

## Reassessing taxonomic relationships in the *Berkheya* clade (Asteraceae, Arctotideae–Gorteriinae): the utility of achene morphology

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### Abstract

The genus *Berkheya* is paraphyletic with the related genera *Cullumia*, *Cuspidia*, *Didelta* and *Heterorhachis* embedded within a broader clade termed the ‘*Berkheya* clade’. As a contribution towards reassessment of species relationships and delimitation of species groupings within the clade, the utility of external achene morphology for supporting natural species groups within the clade was evaluated. Achenes from 68 species and nine infraspecific taxa were examined, including representatives of each genus currently recognised in the *Berkheya* clade. Achene pubescence and pappus characteristics (e.g., scale shape and number of scales within a series) were indicated to be particularly variable and thus less reliable for assessment of species relationships. Of the currently recognised genera, only segregation of *Cullumia* was supported by achene and pappus characteristics. Species groupings implicit in Roessler’s infrageneric classification of eight series within *Berkheya* were to a large extent supported. However, in particular, series *Speciosae* was indicated to be a disparate species grouping and the distinction of series *Angustae* and *Cruciatae* is not supported by achene and pappus morphology. Achene and pappus characteristics suggest several novel species groupings (e.g. an affinity between *Berkheya*

*cuneata*, *B. ferox* and *B. spinosa*). Although the taxonomic sampling in published molecular phylogenetic analyses is limited, achene and pappus morphology partially supports clades resolved in prevailing phylogenetic reconstructions for the clade. More complete sampling in future molecular phylogenetic analyses is required to test novel species affinities suggested in the present study to explore the evolution of the achene and pappus in the *Berkheya* clade.

**Key words:** African flora, Compositae, fruit, taxonomy

## Introduction

The tribe Arctotideae contains approximately 220 species with a predominantly southern African distribution. Two subtribes are recognized, namely Arctotidinae (ca. 80–90 species) and Gorteriinae (ca. 135 species) (Karis *et al.* 2009). The last full revision of the Gorteriinae was by Roessler (1959), whose generic treatment is still followed (Karis 2007; Karis *et al.* 2009). Recent molecular phylogenetic studies (Funk *et al.* 2004; Funk & Chan 2008) and a morphological cladistic analysis (Karis 2006) indicate that the Gorteriinae consists of two sister groups. The ‘*Berkheya* clade’ consists of *Berkheya* Ehrhart (1784: 303) (ca. 80 species), *Cullumia* R.Br. in Aiton (1813: 137) (15 species), *Cuspidia* Gaertner (1791: 454) (one species), *Didelta* L'Héritier de Brutelle (1786: 55) (two species) and *Heterorhachis* Walpers (1847: 278) (two species). The ‘Gorteria clade’ currently comprises the genera *Gorteria* Linnaeus (1759: 1229), *Gazania* Gaertner (1791: 451) and *Hirpicium* Cassini (1820: 27) (Karis 2007; Karis *et al.* 2009).

In the most recent monograph of *Berkheya*, Roessler (1959) accepted 72 species divided into eight series. Seven species and three infraspecific taxa of *Berkheya* and one *Heterorhachis* species have been described subsequently (Roessler 1960, 1973; Hilliard & Burt 1975, 1985, 1989; Manning *et al.* 2010; Manning & Goldblatt 2012; Bergh & Helme 2014). Recent molecular phylogenetic studies indicate that *Berkheya* is paraphyletic as it is currently circumscribed with *Cullumia*, *Cuspidia*, *Didelta* and *Heterorhachis* nested within *Berkheya* (Funk *et al.* 2004; Funk & Chan 2008; Phaliso 2013). These studies also indicate that Roessler’s infrageneric classification of *Berkheya* requires reassessment, although interpretation is hindered by insufficient sampling for this purpose. Therefore, a thorough reappraisal of species relationships and generic circumscription within the *Berkheya* clade is required.

*Berkheya* was established by Ehrhart (1784), based on *B. fruticosa* (Linnaeus 1753: 829) Ehrhart (1784: 138) (*Atractylis fruticosa* L.) as the type. Subsequently, among major botanical works, L'Héritier de Brutelle (1786) established *Didelta* based in large part on capitulum morphology. Under the *Character essentialis* for the genus L'Héritier de Brutelle noted the differences in floret type and sex functionality within a capitulum (neuter ligulate ray florets, sterile central disc florets, and perfect lateral disc florets), the seeds are embedded, the receptacle is separable, and that the receptacle segments are hardened at fruiting. Gaertner (1791) established *Cuspidia* based on the morphology of the involucre bracts and receptacle, floret types and their sex functionality, monomorphic achenes, and 'plumose' pappus. *Rohria* Vahl (1791: 15) was established for two species (those currently known as *Berkheya carlinoides* (Vahl 1791: 17) Willdenow (1803: 2275) and *B. herbacea* (Linnaeus 1782: 381) Druce (1917: 608)) with a honeycombed receptacle surface, multimeric pappus, and sterile ray florets. *Stobaea* Thunberg (1800: 141) was erected for nine newly described species, but the characters by which the genus was distinguished were not stated. A glabrous achene and absence of a pappus were emphasised to support the segregation of *Cullumia* (Aiton 1813). *Stephanocoma* Lessing (1832: 56) was segregated for two species (those currently known as *Berkheya carduoides* (Lessing 1832: 56) Hutch. in Fourcade (1932: 86) and *B. decurrens* (Thunberg 1793: 104) Willdenow (1803: 2276)) with achenes bearing a uniseriate, coronate pappus. *Heterorhachis* was established on the basis of receptacle morphology (Schultz Bipontinus 1844; Walpers 1847). Harvey (1865) maintained *Berkheya* and *Stobaea* as distinct genera, distinguished by the acuminate, acute or taper-pointed pappus versus 'blunt' pappus, respectively. Harvey also retained *Stephanocoma* but noted that it was distinguished only in possessing a coronate pappus.

