Revision of the emblematic Malagasy tree genus Tsebona (Sapotaceae) with the description of a second species

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Revision of the emblematic Malagasy tree genus Tsebona (Sapotaceae) with the description of a second species

Carlos G. Boluda, Roger Lala Andriamiarisoa, Yamama Naciri & Laurent Gautier

Abstract

BOLUDA, C.G., R.L. ANDRIAMIARISOA, Y. NACIRI & L. GAUTIER (2024). Revision of the emblematic Malagasy tree genus Tsebona (Sapotaceae) with the description of a second species. *Candollea* 79: 117–128. In English, English and French abstracts. DOI: http://dx.doi.org/10.15553/c2024v791a7

The Malagasy endemic genus *Tsebona* Capuron hitherto monotypic with a species from the northeast, was hitherto distinguished from all other *Sapotaceae* by two striking characters: the androecium with five bundles of three (sometimes four) stamens, and the seed scar covering more than 60 % of the seed surface. However, a recent collection from the southeast has a similar androecium, but a seed of a different shape and with a smaller scar covering c. 40 % of its surface. Morphological, geographical and genetic data confirm that this specimen represents a new "Critically Endangered" [CR] species of *Tsebona*, restricted to the southeast of Madagascar. We name it *T. mpanjaka* Boluda & L. Gaut. and provide a revision of the genus, including original line drawings, field photographs, and conservation assessments for the two species, as well as a distribution map. We also designate a lectotype for *T. macrantha* Capuron.

Résumé

BOLUDA, C.G., R.L. ANDRIAMIARISOA, Y. NACIRI & L. GAUTIER (2024). Révision du genre d'arbre emblématique malgache Tsebona (Sapotaceae) avec la description d'une deuxième espèce. *Candollea* 79: 117–128. En anglais, résumés anglais et français. DOI: http://dx.doi.org/10.15553/c2024v791a7

Le genre endémique malgache *Tsebona* Capuron, qu'on croyait monotypique avec une espèce du Nord-Est, se distinguait au sein des *Sapotaceae* par deux caractères importants: son androcée constitué de cinq faisceaux de trois (parfois quatre) étamines et la cicatrice de sa graine couvrant plus de 60 % de sa surface. Une récolte récente du Sud-Est présente un androcée similaire, mais une graine de forme différente avec une cicatrice recouvrant c. 40 % de sa surface. Les données morphologiques, géographiques et génétiques confirment qu'il s'agit bien d'une espèce nouvelle «En danger critique» [CR] du genre *Tsebona*, à distribution restreinte au sud-est de Madagascar. Nous nommons cette espèce *T. mpanjaka* Boluda & L. Gaut. et proposons une révision du genre avec dessins au trait, photographies de terrain et évaluation du risque d'extinction pour ces deux espèces, ainsi qu'une carte de distribution. Un lectotype est aussi désigné pour *T. macrantha* Capuron.

Keywords

SAPOTACEAE – *Tsebona* – Madagascar – Lectotypification – New species

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Introduction

Madagascar is one of the richest biodiversity hotspots of the world, harboring more than 12,000 native vascular plant species with 85% endemics (Gautier et al., 2022a). Its ancient isolation from Africa and Asia, estimated to have occurred 90 million years ago (Raval & Veeraswamy, 2003), produced a spectacular ratio of endemism not only at the species level, but also at the generic and even family levels (Callmander et al., 2011). The family *Sapotaceae* in Madagascar is not an exception with 99% of endemic species (Gautier et al., 2022b). Among the 10 genera present on the island, two are subendemic (*Labourdonnaisia* Bojer and *Labramia* A. DC., with representatives either in the Mascarenes or in the Comoros, respectively) and three are strictly endemic (*Bemangidia* L. Gaut., *Capurodendron* Aubrév., and *Tsebona* Capuron). These three genera are the unique members of the endemic tribe *Tseboneae* (Gautier et al., 2013). This tribe originated around 45–52 million years ago (Boluda et al., 2022), and its closest relative, *Northia* Hook. f., is endemic to the Seychelles (Boluda et al., 2023), suggesting a vicariant origin of the lineage following the tectonic plate movements (Warren et al., 2010).

The genus *Tsebona* was hitherto well characterized among all *Sapotaceae* by its polystemonous flowers with androecium arranged in five bundles of three to four stamens, and its seed scar covering more than 60% of the seed surface (Aubréville, 1974); neither of these character states have been observed in the related genera *Bemangidia* and *Capurodendron*. The only described species, *Tsebona macrantha* Capuron, reaching up to 35 m high, ranges among the tallest trees of Madagascar's lowland moist evergreen forests, and is distributed in the northeast. A recent collection from southeastern Madagascar with pre-anthesis flowers and mature fruits (*Andriamiarisoa & Randriatahina 2582*) matches the vegetative and floral characteristics of *T. macrantha* but has seeds with a different shape and a scar covering a surface clearly smaller than the testa.

Based on morphology, molecular data and geography, we demonstrate that the southern collection belongs to the genus *Tsebona* and represents an undescribed species.

Materials and methods

All *Tsebona* specimens deposited in G, K, MO, P, TAN, and TEF herbaria were examined, representing a total of 32 collections, 31 of which were identified as *Tsebona macrantha*. The collection *Andriamiarisoa & Randriatahina 2582*, with mature fruits and pre-anthesis flower buds, was the only one that could not be assigned to this species. It was selected for the phylogenetic reconstruction, together with three *T. macrantha* collections representing the variability of the species in terms of leaf shape, number of secondary veins, and indumentum density (*Randrianaivo & Boluda 3131*, *3149*; *Gautier et al. 5509*).

The phylogenetic reconstruction was adapted from that presented in Boluda et al. (2023). DNA was extracted using the CTAB method with chloroform (Russell et al., 2010; Souza et al., 2012), either from fragments of dry leaves sampled from herbarium specimens or from material collected in the field and dried in silica-gel. DNA fragment sizes for each extraction were estimated using a 2200 TapeStation (Agilent, Santa Clara, CA, U.S.A.).

