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The rediscovery of *Billburttia vaginoides*, with notes on the morphology, anatomy, traditional uses and conservation status of the genus *Billburttia* (Apieae, Apiaceae)

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Abstract

Recent field studies of the hitherto poorly known Madagascar-endemic genus *Billburttia*, including the rediscovery of the rare *B. vaginoides*, have resulted in a more complete understanding of the morphology, anatomy, ethnobotany and conservation status of the two aromatic shrubby species from Central Madagascar. *Billburttia capensoides* is relatively common and occurs in rocky places from Andringitra to Arivonimamo, while *B. vaginoides* is a rare species known only from around the Akaratra massif. The two species can easily be distinguished from one another by their habit (erect or spreading branches) and leaflet shape (obovate to linear-elliptic or filiform-linear). The leaves have numerous secretory canals; organic crystals occur in the epidermis, ground tissue and vascular bundles. Studies of fresh fruits confirmed the lack of wings, the position of the vascular bundles in the tips of the ribs and especially the reported presence of unusual acicular crystals in sheaf-like and spherical aggregates (not only in the epidermis, but also in groups close to the epidermis and around the vascular bundles). X-ray microanalysis showed that the crystals do not contain calcium, only carbon and oxygen, indicating that they are organic and precipitate during drying or fixation in alcohol. Updated descriptions of the morphology and anatomy are presented, together with a key to the species and notes on their traditional uses, conservation status and known geographical distributions.

Keywords: fruit anatomy, key, leaf anatomy, Madagascar, medicinal uses, stem anatomy

Introduction

The Apiaceae of Madagascar have remained poorly studied despite their importance in the evolutionary history of the family and their uses in traditional medicine (Van Wyk *et al.* 1999 & 2013, Van Wyk 2001). *Billburttia* Magee & B.-E.van Wyk (in Magee *et al.* 2009a: 241) is one of 11 Apiaceae genera endemic to Madagascar (Magee *et al.* 2009a), all of which belong to the subfamily Apioideae (Sales *et al.* 2004). Humbert (1956) placed these species in the genus *Peucedanum* Linnaeus (1753: 245). Later, morphological and anatomical studies showed that all Malagasy and African *Peucedanum* species are unrelated to Eurasian *Peucedanum* and were, therefore, accommodated in six African endemic genera of the tribe Tordylieae (Magee *et al.* 2008a,b & 2009a, Winter *et al.* 2008, Magee 2009). The two Malagasy peucedanoids, however, turned out to be unrelated, not only to *Peucedanum*, but also to all other African peucedanoids (Magee *et al.* 2009a).

Billburttia was therefore described, on the basis of morphological, anatomical and molecular data, as a distinct new genus of Apiaceae (Magee *et al.* 2009a, Van Wyk *et al.* 2013). The species closely resemble members of the South African genus *Notobubon* B.-E.van Wyk (in Winter *et al.* 2008: 355) (tribe Tordylieae), but molecular systematic studies (based on ITS and the rps16 intron) placed it firmly in the tribe Apieae (Magee *et al.* 2009b, Van Wyk *et al.* 2013). The remarkable superficial similarity between *B. capensoides* Sales & Hedge (in Magee *et al.* 2009a: 242) and *Notobubon* species such as *N. laevigatum* (Aiton 1789: 352) Magee (in Winter *et al.* 2008: 356) misled even experienced Apiaceae taxonomists such as the late Bill Burtt (for whom the genus was named), who considered the plant to be conspecific with *N. laevigatum*.

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Billburttia vaginoides Sales & Hedge (in Magee *et al.* 2009a: 243), a poorly known species thought to be possibly extinct (Van Wyk *et al.* 2013), has recently been re-collected for the first time since 1957. The availability of fresh vegetative and reproductive material allows for an improved description of the morphology and anatomy of this species and comparisons with *B. capensoides*, the only other species in the genus. The aim of this paper is also to describe the ethnobotanical uses of *Billburttia* species and to re-assess the conservation status, ecology and geographical distribution of the two species.

Materials and methods

Morphology:—Newly collected herbarium material was studied and compared with photographic images and specimens from the collections of BM, CNARP, E, K, MO, P, S and TAN (herbarium abbreviations according to the International Plant Science Center—Index Herbariorum, http://sweetgum.nybg.org/science/ih/). Voucher specimens are housed in TAN and CNARP—*B. capensoides*: Amboniriana-Morarano, district of Arivonimamo, *Rakotonandrasana & Ratrimosaona 1494*; *B. vaginoides*: Ankotrokotroka-Ramainandro, district of Faratsiho, *Rakotonandrasana & Ratrimosaona 1495*. Both species were studied and photographed in their natural habitats by the first author.

Anatomy:—Young stems, leaves and fruits of the two species were collected in Madagascar in October 2015 (vouchers specimens as listed above) and preserved in formaldehyde-acetic acid-alcohol (FAA). The material was infiltrated and embedded in glycol methacrylate as described in Feder & O'Brien (1968), except that the final infiltration was extended to five days. Transverse sections, about 3–5 µm thick, were cut in the middle of the structures using a Porter-Blüm ultramicrotome. Leaf and fruit sections were examined for the presence of crystals under an Olympus CX41 polarizing light microscope, after which they were stained according to the periodic acid Schiff/toluidine blue (PAS/TB) staining method of Feder & O'Brien (1968) and mounted in Entellan.

The morphology and chemical composition of the crystals occurring in plant tissues were investigated by scanning electron microscopy (SEM; TESCAN, soft—VegaTS) at the central analytical facility (Spectrum) of the University of Johannesburg. For SEM examination, the FAA-fixed samples were freeze-dried, mounted on aluminium stubs with double-sided carbon tape and coated with gold for additional analyses. Energy dispersive X-ray spectroscopy (EDS) was done on gold-coated samples to determine the elemental composition of crystals. Elemental and chemical analyses (EDS) of a sample by SEM were made using Oxford Instruments with AZtec software. The voltage for the EDS analysis was 20 kV.

Ethnobotany:—Voucher specimens and photographs were shown to three traditional healers, one traditional midwife and nine elderly persons living around the collection sites in order to record the local uses of the two plants. All informants were asked for their prior informed consent before starting interviews. This research was approved by the scientific committee board of the National Centre for Applied Pharmaceutical Research (CNARP) and a research permit (Permit No. 98/14/MEEF/SG/DGF/DCG.SAP/SCB) was issued by the Malagasy Ministry of the Environment, Ecology and Forests.

