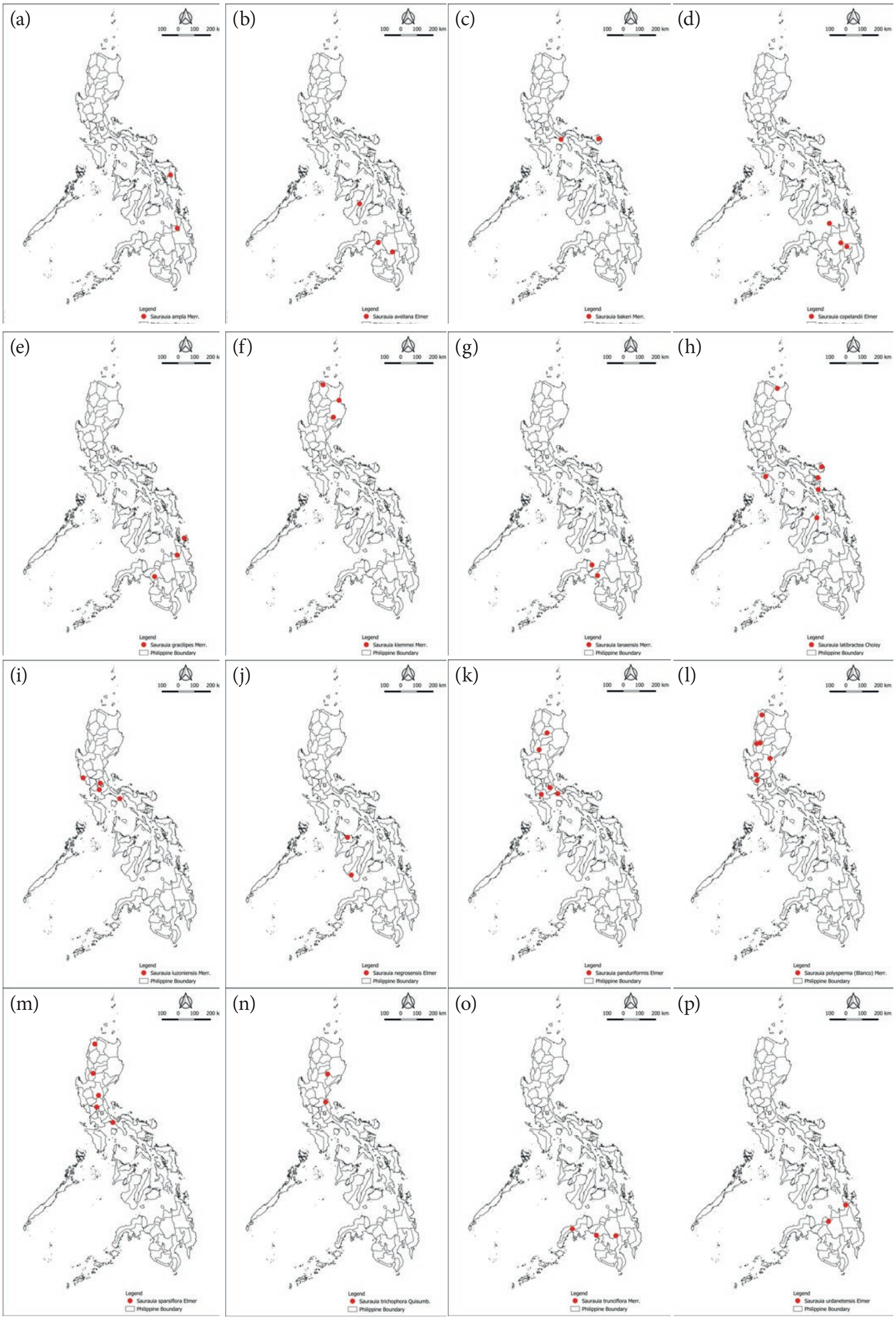


Figure 6. Species distribution map of selected species: (a) *S. ampla*, (b) *S. avellana*, (c) *S. bakeri*, (d) *S. copelandii*, (e) *S. gracilipes*, (f) *S. klemmei*, (g) *S. lanaensis*, (h) *S. latibractea*, (i) *S. luzoniensis*, (j) *S. negrosensis*, (k) *S. panduriformes*, (l) *S. polysperma*, (m) *S. sparsiflora*, (n) *S. trichophora*, (o) *S. trunciflora*, (p) *S. urdanetensis*, based on the CDFP.

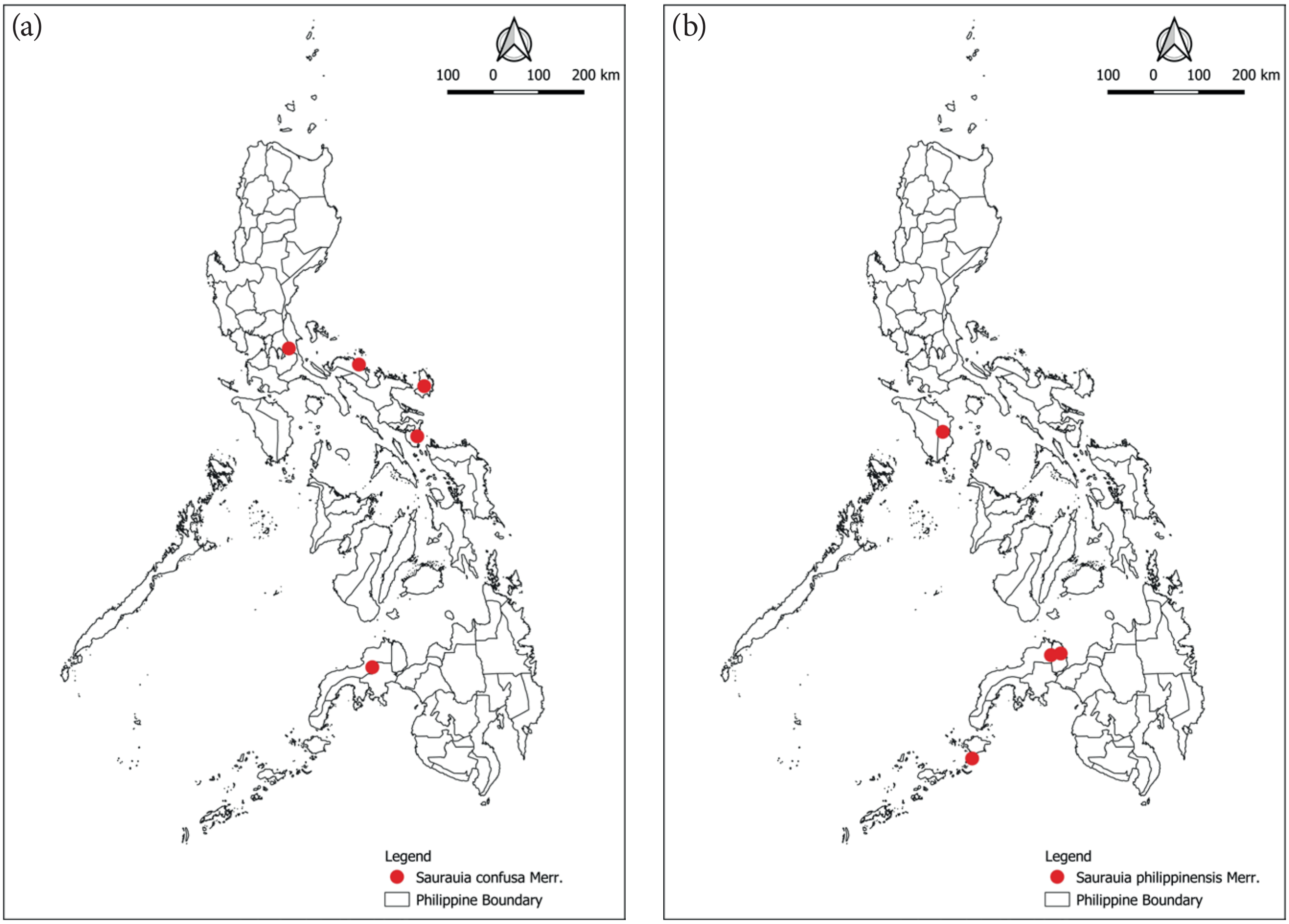


Figure 7. Species distribution map of (a) *Saurauia confusa*, and (b) *Saurauia philippinensis* based on the CDFP.

