ORIGINAL ARTICLE

Ezosciadium (Apiaceae): a taxonomic revision of yet another early diverging South African apioid genus

A. R. Magee · B.-E. van Wyk · P. M. Tilney · S. R. Downie

Abstract The hitherto poorly known Cape endemic genus *Ezosciadium* (Apiaceae) is revised. This genus is highly distinctive and can be distinguished from other annual genera of the region by its pilose vegetative and reproductive organs, the sessile compound umbels with conspicuously unequal rays, the non-inflexed petal tips, the relatively small, highly-inflexed stamens which appear almost sessile, and the prominent carpophores which persist on the plant. The fruit are unusual in the presence of druse crystals around the carpophore and tanniniferous substances in the epidermal cells of the ribs. The phylogenetic position of the genus within the subfamily Apioideae was assessed using *rbc*L, *rps*16 intron (2 new accessions) and nrITS (1 new accession) sequence data. *Ezosciadium capense* was found to form part of an early diverging lineage within the subfamily, sister group to the *Annesorhiza* clade and possibly also closely related to the genera *Molopospermum* and *Astydamia*. A comprehensive taxonomic revision, including typification, detailed descriptions, geographical range and illustrations, is presented.

Keywords Crystals · *Ezosciadium capense* · ITS · *rbc*L · *rps*16 intron · Fruit anatomy · Morphology · Phylogeny

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Introduction

Ezosciadium B.L. Burtt is a poorly known and collected monotypic genus endemic to the Eastern Cape Province of South Africa. As with many other small African endemic Apiaceae, its correct placement within the new emerging tribal classification of the family remains untested by molecular data (Calviño et al. 2006). The genus was included in a large *rbc*L analysis by Forest et al. (2007) in assessing the phylogenetic diversity of the Cape Flora. Although the genus occupied a position sister group to *Annesorhiza* Cham. and Schltdl. and *Itasina* Raf., the limited sampling of the family did not allow for any conclusions to be drawn on possible tribal affinities. *Ezosciadium capense* is traditionally placed in the large tribe Apieae (Pimenov and Leonov 1993). De Candolle (1830) and Sonder (1862) included it within the genus *Helosciadium* W.D.J. Koch, a genus now placed in the tribe Oenantheae (Downie et al. 2001). Wolff (1927) followed Ecklon and Zeyher (1837) in recognising the genus as distinct and postulated an affinity rather to *Sonderina* H. Wolff or *Apium* L., genera traditionally placed in the tribe Apieae. Recent molecular systematic studies, however, have shown *Sonderina* to form part of an African peucedanoid clade together with *Dasispermum* Raf. and other African species of tribe Tordylieae previously treated in the genus *Peucedanum* L. (Winter et al. 2008). As a result, the three genera with which *Ezosciadium* is traditionally associated are scattered in various tribes throughout the subfamily Apioideae. This paper is aimed at ascertaining the phylogenetic placement of the genus within the subfamily using both DNA sequence and anatomical data as well as providing the first comprehensive taxonomic revision, including nomenclature, typification, detailed description, geographical distribution

and illustrations, of this anomalous and poorly known African genus.

Materials and methods

Taxonomic study

Specimens from the following herbaria were studied: BM, K, NBG, PRE and S. From this material, together with information from Leistner and Morris (1976), the recorded distribution of *E. capense* was ascertained and mapped. All illustrations (Fig. 1) 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

Fruit from three specimens of *E. capense*, representing three of the four known populations, were rehydrated in order to study the anatomy. This material included both

Fig. 1 *Ezosciadium capense*. **a** Portion of a flowering stem, **b** cotyledon, **c** first leaf, **d** mature leaf, **e** mature fruit, **f** transverse section through the mature fruit, **g** and **h** petals in dorsal view (**g**) and in ventral view (**h**), **i** stamens in dorsal view. **a**, **e** and **f** from *Acocks 20021* (PRE); **b–d** and **g–i** from *Fries et al. 1156* (PRE)

immature fruit, from *Fries* et al. *1156* (PRE), as well as mature fruit, from *Acocks 20021* (PRE) and *Goldblatt & Porter 12578* (NBG). Following rehydration, the fruit were placed in FAA for a minimum of 24 h and subsequently treated according to a modification of the method of Feder and O' Brien (1968) for embedding in glycol methacrylate (GMA). This modification involves a final infiltration in GMA of 5 days. Transverse sections, about 3 μm thick, were cut using a Porter–Blüm ultramicrotome. The 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). Crystals tend to be dissolved during this procedure, hence the need to study unstained sections.

DNA sampling, isolation, amplification and sequencing

In order to assess the phylogenetic position of *Ezosciadium*, DNA sequences of the chloroplast genes *rps*16 intron and *rbc*L were compiled using available sequences from GenBank. Due to the high divergence and difficulty in aligning the nuclear ribosomal DNA internal transcribed spacer (ITS) region across the subfamily, a reduced sampling was compiled representing the clade within which *Ezosciadium* was shown to be placed based on results of phylogenetic analyses of the aforementioned chloroplast genes. Sources of material used in the study are listed in the Appendix. In total, 52 accessions of *rps*16, 19 accessions of *rbc*L, and 12 accessions of ITS were considered for phylogenetic study. For the broader analyses using plastid markers, representatives of both tribes of subfamily Saniculoideae as well as many of the major clades and tribes of subfamily Apioideae were included. The genus *Hermas* L., a member of subfamily Azorelloideae, was selected as outgroup for the chloroplast datasets, based on previous molecular analyses (Calviño et al. 2006), while the ITS dataset was rooted using *Lichtensteinia obscura* based on the results of the chloroplast analyses.