Conservation status assessment:—Conservation status was assessed using the IUCN Red List Categories and Criteria Version 3.1 (IUCN 2001).

Results and discussion

Characters and character states

Habit:—The two *Billburttia* species are both highly aromatic sprouting shrubs—they are not killed by fire, but resprout from a woody base (Fig. 1). Stems are unbranched except in the region of the inflorescence, and are blue-green or reddish and glabrous. *Billburttia capensoides* is an erect shrub with virgate stems up to 2.0 m tall, while *B. vaginoides* is a shrub or subshrub with spreading stems, 0.6 m (Magee *et al.* 2009a) to 1.5 m tall. Both species have vertical or oblique, thick to very thick woody rhizomes.

Stem anatomy:—Stems are glabrous, round to oval in transverse outline and not ribbed (Figs 2A1, 2B1). The cuticle is thick (ca. 4–7 µm). The epidermal cells are square to round or oval, with thickened outer and inner periclinal cell walls. The cortical collenchyma is in clusters of 3–10 cell layers, with lamellar outer layers and angular to lacunar inner layers, alternating with zones of chlorenchyma in 3–6 layers of isodiametric to slightly tangentially elongated cells. The cortical parenchyma consists of 4–7 layers of thin-walled isodiametric cells of 20–60 µm in diameter (up to

80 µm in *B. vaginoides*), with large intercellular spaces. Sheaf-like to spherical aggregates of acicular crystals occur in the epidermis, the cortical parenchyma and collenchyma, in phloem cells, as well as in intercellular spaces. Large secretory canals of 40–80 µm in diameter (up to 90 µm in *B. capensoides*) occur in the cortical parenchyma, associated with some vascular bundles and cortical collenchyma clusters. Solitary protophloem fibers are present in *B. vaginoides*, but not in *B. capensoides*. Vascular bundles are collateral. The pith consists of thin- to thick-walled isodiametric parenchyma cells of 40–80 µm in diameter (up to 130 µm in *B. vaginoides*), with large intercellular spaces. No crystals are present in the pith parenchyma cells. Medullary vascular bundles are absent. No secretory canals occur in the pith (Figs 2A1, 2B1).

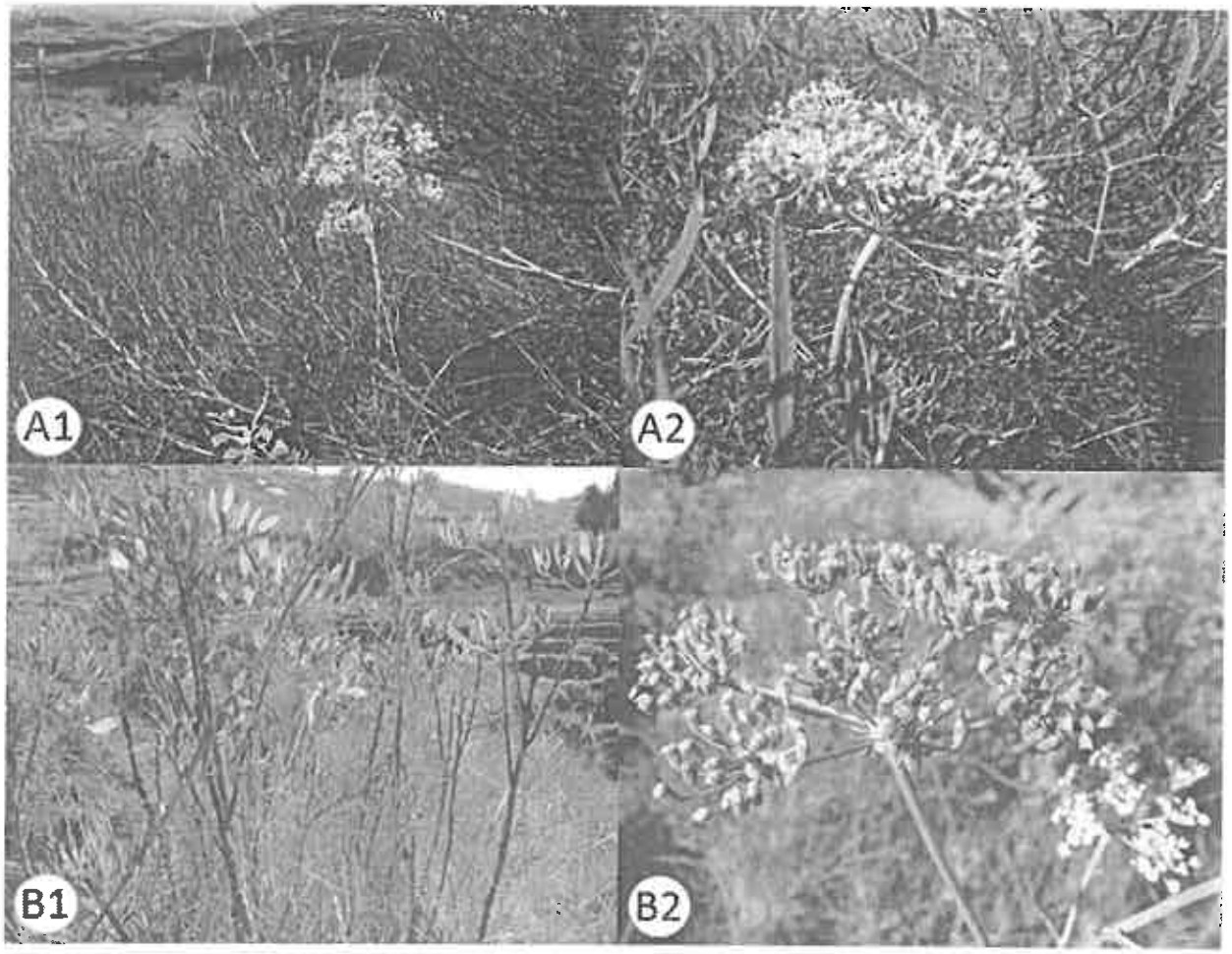

FIGURE 1. Habit, leaves and inflorescences of *Billburttia* species. A. *B. vaginoides*. A1. Spreading habit. A2. Leaves and inflorescence. B. *B. capensoides*. B1. Erect habit. B2. Leaves and inflorescence. Photographs by S.R. Rakotonandrasana.