Jr. (2013) in their vegetation assessment studies, wherein they reported the occurrence of *S. bontocensis* in various forest areas across the Cordillera Mountains. Furthermore, Isaac et al. (2017) argued that the declining trend in species population sizes and suitable habitats, due to deforestation, increases the probability of extinction, especially for restricted endemic species. This suggests that the *Saurauia* species in the mountainous region of the Cordilleras are more vulnerable to climate and environmental changes due to the high deforestation rate in the region as recorded in the study of Daipan and Franco (2022).

Saurauia species with wider geographical distribution in the country

There are 20 *Saurauia* species with a wide distribution or found at least in two (2) or more regions in the country (Figure 6). These are *S. ampla, S. avellana, S. bakeri, S. clementis, S. confusa, S. copelandii, S. elegans, S. gracilipes, S. klemmei, S. lanaensis, S. latibractea, S. luzoniensis, S. negrosensis, S. panduriformis, S. philippinensis, S. polysperma, S. sparsiflora, S. trichophora, S. trunciflora, S. urdanetensis.* The result of the species distribution mapping could serve as an important input in the Red List (threatened species) assessments since they help determine some of the parameters for the inspections by allowing for the calculation of area and the extent of occurrence (IUCN 2014). Aside from that, the results of this study may provide insight into or answers to several taxonomic uncertainties in this genus. For example, the species distribution maps of *Saurauia* species generated in this study represent the known limits or occurrence of a specific *Saurauia* species' range as a provincial or regional boundary, or even islands – thus, any sites outside these range (islands) is unlikely to contain the species. According to Whittaker and Fernández-Palacios (2007) and Kier et al. (2009), islands are known as hotspots for range-restricted species, with high levels of endemism such as the *Saurauia* species in the country. This could indicate that the *Saurauia* species found in Mindanao has a low to zero chance of being discovered in the island of Luzon, or vice versa. As a result, the information presented in this work could be useful in other taxonomic and/or systematic research. However, proper plant systematic research such as comparative analysis of leaf architectural characters of *Saurauia* species should still be performed to delineate confusing species (Daipan et al. 2022). Examples of *Saurauia* species that are distributed in both the islands of Luzon and Mindanao are the *S. confusa* (Figure 7a) and *S. philippinensis* (Figure 7b). It is probable that *S. confusa* may not exist on the island of Mindanao, whereas *S. philippinensis* is only known to exist on the island of Mindoro. These are just a few examples of possible uses of this species distribution mapping in taxonomic investigations.

Climatic type of the different Saurauia species

There are four types of climate in the country according to the Corona's climatic classification (Figure 8). These are the Types I, II, III, and IV. Type I climate has two distinct seasons: dry from November to April and wet the rest of the year. The rainy season lasts from June to September. There is no dry season in Type II, with a very noticeable maximum rain period from December to February. There isn't a single dry month in the year. Type III has no clearly defined maximum rainy season, with a brief dry season lasting only one to three months, either from December to February or from March to May. The rainfall in Type IV is almost uniformly distributed year-round.

The findings of the overlay analysis between the species distribution of the genus *Saurauia* and the climatic type layer revealed that 16 *Saurauia* species occur in places with Type I climate, 22 species can be found in areas with Type II climate, while Type III and Type IV climates have 24 and 21 *Saurauia* species, respectively (Figure 9). *S. elegans* was observed to be distributed across all the four climatic types in the country. This may infer that this species is the most adaptable *Saurauia* to different climatic types. There are also species that are distributed or that occurred in areas with at most three (3) different climatic types, these are *S. clementis* (with climatic types II, III, and IV); *S. latibractea* (types II, III, and IV); *S. panduriformes* (types I, III, and IV); and *S. philippinensis* (types I, II, and III). It is plausible to conclude that these *Saurauia* species are less vulnerable to climate change compared to the *Saurauia* species with only one type of climate, since they can thrive in different habitats with different climatic types in the country. There are 35 *Saurauia* species that are restrained in a single type of climate, these are the *S. abbreviata*, *S. alvarezii*, *S. ampla*, *S. avellana*, *S. bicolor* , *S. cordata*, *S. denticulata*, *S. elmeri*, *S. erythrotricha*, *S. fasciculiflora*, *S. gigantifolia*, *S. glabrifolia*, *S. involucrate*, *S. klemmei*, *S. knemifolia*, *S. leytensis*, *S. loheri*, *S. longistyla*, *S. macgregorii*, *S. merrillii*, *S. mindorensis*, *S. oblancilimba*, *S. oligantha*, *S. oligophlebia*, *S. palawanensis*, *S. panayensis*, *S. samarensis*, *S. sampad*, *S. sibuyanensis*, *S. sorsogonensis*, *S. tayabensis*, *S. trichophora*, *S. wenzelii*, *S. whitfordii*, and *S. zamboangensis*. These species should be given attention in the evaluation and/or updating of the list of threatened species in the country, if not in the IUCN red list.

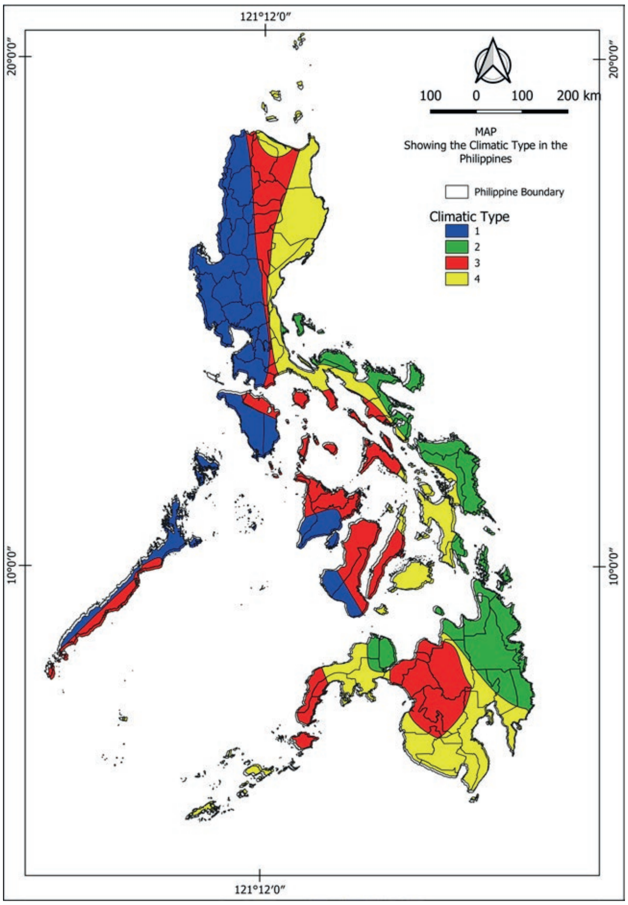
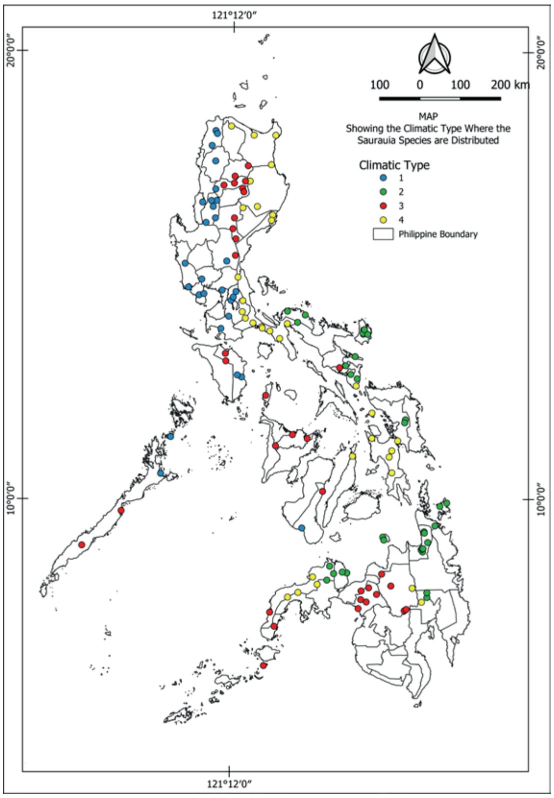