Genomic libraries were prepared for each sample using the KAPA HyperPrep Kit (Roche, Basel, Switzerland), with a Bst Polymerase for Large Fragments (New England Biolabs, Ipswich, MA, U.S.A.) and labelling each specimen with dual indexed primers (Microsynth, Balgach, Switzerland; Christe et al., 2021). The constructed libraries were pooled together in equimolar proportions and 794 protein-coding genes were captured following the protocol of Christe et al. (2021), with a hybridization step with specific biotinylated oligonucleotide probes complementary to the targeted loci. Hybridized sequences were retained on streptavidin covered magnetic beads while all non-target DNA was washed away. Captured DNA was amplified in an 11-cycle PCR using Illumina primers provided by the KAPA HyperPrep Kit and sequenced in an Illumina HiSeq 4000 machine (2 × 100 bp paired-end). For more information about the laboratory and the bioinformatic pipeline see Christe et al. (2021).

From the 794 obtained protein-coding genes, we excluded those with putative signals of paralogy using HybPiper (Johnson et al., 2016), and retained 638 genes for the phylogeny. DNA sequences were aligned using MAFFT version 7 (Katoh & Standley, 2013), and positions with more than 20% of missing data were removed using trimAl v.1.4 (Capella-Gutiérrez et al., 2009).

The phylogenetic reconstruction was made generating one Maximum Likelihood gene tree for each of the selected loci using RAXML v.8.2.4 (Stamatakis, 2014), and then inferring the species tree using Astral-II, a method based on the multispecies coalescence (Mirarab et al., 2014; Mirarab & Warnow, 2015). To have an estimation of the genetic distance among clades (Astral-II does not display branch lengths as genetic distance), we performed an additional reconstruction using only RAXML on a concatenated dataset.

Results

The ASTRAL phylogenetic reconstruction is congruent for all supported nodes with the ones from the concatenated matrix using RAXML (Fig. 1). The genera *Bemangidia*, *Capurodendron*, and *Tsebona* are highly supported as monophyletic although their relationships are not well-resolved, as it was obtained in previous phylogenies (Christe et al., 2021; Boluda et al., 2022). *Bemangidia* appears unsupported as sister to *Tsebona* in ASTRAL, while it appears, with a moderate

support, sister to *Capurodendron* in RAXML. However, as data concatenation violates the assumption that different loci may have different evolutionary histories, ASTRAL results should be considered more accurate, reflecting uncertainty in the topology of the tree. Branch lengths among specimens are very similar in both phylogenetic reconstructions (Fig. 1). In both analyses, *Andriamiarisoa & Randriatahina 2582* is retrieved as sister to the three specimens belonging to *Tsebona macrantha*.

Morphologically, *Andriamiarisoa & Randriatahina 2582* presents glabrescent obovate leaves 14–19 cm long, with 12–14 secondary veins, while the common *Tsebona macrantha* phenotype has obovate to oblanceolate pubescent leaves 17–60 cm long, with 16–30 secondary veins. *Tsebona macrantha* can also display smaller obovate glabrous leaves, sometimes with less secondary veins, a phenotype that would embrace the leaf morphology of the new species. The flowers of *Andriamiarisoa & Randriatahina 2582* are nearly identical to those of *T. macrantha*, including the characteristic androecium arrangement in five bundles of three to four stamens, although they are slightly smaller in size and have staminodes with an entire margin (irregularly toothed or lobed in *T. macrantha*). Following our molecular results, another specimen has been collected at full anthesis, but not yet available to us. However, due to a field photograph, we can state that there is no significant difference between the size of the open flowers and those in a state of pre-anthesis.

The diagnostic character of the new *Tsebona* species is the seed shape and its scar extension. While in *Andriamiarisoa & Randriatahina 2582* the seed is narrowly ellipsoid with a scar occupying c. 40 % of the seed surface (restricted to the ventral side), in *T. macrantha* the seed is ovoid, with the scar occupying more than 60 % of the seed surface (including both the ventral and lateral sides and the margins of the dorsal area).

All known *Tsebona macrantha* specimens have been collected in the northern of the Eastern Phytogeographical Domain of Madagascar (sensu Humbert, 1955), between 14° to 19°S, whereas the single known individual of the new species was collected at 23°26'S in southeastern Madagascar.

Discussion

In the context of a revision of the family *Sapotaceae* in Madagascar, we frequently found distant phylogenetic clades containing specimens displaying very similar vegetative and floral characters but having different geographical distributions. In such cases of convergent evolution within *Sapotaceae*, seeds frequently provide informative characters for species delimitation, especially regarding the seed shape and size, and the shape and extension of the scar. These characters have been helpful to separate species morphologically similar in *Capurodendron* (Boluda et al., 2022, 2023), *Mimusops* L., and *Sideroxylon* L. (unpubl. data) and are also useful to circumscribe infrageneric clades (Boluda et al., 2022). This is also the case in *Tsebona*, where vegetative and most floral characters of *Andriamiarisoa & Randriatahina 2582* overlap with those of *T. macrantha*, though the seeds are clearly different.

The genus *Tsebona*, as circumscribed thus far, was well-characterized by its exceptional androecium arranged in five bundles of three to four stamens and the seeds with the scar occupying more surface than the testa (Capuron, 1962), a combination of characters not observed in other members of the family. However, the collection *Andriamiarisoa & Randriatahina 2582*, which displays the same androecium arrangement, produces seeds with the scar surface smaller than the testa as in other related taxa such as *Bemangidia lowryi* L. Gaut. and *Capurodendron* species. Based on this striking and distinctive seed character, we here describe the new species *Tsebona mpanjaka* Boluda & L. Gaut. Such taxonomic decision is congruent with the phylogenomic reconstruction obtained (Fig. 1), where the specimen is retrieved as sister to all *T. macrantha* accessions. We additionally provide a revision of *Tsebona* with both the description of the genus and *T. macrantha* amended.

Taxonomy

Tsebona Capuron in Adansonia, sér. 2, *2*: 122. 1962.