Leaves:—Leaves are cauline, persistent and strongly aromatic. Petioles are terete, sheathing almost along their entire length, blue-green or reddish and glabrous. The lamina is 1- to 3-pinnate, concolorous, glabrous and glaucous, with the leaflets decreasing in size towards the apex. Leaflets are variable in shape (obovate, linear elliptic to elliptic or filiform-linear) and the margins are weakly serrate to entire. Leaflet bases are narrow to broad and cuneate and the apices are acute to mucronulate. There is only a single midrib (*B. vaginoides*) or three to five veins arising from the leaf base. They are less conspicuous on the lower side than on the upper side when fresh, but quite prominent on both sides when dry. The leaves differ from those of the African peucedanoid *Notobubon laevigatum* by the lack of a prominent midrib and well-developed lateral venation (Magee *et al.* 2009a).

Leaf anatomy:—Leaves are glabrous. Leaf blades are isobilateral, 300–600 µm thick, with palisade mesophyll beneath the upper and lower epidermis, usually also with spongy mesophyll in the innermost regions. The adaxial epidermal cells do not differ from the abaxial ones in shape and size; these cells are square to rectangular in transection (Figs 2A2, 2B2), and polygonal in surface view (Figs 2A3, 2B3), with straight to somewhat curved anticlinal walls, and slightly thickened outer and inner cell walls of 5–15 µm thick. The outer walls of the epidermal cells are covered by a cuticle ca. 2–4 µm thick. Hypodermal cells are absent. The palisade mesophyll consists mostly of two cell layers along

the abaxial and adaxial leaf sides in *B. capensoides*, and of one cell layer in *B. vaginoides*. Palisade cells are upright to long upright [height/width ratio 1.5 to 3 (up to 7)]. The spongy mesophyll is in 1–4 cell layers in *B. capensoides* and in 2–8 cell layers in *B. vaginoides*. Vascular bundles are sheathed by 1 or 2 layers of parenchymatous to slightly collenchymatous cells. Sheaths of neighbouring bundles are commonly interconnected by lateral extensions of larger (70–140 µm in diameter) parenchyma cells. In addition, abaxial and adaxial extensions are associated with the sheaths of larger vascular bundles. Vascular bundles are collateral, rounded, with somewhat tangentially extended phloem and xylem zones, usually accompanied with large secretory canals (35–80 µm in diameter in *B. vaginoides* and 45–90 µm in *B. capensoides*) on the abaxial side and with considerably smaller canals (20–30 µm in diameter in *B. vaginoides* and 35–50 µm in *B. capensoides*) associated with adaxial extensions (Figs 2A2, 2B2).

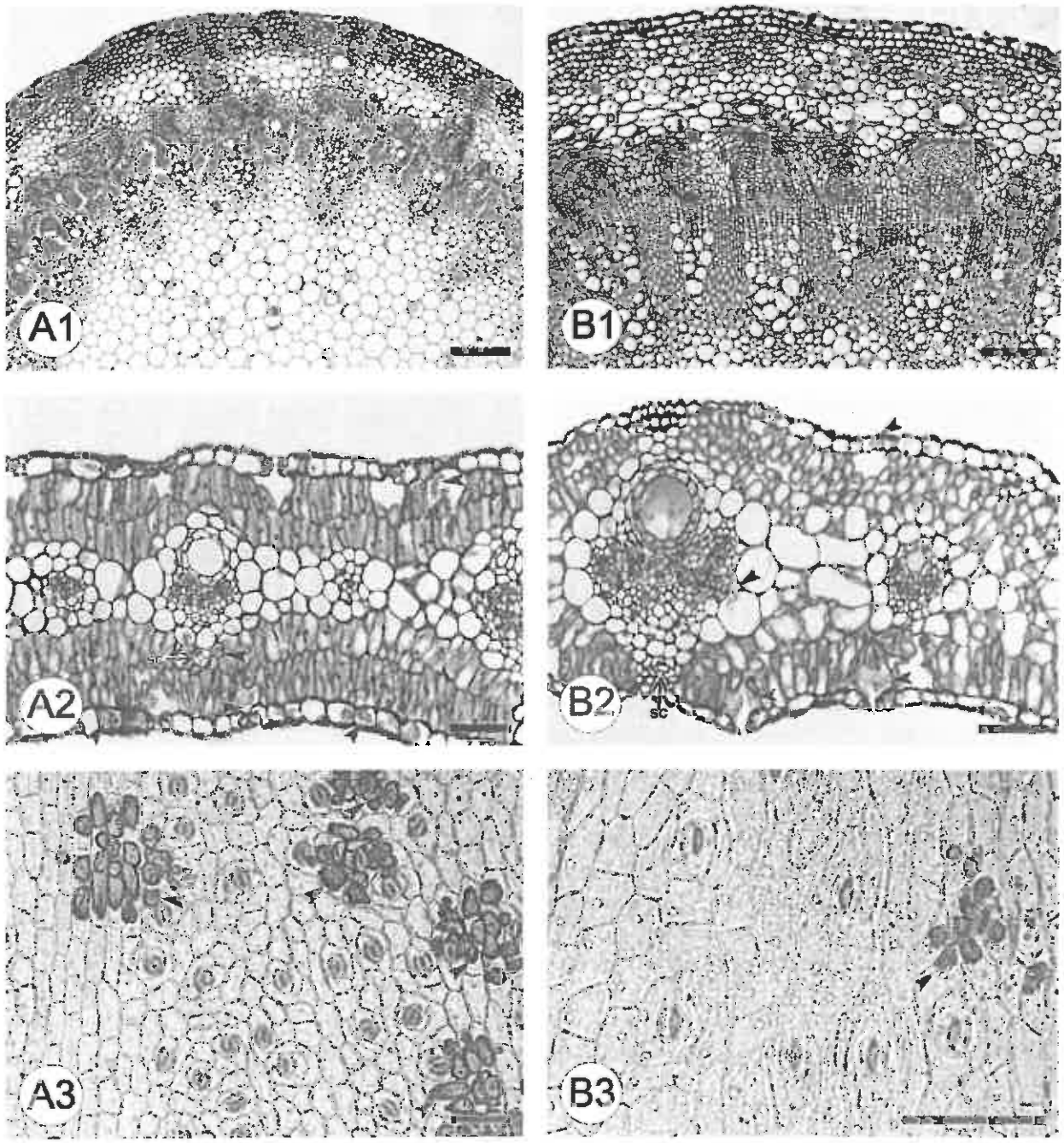

FIGURE 2. Stem and leaf lamina anatomy. A. *Billburttia capensoides* (*Rakotonandrasana & Ratrimosaona 1494*, TAN, CNARP). B. *B. vaginoides* (*Rakotonandrasana & Ratrimosaona 1495*, TAN, CNARP). A1 and B1. Transverse sections of stems; pf—protophloem fibers. A2 and B2. Transverse sections of leaf lamina; sc—small secretory canals associated with adaxial extensions of sheaths around vascular bundles. A3 and B3. Surface view of abaxial epidermis of leaf lamina. Black arrowheads mark the aggregates of acicular crystals. Scale bars: A1 = 500 µm; A2. B1, B2 = 200 µm: A3, B3 = 100 µm.

