ORIGINAL ARTICLE

Billburttia, a new genus of Apiaceae (tribe Apieae) endemic to Madagascar

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Received: 27 January 2009/Accepted: 14 September 2009/Published online: 9 October 2009 © Springer-Verlag 2009

Abstract The genus *Billburttia* is described to include two new species endemic to Madagascar, B. capensoides and B. vaginoides. Both species were tentatively placed within the problematic genus Peucedanum L. (as Peucedanum sp. A and Peucedanum sp. B) based on their dorsally compressed fruits lacking prominent dorsal ribs and with winged marginal ribs. Recently, however, the African members of *Peucedanum* have been shown to be only distantly related to the type of the genus and have therefore been segregated into six African endemic genera. While the Malagasy species appear superficially similar to members from one of these segregate genera, viz. Notobubon, they differ markedly in their fruit anatomical characters, notably a narrower commissure, six commissural vittae, vascular tissue in the tip of the ribs and sphaerocrystals distributed in and around the epidermis. The latter two characters apparently represent apomorphies for

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S. R. Downie Department of Plant Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA *Billburttia.* The non-peucedanoid affinity of the genus, as suggested by the fruit anatomical data, was confirmed using ITS and *rps16* intron sequences. Both parsimony and Bayesian analyses of these data place *Billburttia* within the tribe Apieae of subfamily Apioideae and not closely related to either *Peucedanum* (Selineae) or the African peucedanoid genera (*Lefebvrea* clade of Tordylieae).

Keywords Billburttia capensoides ·

Billburttia vaginoides · Fruit anatomy · ITS · New genus · New species · *Peucedanum · rps16* intron · Sphaerocrystals

Introduction

Although the family Apiaceae is relatively poorly represented in Madagascar [15 genera (11 endemic) and 31 species (10 endemic)], many of the Malagasy taxa represent important components of early diverging lineages of subfamily Apioideae (Van Wyk et al. 1999; Calviño et al. 2006; Calviño et al., in preparation). These species display a diverse range of unusual habit and fruit anatomical characters (Sales et al. 2004). Earlier treatments under-estimated the total number of endemic genera, largely due to inadequate material, and many of the isolated Malagasy species had been included in African genera. Humbert (1956) placed eight of the woody Malagasy species in the arboreal African genus Heteromorpha Cham. and Schltdl. However, after extensive morphological and anatomical studies, they were later excluded from Heteromorpha (Winter et al. 1993; Winter and Van Wyk 1994, 1996) and largely accommodated in four new Malagasy endemic genera (Van Wyk et al. 1999), with one of the species transferred to the tropical African genus Pseudocarum C. Norman. Sales and Hedge (2009) recently described five new Malagasy species. Two of these species were tentatively placed within the problematic genus Peucedanum L. (Sales et al. 2004; "Peucedanum sp. A" and "Peucedanum sp. B"). The former species, described later, has been relatively well-collected but has consistently been misidentified as part of the "Peucedanum capense (Thunb.) Sond. Complex" (Burtt 1991) due to surprising morphological similarities. Recent detailed studies on African Peucedanum have shown that all the species on the continent are unrelated to Eurasian Peucedanum and have, therefore, been accommodated in six African endemic genera (Magee et al. 2008a, b, 2009a; Winter et al. 2008). As a result, the species in Madagascar represented the last remaining challenge to the so-called Peucedanum problem in the Afro-Malagasy region. The goals of this paper are therefore to determine the phylogenetic position and generic affinities of the as-yet-undescribed Malagasy peucedanoid species using fruit anatomical and DNA sequence data and to formally describe these two species.

Materials and methods

Taxonomic study

Herbarium specimens from the collections at BM, E, K, MO, P and S were studied. Line drawings were made by the first author with the aid of a camera lucida attachment on a Zeiss compound microscope or a Wild M3Z stereomicroscope.