Typus: *Tsebona macrantha* Capuron

Trees up to 35 m high, more than 1 m DBH; bole generally straight, unbranched, slash with white latex, crown sparsely branched, usually displaying a marked Aubréville branching pattern. *Leaves* alternate, clustered at the twig apices, leaving conspicuous scars when shed; stipules present, rusty-pubescent, early caducous; petioles rusty-pubescent at least when young; lamina rusty-pubescent when developing, pubescent or glabrescent later, secondary veins regularly spaced, very prominent on the lower surface, usually rusty-pubescent, intersecondary veins absent, tertiary ones percurrent. *Flowers* axillary, among or below the leaves; pedicels rusty-pubescent, pendulous. *Calyx* quincuncial with 5 sepals, the three external ones much more coriaceous than the two internals, all densely tomentose dorsally, the involute margins of the external ones impressing a median groove on the internals. *Corolla* gamopetalous, whitish to cream; tube short, glabrous, with 5 lanceolate lobes contorted in bud, overlapping to the right. *Stamens* in five bundles of 3(–4), opposite to the corolla lobes; filaments short, attached at the top of the corolla tube; anthers of each bundle hirsute, connivent and united among them by the indumentum, narrowly lanceolate to sublinear, attached to the filaments at ⅕ of their length. *Staminodes* 5, alternipetalous, entire to irregularly lobed, bent over the ovary but not concealing it, with the tips arched up. *Ovary* superior, flattened. *Fruit* a large berry up to 10 × 11 cm, irregularly covered by brownish tomentum. *Seeds*

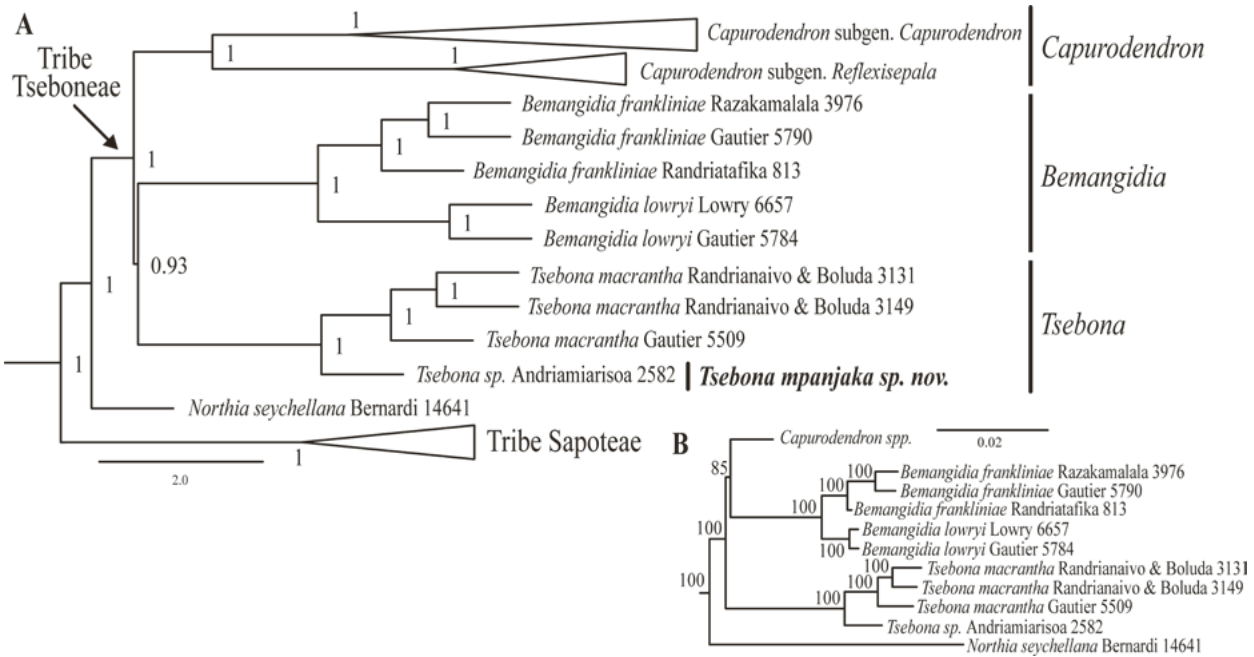

Fig. 1. – A. Phylogenetic tree reconstruction from ASTRAL using 638 protein-coding genes. Note that ASTRAL only calculates internal branch lengths and that tip lines are artificially fixed at the same length for all specimens. The node labels represent ASTRAL support values given as posterior probabilities (PP); **B.** Phylogenetic tree reconstruction from RAXML using the same dataset as ASTRAL but with all genes concatenated. The node labels indicate the bootstrap support. Collector numbers are indicated after the species name, except for *Capurodendron* and tribe *Sapoteae* for which they can be found in Boluda et al. (2022). BioSample number for sequence accessions are given in Boluda et al. (2022, 2023).

1–5(–6) per fruit, large, up to 6 cm long, with a broad ventral scar covering 40–80 % of seed surface.

Distribution and ecology. – *Tsebona* is a genus endemic to Madagascar, restricted to the lowland moist evergreen forests of the Eastern Phytogeographical Domain (sensu Humbert, 1955).

Key to the species of Tsebona

1. Seed more or less ovoid, 4.5–6 × 3.4–4 cm, with the scar ventral and lateral, occupying more than 60 % of the seed surface; lamina obovate to oblanceolate, 17–60 cm long, with (11–)16–30 pairs of secondary veins; northern half of eastern Madagascar ..... ***T. macrantha***

1a. Seed ellipsoid, c. 6 × 2–2.5 cm with the scar only ventral, occupying c. 40 % of the seed surface; lamina obovate, 14–19 cm long, with 12–14 pairs of secondary veins; southeastern Madagascar ..... ***T. mpanjaka***

Tsebona macrantha Capuron in Adansonia, sér. 2, 2: 122. 1962 (Fig. 2, 3).

Lectotypus (designated here): **Madagascar. Reg. Analanjirofo [Prov. Toamasina]:** Ambodirafia, [17°10'S 49°25'E], 75 m, 17.XII.1957, old fl. & fr., *Service Forestier 17875* (P [P04592812]!; isolecto-: K!, P [P04592808, P04592811]!). Holotypus: Reg. SAVA [Prov. Antsiranana]: Farahalana, Anolakely, Canton de Farahalana, Sambava, s.d., fl., *Service Forestier 7100* (P, not traced).