Stomata (Figs 2A3, 2B3) are anomocytic and situated mostly level with the outer epidermal cell walls. They are scattered and their density is nearly equal on both leaf surfaces, but they are distinctly more abundant in *B. capensoides* [59–80 (mean 69.3) per mm² on the adaxial side and 61–78 (mean 67.3) per mm² on the abaxial side] than in *B. vaginoides* [31–42 (mean 36.9) per mm² adaxially and 31–43 (mean 37.1) per mm² abaxially]. Sheaf-like and spherical aggregates of acicular crystals occur in the epidermis, the ground tissue and intercellular spaces, and in the xylem and phloem cells of the vascular bundles.

Petiolules (Figs 3A1, 3B1) are round to oval in transverse outline, not ribbed, and have an adaxial groove in *B. vaginoides*. The cuticle is thin (ca. 2–3 μm). Trichomes are absent. Epidermal cells are square to round or oval, and sometimes papillate with thickened outer periclinal cell walls. Stomata are only associated with the subepidermal chlorenchyma and are level with the surface. The subepidermal chlorenchyma is discontinuous, being interrupted with collenchyma, and consists of 3–5 layers of isodiametric to slightly radially elongated cells with large intercellular spaces. The collenchyma is in clusters of 5–11 cell layers, with lamellar outer layers and angular inner layers, usually associated with the vascular bundles. The ground tissue consists of isodiametric parenchyma cells without chloroplasts, 40–110 μm in diameter. Vascular bundles are arranged in one concentric circle of 9–11 bundles, with one or two medullary bundles. Peripheral bundles are collateral, with normal or inverted orientation, with a flat concave arc of phloem and cup-shaped xylem; sclerenchyma caps are absent. Medullary bundles are semi-amphivasal to amphivasal. Small (15–25 μm in diameter) secretory canals are present in the phloem; somewhat larger (30–50 μm in diameter) secretory canals occur in the ground tissue of *B. capensoides* and, rarely, in *B. vaginoides*. Sheaf-like and spherical aggregates of acicular crystals occur in the epidermal and ground tissue cells, and in the phloem and xylem cells of the conductive bundles.

Petiolar bases (Figs 3A2, B2) lack trichomes. Abaxial epidermal cells are rounded, sometimes papillate, and have evenly thickened walls; the cuticle is thin (ca. 2–3 μm). The abaxial subepidermal chlorenchyma is discontinuous, interrupted with collenchyma, and consists of 3–5 layers of isodiametric to slightly radially elongated cells. The abaxial collenchyma is in clusters of 3–8 cell layers, with lamellar outer layers and angular to lacunar inner layers, and is associated with the vascular bundles. Adaxial epidermal cells are flattened and rectangular in transection (10–20 μm in width), thin-walled, and without a cuticle. The adaxial collenchyma is in one lamellar to angular continuous layer of flattened to rectangular cells (15–30 μm in width). The ground tissue consists of isodiametric parenchyma cells without chloroplasts, the cells being 30–140 μm in diameter, with large intercellular spaces. Vascular bundles are collateral, with a flat concave arc of phloem and cup-shaped xylem, and lack sclerenchyma caps. Small (15–25 μm in diameter) secretory canals occur in the phloem; somewhat larger (20–50 μm in diameter) ones occur in the ground tissue. In addition, large secretory canals (40–90 μm in diameter) are arranged between the peripheral vascular bundles and the subepidermal collenchyma clusters. Sheaf-like and spherical aggregates of acicular crystals occur in the cells of the epidermis, ground tissue (collenchyma and parenchyma), phloem and xylem (Figs 3A2, B2).

Inflorescences and flowers

Synflorescences are glabrous and vary from compound umbels to panicles of compound umbels bearing yellow flowers (Fig. 1). The lateral umbels are in part functionally male.

Fruits

For descriptions of mature fruits see Magee *et al.* (2009a,b) and Van Wyk *et al.* (2013). Immature fruits in transverse section are slightly dorsally compressed and homomericarpic (Figs 3A3, 3B3). All the ribs are very well developed. The epidermal cells stain darker than the other cells and striae are already visible on the surface. The mesocarp is up to about nine cell layers thick and is parenchymatous. Endocarp cells are periclinally elongated. Developing vascular tissue occupies a prominent position in each rib. Rib oil ducts are absent. Each mericarp has about 11 conspicuous vittae of varying diameters (Figs 3A3, 3B3). Sheaf-like and spherical aggregates of acicular crystals are found in clusters in the mesocarp towards the epidermis (Fig. 4) and in the epidermis. The broad commissure is clearly evident.

Crystals

The occurrence of aggregated acicular crystals in the intercellular spaces and xylem cells (Figs 3A3, 3B3, 4) suggests that these crystals are formed by precipitation of organic substances interacting with ethanol used for fixation of plant samples. Similar artefacts have been reported by Kotina *et al.* (2015) in the bark tissues of some South African Fabaceae.

To confirm these observations, the elemental composition of the crystals from the mesocarp of fruits of both *Billburttia* species was also studied by X-ray microanalyses (EDS) (Fig. 5). This technique shows the elements that are

present and their relative proportions (but it should be kept in mind that the method is qualitative only and that relative proportions may be misleading, especially for biological samples). For the aggregated acicular crystals, EDS analysis confirmed that these crystals contain only carbon and oxygen, with no minerals or other heavier elements (excepting the gold used for coating).