An aliquot of total DNA from *E. capense* was obtained from the Leslie Hill Laboratory at Kirstenbosch Botanical Garden, South Africa. The *rps*16 intron was amplified using the primers of Oxelman et al. (1997), while the ITS region was amplified using the Sun et al. (1994) AB101 and AB102 primers. Amplified PCR products were purified using a QIAquick PCR purification kit (Qiagen Inc.) according to the manufacturer's instructions and directly sequenced on a 3130 *xl* Genetic Analyzer (Applied Biosystems Inc.) using BigDye Terminator version 3.1 (Applied Biosystems Inc.). The newly obtained ITS and *rps*16 intron sequences of *E. capense* from *Goldblatt & Porter 12578* (NBG) and *Stoibrax dichotomum* from
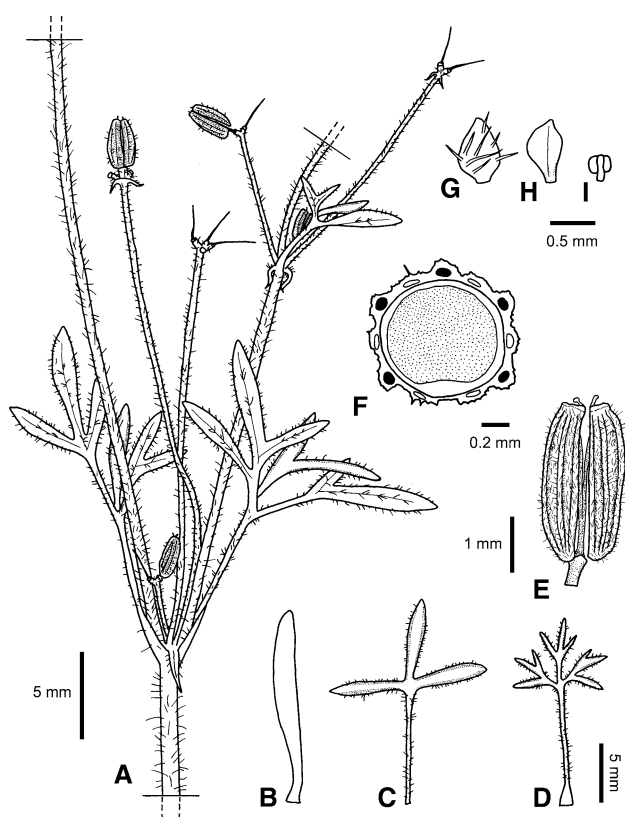
Sanchez-Mata & Molina Abril s.n. (K) were submitted to Genbank.

Sequence alignment and phylogenetic analyses

For each data set, complementary strands were assembled and edited using Sequencher version 3.1.2 (Gene Codes Corporation) and aligned manually in PAUP* version 4.0b10 (Swofford 2002), with gaps positioned so as to minimise nucleotide mismatches. Data sets are available on request from the corresponding author. Phylogenetic analyses were conducted using the maximum parsimony (MP) algorithm of PAUP*. Character transformations were treated as equally likely (Fitch parsimony; Fitch 1971). Tree searches were performed using a heuristic search with 1,000 random sequence additions, tree bisection-reconnection (TBR) branch swapping and the MULPARS option in effect. A limit of ten trees per replicate was set to reduce the time spent on swapping in each replicate. Internal support was assessed with 1,000 bootstrap replicates (Felsenstein 1985) using TBR swapping and holding ten trees per replicate. Only values greater than 50% are reported.

Bayesian inference (BI) was performed on the *rps*16 intron dataset using MRBAYES v. 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The TrN+G model, as indicated to be the best model by MODELTEST v. 3.06 (corrected AKAIKE information criterion; Posada and Crandall 1998), was implemented. The analysis was performed for 1 million generations of Monte Carlo Markov Chains and a sampling frequency of ten. The resulting trees were plotted against their likelihoods in order to determine where the likelihoods converge on a maximum value. All the trees before this convergence were discarded as the 'burn-in' phase. The remaining trees were imported into PAUP* and a majority rule consensus tree was produced in order to show the posterior probabilities (PP) of all observed bi-partitions (only PPs above 0.5 are reported).

Results and discussion

Morphology

Ezosciadium is a markedly pilose (Fig. 1a), erect annual herb up to 0.35 m tall. The leaves are cauline, with the lower ones prominently tri-lobed (Fig. 1c) and the upper ones digitately compound (Fig. 1d). The cotyledons are simple and narrowly oblanceolate (Fig. 1b), fitting the L type, as defined by Cerceau-Larrival (1962) as commonly found in the subfamily Apioideae. The very sparse, compound umbels are sessile and borne in the leaf axils. They are composed of 2 to 4 markedly unequal rays (Fig. 1a) that are of diagnostic value amongst African annual genera. The flowers are also unusual in that the petals are not inflexed at their tips (Fig. 1h) and the relatively small stamens remain highly inflexed, so that they appear almost sessile (Fig. 1i).