Fruit anatomy

Fruits from two specimens of Peucedanum sp. A (Humbert 3634, P; Rakotozafy 631, P) and one specimen of Peucedanum sp. B (Decary 7583, P) were rehydrated for anatomical study. Following rehydration, the fruits were fixed in FAA for a minimum of 24 h and subsequently embedded in glycol methacrylate (GMA) according to a modification (final infiltration in GMA of 5 days) of the method of Feder and O'Brien (1968). Transverse sections of about 3 µm thick were made using a Porter-Blüm ultramicrotome. These sections were examined for the presence of crystals using a light microscope, after which they were stained according to the periodic acid Schiff/toluidine blue (PAS/ TB) staining method of Feder and O'Brien (1968). To study the three-dimensional structure of the vittae, boiling water was poured over the fruits which were then left to cool and soak for 24 h. The exocarp was peeled off while keeping the fruit submerged in water to prevent desiccation.

Molecular data

Total DNA was extracted from herbarium specimens of *Peucedanum* sp. A (three specimens) and *Peucedanum* sp.

B (one specimen) using the PureLink Plant Total DNA Purification Kit (Invitrogen, Carlsbad, CA, USA). For amplification of the nuclear ribosomal DNA internal transcribed spacers (ITS) and chloroplast DNA rps16 intron region, we used the primers and amplification methods of Downie and Katz-Downie (1996, 1999). Successfully amplified PCR products were purified according to the ExoSAP protocol of Werle et al. (1994) using 5 U of Exonuclease I (New England Biolabs, Ipswich, MA, USA) and 0.5 U of Shrimp Alkaline Phosphatase (Promega, Madison, WI, USA). Sequencing reactions were carried out using the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems) and sequenced using either an ABI (Applied Biosystems) 3130 XL or 3730 XL sequencer. Complementary strands were assembled and edited using Sequencher version 3.1.2 (Gene Codes) and manually aligned in PAUP* (Swofford 2002), positioning gaps so as to minimise nucleotide mismatches. Sources of material used in the study are provided in the Appendix. All newly obtained sequences have been deposited in GenBank.

Phylogenetic analyses

To assess the phylogenetic positions of the two species, newly obtained ITS (three accessions of Peucedanum sp. A and one accession of Peucedanum sp. B) and rps16 intron (two accessions of Peucedanum sp. A) sequences were incorporated into the respective ITS and rps16 intron data matrices of Magee et al. (2009b). These matrices include representatives of most of the relevant major clades of the "apioid superclade," including a representative sampling of the Lefebvrea clade (Magee et al. 2009b), which comprises many African peucedanoid genera (Winter et al. 2008). Preliminary analyses indicated an affinity between the two new Malagasy species and the tribe Apieae, therefore all available accessions of the latter were also included. The trees were rooted with taxa from tribes Smyrnieae and Oenantheae based on the results of Downie et al. (2001). In total, 150 accessions of ITS and 60 accessions of rps16 were considered for the phylogenetic study. Data sets are available on request from the corresponding author. Parsimony analyses (MP) were conducted using PAUP* with character transformations treated as unordered and equally weighed (Fitch parsimony; Fitch 1971). Tree searches were performed using a heuristic search with 500 random sequence additions, tree bisectionreconnection (TBR) branch swapping, and the MULPARS option in effect, but saving no more than five of the shortest trees from each search. These equally parsimonious trees were then used as starting trees for TBR branch swapping (MULPARS and STEEPEST DESCENT in effect) with the maximum number of trees saved set at 12,000; these trees

were permitted to swap to completion (Downie et al. 1998). Bootstrap percentage values (BP; Felsenstein 1985) were determined from 500,000 replicate analyses using fast stepwise addition of taxa. Only values greater than 50% are reported, and the following scale was used to evaluate support percentages: 50-74%, weak; 75-84%, moderate; and 85-100%, strong. Appropriate models of nucleotide evolution were selected for each dataset using MODEL-TEST version 3.06 (corrected AKAIKE information criterion; Posada and Crandall 1998). Bayesian inference (BI) was performed for 4 million generations of Monte Carlo Markov Chains with a sampling frequency of 100 using MRBAYES version 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). Suboptimal trees were discarded as the 'burn-in' phase (25%), and from the remaining trees a majority rule consensus was produced using the "sumt" command of MRBAYES. Only posterior probability (PP) values greater than 0.5 are reported, and the following scale was applied: 0.50-0.84, weak; 0.85-0.94, moderate; 0.95-1.0, strong.