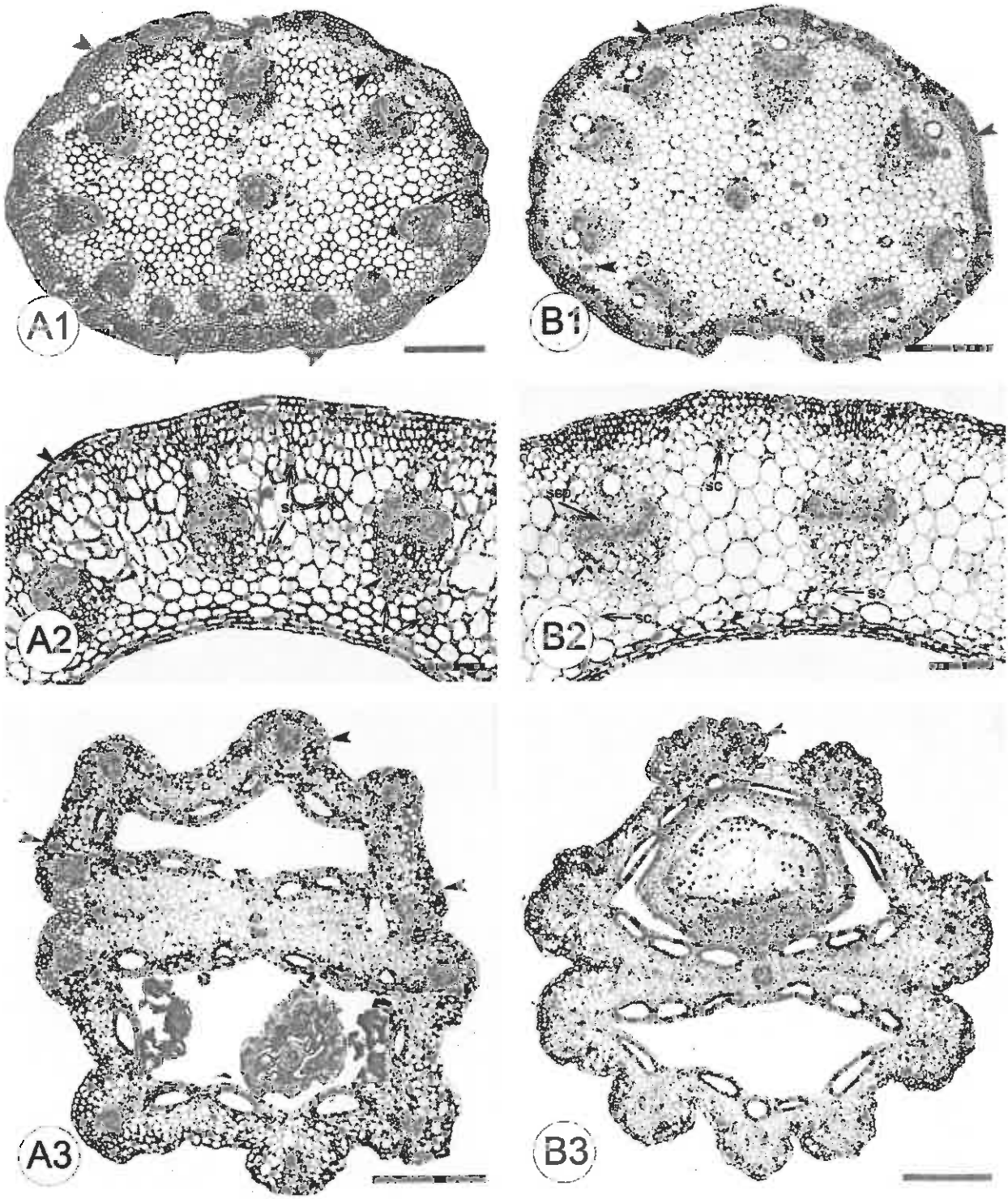

FIGURE 3. Petiole and fruit anatomy. A. *Billburttia capensoides* (*Rakotonandrasana & Ratrimosaona 1494*, TAN, CNARP). B. *B. vaginoides* (*Rakotonandrasana & Ratrimosaona 1495*, TAN, CNARP). A1 and B1. Transverse sections of petiolules. A2 and B2. Transverse sections of petiolar bases; sc—small secretory canals in ground tissue, scp—small secretory canal in phloem. A3 and B3. Transverse sections of fruits. Black arrowheads mark the aggregates of acicular crystals. Scale bars: A1, A3, B1, B3 = 500 μm; A2, B2 = 200 μm.

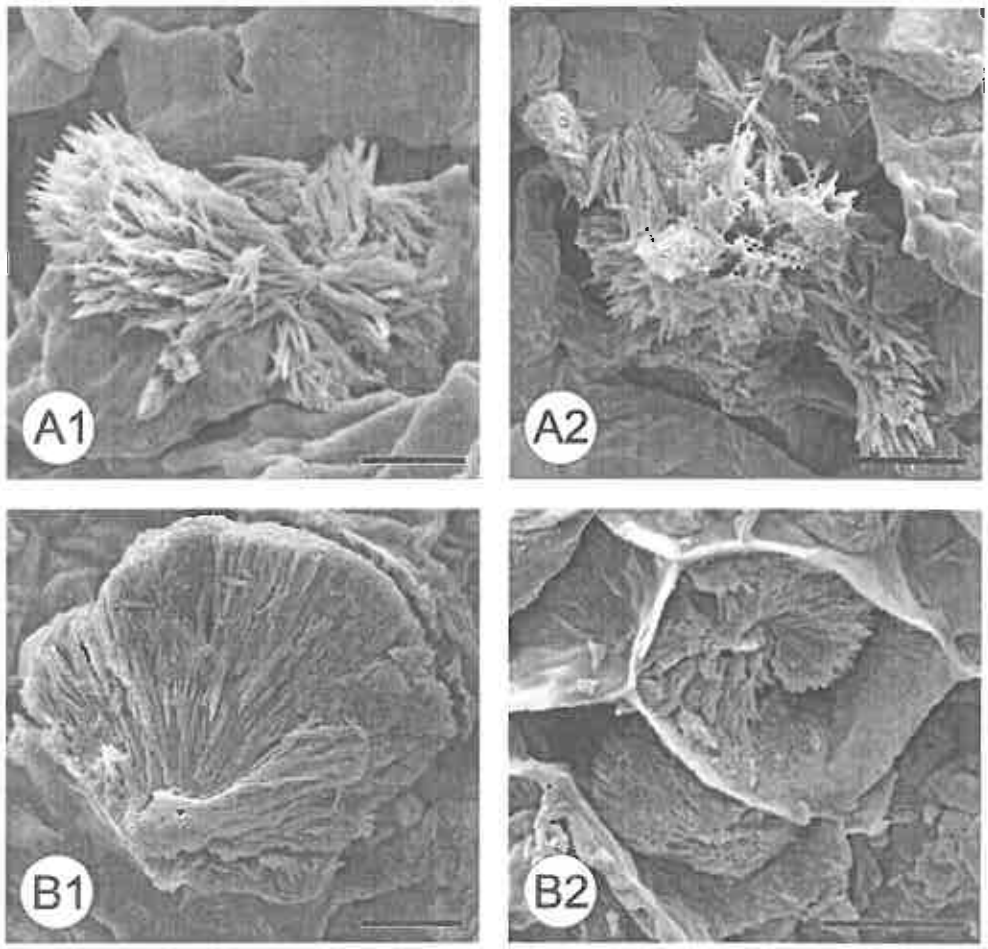

FIGURE 4. Scanning electron micrographs showing the morphology of aggregated acicular crystals in mesocarp cells. A1 and A2. *Billburttia capensoides* (*Rakotonandrasana & Ratrimosaona 1494*, TAN, CNARP). B1 and B2. *B. vaginoides* (*Rakotonandrasana & Ratrimosaona 1495*, TAN, CNARP). Scale bars: A1, B1 = 5 µm; A2, B2 = 10 µm.