Figure 8. Climatic type in the Philippines based on the Modified Corona's Climatic Classification

The climatic data for each of the *Saurauia* species generated in this paper could serve as an important input in the habitat suitability studies and even in the species-level conservation planning. Although the current climatic assessment was based solely on the CDFP website at the time of reporting. Similar species may be



Species	I	II	III	IV
S. abbreviata				✓
S. alvarezii			✓	
S. ampla		✓		
S. avellana			✓	
S. bakeri		✓		✓
S. bicolor				✓
S. bontocensis	✓		✓	
S. cinnamomea	✓			✓
S. clementis		✓	✓	✓
S. confusa		✓		✓
S. copelandii		✓		✓
S. cordata		✓		
S. denticulata		✓		
S. elegans	✓	✓	✓	✓
S. elmeri				✓
S. erythrotricha		✓		
S. fasciculiflora	✓			
S. gigantifolia		✓		
S. glabrifolia		✓		
S. gracilipes		✓	✓	
S. involucrata		✓		
S. klemmei				✓
S. knemifolia	✓			
S. lanaensis		✓	✓	
S. latibractea		✓	✓	✓
S. leytensis				✓
S. loheri	✓			
S. longipedicellata			✓	✓

Species	I	II	III	IV
S. longistyla			✓	
S. luzoniensis	✓			✓
S. macgregorii			✓	
S. merrillii				✓
S. mindorensis	✓			
S. negrosensis	✓		✓	
S. oblancilimba	✓			
S. oligantha			✓	
S. oligophlebia		✓		
S. palawanensis			✓	
S. panayensis			✓	
S. panduriformis	✓		✓	✓
S. papillulosa	✓		✓	
S. philippinensis	✓	✓	✓	
S. polysperma	✓		✓	
S. samarensis		✓		
S. sampad		✓		
S. sibuyanensis			✓	
S. sorsogonensis		✓		
S. sparsiflora	✓			✓
S. tayabensis				✓
S. trichophora				✓
S. trunciflora			✓	✓
S. urdanetensis		✓	✓	
S. vanoverberghii	✓		✓	
S. wenzelii				✓
S. whitfordii		✓		
S. zamboangensis			✓	

Figure 9. Climatic type of the different *Saurauia* species in the Philippines.

found in other locations, extending the species' range. This may also increase the climate suitability of species. This information could also help in some taxonomic research in identifying confusing species based on their climatic type since climate is widely recognized as one of the most important factors of species distribution, and plants are sensitive to bioclimates, which dictate plant habitats (Kwon et al. 2012).

CONCLUSIONS

Species distribution mapping of *Saurauia* in the Philippines was conducted using the occurrence information from the checklist of vascular plants obtained from the CDFP. Although the distribution data was only based on the CDFP website, this paper provided a relevant information on the patterns of species distribution of the genus *Saurauia* in the country. The findings revealed that 29 species have limited distribution at the provincial level at present, while seven (7) species are only found in a specific region. It is plausible to conclude that these species are the most threatened *Saurauia* in the country on the basis of their endemicity or extent of occurrence. Furthermore, 35 *Saurauia* species are confined to a single type of climate, which may infer that they are the most vulnerable to climate change due to their inability to adapt to other climatic types. The *Saurauia* species identified in this study with a single provincial or regional distribution at present and restrained in only one type of climate should be prioritized and given special consideration in terms of species conservation. Finally, the information and data derived in this paper could serve as a baseline for assessing conservation status, habitat modelling studies, species-level conservation planning, and understanding climate change impacts.

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The ginger genus *Burbidgea* confirmed in the flora of the Philippines

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Abstract. The facultative epiphytic ginger genus *Burbidgea* harbours six species hitherto thought to be endemic in Borneo. Recent fieldwork in Zamboanga region, Mindanao, Philippines, resulted in the collection of a ginger, which we identified as *Burbidgea nitida*. The species, however, was already collected in Zamboanga region in 1919 but the evidence remained misidentified for a long time as *Hedychium*. Including the *Burbidgea* in the flora of the Philippines, increases the number of native ginger genera of the country to 17. Based on the recent collections, a complete description of *B. nitida* in the Philippines is provided here including illustrations and notes on ecology and a local name. We lectotypify *Burbidgea nitida*, the type of the genus, and clarify the overlap of native ginger genera and species between Borneo and Philippines.

Keywords: Borneo endemics, *Burbidgea nitida*, Huxley's Line, Zamboanga.

INTRODUCTION

The Zamboanga region in the southwestern part of the Philippines has a unique biogeographical history (Dickerson et al. 1928). The region including the islands of Basilan, Sulu, and Tawi-Tawi has a diverse flora and fauna with a strong Bornean affinity (Valejo 2011). The understanding of the biodiversity of this region is poorly known because the ongoing insurgence has made exploration difficult in the past decades.

The number of ginger genera in the Philippines increased in the past few years as a result of taxonomic revisions and recent discoveries performed by several Filipino botanists. In 2022, Mazo et al. reported the occurrence of the genus *Sulettaria* A.D.Poulsen & Mathisen in the Philippines, resulting in a total of 18 ginger genera (Pelser et al. 2011 onwards) including *Curcuma* L. and *Kaempferia* L. that are not native.

In 2021, an epiphytic ginger was collected in the municipality of Leon B. Postigo, Zamboanga del Norte, Philippines. The taxon was found to be a member of the genus *Burbidgea* Hook.f. This genus was thought to be endemic to Borneo (Smith 1972) and therefore represents a new genus record for the Philippines. After reviewing protologues and other published papers as well as examining herbarium collections including types, we identified the species as *Burbidgea nitida* Hook.f. In the present paper, we formally report the new distribution record of *Burbidgea* to the Philippines and provide detailed information based on the recent material. This increases the number of ginger genera (including non-native) of the country to 19.

MATERIAL & METHODS

The collections were made in the field following standard methods ensuring flowers were pickled in 70% ethanol. Relevant herbarium specimens deposited at E, K, L, PNH, SING, SAR, US, digital images accessed on Zingiberaceae Resources Centre (Newman et al., 2005 onwards) and JSTOR Global Plants (https://plants.jstor.org/), and publications by Smith (1972, 1984) were consulted for identification. The description made in this paper is based on living material, dried specimens, and photographs.

TAXONOMIC TREATMENT

Burbidgea Hook.f. in Bot. Mag. 105: t. 6403. 1879.

Type: *Burbidgea nitida* Hook.f.