Results and discussion

Morphology

Peucedanum sp. A and *Peucedanum* sp. B are glabrous shrubs or subshrubs up to 2 m tall with cauline, glaucous leaves. They are easily distinguished from each other by differences in their ultimate leaf segments. In *Peucedanum* sp. A these are linear-elliptic to elliptic (Fig. 1a) and closely resemble those of *Notobubon laevigatum* (Aiton) Magee (Fig. 1i), from which it can be distinguished by the lack of a prominent midrib and well-developed lateral venation. The ultimate leaf segments of *Peucedanum* sp. B, in contrast, are filiform to linear with only the main vein visible (Fig. 1b). The relatively small fruits (Fig. 1g) are superficially similar to those found in species of *Notobubon* B.-E. van Wyk (Fig. 1l), but differ in the prominent dorsal ribs and several other important details, as described below.

Fruit anatomical description

In transverse sections (Fig. 1c–e) the fruits of *Peucedanum* sp. A and *Peucedanum* sp. B are slightly dorsally compressed and homomericarpic. The marginal ribs are expanded into narrow or scarcely developed wings and the dorsal ribs are very prominent. The commissure differs notably from that of species of both Eurasian *Peucedanum* (Fig. 1k) and the African peucedanoid taxa (e.g. *Notobubon*, Fig. 1j) in that it extends only to the base of each marginal wing (and not to the tip of each wing, as in the

latter genera). The epidermal surface is striate and the epidermal cells are periclinally elongated. There is some lignification of the mesocarp in the ribs and the cells of the endocarp are parenchymatous and periclinally elongated. The vascular tissue is located at the tip of the dorsal and marginal ribs (Fig. 1c-e) and as such is an unusual and diagnostic character for both species. Rib oil ducts were not observed in the mature fruits. The mericarp has 10-12 regular vittae. Along the commissural length there are six vittae of unequal length, while in each vallecula one or two vittae may be present. Crystals were observed in groups close to the epidermis and around the vittae in unstained sections of both species (Fig. 1f). These crystals were initially thought to be calcium oxalate druses, as reported in some taxa of the Apioideae-Saniculoideae clade (Rompel 1895; Drude 1897–1898; Van Wyk and Tilney 2004; Liu et al. 2006, 2007; Magee et al. 2008c). However, the crystals did not dissolve during staining with PAS/TB, thus indicating that they were not composed of calcium oxalate. On closer examination of both stained and unstained transverse sections these structures were identified as sphaerocrystals of unknown composition, which apparently have never before been reported in Apiaceae and thus represent a potentially useful diagnostic character at the generic level.

ITS and rps16 intron data

The ITS dataset included 634 unambiguously aligned nucleotide positions of which 384 were variable and 321 parsimony informative. MP analyses resulted in the preset maximum tree limit of 12,000 trees, each of 2,207 steps [consistency indices (CI) of 0.30 without uninformative characters; retention index (RI) of 0.73]. MODELTEST selected the GTR+I+G model of evolution for use in the BI analysis. The MP strict consensus and BI majority-rule consensus trees (Fig. 2a) were visually largely topologically congruent and retrieved the same clades as those reported by Magee et al. (2009b). The clade comprising the two new species (three accessions of Peucedanum sp. A and one accession of *Peucedanum* sp. B) was strongly supported (PP 1.0, BP 100) and formed part of tribe Apieae (PP 1.0, BP 84). The two species were sister group to a clade comprising Sclerosciadium nodiflorum Coss., Ammi majus L. and Petroselinum crispum (Mill.) Fuss (PP 0.91, BP 61).