Fruit anatomy

The fruit are distinctly pilose and oblong in shape (Fig. 1e). They are borne on a partly bifid carpophore (Fig. 1e), one half of which remains attached to the umbellate rays even after the fruit have fallen off (Fig. 1a). In transverse section (Fig. 2a, 1f) the fruit are similar to many other apioid genera. They are isodiametric and homomericarpic with five equal, prominent ribs. The commissure is very narrow, being confined to the carpophore region. The epidermal
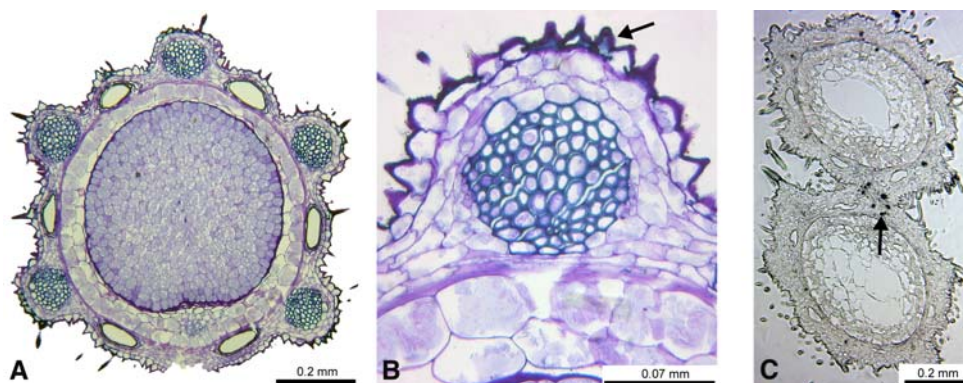
Fig. 2 Transverse sections through the fruit of *Ezosciadium capense*. **a** Mature mericarp, **b** rib of mature fruit with epidermal cells containing tanniniferous substances (indicated with *arrow*), **c** immature fruit showing presence of druse crystals around the carpophore (crystals indicated with an *arrow*, similarly dark structures located at the base of the ribs are highly lignified vascular tissue). **a** and **b** from *Goldblatt & Porter 12578* (NBG), **c** from *Fries* et al. *1156* (PRE)

cells are bottle-shaped and extend into hairs; those of the ribs contain tanniniferous substances (Fig. 2b). This is unusual in Apiaceae, but their presence has been recorded in *Hydrocotyle* L. and *Trachymene* Rudge (Liu 2004). The mesocarp is composed of parenchymatous cells that are usually periclinally elongated. The endocarp is also parenchymatous, the cells being narrow and strongly periclinally elongated. The vascular tissue occupies a large portion within each of the five ribs and is distinctly circular. Rib oil ducts were not observed in the fruit. The vittae are arranged as in most members of the subfamily Apioideae—two commissural and four vallecular. The endosperm is isodiametric and flattened to slightly concave on the commissural face. The testa is prominent in transverse section and may be comprised of up to three layers of cells especially in the commissural region, with the outer periclinal cell walls of the outermost layer being conspicuously thickened.

Druse crystals, restricted to the region surrounding the carpophore in the commissural area (Fig. 2c), were found to be present in all fruit of *Ezosciadium* examined. The presence of scattered druse crystals in the mesocarp (i.e. with crystals occurring around the seed and not restricted to the carpophore region) has a high predictive value for determining ancestral lineages within Apioideae (Van Wyk and Tilney 2004). This type of crystal distribution is known to occur in many of the early diverging branches of the Apioideae–Saniculoideae clade (Van Wyk and Tilney 2004), including most members of the Saniculoideae (Liu et al. 2007), all genera of the *Annesorhiza* clade (i.e. *Annesorhiza*, *Chamarea* Eckl. and Zeyh., *Itasina*), the tribe Heteromorpheae, as well as *Molopospermum* W.D.J. Koch (Liu et al. 2006) and *Astydamia* DC. (Liu pers. comm.). In the remaining Apioideae, crystals are usually completely absent but, for example, are frequently present around the carpophore in some members of the tribe Scandiceae, such as species of *Anthriscus* Pers., *Caucalis* L., *Chaerophyllum* L., *Daucus* L., *Osmorhiza* Raf., *Scandix* L. and *Torilis* Adans. (Drude 1897–1898). The presence of druse crystals, even though restricted to the carpophore region in *Ezosciadium*, may suggest an affinity to the early diverging lineages of the subfamily as shown by analyses of all three molecular data sets (Fig. 3a–c).