A genus of herbaceous perennials, terrestrial or epiphytic plants currently represented by six species which are native to Borneo (Smith 1972; Neo et al. 2020; Newman & al. 2005–). The present paper extends its distribution to include the Philippines. The genus is morphologically most closely related to *Riedelia* Oliv. in New Guinea but can be distinguished by having a persistent calyx, a labellum that is much longer than broad and bilobed in the upper third, and narrow and much elongated fruits. Smith (1972) divided *Burbidgea* species in two groups based on the shape of the dorsal petal and the bilobed part of the labellum.

Burbidgea and three other genera (*Pleuranthodium* (K.Schum.) R.M.Sm., *Riedelia* and *Siamanthus* K.Larsen & J.Mood) are placed in the tribe Riedelieae characterized by long, slender silique-like capsules that open by longitudinal slits to the base (Kress et al. 2002; Smith 1972; Larsen and Mood 1998).

Burbidgea nitida Hook.f., Bot. Mag. 105: t. 6403. 1879.

Type: Cultivated at Veitch' nursery, 1878, *F.W.T. Burbidge s.n.*, lectotype K000292495!, designated here. Origin: Borneo, Malaysia, Sarawak, Fifth Division, between Lawas and Trusan River, 300–460 m, 1877, *F.W.T. Burbidge s.n.*

Description

Description based on Philippine collections: Epiphytic herb in dense clump. Rhizome 8.5–11.4 mm in diameter, yellowish green externally, cream internally, scales thick and fleshy, 8–13 mm long, glabrous, light brown. Leafy shoot 45–55 cm long, arching, with ca. 12 leaves per shoot; sheath glabrous, sparsely scabrid at the margin, pale green; ligule oblong, 4.5–6.5 × 3–6 mm, glabrous, mid-green, apex emarginate; petiole 3–7 mm long, glabrous, mid-green; lamina elliptic, 12–21 × 4–6 cm, glabrous on both sides, coriaceous, smooth, mid-green, base obtuse, margin entire, apex acuminate. Inflorescence terminal to the leafy shoot; free part of peduncle 1.8–4.5 cm long, glabrous, maroon to bright red; spike hemispherical, monopodial, 7–10 × 4–5.6 cm, with 12–20 flowers, 2–6 open at a time; basal bracts solitary, caducous, elliptic to ovate, 4.8–5 × 1.8–2 cm, glabrous, light orange tinged white, apex mucronate; rachis unbranched, 3.5–4 cm long, bright red, glabrous; fertile bracts and bracteoles absent; pedicel 3–4 mm long, puberulent, bright red; calyx 12–18 mm long, sparsely puberulent, bright red, apex 3-dentate; corolla tube 20–23 mm long, glabrous outside, inside puberulous in upper half, bright red to orange, lighter in the upper part; corolla lobes glabrous on both sides, orange to bright red, dorsal lobe ovate, 20–25 × 10–15 mm, apex mucronate, lateral lobes narrowly ovate, 20–25 × 8–11 mm, apex shortly mucronate; labellum spatulate, 11–15 mm long, the base forming a short 6–11 mm semi-tube clasping the filament, apex deeply bilobed, lobes ovate and petaloid, 3–5 × 3–4 mm, glabrous, yellow to orange (with age), apex rounded; lateral staminodes absent; stamen 18–20 mm long when flattened; filament 1.2–1.5 mm long, glabrous, yellow to orange; anther linear, 7–10 × 0.5–1 mm, connective tissue pubescent, orange; thecae 7–10 × 0.4–0.6 mm, glabrous, white to pale yellow; anther crest cuneiform, petaloid, 5–6 × 4–5 mm, glabrous except for puberulous base, light orange or red, margin undulate; style 3–3.5 cm long, puberulent, white; stigma c. 0.8 mm wide, ostiole rounded triangular, margin with erect hairs, crest c. 0.5 mm long rounded entire, white; epigynous glands 2, linear, 2–3 mm long, glabrous, cream, apices rounded, laterally flattened; ovary narrowly cylindric, 6–8 × 2–3 mm, pubescent,

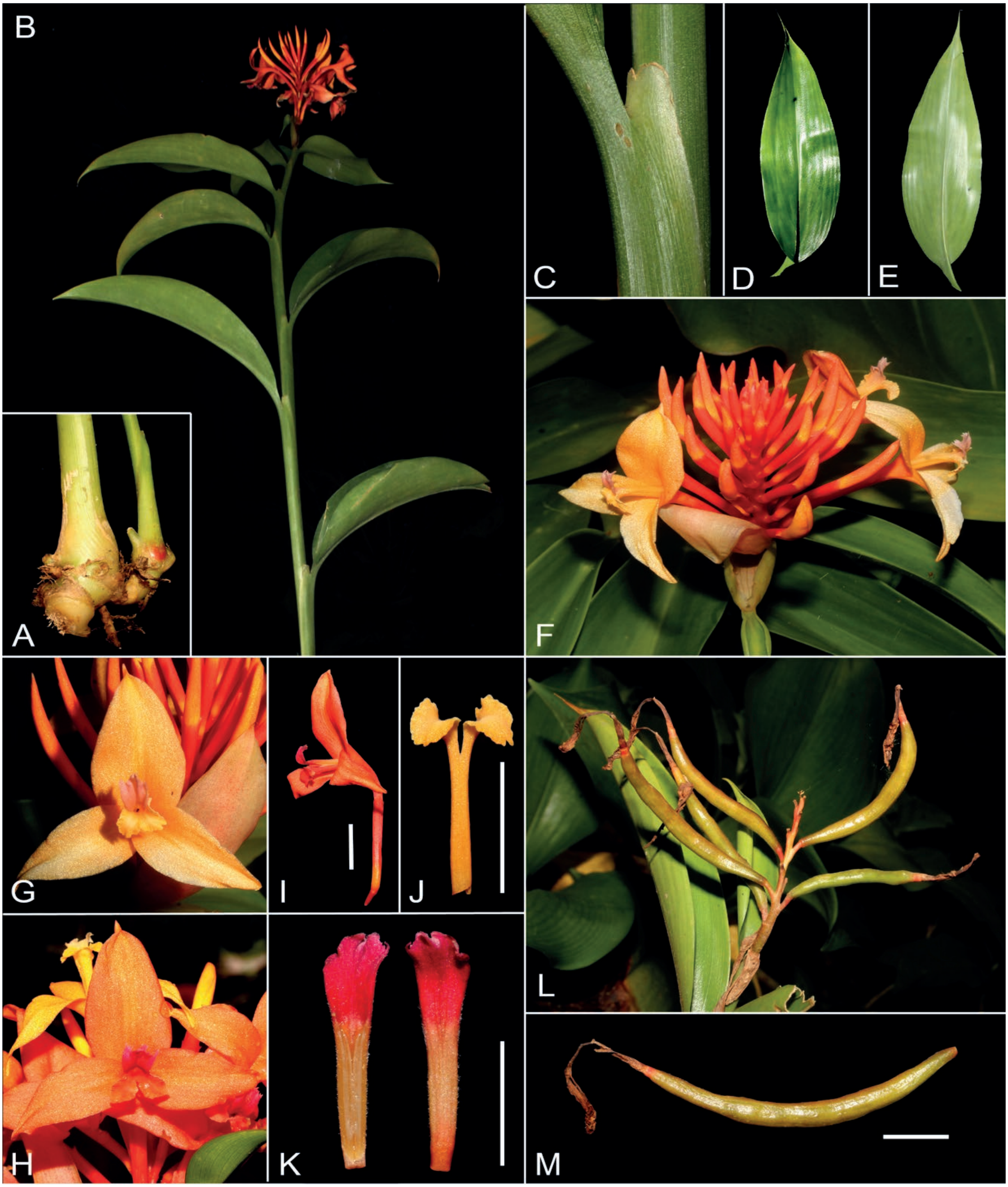

Figure 1. *Burbidgea nitida*. **A.** Base of the leafy shoot. **B.** Habit. **C.** Ligule. **D.** Leaf abaxial surface. **E.** Leaf adaxial surface. **F.** Inflorescence. **G.** Flower anthesis with light orange corolla lobes and labellum. **H.** Flower with red-orange corolla lobes and labellum. **I.** Flower removed (side view). **J.** Labellum. **K.** Stamen (front & back view). **L.** Infructescence. **M.** Fruit. Based on *K.R.F. Mazo 46* (A-K) and *K.R.F. Mazo 91* (L-M). Photos by: K.R.F. Mazo. Scale bars = 1 cm.