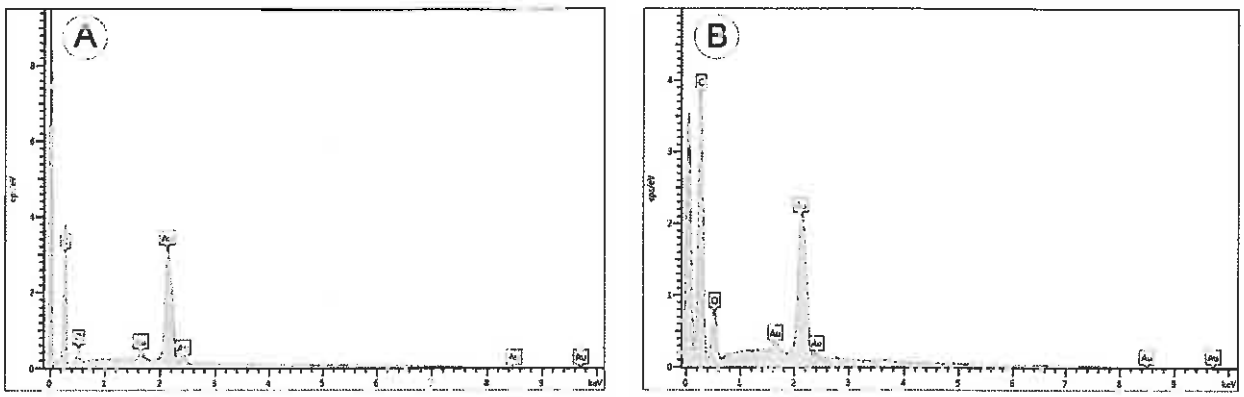

FIGURE 5. Elemental analysis of the aggregated acicular crystals in the mesocarp performed by energy-dispersive X-ray spectroscopy (EDS). The EDS spectra show only carbon (C) and oxygen (O) peaks, indicating organic crystals. A. *Billburttia capensoides*. B. *B. vaginoides*.

Taxonomy

Billburttia **Magee & B.-E.van Wyk** in Magee *et al.* (2009a: 223); Van Wyk *et al.* (2013: 114).

Type:—*B. capensoides* Sales and Hedge

A detailed description of the genus is given by Magee *et al.* (2009a).

Billburttia is a genus of two species, endemic to Madagascar and restricted to the central part of the country.

Diagnostic characters:—*Billburttia* can be distinguished from other peucedanoid species by its leaflets lacking a prominent midrib, but with well-developed lateral venation. Fruit characters are also distinct, especially the six commissural vittae (vs. the normal two), the ribs with vascular bundles located near the tips (vs. near the base of the ribs), and the presence of acicular crystals in sheaf-like and spherical aggregates. The width of the commissure is also a very important diagnostic character according to Magee *et al.* (2009a). It extends only to the base of each marginal wing/rib (and not to the tip of each wing as in peucedanoids).

Key to the species

1. Stems erect; leaflets broad, more than 4 mm (up to 15 mm) wide, obovate, linear-elliptic to elliptic, with 3 to 5 veins..... *Billburttia capensoides*
- Stems spreading; leaflets narrow, usually less than 2 mm (up to 4 mm) wide, linear-filiform, with a single vein ..... *Billburttia vaginoides*

***Billburttia capensoides* Sales & Hedge** in Magee *et al.* (2009a: 242); Van Wyk *et al.* (2013: 114).

Type:—MADAGASCAR, Ouest du massif d'Andringitra, Sept 1911, *Perrier de la Bâthie 6807* (holoype P!).

Shrub or subshrub. Stems 1–2 m, erect, c. 20 mm thick at base. Leaves 1- to 3-pinnate; rachis 60–150 mm; leaflets in 3–5 pairs; basal leaflets sometimes not divided, lateral leaflets elliptic to linear elliptic, 20–63(–70) × 5–15 mm, terminal leaflet obovate, 25–40 × 3–15 mm; margins weakly serrate; base narrow to broad and cuneate, with 3–5 basal veins; apex acute to mucronulate. Primary umbel 70–130 mm wide; rays 12–28(–40), 10–55 mm long; bracts 3–16 mm long; raylets 15–30, 4–8(–14) mm long; bracteoles 1.5–3.0(–12) × 1.0–1.5(–3) mm. Petals ovate, 2.0–2.1 × 1.1–1.5 mm; stamens up to 3 mm. Fruit ovate to narrowly ellipsoid, 3.5–4.5 × c. 1.5 mm, ribbed, but not winged.

Diagnostic characters:—*Billburttia capensoides* is distinguished from *B. vaginoides* by the erect (not spreading) habit and the much broader, obovate or linear-elliptic to elliptic shape of the leaflets, each with three to five well-developed basal veins (not linear-filiform, with a single midrib).

Distribution and habitat:—Known only from the central part of Madagascar, *B. capensoides* is apparently rare and localized. It occurs from Andringitra to the Arivonimamo massif, at elevations of 1 000–2 658 m (Fig. 6). The species grows in fertile red clay soil on volcanic rock or in infertile lateritic clay soil. The habitat is undisturbed areas in the rocky evergreen sclerophyllous woodland and open grassland, with *Leptolaena* Thouars (1807: 41, t11) spp., *Uapaca bojeri* Baillon (1874: 176) and *Helichrysum* Miller (1754: [462]) spp. It can also withstand low levels of anthropogenic disturbance and sometimes grows in open rocky grassland in competition with *Helichrysum* species.

Phenology:—Flowering occurs in July to September and fruiting from September to October.