Trees up to 35 m high, more than 1 m DBH; bole straight, generally unbranched for ⅔ of its length, top sparsely branched, bark relatively thin, greyish brown, longitudinally fissured, inner bark yellowish to pale brown, external wood pinkish-brown, with white latex; branches displaying a marked Aubréville branching pattern with 1.3–3.5 cm diam. ultimate twigs, covered by a dense rusty tomentum when growing, soon glabrescent, greyish brown, with conspicuous scars of fallen leaves and flowers. *Leaves* alternate, clustered at the twig apices; stipules narrowly triangular, 6–12 × 2 mm, rusty-pubescent dorsally, pubescent to subglabrous ventrally, early caducous; petiole 40–100 × 2.5–5.0 mm, terete, rusty-pubescent, glabrescent when old; lamina obovate to oblanceolate, 17–60 × 6.5–20 cm, chartaceous to coriaceous, base cuneate, rounded or subcordate, apex rounded to retuse, upper surface glabrous at maturity, lower surface sparsely pubescent or glabrous at maturity; midrib even to depressed on the upper surface, strongly protruding on the lower surface, glabrous to pubescent; leaf venation brochidodromous to eucamptodromous, with (11–)16–30 regularly spaced secondary veins even to depressed on upper surface, strongly protruding on lower surface, densely rusty-tomentose to nearly glabrous, diverging from the midrib at an angle of 45°–70°, straight to arching

Fig. 2. – *Tsebona macrantha* Capuron. **A.** Trunk with scars made by local people to obtain latex, with CGB holding a fruit; **B.** A branch holded by R. Randrianaivo (MO); **C.** Brachyblast; **D.** Old flower with a petal removed showing the stamens; **E.** Ventral side of a corolla opened, showing stamens and staminodes; **F.** Fruits and seeds; **G.** Dorsal view of a seed showing the smooth testa; **H.** Ventral side of a seed entirely covered by the scar.
[**A, F:** *Randrianaivo & Boluda 3019*; **B:** *Gautier et al. 5509*; **C:** *Randrianaivo & Boluda 3139*; **D, E:** *Randrianaivo & Boluda 3131*; **G, H:** *Service Forestier 27675*] [Photos: **A, C–F:** C. Boluda; **B, G, H:** L. Gautier]

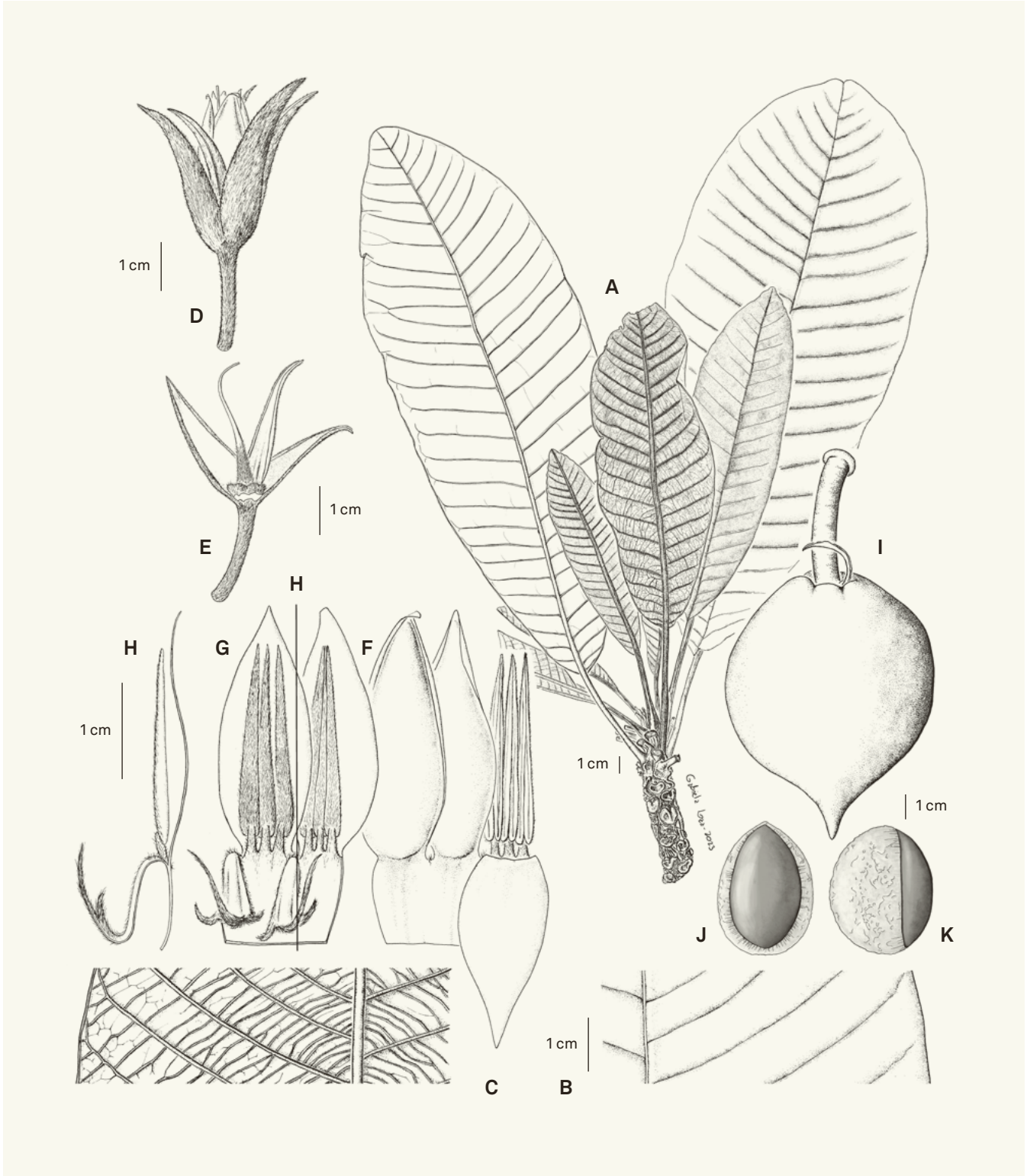

Fig. 3. – *Tsebona macrantha* Capuron. **A.** Twig apex; **B.** Leaf, adaxial side; **C.** Leaf, abaxial side; **D.** Flower, lateral view; **E.** Flower with two sepals and corolla removed showing the gynoecium; **F.** Corolla fragment, seen from outside; **G.** Corolla fragment, seen from inside; **H.** Corolla section showing lobe, stamen and staminode; **I.** Fruit**; J.** Seed, dorsal view showing the shiny portion of the testa; **K.** Seed, lateral view.
[**A–C:** *Randrianaivo & Boluda 3149*, G; **D–H:** *Randrianaivo & Boluda 3131*, G; **I:** *Randrianaivo & Boluda 3019*, G; **J, K:** *Service Forestier 27675*, P]
[Drawings: G. Loza]