Table 1. Species of Zingiberaceae occurring in both Borneo and the Philippines.

Species
Etlingera brevilabrum (Valeton) R.M.Sm.
Etlingera coccinea (Blume) S.Sakai & Nagam.
Etlingera fimbriobracteata (K.Schum.) R.M.Sm.
Etlingera sessilanthera R.M.Sm.
Geocharis fusiformis (Ridl.) R.M.Sm.
Globba francisci Ridl.
Hornstedtia havilandii (K.Schum.) K.Schum.
Plagiostachys albiflora Ridl.
Sulettaria longituba (Ridl.) A.D.Poulsen & Mathisen

orange. Infructescence 4.5–9 cm long; fruits narrowly elongated, 30–46 × 3–5 mm, sparsely pubescent, yellow-green becoming greenish red when mature, remnant of calyx persistent; seeds fusiform, 4.5–5 mm, brown with white aril; aril transparent white, 0.06 mm thick, covering the seed.

Etymology

The genus is named in honour of the British explorer, Frederick William Burbidge (1847–1905), who discovered *Burbidgea nitida* during an expedition to Borneo. The epithet means polished or glossy referring to the shiny leaf blades.

Vernacular name

Locally known as *lakimag* by the Subanen people of Zamboanga del Norte. Uses not recorded.

Ecology and distribution

In the Philippines, *Burbidgea nitida* is only confirmed recently in the provinces of Zamboanga del Norte and Zamboanga Sibugay, both in the Zamboanga Region. The locality, Mt. Tubuan, where *M. Ramos & G.E. Edaño 36732* was collected, is impossible to place on a current map; most probably the name of the mountain is no longer used, so we are not sure in which province this is in the Zamboanga Region.

During fieldwork, only one population was observed growing epiphytically on small trees in shaded secondary lowland evergreen rainforests at 380–400 m.

Notes

The closest genus in terms of morphology in the Philippines is *Hedychium*, which is represented by two species in the country. It is therefore not surprising that the two collections at Kew by *M. Ramos & G.E. Edaño* (*36732* and *36882*) from 1919 were initially identified as *Hedychium*. Even though these collections included flowers (*36882*) and fruits (*36732*), the identification was not updated for a very long time when the labels were edited with an ink pen, '*Hedychium*' crossed out and '*Burbidgea nitida*' added. The handwriting matches that of Rosemary M. Smith and her undated annotations must have been done after her two papers on the genus in 1972 and 1984, none of which mentioned an occurrence in the Philippines. They were not reported in any later publication but the records did become available online (Newman & al. 2005–). Our study based on collections from the same plant of flowers and fruits confirms her identification.

The original material at Kew herbarium placed in a type folder consists of three sheets (K000292494, K000292495, K000292496) representing at least two gatherings from the plant cultivated at Veitch' nursery originally collected by F.W.B. Burbidge in Borneo in 1877. It is not written who actually collected the material in the nursery but it may very well be Burbidge himself as Hooker (1879), when describing the new genus, mentioned the pencil sketch made by Burbidge (attached to the sheet barcoded K000292495). This beautiful sketch clearly matches the flower details of the plate (Hooker 1879) and unlikely to have been done during field conditions in Borneo. A note in pencil attached to the third sheet (K000292496) says that 'These are not good specimens being the weak second growths of the season' and thus obviously an observation made during cultivation. Hooker indeed clarified that the plant flowered twice within the first year after having been brought into cultivation. This would make the year of collection of the cultivated material 1878 as somebody has also added in pencil to K000292494. We designate here K000292495 as the lectotype as it is the most informative.

When Burbidge collected this species later to be named in his honour in Borneo, he only found one population in shady forest (Hooker 1879; Veitch 1906), similar to the recent collection made in Philippines but whereas the former was thriving on moist rocks the latter was epiphytic. The labels of most other Bornean collections we have examined state that the plant was terrestrial. Maybe future collections in the Philippines will reveal that this species may also grow terrestrially there.

Borneo harbours native 24 ginger genera and 260 species whereas the Philippines has 17 genera and 127 species (including *Burbidgea nitida*). Currently, 14 native ginger genera but only nine species overlap between the two areas (Table 1), three of which are in Palawan, the other six crossing the Huxley's line (extension of Wallace's Line to the Philippines). Therefore, it is not surprising that the genus *Burbidgea* also crosses Huxley's line even though it would have been more likely that a taxon in Borneo is also found in other parts of Sundaland (Java, Sumatra or the Malay Peninsula).

Even though *Burbidgea* is here documented for the Philippines and Mood et al. 2020 synonomised *Haplochorema* K.Schum. in *Boesenbergia* Kuntze, three other ginger genera still remain endemic in Borneo: *Borneocola* Y.Y.Sam (Sam et al. 2016 convincingly separated this genus from *Scaphochlamys* Baker using molecular and morphological data), *Epiamomum* A.D.Poulsen & Škorničk. and *Myxochlamys* A.Takano & Nagam. Surprisingly, none of these genera were listed in Neo et al. 2020.

Specimens examined

Cultivated plant at Veitch' nursery, collected as the lectotype from the same plant originally from Borneo, 1878, *F.W.T. Burbidge s.n.*, (K, syntypes K000292494!, K000292496!, likely not collected the same date). Philippines, Mindanao. Zamboanga District, Mount Tubuan, October 1919, *M. Ramos & G.E. Edaño 36732* (K!, US 00336046!); same District, Malangas, November 1919, *M. Ramos & G.E. Edaño 36882* (K!, US 00336047!); Zamboanga del Norte. Cultivated plant at barangay Tinuyop, Leon B. Postigo, (4 km from the wild locality), 300 m, 3 July 2021, *K.R.F. Mazo 46* (PNH, acc. no. 258613!); 24 October 2021, *K.R.F. Mazo 91* (FEUH 004111! [spirit material only]); plant originally collected at Leon B. Postigo, Barangay Tinuyop, near Lunganitan, 8°3'40.29"N, 122°56'11.69"E, 640 m, 25 March 2021, *K.R.F. Mazo s.n.*

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Ancient woodland indicator species: can old herbarium specimens supplement recent records to inform ecological management?

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Abstract. Old herbarium specimens have become increasingly well-recognised as a rich source of ecological baseline data. For long-continuity plant communities, such as ancient woodland, these records may be particularly important for present day ecological management. To evaluate this potential, searches for pre-1950 Ancient Woodland Indicator (AWI) herbarium specimens collected in East Gloucestershire, UK, were conducted using digital open access sources and the physical Royal Agricultural University herbarium. In total 305 specimens were retrieved from twelve herbaria, with small regional collections being particularly important sources. The earliest specimen dated to 1834. There was a significant association between old specimen availability and year of collection, due to a peak in the late-1800s and early-1900s. Over half of the AWI species for the region were represented, although some taxonomic bias was evident. To determine if old AWI specimens contributed any new location records, 246 unique specimens with detailed georeferences were mapped and compared to the locations of 1950-1999 and 2000-2021 biological records. One third of the pre-1950 specimens had not been recorded in the same locality since collection of the old specimen, indicating either a gap in recent records or floristic change. However, length of time since specimen collection was not a predictor of a 1950-2000 or 2000-2021 record in the same locality. Overall, it is highly recommended that policy-makers, land managers, and field surveyors consult old AWI herbarium records for ancient woodland identification, management, and restoration.