Vernacular names:—*Tsilaninosy* (Bara dialect), *Hazompitatra* (Merina dialect).

Uses:—Our ethnobotanical survey showed that a leaf decoction is traditionally used to stimulate lactation and to treat abdominal pains. A decoction of the whole plant may also be inhaled to treat influenza and headache. Decoctions of the aerial parts are taken to prevent neonatal jaundice. According to Boiteau *et al.* (1999), a decoction of the plant is reputed to be effective in treating colic.

Conservation status:—Based on 14 specimens collected in 14 different localities, nine subpopulations were investigated, four of which are inside three protected areas. These are Andringitra National Park and two newly protected areas (Itremo and Manjakatompo). The extent of occurrence (EOO) area is 25 178 km² (IUCN 2001). However, subpopulations outside the protected areas are subjected to anthropogenic pressure (agriculture and grazing by livestock). In addition, the area of occupancy (AOO) is low (only 126 km²) (IUCN 2001). The proposed conservation status is NT (Near Threatened).

Specimens examined:—MADAGASCAR, Antananarivo: without locality, without date, *Baron 340* (P); Central Madagascar, Oct 1882, *Baron 2021* (BM); Faratsiho, Ankaratra, Jan 1955, *Bosser 7563* (P); Ankaratra, pente rocailleuse [rocky slope], 2200 m, Nov 1955, *Bosser 8623*, (P); Ankisatra, 27 May 1889, *Catat 420* (P); Station forestière de Manjakatompo [Manjakatompo Forest Station] near sommet Nosiarivo, in massif l'Ankaratra [Ankaratra massif], 27 Jan 1975, *Croat 28892A* (P); Massif du Tsiafajavona [Tsiafajavona massif], 2200–2600 m, 16 May 1938, *Decary 13400* (P); Nord Betsileo, Antsirabe [north of Betsileo, at Antsirabe], Aug 1880, *Hildebrandt 3571* (BM, K, P); Pentes nord de Vohitra, Antsirabe [north slope of Ivohitra, Antsirabe], March 1934, *Jard. Bot. Tananarive 82-1* (P); Tsiafajavona, 10 May 1938, *Jard. Bot. Tananarive 3644* (P); Env. d'Antsirabe, 1500 m, Oct 1913, *Perrier de la Bâthie 6792* (P); Manjakatompo Station Forestière [Manjakatompo Forest Station], 2320 m, 28 Oct 2003, *Phillipson, Schatz, Schmidt & Rabehevitra 5636* (P, TAN); Ambatobe, 6 km ouest de la ville de Manalalondo [6 km west of the town of

Manalalondo], 1685 m, 19 Sept 2015, *Rakotonandrasana et Ratrimosaona 1494* (TAN, CNARP); Ankaratra, *Rousson s.n. 1890* (P); Distr. Betafo, sommet du pic de Vohimalaza près Betafo [summit of Vohimalaza], 1700 m, 18 Nov 1912, *Viguier & Humbert 1357* (P); Distr. Betafo, dans la coulée de laves de l'Iantsifitra [in the lava flow of Iantsifitra], c. 1450 m, 18 Nov 1912, *Viguier & Humbert 1386* (P); Betafo, monts Vararata [Vararata mountains], c. 2000 m, 19 Nov 1912, *Viguier & Humbert 1617* (P); Prov. Itasy, distr. Kitsamby, sur le flanc ouest de l'Ankaratra, entre Ambatofotsy et le Tsiafajavona [on the western slope of Ankaratra, between Ambatofotsy and Tsiafajavona], c. 2200 m, 17 Nov 1912, *Viguier & Humbert 1727* (P); Antsirabe, Dec 1913, *Waterlot s.n.* (P). Fianarantsoa: 6 km ouest Itremo [6 km, west of Itremo], 14 Feb 2009, *Andriamahay & Rakotoarisoa SNGF 2219* (TAN); Ambatofinandrahana, sur la valée d'Ananiloha [in Ananiloha valley], 1475 m, 7 Feb 2001, *Andriamihajarivo et al. 37* (TAN); Ambositra to Ambatofinandrahana on R.N. 35, 1600 m, 11 March 1992, *Clement, Phillipson & Rafamantanantsoa 2009* (TAN); Itremo, Vicinity of col de l'Itremo, massif de l'Itremo [Itremo massif], 1300–1400 m, 27 Jan 1975, *Croat 29892* (P, TAN); Route de l'Itremo [on the way to Itremo], 7 July 1966, *Debray 2015-D* (CNARP); Environs d'Ambatofinandrahana [around Ambatofinandrahana], 1600–1700 m, 17 Feb 1938, *Decary 13035* (P); Ambositra, dans les bois de Tapia [in the Tapia wood], 2 Feb 1942, *Decary 17248* (P); Ambatofinandrahana, 3 Feb 1942, *Decary 17356* (P); East margin of Itremo massif, 1290 m, 24 Nov 1993, *Du Puy, Labat & Andriantiana M660* (P, TAN); Andringitra, plateau d'Andohariana [Andohariana plateau], c. 2000 m, 14 Nov 1970, *Guillaumet 3588* (P); Andringitra, Riambavy, près du cascade [next to the waterfall], 2000 m, 1934–1935, *Heim s.n.* (P); Riviere Menaloha [Menaloha River], 11 Dec 1944, *Homolle s.n.* (P); Env. de Miarinarivo, SE d'Ambalavao [Around Miarinarivo, south eastern of Ambalavao], 1000–1400 m, 23 Nov 1923, *Humbert 3634* (P); Env. d'Ambatofinandrahana [Around Ambatofinandrahana], 1400–1500 m, 16 Jan 1955, *Humbert & Capuron 28129* (P); Env. d'Ambatofinandrahana [around Ambatofinandrahana], March 1960, *Keraudren 218* (P); W du massif d'Andringitra [west of Andringitra massif], 1000 m, Sept 1911, *Perrier de la Bathie 6807* (P); Itremo massif, W of Ambatofinandrahina, along road to Col d'Itemo, 1540 m, 9 Nov 2002, *Porter P. Lowry II et al. 5841* (TAN); Réserve Naturelle Intégrale no. 5, près de la montagne rocheuse Vangomena, plateau de Sonindrana [near the rocky mountain Vangomena, Sonindrana plateau], 2010 m, 23–24 Sept 1994, *Rakotovao 52* (P, TAN); Itremo, 7 June 1966, *Rakotozafy 631* (P, TAN); Ambalavao, Sendrisoa, Antanifotsy, pic Boby d'Andringitra [Boby summit of Andringitra], 2658 m, 29 Oct 1994, *Razafindrabe 193* (P, TAN); Imaitso, June 1973, *Razafindrambao 824* (CNARP); Distr. Ambalavao, canton Sendrisoa [District of Ambalavao, Sendrisoa], 2278 m, 20 Oct 1957, *Resérves Naturelles 2278* (P); Eastern margin of the Itremo massif, 1580–1700 m, 10 March 2000, *Schatz et al. 3962* (TAN).