upwards; intersecondary veins absent; tertiary veins percurrent, mainly composed of regularly spaced subparallel veins connecting the secondaries. *Flowers* axillary, among the leaves; pedicel rusty-pubescent, pendulous, 30–65 × 2–3 mm. *Calyx* quincuncial with 5 sepals, the three external ones broadly triangular, 26–36 × 8–13 mm at base, coriaceous, nearly navicular, densely tomentose dorsally, margins pubescent to glabrous, involute and impressing a groove on the internal sepals, yellowish-green below the trichomes, ventrally glabrous or with some sparse trichomes towards the margins, the two internal sepals subtriangular to ovate-lanceolate, with membranaceous margins, cucullate, tomentose on the external surface, with a median longitudinal groove, glabrous ventrally, yellowish-green. *Corolla* gamopetalous, whitish to cream; tube 4–12 mm long, glabrous, with five lanceolate lobes 20–24 × c. 8 mm, contorted in bud, overlapping to the right. *Stamens* in five bundles of 3(–4), opposite to the corolla lobes; filaments short, c. 3 × 0.8 mm, white, hirsute, attached at the top of the corolla tube; anthers of each bundle hirsute, connivent and united among them by the indumetum, narrowly lanceolate to sublinear, 15–17 × 1.5 mm long each, attached to the filaments at ⅕ of their length, base cordate, connective extending above the thecae in a c. 3 mm mucro. *Staminodes* 5, alternipetalous, white, c. 10 mm long, hirsute with a basi-central glabrescent sector, irregularly laciniate, with the central lacinia longer, narrowly triangular, 5–6 × 1.5–2.5 mm, bent inwards with the central lacinia arched up. *Ovary* superior, flattened, frequently 5-lobed, c. 9 mm diam., 1.5 mm high, hirsute; style 25–40 × 1–2 mm, whitish-green, hirsute towards the base, tapering towards the apex. *Fruits* borne on a c. 6 × 0.7–1 cm woody pedicel slightly inflated proximally, a large globose apiculate berry, frequently asymmetrical depending on the number of developed seeds, up to 10 × 11 cm, greenish-yellow, covered irregularly with a brownish tomentum; pulp light yellow to cream, with copious white latex especially at the base and the tip, inner pulp surrounding the seeds fibrous; calyx persistent or not. *Seeds* 1–5(–6) per fruit, globally ovoid in dorsal view but with shape affected by the number of seeds per fruit, 4.5–6 × 3.4–4.0 cm, dorsal side convex, ventral side frequently more flattened; testa shiny, only covering the central sector of the dorsal side, scar rugose, covering the remaining 60–80 % of the seed surface.

Vernacular names. – "Tsebo", "Tsebona", "Tsebona malotra", "Tsetsebona", "Taolandoha" (Capuron, 1962).

Uses. – According to local population, the sticky latex is used to catch birds and mice. It is usually extracted performing bark slashes, which explains why the trunks of *Tsebona macrantha* are often marked with numerous scars.

Distribution, ecology and phenology. – *Tsebona macrantha* ranges among the high canopy trees of the lowland moist evergreen forests of the northern half of the Eastern Phytogeographical Domain of Madagascar, from north of Sambava to Vatomandry, 14° to 19°S (Fig. 4). Flowers are reported from January to May and mature fruits from November to February.

Conservation status. – "Near Threatened" [NT] under Criterion B2ab(iii) (see Faranirina, 2021).

Notes. – The type collection (*Service Forestier 7100*) could not be traced in P. A search in the main other herbaria where specimens of the *Service Forestier de Madagascar* are generally deposited (G, K, MO, TEF) yielded no results. Likewise, four further collections cited by Capuron (1962), and also mentioned in the *Flore de Madagascar et des Comores* (Aubréville, 1974), are also missing: Mananara Nord NP, s.d., *Service Forestier 7-R-107*; Lohanantsahabe, s.d., *Service Forestier 51-R-188*; Masoala NP, s.d., *Service Forestier 84-R-140*; Somisika, s.d., *Service Forestier 173-R-199*.

According to ICN Art. 9.11 and 9.12 (Turland et al., 2018), a lectotype is therefore designated here among the located paratypes cited in the protologue. The specimen P04592812 corresponding to the collection *Service Forestier 17875* is designated here as the lectotype of the name *Tsebona macrantha* because it has old flowers and mature fruits; two duplicates are also kept at P and one at K.