Keywords: Ancient forest, connectivity, continuity forest, herbaria, inventory, natural history, restoration, old growth forest.

INTRODUCTION

Ecological records >50 years old have previously been overlooked in their relevance to present-day conservation action (Willis et al. 2007). Recently, increased digitisation and accessibility have greatly increased interest in the application of old herbarium data to biodiversity conservation (e.g. James et al. 2018; Hedrick et al. 2020; Albani Rocchetti et al. 2021; Baldini et al. 2022; Heberling 2022). Herbaria often provide the earliest species distribution data available to inform current ecological decision-making (Meineke et al. 2018;

Lang et al. 2019). In particular, preserved specimens and georeferenced metadata (taxonomic identification, collection date, and locality) can provide useful baseline biogeographical data for spatio-temporal studies (Lavoie 2013; Le Bras 2017).

Ancient woodland in the UK is land that has been continuously wooded since at least 1600 (1750 in Scotland) (Reid et al. 2021). This comprises four land-use types: ancient semi-natural woodland; plantations on ancient woodland sites; ancient wood pasture and parkland; infilled ancient wood pasture and parkland (for definitions see DEFRA et al. (2022)). These vary in terms of canopy openness, floristic composition, silvicultural or pastoral uses, and planted or natural regeneration, but the uniting factor is centuries-long wooded continuity (Peterken 2018). Since the date threshold, and often long before, the land has been wooded, and even if felled, replanted without any other intervening land-use: it is therefore uninfluenced by agricultural inputs or cultivation (DEFRA et al. 2022). As such, ancient woodland has a high conservation value and is widely considered to be irreplaceable (UK Government 2021; Reid et al. 2021). The ancient woodland concept arose to distinguish this important habitat - along with its distinctive ecology, ecosystem services, and cultural heritage - from newer woodlands established on other land-uses (Peterken 2018). Concepts analogous to ancient woodland are recognised internationally (e.g. Kirca et al. 2018; McMullin and Wiersma 2019).

Ancient woodland has been subject to habitat loss and change, covering just 2.5% of the UK's land area today (Reid et al. 2021). From the 1800s to mid-1900s many ancient woodlands were degraded or lost due to the enclosure of the countryside, shifts in land use during the agricultural and industrial revolutions, and the introduction of modern forestry practices (Rotherham 2022). Most notably, between the 1950s and 1980s, 39% of ancient semi-natural woodland area was converted to plantation forestry and a further 9% was lost to agriculture (Reid et al. 2021). Even today, with its value well-known, threats from infrastructure and development exist with the addition of new environmental and climatic challenges (Rackham 2008; Razzaque and Lester 2021). In response, the legal protection of ancient woodland features prominently in the UK National Planning Policy Framework and forestry policy, as does responsibility for its restoration and connectivity (DEFRA et al. 2022; Ministry of Housing, Communities and Local Government 2019; UK Government 2021). Therefore, accurate identification of ancient woodland is necessary to meet legislative requirements as well for its intrinsic value.

Ancient Woodland Indicator (AWI) species richness has long been one of the evidence source used in the identification of ancient woodlands (Peterken, 1974). These are vascular plant species that are particularly, but not exclusively, associated with ancient woodland, for example English Bluebell (*Hyacinthoides non-scripta*), Herb Paris (*Paris quadrifolia*), and Lily-of-the-Valley (*Convallaria majalis*) (Glaves et al. 2009). Numerous studies have evidenced a strong affinity between these understorey plant species and ancient woodland (e.g. Peterken 1974; Wulf 1997; Kelemen et al. 2014; Swallow et al. 2020). They typically exhibit life traits associated with habitat continuity such as long lifespan, late maturity, and rhizomatous regeneration (Hermy et al. 1999). In addition, many have short-distance dispersal strategies, and do not easily colonise more recently established woodland (Hermy et al. (1999). As such, AWI species lend themselves to uses beyond their initial purpose, including site prioritisation for defragmentation and protected area planning (Dyderski et al. 2017) or as target species for conservation management (Brown et al. 2015). The AWI concept is embedded in policy and practice but its strengths and limitations should be noted (Rotherham 2011; Sansum and Bannister 2018; Webb and Goodenough 2018).

Currently, old AWI herbarium specimens are not explicitly listed among the evidence sources for ancient woodland identification in the UK (Glaves et al. 2009) nor the Ancient Woodland Inventory (Sansum and Bannister 2018). However, they have contributed to the creation of regional AWI lists (Glaves et al. 2009). As ancient woodland is a long continuity ecosystem, the inclusion of old herbarium AWI records along with recent biological records and survey data could be extremely valuable. This approach would account for the past vegetation community that may have more clearly supported the evidence for ancient woodland status, for example if an AWI species had become locally extinct due to habitat change. Old AWI herbarium records have been successfully employed in a small number of studies to identify former ancient woodland sites by corroborating recent biological records and old cartographic evidence (e.g. Rotherham 2022). Although herbarium specimen mapping has been widely-employed in biogeographical studies (Lavoie 2013), it remains an under-explored and under-utilised technique in the context of ancient woodland. Seemingly no systematic analysis of its application has previously been undertaken.

The aim of this study was to examine the potential of old AWI herbarium specimens to inform ancient woodland identification and ecological management at a landscape scale. To achieve this, the objectives were twofold: (1) to investigate the availability of old (pre-

1950) AWI specimens and their temporal and taxonomic coverage; (2) to analyse the proportion of these specimens with a recent (1950–1999 or 2000–2021) biological record in the same locality.

MATERIALS AND METHODS

The study focused on Vice-county 33 (VC33), East Gloucestershire, located in the South-West of the UK (Figure 1). Vice-counties were created in 1852 for the purpose of biological recording and their boundaries have since remained constant. VC33 is centred on 51.49 N, 1.58 W (Figure 1), elevation 20-275 metres above sea level, with dominant geological substrates of oolitic Limestone and Lias clay (British Geological Survey 2021).

AWI species for VC33 were determined from standard lists for South-West England (Rose 1999) and neighbouring counties Avon, North Somerset, South Gloucestershire, and Worcestershire (Kirby 2004), as compiled by Glaves et al. (2009) This selection method aligned with Swallow et al. (2020). Taxonomic names followed Stace (2019).

Figure 1. Location of Vice-county 33 East Gloucestershire, UK.

Old AWI specimens were defined as pre-1950 as this pre-dates major shifts in countryside management associated with mid-1900s agricultural intensification and extensive felling of ancient semi-natural woodland for plantation forestry. To obtain pre-1950s VC33 AWI specimens, searches for published digitised herbarium accessions were conducted primarily via the Herbaria United database (2006–) (herbariaunited.org) and a small number via the Global Biodiversity Information Facility database (2001–) (GBIF.org) (as available on 31/05/2021), as well as inspection of the unpublished accessions list of the Royal Agricultural University herbarium. To account for nomenclatural changes, synonyms were included in searches. Respective digital and physical accessions were retrieved and a metadata list (identification, location and date) was created. At this stage, all specimens, including duplicates, were included.

Unique records with sufficiently detailed georeference data were mapped in QGIS (QGIS.org 2020). When species-location-year duplicates existed across the herbaria, only one was retained for mapping and analyses. For species-location duplicates, only the most recent was used. Named point landmarks or properties could be often assigned with greater than 1km^2 accuracy. Names of area features such as large woodlands were taken as the centre point of that location (Aedo et al. 2015).