rps16 intron dataset

The *rps*16 intron dataset included 1,113 characters of which 304 were variable and 169 parsimony informative. The MP analysis (Fig. 3a) resulted in 2,208 equally most parsimonious trees with a tree length (TL) of 534 steps, a consistency index (CI) of 0.70 (including uninformative characters) and a retention index (RI) of 0.85. The overall

topologies obtained from both the MP and BI analyses were similar, with the general branching order similar to that found in previous analyses (Calviño et al. 2006). Amongst the early diverging lineages of Apioideae the genus *Lichtensteinia* Cham. and Schltdl. formed the earliest branching clade (BP 98; PP 1.0). Following was the lineage comprised of the *Annesorhiza* clade plus *Ezosciadium* and *Molopospermum* (BP 65; PP 0.91). In the BI tree, *E. capense* grouped with *Molopospermum* (PP 0.67), sister group to the *Annesorhiza* clade (*Annesorhiza*, *Chamarea* and *Itasina*). The tribe Heteromorpheae (BP 100; PP 1.0) was a subsequent sister group, followed by the tribes Bupleureae (BP 97; PP 1.0) and Pleurospermeae (BP 100; PP 1.0). Higher up on the trees the tribes Oenantheae (BP 98, PP 1.0), Smyrnieae, Scandiceae, and other members of the Apioid superclade, including the tribe Apieae (BP 91, PP 1.0), were retrieved. The placement of *Chamaesciadium acaule* differed in the two analyses. In the MP tree, it was found to be sister group to a clade comprising Apieae, Echinophoreae, Selineae and Tordylieae, while in the BI tree (not shown) it was sister group to a clade comprising Echinophoreae, Selineae and Tordylieae (PP 1.0).

rbcL dataset

The *rbc*L dataset included 1,238 characters of which 126 were variable and 57 parsimony informative. The MP analysis (Fig. 3b) resulted in three equally parsimonious trees with a TL of 176 steps, a CI of 0.76 and a RI of 0.75. The overall topology of the MP trees retrieved in this analysis was found to be similar to that shown in the *rps*16 intron trees. Although *rbc*L sequences for *Molopospermum* and *Astydamia* were not available, the position of *E. capense* as sister group to the *Annesorhiza* clade (represented in this analysis by *Annesorhiza* and *Itasina*) is moderately supported (BP 84).

ITS dataset

The ITS dataset included 638 characters of which 136 were variable and 57 parsimony informative. The MP analysis (Fig. 3c) resulted in a single parsimonious tree with a TL of 220 steps, a CI of 0.78 and a RI of 0.52. In this tree, *E. capense* is sister group to the clade of *Astydamia* and *Molopospermum* (BP 54) which, in turn, is sister group to the *Annesorhiza* clade.

Basal African Apiaceae

Burtt (1991) argued that the southern African taxa of Apiaceae are of importance beyond their relatively small size. Recent molecular systematic studies have indeed
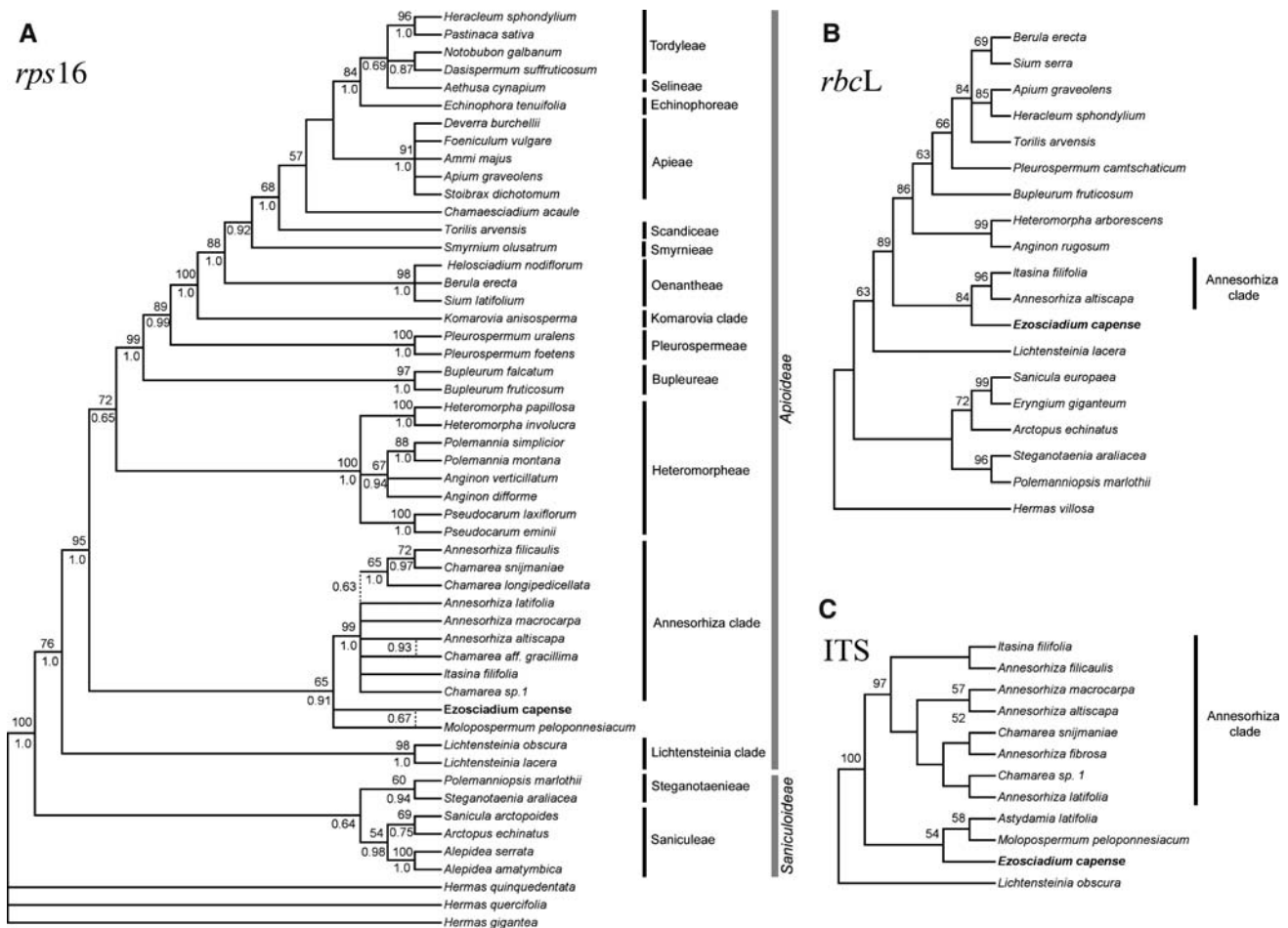
Fig. 3 a Strict consensus tree of the 2,208 equally most parsimonious trees with a length of 534 steps, based on the MP analysis of *rps*16 sequence data (CI = 0.70 and RI = 0.85). BP's are given above the branches and PP's are given below the branches. Branches supported only in the BI tree are indicated as *dotted lines*, while those that differed in the BI tree are indicated as *grey lines*. **b** Strict consensus tree of the 3 equally most parsimonious trees with a length of 176 steps, based on MP analysis of *rbc*L sequence data (CI = 0.76 and RI = 0.75). **c** Single most parsimonious tree with a length of 220 steps, based on ITS sequence data (CI = 0.78 and RI = 0.52)