Additional specimens examined. **Madagascar. Reg. SAVA [Prov. Antsiranana]:** Makirovana-Tsihomanaomby, 14°10'15"S 49°57'18"E, 400 m, 4.V.2010, fl., *Birkinshaw et al. 1825* (MO, P, TAN); Antanandavahely, Cap Est, 15°19'29"S 50°18'17"E, 120 m, 21.XI.2017, ster., *Gautier 6017 et al.* (G, TAN); ibid., 15°16'47"S 50°20'40"E, 290 m, 22.XI.2017, fr., *Gautier et al. 6032* (G, P); Sahafary, Cap Est, 15°17'S 50°22'E, 1997, imm. fr., *Randrianaivo & Bernard 116* (MO, P, TAN); Maroanbihy, [14°25'30"S 49°42'30"E], 14.XII.1961, buds & fr., *Réserves Naturelles 11760* (K, MO, P, TEF); Anoviara, [14°42'S 49°50'E], old fl. & imm. fr., *Service Forestier 10614* (P); Ambalanirana, [13°51'S 50°00'E], 9.VIII.1955, buds & fr., *Service Forestier 14252* (P, TEF); Ambatobiribiry, [14°11'S 50°06'E], 100–330 m, 9.IV.1967, fl. & fr., *Service Forestier 27675* (P, TEF). **Reg. Analanjirofo [Prov. Toamasina]:** Ambanizana, Masoala National Park, 15°37'54"S 49°58'39"E, 275 m, 30.XI.2010, fr., *Gautier et al. 5509* (G, K, MO, P); Fampanambo, Maroantsetra, 15°22'35"S 49°37'45"E, 100 m, V.1999, fl., *Rabenantoandro et al. 85* (G, K, MO, P, TAN); Mananara Nord National Park, 16°18'50"S 49°47'21"E, 289 m, 1.II.2018, fr., *Randrianaivo & Boluda 3019* (G, TEF); Distr. Toamasina II, Comm. de Mahavelona-Foulpointe, Fkt. de Morarano, forêt d'Analalava AP, 17°42'51"S 49°27'18"E, 54 m, 12.II.2018, fl., *Randrianaivo & Boluda 3131* (G, MO, P, S, TEF); Anena, [15°52'S 49°41'E], 27.I.1954, ster., *Service Forestier 8947bis* (P, TEF). **Reg. Atsinanana [Prov. Toamasina]:** Tamatave, VII.1894, ster., *Jaillet s.n.* (P); Corridor Ankeniheny-Zahamena, Ambalabe, 19°08'12"S 48°33'18"E, 19.I.2012, ster., *Rakotoarivelo et al. 601* (TAN); Betampona, 17°55'25"S 49°11'58"E, 400 m, 7.IV.2010, ster., *Rakotovao et al. 5201* (MO, TAN); Betampona, 17°55'28"S 49°12'00"E, 414 m, 16.II.2018, ster., *Randrianaivo & Boluda 3139* (G, TEF); Betampona, 17°54'59"S 49°12'03"E, 551 m, 16.II.2018, ster., *Randrianaivo & Boluda 3149* (G, TEF); Corridor Ankeniheny-Zahamena, 19°08'28"S 48°32'19"E, 23.IV.2017, imm. fr., *Razanatsima et al. 1672* (G, P, TAN); Betampona, [17°55'S 49°13'E], s.d., ster., *Service Forestier 8581bis* (P, TEF). **Sine loco:** s.d., seeds, *Service Forestier 12252* (P); s.d., seeds, *Service Forestier 34-R-197* (P); s.d., ster., *Service Forestier s.n.* (P).

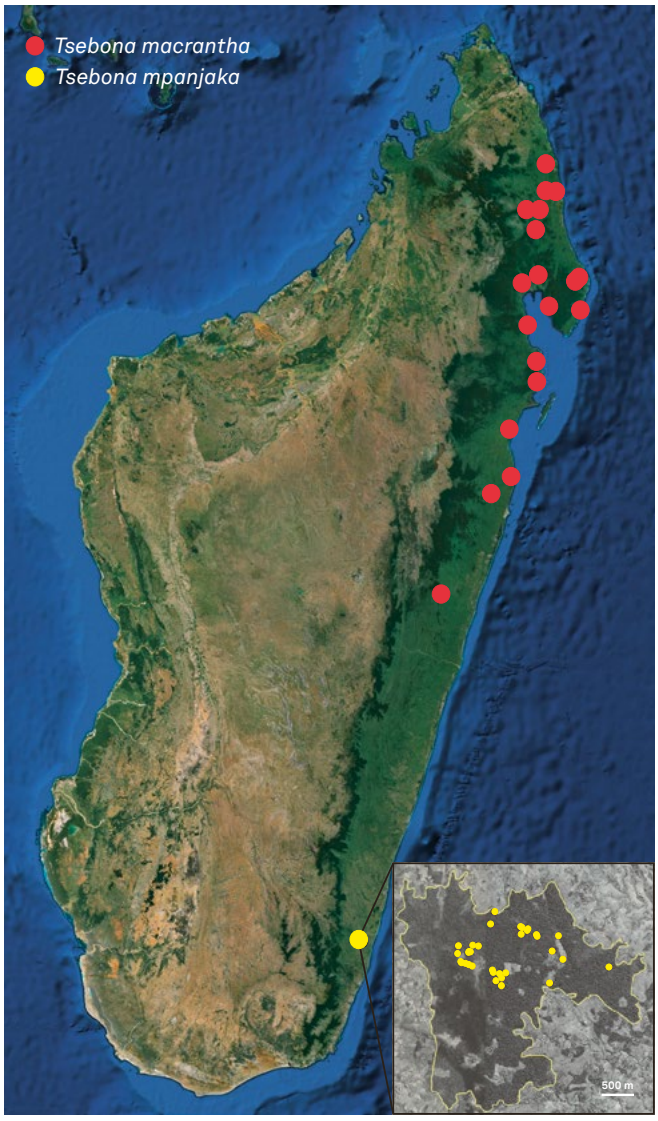

Fig. 4.– Map showing the known distribution of *Tsebona macrantha* Capuron (red dots) and *T. mpanjaka* Boluda & L. Gaut. (yellow dots; based on two collections plus a field census), with a zoom of the Agnakatrika forest, delimited by a yellow line, in the Ankarabolava-Agnakatrika Protected Area.

Tsebona mpanjaka Boluda & L. Gaut., **sp. nov.** (Fig. 5, 6).

Holotypus: Madagascar. Reg. Atsimo-Atsinanana [Prov. Fianarantsoa]: distr. Vaingaindrano, comm. Tsianofana, Fkt. Abaronga, village d'Androzabe, Agnakatrika, forêt d'Ankarabolava-Agnakatrika, 23°26'42"S 47°30'25"E, 124 m, 8.XII.2020, fl. buds & fr., *Andriamiarisoa & Randriatahina 2582* (G [G00390645]!, iso-: MO!, P!, TAN!).