The *rps16* intron dataset included 865 unambiguously aligned nucleotide positions of which 179 were variable and 98 parsimony informative. MP analyses resulted in the preset maximum tree limit of 12,000 trees, each of 277 steps (CI of 0.75 and 0.63, with and without uninformative characters respectively; RI of 0.86). MODELTEST selected the K81uf+G model of evolution for use in the BI



Fig. 1 Diagnostic morphological and anatomical characters of *Billburttia* (**a**–**h**) in comparison to the vegetatively similar *Notobubon laevigatum* (**i**, **j**, **l**, **m**) and the type species of *Peucedanum* (*P. officinale*) (**k**). **a** and **b** Ultimate leaf segment of *Peucedanum* sp. A (**a**) and *Peucedanum* sp. B (**b**). **c**–**e** Transverse sections through the fruits of *Peucedanum* sp. B (**c**) and *Peucedanum* sp. A (**d**, **e**). **f** Marginal wing of *Peucedanum* sp. A showing the presence of sphaerocrystals (indicated with *arrows*) in and around the epidermis. **g** and **h** Mature mericarp of *Peucedanum* sp. A in dorsal view (**g**) and

in commissural view (**h**) showing six commissural vittae of unequal length. **i** Ultimate leaf segment of *N. laevigatum*. **j** and **k** Transverse sections through the fruits of *N. laevigatum* (**j**) and *P. officinale* (**k**). **l** and **m** Mature mericarp of *N. laevigatum* in dorsal view (**l**) and in commissural view (**m**) showing two commissural vittae. **a** *Du Puy* et al. *M660* (P), **b** *Perrier de la Bâthie 6799* (P), **c** *Decary 7583* (P), **d** *Rakotozapy 631* (P), **e** and **f** *Humbert 3634* (P), **g** and **h** *Humbert 1727* (P), **i**, **l** and **m** *Magee* 7 (JRAU), **j** *Botha 3547* (PRE), **k** *PE* 328993. Scale: **a**, **b**, **g**, **h**, **i** = 1 mm; **c–e**, **j**, **k** = 0.5 mm; **f** = 0.2 mm

analysis. The MP strict consensus tree and the BI majorityrule consensus trees (Fig. 2b) were visually largely topologically congruent and retrieved the same clades as those reported by Magee et al. (2009b), although the topologies recovered within the clade comprising tribe Apieae differed slightly as discussed below. The position of the Malagasy species, *Peucedanum* sp. A, within tribe Apieae (PP 0.97, BP < 50) was confirmed (Fig. 2b), although *rps16* intron data for the closely related *Peucedanum* sp. B was unavailable due to amplification difficulties. In both the BI and MP consensus trees, the two accessions of *Peucedanum* sp. A formed a clade which was sister group to *Stoibrax dichotomum* (L.) Raf. (PP 0.85, BP < 50). However, in the BI consensus tree *Peucedanum* sp. A and *Stoibrax dichotomum* formed part of a larger, weakly supported clade (PP 0.82) including *Deverra* DC., *Ammi majus*, *Petroselinum crispum*, *Apium graveolens* L. and *Naufraga balearica* Constance and Cannon (BI 0.82), while in the MP strict consensus tree *Peucedanum* sp. A and *Stoibrax* Raf. formed part of a clade (BP < 50%) comprising *Anethum graveolens* L. and *Foeniculum vulgare* Mill. Fig. 2 Portions of Bayesian inference trees, showing the position of the Malagasy peucedanoid species within the tribe Apieae, based on ITS (a) and *rps16* intron (b) sequence data. Posterior probability (PP) values are presented *below the branches*. Bootstrap percentage (BP) values from parsimony analysis are presented *above the branches*. BP values below 50% are not indicated. Branches supported only in the BI are indicated by *dashed lines*