shown that African genera are often sister to (or basally divergent within) other major lineages and therefore essential to understanding of relationships within the family as a whole. The basally divergent position of African genera was first shown by early molecular systematic studies which suggested that *Heteromorpha* Cham. and Schltdl. and *Anginon* Raf. formed basal-branching lineages within the subfamily Apioideae (Downie et al. 1996, 1998; Plunkett et al. 1996a, b). Downie and Katz-Downie (1999) subsequently retrieved a broader *Heteromorpha* clade, comprising numerous woody African genera (*Anginon*, *Dracosciadium* Hilliard and B.L. Burtt, *Glia* Sond., *Heteromorpha* and *Polemannia* Eckl. and Zeyh.). This clade was later recognised by Downie et al. (2000b) as the tribe Heteromorpheae. More recently, an analysis focusing on the phylogenetic position of African Apiaceae (Calviño et al. 2006) indicated that several other

African genera occupied early diverging positions within the Apioideae. *Lichtensteinia* was shown to be the most early diverging lineage sister group to other genera of the subfamily Apioideae. Successively sister to the *Lichtensteinia* clade was a clade comprising largely African herbaceous genera, called the *Annesorhiza* clade (comprising *Annesorhiza*, *Chamarea*, and *Itasina*). This *Annesorhiza* clade was closely related to *Molopospermum* and *Astydamia*, however, these relationships were not very strongly supported. Even within the subfamily Saniculoideae, its relatively small African contingent has been shown to be basally divergent. Downie and Katz-Downie (1999) demonstrated that two woody African genera (viz., *Steganotaenia* Hochst. and *Polemanniopsis* B.L.Burtt) traditionally placed within the Apioideae, formed a sister group relationship with the subfamily Saniculoideae. These two genera have subsequently been included in an

expanded Saniculoideae by Calviño and Downie (2007) as the tribe Steganotaenieae. Furthermore, within their tribe Saniculeae two African endemic genera (*Alepidea* Delar. and *Arctopus* L.) have now also been shown to occupy early diverging positions, both successively sister to the remaining genera (Calviño and Downie 2007; Magee et al. 2008). In this study, we report on yet another early diverging branch of subfamily Apioideae from South Africa. In the results of all molecular analyses, *E. capense* shows a close affinity with the *Annesorhiza* clade, the latter representing a group of deciduous, perennial herbs endemic to southern Africa. Such a relationship between *Ezosciadium* and the *Annesorhiza* clade has also been proposed independently (C. Calviño, unpublished data) on the basis of MP analysis of cpDNA *trn*Q-*rps*16 sequences obtained from one of the same collections examined herein (*Goldblatt & Porter 12578*, MO). However, in all cladograms currently available for the group, the relationships among *Ezosciadium*, the *Annesorhiza* clade, the European genus *Molopospermum*, and the Canary Islands endemic genus *Astydamia* are not yet clear and demand further study.

It seems that the basally divergent lineages are morphologically as diverse as the more derived lineages as they include woody and herbaceous elements, with various leaf and fruit types. It is therefore not easy to ascertain the phylogenetic position of most genera without molecular systematic evidence. The lineage comprised of the *Annesorhiza* clade, *Astydamia*, *Ezosciadium* and *Molopospermum* has no obvious morphological synapomorphies, but the combined presence of heteromericarpic fruit (in *Annesorhiza* and *Molopospermum*), scattered druse crystals in the mesocarp, and the highly lignified vascular bundles (in *Annesorhiza* and *Ezosciadium*) do, to some extent, provide support for this clade.