Billburttia vaginoides **Sales & Hedge** in Magee *et al.* (2009a: 243); Van Wyk *et al.* (2013: 114).

Type:—MADAGASCAR, Flanc E du Mt. Tsiafajavona, March 1921, *Perrier de la Bâthie 13557* (holotype P!; isotype P!).

Shrub or subshrub. Stems spreading, 1.0–1.5 m, c. 15 mm thick at base. Leaves 1- to 3-pinnate; rachis 70–130(–140) mm; leaflets in 4–6 pairs; basal leaflets always divided, lateral leaflets linear, 25–45 × 2(–4) mm, terminal leaflet linear (15–)20–35 × 1.5–2.0(–4) mm; margins weakly serrate; base narrow to broad and cuneate; with a single midrib; apex acute to mucronulate. Primary umbel 50–100 mm wide; rays 12–30(–80), 15–50 mm long; bracts 3–15 mm long; raylets 15–30, 4–12 mm long; bracteoles 1.5–10.0 × 1–2 mm. Petals ovate, 1.8–2.0 × 1.0–1.2 mm; stamens 2.5–4.0 mm. Fruit narrowly ellipsoid, 3.0–3.5 × 1.5 mm, prominently ribbed, but not winged.

Diagnostic characters:—*Billburttia vaginoides* is easily distinguished from *B. capensoides* by the spreading habit, and the linear-filiform leaflets occurring in 4 to 6 pairs, each with a single vein (midrib).

Distribution and habitat:—The species is stated to be in danger of extinction, according to notes by Perrier de la Bâthie on his specimen collected on Ankaratra summit in 1921. The last collection cited by Magee *et al.* (2009a) was made in 1939 and is housed at the Paris herbarium (P). A full investigation at the TAN herbarium showed that Bosser deposited a specimen of that species collected around Behenjy in 1957. The species was recently rediscovered by botanists of CNARP during field collecting of aromatic plants around Arivonimamo. One of us (BEVW) helped to identify the specimen. The field work revealed that only a few individuals are currently known to occur at Ambohitrambo (Arivonimamo district) and at Ankotrokotroka (Faratsiho district) (Fig. 6). The habitat at Arivonimamo and Behenjy comprises rocky, evergreen, sclerophyllous woodland, which is highly disturbed and currently dominated by *Leptolaena pauciflora* Baker (1883: 96) and *Uapaca bojeri.* However, at Faratsiho, *B. vaginoides* grows in volcanic soil in open, rocky grassland.

Phenology:—Flowering occurs in April to June; fruiting in May to August.

Vernacular name:—*Volotaratsintsina* (Merina Dialect).

Uses:—According to local people, the plant is used to reduce fever, and decoctions of the stems and leaves are taken to treat convulsions. A decoction of the plant is said to be soporific (i.e. it induces drowsiness or sleep).

FIGURE 6. The known geographical distribution of *Billburttia capensoides* (dots) and *B. vaginoides* (triangles).

Conservation status:—The species is rare and was previously known only from a few herbarium collections made between 1890 and 1957. Based on five specimens collected in five different localities, five subpopulations were investigated and all of them occur outside protected areas. The extent of occurrence (EOO) is 2081 km² and the area of occupancy (AOO) is 45 km² (IUCN 2001). The total known population is less than 250 mature individuals. Populations are subjected to anthropogenic pressure, including agriculture, grazing by livestock and wildfires. The proposed conservation status is CR (Critically Rare) [c2 (aii)]. Conservation of the natural ecosystem in which this species occurs should be encouraged. Transplantation of seedlings into protected areas is needed in order to improve seedling survival and increase the chance of future successful germination. *Ex situ* cultivation and research on propagation are also of great importance.

Specimens examined:—MADAGASCAR, Antananarivo: Prov. Emirina, without date, *Bojer s.n.* (P); Route du sud, pseudo steppe sur rocaille [on the way to the south, pseudo steppe on rock], June 1957, *Bosser 13204* (TAN); Vallée de l'Ikopa NO d'Ankazobe [Ikopa valley, northwest of Ankazobe], 13 March 1930, *Decary 7564* (P); Tampoketsa, au NE de Fenoarivo [Tampoketsa, northeast of Fenoarivo], 16 March 1930, *Decary 7583* (BM, P); Behenjy (Imerina) sur rocaille humide [on wet rock], 11 March 1930, *Decary 13893* (P, TAN); Ambohimasimbola, 27 April 1939, *Jard. Bot. Tananarive 4261S* (P); Ankaratra, vers 2200 m, April 1914, *Perrier de la Bâthie 6795* (P); Mt Tsiafajavona, 2000 m, April 1914, *Perrier de la Bâthie 6799* (P); Flancs est de mt Tsiafajavona, vers 2200 m, type de l'espèce [east slope of Tsiafajavona mountain, around 2200 m, typus], April 1914, *Perrier de la Bâthie 13557* (P); Ambohitrambo, 8 km au N d'Arivonimamo [Ambohitrambo, 8 km north of Arivonimamo], 1406 m, 13 Aug 2014, *Rakotondrafara et al. 1424* (TAN, CNARP); Ankotrokotroka, Faratsiho, 1454 m, 19 Sept 2015, *Rakotonandrasana et Ratrimosaona 1496* (TAN, CNARP); Région centrale d'Ankaratra [Central region of Ankaratra], *Rousson s.n.* 1890 (P).

Conclusion

Billburttia is a poorly known genus of two aromatic and medicinal plant species restricted to the central part of Madagascar. Medicinal uses and some vernacular names are recorded for the first time. Anatomical details of the stems, leaves and fruits are presented, based on recent collections. The known geographical distribution of the two species has been mapped, after carefully verifying the localities. *Billburttia vaginoides,* previously considered to be possibly extinct, has been rediscovered more than 50 years after the last collection in 1957. In contrast to *B. capensoides*, which is a relatively common species, *B. vaginoides* is rare and occurs only outside protected areas. It is threatened with extinction and conservation measures should be implemented as a matter of urgency.

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