Phylogenetic position

The results of our phylogenetic analyses show that the two new Malagasy peucedanoid species are not closely related to either Eurasian Peucedanum or any of the African peucedanoid taxa as suggested by previous authors based on superficially similar leaf and fruit morphological characters. Analyses of both ITS and rps16 intron data strongly place the Malagasy peucedanoids within tribe Apieae (Fig. 2). Although Peucedanum sp. B was not included within the analyses of the rps16 intron dataset, it is clearly closely related to Peucedanum sp. A based on morphological, anatomical and ITS sequence data. It is unclear whether sister group of the Malagasy peucedanoids is Stoibrax (as suggested by cpDNA) or the clade comprising Ammi, Petroselinum and Sclerosciadium (as nrITS sequence data suggests). This discrepancy may be due to low resolution within the rps16 intron dataset or it may be the result of hybridization and introgression. Although the fruits of the Malagasy peucedanoids are superficially similar to the previously mentioned peucedanoid genera, they are markedly dissimilar in their anatomy and, based on the results of the molecular analyses, rather allied to genera within the tribe Apieae. Comparisons of the Malagasy peucedanoids with the other known Apieae genera, clearly indicate that these taxa represent a distinct new genus easily recognised by a combination of the woody habit, the persistent cauline leaves, the dorsally compressed narrowly winged fruits, and several fruit anatomical characters, namely the presence of six commissural vittae, ribs with the vascular bundles located near the tips, and sphaerocrystals around the epidermis and vittae. The other genera of the tribe typically comprise herbs with radical, annual leaves and isodiametric rather unspecialised fruits.

Taxonomic treatment

Billburttia Magee and B.-E. van Wyk, gen. nov. Forma foliorum, mericarpiorum et habitu Peucedanum L. et

praecipue Notobubon B.-E. van Wyk similis sed ab eorum vittis vallecularibus 6, margine mericarpiorum indistincto, commissuris angustis, sphaerocrystallis in regione epidermidis bene differt. TYPE: B. capensoides Sales and Hedge.

Shrub or subshrub, clump-forming, glabrous, 0.5-2.0 m tall. Rootstock thick to very thick, woody, vertical or oblique. Stems erect, branched in region of inflorescence, terete, distinctly and finely ridged, solid, blue-green or reddish. Leaves cauline, persistent, 1- to 3-pinnate. Ultimate leaf segments filiform-linear or linear-elliptic to elliptic; margins entire; base narrow to broad and cuneate; apex acute to mucronulate; firm to thick-textured; concolorous, glabrous to glaucous; midrib and lateral veins equally developed or only midrib prominent, flush with or impressed. Petioles terete, sheathing almost along their entire length. Synflorescence a panicle of compound umbels, primary umbel 1, terminal, compound, composed largely of hermaphroditic flowers; lateral umbels 6, compound, smaller, composed largely of functionally male flowers; peduncle more than two times longer than the diameter of the primary umbel; striate. Primary umbel rounded; rays 15–30, 15–40 mm long, ±equal, glabrous; involucre present; bracts numerous, linear-oblong, unequal, apex acute to acuminate, glabrous, papery; raylets glabrous, glaucous; involucel present; bracteoles numerous, linear-oblong, unequal, apex acute to acuminate, ±united at base, glabrous, papery, shorter than raylets; umbellules many-flowered. Flowers pentamerous, predominantly hermaphrodite, those in the lateral umbels usually functionally male; sepals minute, apex truncate to acuminate, glabrous; petals 1.5 mm long, greenish-yellow, yellow or white, elliptic to obovate, acuminate, with inflexed tips, glabrous; stylopodium broadly conical; styles 2, short; ovary bilocular. Fruit slightly dorsally compressed, narrowly ellipsoid or oblong-ellipsoid, $3.5-4.5 \text{ mm} \times \text{c}$. 1.5 mm, glabrous; mericarps homomorphic; median and lateral ribs prominent; marginal ribs narrowly winged, not saccate at base; styles becoming strongly reflexed up to or beyond the base of the stylopodium; commissural vittae 6; vallecular vittae 4–6, solitary or in pairs; commissure broad, extending to the base of each wing; carpophore bipartite. Endosperm face plane.