Taxonomic treatment

Ezosciadium B.L. Burtt in Edinb. J. Bot. 48(2):207, 268 (1991). *Trachysciadium* H. Wolff in Pflanzenr. Heft 90: 108 (1927), non *Trachysciadium* (DC.) Eckl. and Zeyh. (1837). TYPE: *E. capense* (Eckl. and Zeyh.) B.L. Burtt. [Note: Burtt (1991) gives a detailed argument for considering *Trachysciadium* as a synonym of *Pimpinella* L. and hence the need for the new generic name, *Ezosciadium*. De Candolle (1830) described *Trachysciadium* as a section of the genus *Helosciadium* to accommodate two Himalayan species now included in *Pimpinella*. The publication of the genus *Trachysciadium* by Ecklon and Zeyher (1837) had been considered valid by some authors (e.g. Wolff 1927), who regarded it as being validated by the combined genericospecific description, and taxonomically independent of *Helosciadium* sect. *Trachysciadium* (in which case the name *Ezosciadium* would be superfluous). We however, prefer to follow Burtt (1991), who argued that there is no reasonable doubt that Ecklon and Zeyher (1837) adopted De Candolle's taxon and simply altered its rank. As such, Burtt's proposed *Ezosciadium* nom. nov. is valid and necessary].

This highly distinctive genus appears to be an isolated basal lineage within Apiaceae subfamily Apioideae sister group to the *Annesorhiza* clade. DNA sequence data also support a possible close relationship with *Astydamia* and *Molopospermum*, as do some morphological and fruit anatomical characters. The genus is endemic to the Eastern Cape Province of South Africa within the eastern part of the Cape Floristic Region.

Ezosciadium capense (Eckl. and Zeyh.) B.L. Burtt in Edinb. J. Bot. 48(2): 207 (1991). *Trachysciadium capense* Eckl. and Zeyh., Enum. Pl. Afric. Austral. 341 (1837); H. Wolff in Pflanzenr. Heft 90: 108 (1927). *Helosciadium capense* (Eckl. and Zeyh.) Sond. in Harv. and Sond., Fl. Cap. 2: 536 (1862). *Trachydium capense* (Eckl. and Zeyh.) Drude in Engl. & Prantl, Pflanzenfam. 3(8): 189 (1898). Type: South Africa. Uitenhage district (3325): 'Coega Kopje' not far from 'Zwartkopsrivier' (–DC), *Ecklon and Zeyher 2196* (SAM!, lectotype here designated; MO, photo!, P, photo!, S!, isolectotypes).

Erect, annual herb, 0.1–0.35 m tall, pilose. *Cotyledons* simple, narrowly oblanceolate, 15 mm × 2 mm, margins entire. *Stem* solitary, dichotomously branched. *Leaves* cauline, 10–37 × 7–26 mm, first leaves tri-lobed, upper leaves digitately compound. *Petioles* 5–18 mm long, sheathing slightly at the base. *Pinnae* 12–24 mm × 5–13 mm, 2- or 3-lobed; lobes linear-oblong to narrowly oblong, 5–12 mm × 1–2 mm, flat, apex acute, margins and venation pilose, venation pinnate. *Umbels* compound, sparse, axillary, sessile (peduncle absent, rays arising directly from each node); involucre present; bracts 2, 2–4 mm long, becoming prominently recurved, lanceolate, apex acute, pilose; rays 2 to 4, unequal in length, with at least one remaining markedly shorter, longer rays 11–31 mm long and shorter ray 3–10 mm long at anthesis, pilose; involucel present; bracteoles 2, 1–2 mm long, becoming prominently recurved, lanceolate, apex acute, pilose; umbellate rays 2 to 4, short, less than 1 mm long at anthesis, pilose. *Flowers* subsessile, pentamerous, hermaphroditic; sepals obsolete; petals yellow, 0.6–0.7 mm long, ovate to obovate, tips not inflexed, acute, septum absent on inner face, pilose on the dorsal surface; stamens with anthers highly inflexed, small, 0.2–0.4 mm tall; ovary densely pilose, stylopodium flat; styles erect, very short. *Fruit* isodiametric, oblong, 2.5–3.0 mm × ±1.5 mm broad; mericarps homomorphic, distinctly pilose; median, lateral and marginal ribs 5, equal, prominent; commissural vittae 2; vallecular vittae 4; commissure very narrow;
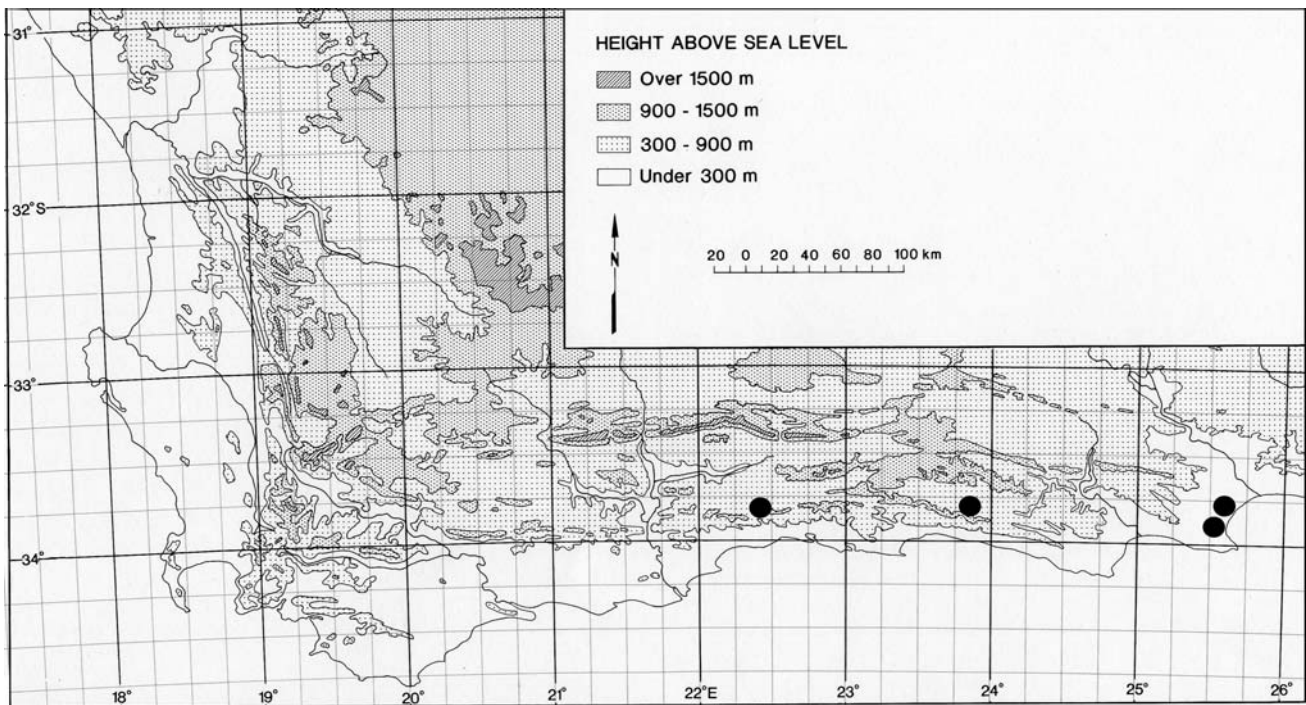
Fig. 4 The known geographical distribution of *Ezosciadium capense*