Tsebona mpanjaka Boluda & L. Gaut. differs from the only other known species in the genus, T. macrantha Capuron, by its ellipsoid seeds c. 6 × 2–2.5 cm (vs. 4.5–6 × 3.4–4.0 cm), with a ventral scar not extending on the lateral and dorsal sides, covering less than 40% of the seed surface (vs. a ventral and lateral scar, covering more than 60% of the seed surface), and the lamina with 12–14 pairs of secondary veins (vs. (11–)16–30 pairs).

Trees up to 30 m high, 40 cm DBH; bole straight, unbranched for c. ¾ of its length, top sparsely branched, bark relatively thin, greyish, longitudinally fissured, external wood with white latex; branches displaying a marked Aubréville branching pattern with 3–4 cm diam. ultimate twigs, covered by a dense rusty tomentum when growing, glabrescent, greyish brown, with conspicuous scars of fallen leaves and protruding scars of fallen flowers and fruits. *Leaves* alternate, in clusters of 10–20 at the twig apices; stipules narrowly triangular to linear, 11 × 1.5 mm, hirsute dorsally with rusty 1.5 mm long trichomes, more or less glabrous ventrally, caducous; petiole 50–65 × 2.0–3.5 mm, swollen up to 7 mm at base, terete, rusty-tomentose with c. 1.5 mm long trichomes concealing the epidermis when young; lamina broadly obovate, 14–19 × 8–11 cm, chartaceous to coriaceous, base obtuse, apex rounded to retuse, upper surface glabrous at maturity, lower surface sparsely pubescent or glabrous at maturity; midrib even to depressed on the upper surface, strongly protruding on the lower surface, glabrous or pubescent; leaf venation eucamptodromous, with 12–14 regularly spaced secondary veins even to depressed on upper surface, strongly protruding on lower surface, rusty-tomentose when young, glabrescent, diverging from the midrib at an angle of 50°–60°, straight but arching upwards on the margin; intersecondary veins absent; tertiary veins percurrent with regularly spaced, sometimes bifurcating, subparallel veins connecting the secondaries. *Flowers* (description based on a pre-anthesis stage supplemented by field images of flowers at anthesis) axillary, among the leaves; bracts broadly ovate, 10 × 7 mm, hirsute on the external surface, glabrous on the basal half of the internal surface, hairy above; pedicel rusty-pubescent, pendulous, 30 × 4 mm. *Calyx* quincuncial with 5 sepals, narrowly triangular, coriaceous, 22–30 × 10–15 mm; the two external ones densely tomentose dorsally, with trichomes shorter than those of the pedicel, margins tomentose to glabrous, involute, impressing a groove on the internal sepals; ventrally glabrous except towards the tip; median sepal densely pubescent on the exposed segment of the dorsal surface, with plicate silvery trichomes, ventrally glabrous except towards the tip; the two internal sepals narrowly triangular to lanceolate, slightly smaller and thinner, with plicate silvery trichomes in the central portion of the dorsal surface, glabrous ventrally. *Corolla* gamopetalous, whitish to cream; tube c. 4–6 mm long, glabrous, with five lanceolate lobes c. 20 × 8 mm, contorted in bud, overlapping to the right. *Stamens* in five bundles of 3, opposite to the corolla lobes; filaments short, white, hirsute, attached at the top of the corolla tube; anthers of each bundle hirsute, connivent and united among them by the

Fig. 5. – *Tsebona mpanjaka* Boluda & L. Gaut. **A.** Trunk; **B.** Branch with a fruit; **C.** Branch with a fruit and flower buds holded by I. Armand (local field assistant); **D.** Flower; **E.** Twig apex with flowers; **F.** Longitudinal cut of a fruit showing the seeds; **G.** Lateral view of a seed; **H.** Ventral (covered by the scar) and dorsal (covered by the testa) view of the seed.
[**A–C, F–H:** *Andriamiarisoa & Randriatahina 2582*; **D, E:** *Randriatahina 320*] [Photos: **A–C**, **F–H:** R.L. Andriamiarisoa; **D, E:** J. Randriatahina]