The locations of pre-1950 specimens were compared to post-1950 digitally available biological records. QGIS was coded to display: (a) pre-1950 herbarium specimen only i.e. no 1950–2021 record in the same locality; (b) most recent record in the same locality 01/01/1950 –31/12/1999, and; (c) most recent record in the same locality 01/01/2000–31/05/2021. Post-1950 mapped digital records were obtained with permission from the Botanical Society of Britain and Ireland (BSBI) Distribution Database (2000–) (https://database.bsbi.org/) (Pescott et al. 2018). Record search criteria included specimens and observations. Taxon aggregates were included but hybrids were excluded. For 2000–2021 records 1km or finer resolution was used. Records located within the 1km square containing a old specimen point record or within the 1km squares covered by an area record or intersected by its boundary were counted as being in the same locality. Where point records were within a contiguous habitat such as a woodland, these were treated as area records. For 1950–1999, most available records were 2km resolution – these were only counted as the same locality when they substantially overlapped a large area feature e.g. large woodland, or if an old specimen point record was central.

To test if the number of old herbarium AWI specimens available was associated with year of collection, a chi-squared test of association was conducted. To establish if year of specimen collection had any bearing on availability of a 1950-2021 or 2000–2021 record in the same locality, a generalised linear model with binary logistic response was applied. SPSS (IBM corp. 2019) was used for all analyses.

RESULTS

In total, 305 AWI specimens dating between 1835 and 1949 from VC33 were obtained from 11 digitised published collections plus the unpublished Royal Agricultural University herbarium (Figure 2). The majority of specimens were housed in Gloucester City Museum (128), followed by the South London Botanical Institute (47), and the Royal Agricultural University (39). The latter herbarium was particularly distinct in the early date of its AWI specimens relative to the other herbaria (Figure 2). This collection held 24 AWI species and all specimens were unique location or date contributions.

Across all herbarium collections, 65 AWI species out of a potential 111 for the region were represented, including forbs, graminoids, pteridophytes, and some ligneous species. The most commonly collected species were of the following Families: Orchidaceae (five species, 45 specimens); Ranunculaceae (five, 42); Violaceae (two, 35) (Appendix 1). Nearly half of the species (32) were represented by only one or two specimens. The most represented species was *Cephanlanthera damasonium* (22 specimens).

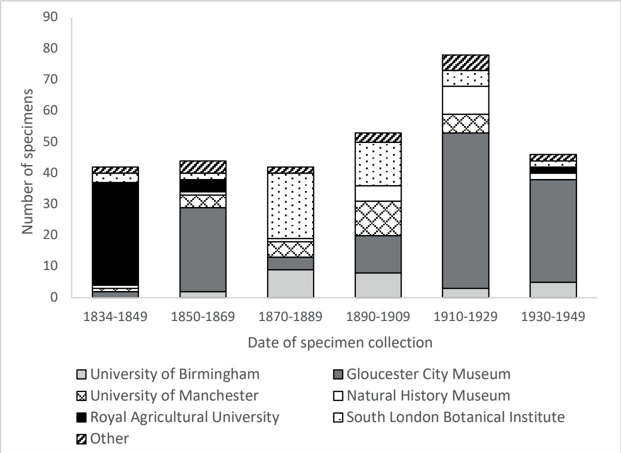

Figure 2. Number of pre-1950 Vice-county 33 Ancient Woodland Indicator specimens in herbarium collections, available via herbariaunited.org and GBIF.org as of 31/05/2021 and previously unpublished Royal Agricultural University specimens (n=305). Other = Royal Botanic Gardens, Kew; Aberystwyth University; University College Dublin; Bolton Museum; Royal Botanic Garden, Edinburgh; University of Pisa. All specimens, inclusive of duplicates of species collected in same location within same date, and specimens collected in same location but in different years.

Of the 305 specimens retrieved, 51 were duplicates and eight specimens were not locatable due to vague georeferences. Mapping of the pre-1950 herbarium specimens revealed a clustered distribution, largely aligned with current ancient woodland configuration (Figure 3). Of the 246 geolocatable herbarium specimens: 80 (32.5%) had no 1950 –2021 record in the same locality; 31 (12.6%) had been most recently recorded in the same locality between 1950 and 1999; 135 (54.9%) had been most recently recorded in the same locality between 2000 and 2021 (Figures 3, 4).

There was a statistically significant association (p=0.030) between year of collection category and number of old AWI specimens retrieved, with more specimens available between 1890 and 1929 (Figure 4 and Table 1). However, year of specimen collection was not a significant predictor of a 2000–2021 record being available for the same locality, nor for the wider time period of 1950–2021 (Table 1).

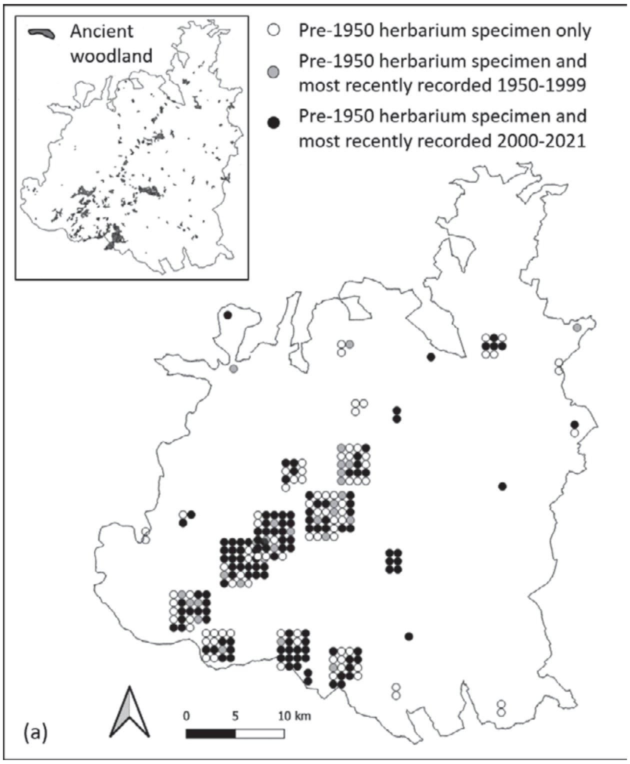

Figure 3. Geolocated pre-1950 herbarium Ancient Woodland Indicator species specimens in Vice-county 33, East Gloucestershire, UK. Each point represents one specimen. Data points are displaced from a central point and displayed in a grid pattern for ease of interpretation. Ancient woodland land cover map (Open Government Licence, Natural England 2021).

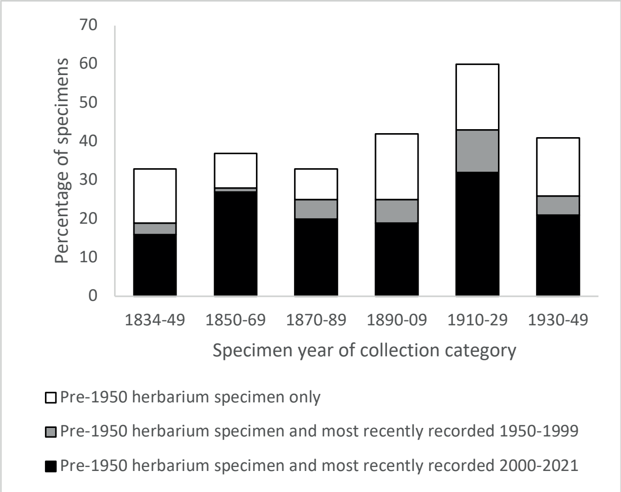

Figure 4. Number of pre-1950 Ancient Woodland Indicator specimens by date of collection (n=246), exclusive of duplicates and vague georeferenced specimens: pre-1950 specimen only with no record in same locality since 1950; most recently recorded in same locality between 1950 and 1999; most recently recorded in the same locality 2000–2021.