carpophore bifid for the upper two-thirds of its length, persisting partly on the plant (Fig. 1).

Diagnostic characters

Ezosciadium capense is a highly distinctive species, easily distinguished from all other annual species by the pilose vegetative and reproductive organs, the sessile compound umbels with a few, markedly unequal rays (Fig. 1a), the non-inflexed petal tip (Fig. 1h), the relatively small, highly inflexed stamens which appear almost sessile (Fig. 1i), and the prominent bifid carpophores, one half of which persists on the plant (Fig. 1a, e).

Distribution and habitat

This poorly collected species is endemic to the Eastern Cape Province of South Africa and is known from only four recorded populations (Fig. 4), three of which have only come to light recently. Originally known only from Coega koppie near Uitenhage, the distribution range has been expanded to include Perdepoort (the westernmost locality), Joubertina and Bethelsdorp.

Additional specimens examined

South Africa, Eastern Cape Province. **–3322 (Oudshoorn)**: Perdepoort, N of Camfer (–CD), *Goldblatt & Porter 12578* (NBG). **−3323 (Willowmore)**: 4 miles N by W of Joubertina (–DD), *Acocks 20021* (PRE). **−3325 (Uitenhage)**: Bethelsdorp, salt pan (–DC), *Fries* et al. *1156* (BM, PRE). **Precise locality unknown**: *Anon s.n.* herb. S sheet 243 (S)