Etymology

The genus is named in honour of Brian Laurence ("Bill") Burtt (1913–2008) of the Royal Botanic Garden Edinburgh (Weber and Noltie 2008) whose paper on southern African Apiaceae (Burtt 1991) has contributed greatly to our understanding of the family in that region. He was also a co-author of the account of Gesneriaceae for *Flore de Madagascar*.

Diagnostic characters

The species of *Billburttia* are superficially similar to some species of *Notobubon* in being small shrubs or subshrubs with a woody rootstock and having relatively small, slightly dorsally compressed fruits. *Billburttia* is, however, easily distinguished from *Notobubon* and members of tribe Apieae by a combination of distinctive fruit characters, namely the narrower commissure (not extending beyond the base of each wing, as in *Notobubon*), the six commissural vittae (vs. two), ribs with the vascular bundles located near the tips (vs. near the base of the ribs), and the presence of sphaerocrystals around the epidermis and vittae.

Distribution

The species are endemic to the central part of Madagascar (Fig. 3).

Key to the genus Billburttia and its species

- 1. Mericarps isodiametric or laterally compressed in transverse section... "all other Malagasy Apiaceae"
- 1. Mericarps slightly dorsally compressed in transverse section... "Billburttia":
- Ultimate leaf segments broad, more than 3 mm wide, linear-elliptic to elliptic; shrub or subshrub, 1–2 m tall ... "B. capensoides (=Peucedanum sp. A)"
- Ultimate leaf segments narrow, less than 1 mm wide, linear-filiform; subshrub, c. 60 cm tall ... "B. vaginoides (=Peucedanum sp. B)"

1. Billburttia capensoides: Sales and Hedge, sp. nov. Herba suffruticosa vel suffrutex, glabra. Caudices verticales. Caules 1–2 m, foliati, erecti, solidi, tenuiter striati. Folia 1 to 2-pinnata, segmentis ellipticis vel lineari-ellipticis, 5–15 mm latis; vaginae amplexicaules ad 2–6 cm longae.



Fig. 3 The known geographical distribution of *Billburttia capensoides* (*circles*) and *B. vaginoides* (*triangles*)

Bracteae 4–8 (–12) mm longae; bracteolae 5–3 \times 1–1.5 mm basin versus plus minusve connatae; radii 12–25, 15–40 mm longi. Mericarpia dorsaliter compressa, basi non saccata jugis aequalibus haud alatis; stylopodium conicum; styli valde reflexi applanati stylopodiis 1.5-duplo longior. TYPE: Madagascar. Ouest du massif d'Andringitra; September 1911; Perrier de la Bâthie 6807 (P!, holotype).

Shrub or subshrub, aromatic. Rootstock very thick, vertical. Stems 1–2 m, c. 0.5 cm wide at base; blue-green or reddish. Leaves 1- or 2-pinnate. Ultimate leaf segments linear-elliptic to elliptic, $20-40 \times c$. 5–15 mm; apex acute to mucronulate; thick-textured; somewhat or evidently glaucous; midrib and lateral veins equally developed. Petiole of lower leaves 20–60 mm long. Primary umbel (60) 100–120 mm wide; rays 12–25, 15–40 mm long; bracts 4–8 (–12) mm long; raylets 15–20, 4–8 mm long; bracteoles $1.5-3 \times 1-1.5$ mm. Fruit narrowly ellipsoid, $3.5-4.5 \times c$. 1.5 mm; styles reflexed beyond the base of the stylopodium. Illustrated in Sales and Hedge (2009).

Diagnostic characters Billburttia capensoides is readily distinguished from the closely related *B. vaginoides* by the broader, linear-elliptic to elliptic ultimate leaf segments with a prominent midrib and well-developed lateral

venation. This much-collected Malagasy species had been previously referred to the superficially similar southern African *Peucedanum capense* (Thunb.) Sond. [=*Notobubon laevigatum* (Aiton) Magee]. The epithet of this new Malagasy species indicates its superficial similarity to the southern African taxon.

Distribution and habitat Dry hillsides, pine woods, dry pine wood, scrubby forest and roadside, sunny places, stream bank in open grassland, damp places; locally abundant, 1,000–2,658 m.