DISCUSSION AND CONCLUSIONS

The herbarium accession search results yielded 305 pre-1950 AWI specimens for the region. Crucially, this was sufficient to detect spatio-temporal patterns and trends, even after duplicates were discounted. Eighty-seven percent of the specimens were digitally accessed, emphasising the importance of herbarium digitisation (Soltis 2017). Specimen availability was reliant on very few herbaria: 94% of specimens were retrieved from just six collections and the remaining six each contributed <10 specimens. Two of the three highest contributors of old AWI specimens were small collections with a regional focus; Gloucester City Museum and the Royal Agricultural University. Small herbaria (<100,000 specimens (Lavoie 2013)) have been well-recognised in the literature for their contribution to biodiversity data (Colombo et al. 2016; Marsico et al. 2020) and the results reinforce that such collections should not be overlooked.

Table 1. Influence of old AWI specimen collection year on (a) number of available old herbarium AWI specimens (n=305), chi-squared test of association, and (b) likelihood of a 2000-2021 or 1950-2021 record in the same locality as the old specimen (n=246), generalised linear model.

	Chi (d.f.)	p
(a)		
Association between number of old herbarium AWI specimens and year category	12.341 (5)	0.030
(b)		
Year of herbarium specimen collection as predictor of 2000–2021 record in same locality	0.454 (1)	0.500
Year of herbarium specimen collection as predictor of 1950–2021 record in same locality	0.243 (1)	0.622

Although the first UK herbarium was established in 1621 (Smith 2018), close to the ancient woodland date threshold of 1600, the earliest specimen retrieved for this study dated to 1834. Specimen availability was fairly even through the mid-late 1800s, but statistical testing showed that temporal coverage of specimens was not evenly distributed over the full 1834-1949 period. This was mainly due to a peak in specimen numbers between 1890 and 1929. This pattern aligns with the wider collecting trend of British and Irish flora, which was most prolific in the late 1800s to early 1900s (Groom et al. 2014).

Old herbarium specimens represent ‘hard won’ data, limited by contemporary transport and recording technologies. They are also finite and the best available data for their time (Meineke et al. 2018). However, it is important to caveat their limitations. Biological recording is well-known to be prone to taxonomic and location biases, over- and under-sampled locations and false absences (Daru et al. 2018; Troudet et al. 2017). Just over half of the possible AWI species for this region were represented at least once among the specimens. However, specimen collection appears to have been influenced by axiophyte theory (Walker et al. 2010) with charismatic plants such as Orchidaceae more frequently collected than, for example, Cyperaceae. In terms of location bias, the majority of the old AWI specimens were collected from larger ancient woodlands. The ancient woodland concept, in its current sense, did not exist at that time (Peterken 2018), but the quality of woodland vegetation may have influenced choice of collection location.

The 32.5% of the old AWI specimens without a record in the same locality between 1950 and 2021 are particularly valuable as they provide potentially unique species-location data. Ancient woodlands are commonly identified using a range of desk-based evidence, including existing biological records, as field surveys are too resource-intensive to carry out on every site (Glaves et al. 2009; Natural England 2022). Therefore, old AWI herbarium data could add a useful evidence source to the UK Ancient Woodland Inventory or similar undertakings at any scale, particularly as herbaria are increasingly digitally available. This technique would be especially applicable when AWI richness thresholds (for example 8, 10, or 12 species (Glaves et al. 2009)) form part of the evidence for ancient status. In the present study, herbarium georeference data was often sufficiently detailed to pinpoint a named woodland. In such situations, the inclusion of old herbarium records could influence the classification of a woodland as ancient, and consequent levels of protection.

Old AWI specimens, with or without a corresponding recent record, may assist in the identification of lost, remnant or overlooked wooded sites. Mapping showed some small clusters of old AWI specimens located outside of named woodlands. Feasibly, some of these could indicate former ancient wooded commons, wood pastures or lost woodlands (Rotherham 2017). These, along with ancient woodland remnants of less than 2 hectares and ancient hedgerows, have been increasingly recognised for their intrinsic value, as well as their potential for connectivity, restoration, or wilding (e.g. Groenewoudt et al. 2022; Lenoir et al. 2021; Rotherham 2017; Sansum and Bannister 2018). Ancient woodland ecological planning depends not only on extant and mapped habitats, but also the historic landscape configuration (Kimberley et al. 2016). The 54.9% of old AWI specimens with a 2000–2021 record in the same locality could represent relict populations of such sites. To triangulate the evidence of 'ancientness', old herbarium records could be applied in combination with a range of other environmental indicators such as the palynological record (e.g. Dark 2021) and soil profile analysis (e.g. Rotherham 2022). Bergès and Dupouey (2019) advocate the exploration of all types of historical ecological documents to better inform ancient woodland management.

It might have been expected that the oldest AWI specimens would be significantly less likely to have a recent record in the same locality due to local extinctions in light of the threats to ancient woodland. However, statistical testing showed that year of herbarium specimen collection was not a significant predictor of whether or not a 1950–2021 or 2000–2021 record was available. In addition, the majority of old AWI specimens did have a corresponding recent record in the same locality, either between 2000 and 2021 (54.9%) or 1950 and 1999 (12.6%). Further, a proportion of the old AWI specimens without a recent record in the same locality may have been false absences. Therefore, known local losses of AWI species are in the minority. This suggests that ancient woodlands have largely maintained the necessary environmental conditions required to support ancient woodland flora: the oldest specimens with corresponding 2000–2021 records indicate a persistent population over around 180 years. Conversely, there is evidence of an extinction debt of over 100 years for woodland plants after habitat alteration (Vellend et al. 2006). A greater length of time and ground-truthing would be required to elucidate these population dynamics.

Old AWI herbarium specimens could also assist in resource allocation for species and habitat conservation prioritization (Kricsfalusy and Trevisan 2014). The use of old herbarium data to assess the likelihood of species presence where no recent record exists has met with success in other ecosystems and species groups (e.g. Lienert et al. 2002; Applequist et al. 2007; Aedo et al. 2015). Old AWI specimens without a corresponding recent record should not be treated as an absence or loss without ground-truthing. Old herbarium data may also guide targeted population surveys for conservation status assessment, for example to designate Red List status. In addition, AWI individual species have been increasingly employed as target species or success indicators of ancient replanted woodland restoration (Palo et al. 2013; Brown et al. 2015), rewilded woodlands (Broughton et al. 2021), and ancient woodland soil translocation mitigation (Craig et al. 2015). Old AWI herbarium records could expand the species presence baseline against which success is measured.

In conclusion, these findings strongly support the use of old AWI herbarium specimens in addition to more recent records for the purposes of ancient woodland identification and management for nature recovery. The digital availability of old herbarium records provides a rich source of high-granularity data that adds to the evidence for ancient status of extant woodlands and may elucidate the locations of lost ancient wooded sites, both of which are important for woodland restoration and connectivity. Further they provide scope for targeted field assessments of scarce or protected AWI species. When using such data, caution should be applied given the biases of biological recording and specimen collection. However, the unique value of old herbarium specimens outweighs these limitations. Future research into the application of old herbarium records to ecological management in other ecosystems and species groups is warranted.

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Appendix 1. Pre-1950 herbarium specimens for Vice-county 33 East Gloucestershire: Family, species and number of specimens, inclusive of duplicates. Total 305 specimens.

Family	Species	Number of pre-1950 herbarium specimens
Adoxaceae	*Adoxa moschatellina*	4
Ranunculaceae	*Anemone nemorosa*	10
Ranunculaceae	*Aquilegia vulgaris*	3
Woodsiaceae	*Athyrium filix-femina*	6
Blechnaceae	*Blechnum spicant*	1
Campanulaceae	*Campanula trachelium*	1
Brassicaceae	*Cardamine amara*	4
Brassicaceae	*Cardamine impatiens*	2
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