Appendix 1

List of taxa included in this study with GenBank accession numbers and authors

ITS	*Annesorhiza altiscapa* Schltr. ex H.Wolff, DQ368830[i]; *Annesorhiza fibrosa* B.-E.van Wyk, DQ368831[i]; *Annesorhiza filicaulis* Eckl. and Zeyh., DQ368832[i]; *Annesorhiza latifolia* Adamson, DQ368833[i]; *Annesorhiza macrocarpa* Eckl. and Zeyh., DQ368835[i]; *Astydamia latifolia* (L.f.) Kuntze, DQ368836[i]; *Chamarea snijmaniae* B.L.Burtt, DQ368839[i]; *Chamarea* sp. 1, DQ368840[i]; *Ezosciadium capense* (Eckl. and Zeyh.) B.L.Burtt, AM982517[n]; *Itasina filifolia* (Thunb.) Raf., DQ368857[i]; *Lichtensteinia obscura* (Spreng.) Koso-Pol., DQ368858[i]; *Molopospermum peloponnesiacum* Koch, AF074335[e], AF074336[e].
*rbc*L	*Anginon rugosum* (Thunb.) Raf., U50222[c]; *Annesorhiza altiscapa* Schltr. ex H.Wolff, AM234812 [l]; *Apium graveolens* L. L01885 L11165[a]; *Arctopus echinatus* L. AY188414[g]; *Berula erecta* (Huds.) Coville, AM234813[l]; *Bupleurum fruticosum* L., D44556[b]; *Eryngium giganteum* M.Bieb., DQ133808[h]; *Ezosciadium capense* (Eckl. and Zeyh.) B.L.Burtt, AM234818[l]; *Heracleum sphondylium* L., AY395540[j]; *Hermas villosa* Thunb., DQ133810[h]; *Heteromorpha arborescens* Cham. and Schltdl., DQ133811[h]; *Itasina filifolia* (Thunb.) Raf., AM234821[l]; *Lichtensteinia lacera* Cham. and Schltdl., AM234822[l]; *Pleurospermum camtschaticum* Hoffm., D44583[b]; *Polemanniopsis marlothii* (H.Wolff) B.L.Burtt, AM234824[l]; *Sanicula europaea* L., DQ133820[h]; *Sium serra* (Franch. and Savat.) Kitag., D44587[b]; *Steganotaenia araliacea* Hochst., EU213518[m]; *Torilis arvensis* Link, AM234827[l].
*rps*16 intron	*Aethusa cynapium* L., AF110539[d]; *Alepidea amatymbica* Eckl. and Zeyh., DQ832338[k]; *Alepidea serrata* Eckl. and Zeyh., DQ832349[k]; *Ammi majus* L., AF164814[f]; *Anginon difforme* (L.) B.L.Burtt, AY838391[e]; *Anginon verticillatum* (Sond.) B.L.Burtt, AY838404[e]; *Annesorhiza altiscapa* Schltr. ex H.Wolff, AY838405[e]; *Annesorhiza filicaulis* Eckl. and Zeyh., AY838407[e]; *Annesorhiza latifolia* Adamson, AY838408[e]; *Annesorhiza macrocarpa* Eckl. and Zeyh., AY838410[e]; *Apium graveolens* L., AF110545[d]; *Arctopus echinatus* L., DQ832351[k]; *Berula erecta* (Huds.) Coville, AF164819[f]; *Bupleurum falcatum* L. AF110566[d]; *Bupleurum fruticosum* L., AF110569[d]; *Chamaesciadium acaule* C.A.Mey., AY838411[e]; *Chamarea longipedicellata* B.L.Burtt, AY838413[e]; *Chamarea snijmaniae* B.L.Burtt, AY838414[e]; *Chamarea* sp.1 AY838415[e]; *Chamarea* aff. *gracillima* (H.Wolff) B.L.Burtt, AY838416[e]; *Dasispermum suffruticosum* (Bergius) B.L.Burtt, AY838417[e]; *Deverra burchellii* (DC.) Eckl. and Zeyh., AY838418[e]; *Echinophora tenuifolia* L. AF164812[f]; *Ezosciadium capense* (Eckl. and Zeyh.) B.L.Burtt, AM982518[n]; *Foeniculum vulgare* Mill., AF110543[d]; *Helosciadium nodiflorum* Koch, AF164820[f]; *Heracleum sphondylium* L., AF164800[f]; *Hermas gigantea* L.f., AY838420[e]; *Hermas quercifolia* Eckl. and Zeyh., AY838421[e]; *Hermas quinquedentata* L.f., AY838422[e]; *Heteromorpha involucrata* Conrath, AF110577[d]; *Heteromorpha papillosa* C.C.Towns., AY838425[e]; *Itasina filifolia* (Thunb.) Raf., AY838426[e]; *Komarovia anisosperma* Korov., AF110555[d]; *Lichtensteinia lacera* Cham. and Schltdl., AY838427[e]; *Lichtensteinia obscura* (Spreng.) Koso-Pol., AY838428[e]; *Molopospermum peloponnesiacum* Koch, AY838432[e]; *Notobubon galbanum* (L.) Magee, AY838435[e]; *Pastinaca sativa* L., AF110538[d]; *Pleurospermum foetens* Franch., AF110559[d]; *Pleurospermum uralense* Hoffm., AF110560[d]; *Polemannia montana* Schltr. and H.Wolff, AF110570[d]; *Polemannia simplicior* Hilliard and B.L.Burtt, AF110571[d]; *Polemanniopsis marlothii* (H.Wolff) B.L.Burtt, DQ832374[k]; *Pseudocarum eminii* H.Wolff, AY838441[e]; *Pseudocarum laxiflorum* (Baker) B.-E.van Wyk, Y838442[e]; *Sanicula arctopoides* Hook. and Arn., DQ832375[k]; *Sium latifolium* L., AF110552[d]; *Smyrnium olusatrum* L., AF110551[d]; *Steganotaenia araliacea* Hochst., DQ832384[k]; *Stoibrax dichotomum* (L.) Raf., AM982519[n]; *Torilis arvensis* Link, AF110548[d].

[a] Olmstead et al. (1992), [b] Kondo et al. (1996), [c] Plunkett et al. (1996b), [d] Downie and Katz-Downie (1999), [e] Downie et al. (2000a), [f] Downie et al. (2000c), [g] Chandler and Plunkett (2004), [h] Andersson et al. (2006), [i] Calviño et al. (2006), [j] Silvertown et al. (2006), [k] Calviño and Downie (2007), [l] Forest et al. (2007), [m] Lahaye et al. (2008), [n] present study

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