Phenology Flowering (August) October–March; fruiting June–September.

Vernacular name tsilaninosy (Rakotovao 52).

Selected specimens examined Baron 340, s. loc. (K, P); 2021, central Madagascar (BM, K). Bosser 7563, Faratsiho, Ankaratra (P). Clement, Phillipson & Rafamantanantsoa 2009, Fianarantsoa, Ambositra to Ambatofinandrahana on R.N. 35, 1600 m (E, MO, P). Croat 28892A, station forestière de Manjakatompo near sommet Hosiarivo, in massif l'Ankaratra (MO); 29892, vicinity of col de l'Itremo, massif de l'Itremo (MO, P). Decary 13035, Ambatofinandrahana, 1700 m (P); 13400, massif du Tsiafajavona, 2200-2600 m (P); 17248, Ambositra, dans les bois de Tapia (P); 17356, Ambatofinandrahana (P). Du Puy, Labat & Andriantiana M660, west of Ambositra, 20° 34'S, 46° 35'E, east margin of Itremo massif, 1290 m (K, P). Guillaumet 3588, plateau d'Andohariana, Andringitra, c. 2000 m (P). Hildebrandt 3571, nord Betsileo, Antsirabe (BM, K, P). Hodgkin & Stansfields.n, without locality (K). Humbert3634, env. de Miarinarivo, SE d'Ambalavao, c. 1,200 m (P). Humbert & Capuron 28129, env. d'Ambatofinandrahana, c. 1450 m (P). Jard. Bot. Tananarive 221, pentes nord de Vohitra, Antsirabe (P); 3644, Tsiafajavona (P). Keraudren 218, env. d'Ambatofinandrahana (P). Moeller, Andriantiana & Haevermans 01-26, Fianarantsoa, Itremo, col d'Itremo, 20° 34'S, 46°34'E (E). Perrier de la Bâthie 6792, env. d'Antsirabe, 1500 m (P); 6807, W du massif d'Andringitra, 1000 m, type de l'espèce (P). Rakotovao 52, Réserve Naturelle Intégrale no. 5, près de la montagne rocheuse Vangomena, plateau de Sonindrana, 2010 m (MO, P). Rakotozafy 631, Itremo (P). Razafindrabe 193, Fianarantsoa, Ambalavao, Sendrisoa, Antanifotsy, pic Boby d'Andringitra, 2658 m (MO, P). Resérves Naturelles 2278 Razafindrakoto, distr. Ambalavao, canton Sendrisoa (P). Viguier & Humbert 1357, distr. Betafo, sommet du pic de Vohimalaza près Betafo, 1700 m (P); 1386, distr. Betafo, dans la coulée de laves de l'Iantsifitra, c. 1450 m (P); 1617, Betafo, monts Vararata, c. 2000 m (P); 1727, prov. Itasy, distr. Kitsamby, sur le flanc ouest de l'Ankaratra, entre Ambatofotsy et le Tsiafajavona, c. 2200 m (P). Waterlot s.n., Antsirabe (P).

2. Billburttia vaginoides: Sales and Hedge, sp. nov. Herba perennis glabra. Caudices obliqui. Caules c. 60 cm, tenuiter striati solidi rubentes. Folia 3-pinnata segmentis filiformibus vel linearibus, c. $30 \times 1-2$ mm, vaginae prominentes amplexicaules, 1.5-6 cm longae prope basin rubentes. Bracteae 3–9 mm longae, inaequales; bracteolae 3–5 mm ad basim plus minusve connatae, radii 15–30, 20– 40 mm, aequales. Mericarpia (immaturae) dorsaliter vix compressa haud alatis, basi non saccata, jugis prominentibus, stylopodium conicum, styli valde reflexi teretes, stylopodio 1–1.5 longior. Affinis B. capensoides. TYPE: Madagascar. Flanc E du Mt. Tsiafajavona; March 1921; Perrier de la Bâthie 13557 (P!, holotype).