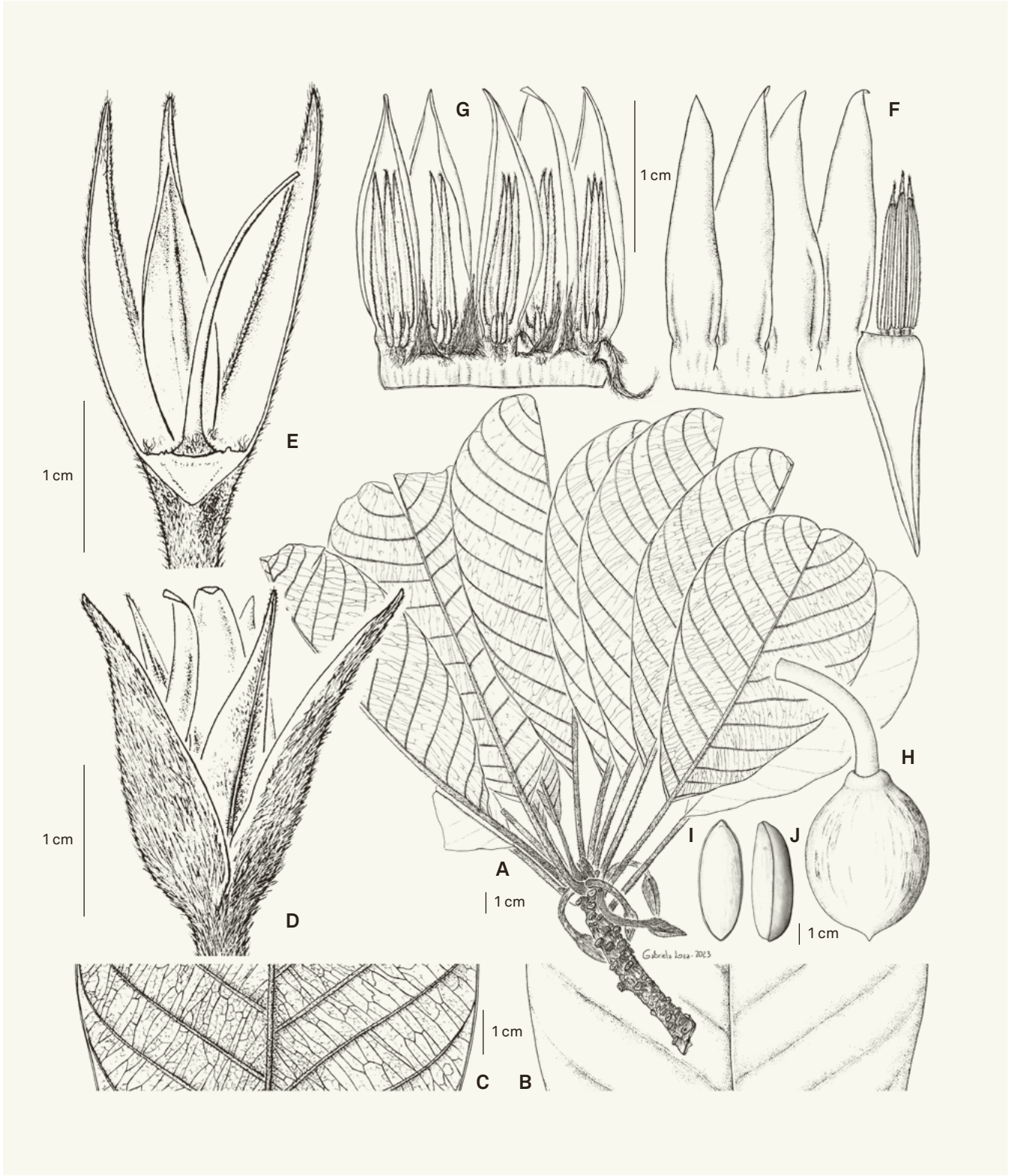

Fig. 6. – *Tsebona mpanjaka* Boluda & L. Gaut. **A.** Twig apex; **B.** Leaf, adaxial side; **C.** Leaf, abaxial side; **D.** Flower, lateral view; **E.** Flower with two sepals and corolla removed showing the ovary; **F.** Corolla, spread and seen from outside; **G.** Corolla, spread and seen from inside; **H.** Fruit; **I.** Seed, ventral side entirely covered by the scar; **J.** Seed, lateral view.
[*Andriamiarisoa & Randriatahina 2582*, G] [Drawings: G. Loza]

indumentum, narrowly lanceolate to sublinear, c. 12 × 1 mm, attached to the filaments at ⅕ of their length, base cordate, connective hirsute, extending above thecae in a c. 1 mm long mucro. *Staminodes* 5, alternipetalous, white, c. 6 mm long, hirsute, narrowly triangular, bent inwards with the tip arched up. *Developing ovary* superior, deeply 5-lobed, shortly pubescent; style c. 20 × 1.5 mm, whitish-green, pubescent at the base, tapering towards the apex, 5-fluted. *Fruits* borne on an enlarged c. 8 × 1 cm pedicel, an ellipsoid berry with an obtuse apex, 9 × 6 cm, covered irregularly with a brownish tomentum; calyx mostly caducous. *Seeds* up to five, narrowly ellipsoid, but with shape affected by the number of developing seeds, c. 6 × 2–2.5 cm, testa shiny, dark brown; scar rugose, beige, ventral throughout the whole length of the seed and covering c. 40 % of the seed surface.

Distribution, ecology and phenology. – *Tsebona mpanjaka* is known only from a single locality on southeastern Madagascar, in the lowland moist evergreen forests of Agnakatrika, in the Ankarabolava-Agnakatrika Protected Area in the Atsimo-Atsinanan region (Fig. 4). A field census was recently conducted in the Ankarabolava-Agnakatrika Protected area and revealed 38 individuals (19 adults 20 cm DBH or more, 13 subadults 10–20 cm DBH, and 6 juveniles < 10 cm DBH). The species is apparently absent from the most degraded parts of Agnakatrika as well as from Ankarabolava. Flowers are reported in July and December, and mature fruits in December.

Conservation status. – With an estimated extent of occurrence (EOO) of 1.65 km^2, and an area of occupancy (AOO) of 12 km^2 in a single location, *Tsebona mpanjaka* has been assessed as "Critically Endangered" [CR B1ab(i,ii,iii,v)+2ab(i,ii,iii,v)], following IUCN Red List Categories and Criteria (IUCN, 2012). It is reported to be frequently logged, and this is the major threat it faces, which might explain its absence in the more degraded southern portion of the protected area (Ankarabolava forest). Seeds have been collected and were grown in a local nursery in 2021 but have not been included in seedbanks so far. Besides, they are most probably recalcitrant, dying if dried.

Etymology. – The epithet *mpanjaka* means queen in Malagasy. With this epithet, we would like to honor the majesty of this tree by calling it the "Queen Tsebona".

Vernacular names. – "Avoa" (*Andriamiarisoa & Randriatahina 2582*), "Jambo" (*Randriatahina 320*).

Notes. – During the field census that was recently conducted by local staff in the protected area where *Tsebona mpanjaka* was discovered, a fertile tree with open flowers was located, collected and photographed, but unfortunately it was not available when the molecular sampling was conducted. Although the authors of the species have not seen the specimen, photographs of the living plant have been used to complement the description of the flower.

While assembling the information on the herbarium collections of *Tsebona*, we found in the Tropicos database [https://tropicos.org/specimen/100311870], a record of a collection labelled as "*Tsebona*" (*Rakotovao 5001*) collected 50 km north of Fort Dauphin, only c. 100 km south of the known locality of *Tsebona mpanjaka*. It is documented to be deposited in TAN herbarium, however it could not be traced either in the mounted collection or in the backlog of unmounted specimens. If the collection really corresponds to a *Tsebona* species, it is very likely to be *T. mpanjaka*. This would increase considerably its distribution toward the south and would entail changes in the conservation assessment.

Paratypus. – Madagascar. **Reg. Atsimo-Atsinanana [Prov. Fianarantsoa]:** distr. Vaingaindrano, comm. Tsianofana, Fkt. Agnalambaka, Androzabe village, Ankarabolava Agnakatrika Protected Area, 23°26'04"S 47°30'21"E, 130 m, 8.XII.2022, fl., *Randriatahina 320* (MO, P, TAN).

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