Subshrub. Rootstock thick, oblique. Stems c. 60 cm, 4–5 mm wide at base; reddish. Leaves 3-pinnate. Ultimate leaf segments filiform to linear, c. $30 \times 1-2$ mm; apex mucronulate; firm-textured; glabrous; only midrib prominent. Petiole of lower leaves 15–20 (–60) mm long. Primary umbel 60–100 mm wide; rays 15–30, 20–40 mm long; bracts 3–9 mm long; raylets 15–22, 4–7 mm long; bracteoles, 3–5 mm long. Fruit [immature] oblong-ellipsoid, c. 3.5 × 1.5 mm; styles reflexed up to or beyond the base of the stylopodium.

Diagnostic characters This species has a very different facies from that of its closest relative, *B. capensoides*, because of its narrower, linear-filiform ultimate leaf segments. The two species grow in similar environments and although in the field they must look clearly different, *B. vaginoides* being a much smaller plant, in most characters they are surprisingly similar. Mature fruit of *B. vaginoides* is unknown and any evidence that this species still exists would be desirable. Our examination of herbarium specimens revealed that Humbert originally recognised material of this taxon as a possible new species and pencilled the name *Peucedanum emirnense* on some sheets.

Distribution and habitat Ravines, edge of streams, damp rocks, gneiss; 2,000–2,200 m. The field-note of *Perrier de la Bâthie 13557* (of 1921) states "Espèce en voie d'extinction". Although some gatherings date from after 1921, there are apparently no collections of the species after 1939.

Phenology Flowering April–June; fruiting March.

Selected specimens examined Bojer s.n., prov. Emirina (P). Decary 7564, vallée de l'Ikopa NO d'Ankazobe (P); 7583, Tampoketsa, au NE de Fenoarivo (BM, P); 13893, Behenjy (P). Jard. Bot. Tananarive 4261S, Ambohimasimbola (P). Perrier de la Bâthie 6795, Ankaratra, vers 2,200 m (P); 6799, mt Tsiafajevona, 2,000 m (P); 13557, flancs est de mt Tsiafajevona, vers 2,200 m, type de l'espèce (P). Rousson s.n., Ankaratra (P). **Acknowledgments** The authors would like to thank the curators and staff of BM, E, K, MO and P, who kindly made their specimens available for study, Prof. A.E. van Wyk (University of Pretoria) for valuable advice, and the University of Johannesburg, the South African Biosystematics Initiative and the National Research Foundation of South Africa for funding.

Appendix

Voucher information for accessions added to the ITS and rps16 intron matrices of Magee et al. (2009b). The information is listed as follows: taxon, GenBank numbers for ITS, rps16 intron, voucher information (for new accessions). Regions for which sequence data are not available are indicated with a dash.

New accessions Billburttia capensoides Sales and Hedge, FM986437, FM986441, Du Puy et al. M660 (P); B. capensoides Sales and Hedge, FM986438, FM986442, Keraudren 119 (P); B. capensoides Sales and Hedge, FM986439, —, Razafindrabe 193 (P); B. vaginoides Sales and Hedge, FM986440, —, Bojer s.n. (P).

Published GenBank accessions Ammimajus L., U78386 (Downie et al. 1998) & U78446 (Downie et al. 1998), —; Anethum graveolens L., —, AF110542 (Downie and Katz-Downie 1999); Deverra denudata (Viv.) R. Pfisterer and Podlech, —, AY838419 (Calviño et al. 2006); Deverra triradiata Hochst. ex Boiss., —, AF164815 (Downie et al. 2000); Diplolophium somaliense Verdc., DQ368843 (Calviño et al. 2006), —; Foeniculum vulgare Mill., AY581806 (Tabanca et al. 2005), —; Foeniculum vulgare Mill., EF421428 (Kersten and Knoess, unpublished), —; Petroselinum crispum (Mill.) Fuss, U78387 & U78447 (Downie et al. 1998), —; Ridolfia segetum Moris, U78384 & U78444 (Downie et al. 1998), —.

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