Lessing (1832) was the first author to propose an infrageneric classification of *Berkheya*, dividing the genus into four subgenera: *Evopis*, *Agriphyllum*, *Basteria* and *Berkheya*. Candolle (1838) partially followed Lessing's classification, subdividing the genus into six sections. Lessing's subgenera *Evopis*, *Agriphyllum* and *Basteria* were retained as sections; subgenus *Berkheya* was renamed sect. *Euberkheya*, and two new sections, sectt. *Trichodes* and *Trichocoma*, were established. Harvey (1865) slightly modified Candolle's classification by merging sectt. *Evopis* and *Agriphyllum* and erecting a new section, *Pseudostobaea*. Each of these infrageneric classifications were based on a combination of morphological characters, but mainly on life form and leaf morphology.

In his revision of Gorteriinae, Roessler (1959) sunk *Stobaea*, *Rohria* and *Stephanocoma* into *Berkheya*, and divided the expanded *Berkheya* into eight series: *Fruticosae* (13 species), *Angustae* (monotypic), *Cruciatae* (monotypic), *Armatae* (6 species), *Speciosae* (9 species), *Rigidae* (14 species), *Subulatae* (11 species) and *Decurrentes* (17 species). A combination of vegetative and reproductive morphological characters was used to distinguish each series. Some character states were shared among multiple series, and some series had multiple states for a specific character. In particular, Roessler emphasized growth form, leaf shape and arrangement, involucre bract morphology (series *Angustae* only), receptacle alveoles, achene length and pubescence, pappus series, pappus scale shape and apex shape, and discoid versus radiate capitulum type.

As a contribution towards reassessment of species relationships and delimitation of species groupings within the *Berkheya* clade, the utility of external achene morphology for indicating natural species groups within the clade was evaluated. Achene morphological characters have proved valuable to aid elucidation of taxonomic relationships in diverse Asteraceae groups (e.g. Grau 1980; Tadesse *et al.* 1995; Dittrich 1996; Vincent & Wilson 1997; Zhang *et al.* 2013), including the sister subtribe Arctotidinae (McKenzie *et al.* 2005). As is evident in the preceding discussion, achene and pappus characters have been emphasised previously to support, either entirely or partially, circumscription of genera in the Gorteriinae. However, a detailed assessment of the taxonomic value of achene and pappus characters in the *Berkheya* clade has not been undertaken previously. In addition, achene and pappus morphology is compared with existing phylogenetic knowledge.

## **Materials and Methods**

Achenes from 67 species and 9 infraspecific taxa were examined, including representatives of each genus currently recognised in the *Berkheya* clade (Karis 2007; Karis *et al.* 2009) and seven of the eight *Berkheya* series circumscribed by Roessler (1959) (Table 1). Achenes were examined from herbarium specimens lodged in the Selmar Schonland Herbarium, Grahamstown (GRA), the Compton Herbarium, Cape Town (NBG), and the National Herbarium, Pretoria (PRE), and from fresh collections. The taxa examined and voucher details are listed in Appendix 1. For comparability, achenes of disc florets were examined; the ray florets are sterile in all Gorteriinae

that bear this floret type, and some taxa have discoid capitula (Karis 2007; Karis *et al.* 2009). Wherever possible, mature achenes were examined; immature achenes were studied only when mature achenes were unavailable and are indicated as such in Appendix 1. Achenes from multiple specimens of a taxon were examined wherever possible to obtain an indication of intraspecific variability. However, sample sizes were usually small (for most taxa <10 specimens per taxon), which reflects the scarcity of herbarium specimens bearing mature achenes that are readily extractable without causing damage to the specimen. As the achenes were dry, no treatment was necessary prior to direct mounting on stubs without pretreatment, sputter-coated with gold-palladium and observation with a JEOL JSM 480 scanning electron microscope (SEM) at the Electron Microscope Unit, Rhodes University, Grahamstown, and a Leica Stereoscan 420 SEM at the Electron Microscope Unit, University of Limpopo, Medunsa campus. Features of external achene and pappus morphology were recorded. Where necessary, supplementary observations were made with a compound microscope. Features that exhibited variability between taxa were assessed for their taxonomic utility. Achene ontogeny was not investigated. Continuous quantitative characters were not recorded, as these might be influenced by growth conditions, stage of achene development, and the proportion of florets within a capitulum that develop a mature achene containing a fertile seed (Breitwieser & Ward 2003).

**Table 1.** Summary of the taxa sampled in this study. The classification follows Roessler (1959) and incorporates taxa described subsequently (Roessler 1960, 1973; Hilliard & Burt 1975, 1985, 1989; Manning *et al.* 2010; Manning & Goldblatt 2012; Bergh & Helme 2014). Intraspecific taxa are indicated in brackets.

Taxon	Total number of species	Number of species sampled
<i>Berkheya</i> series Fruticosae	13(3)	11
Series Angustae	1	0
series Cruciatae	1(1)	1(1)
series Armatae	6	5
series Speciosae	10(6)	7(2)
series Rigidae	17(8)	13(3)
series Subulatae	11(3)	4(1)
series Decurrentes	19(1)	15(1)
<i>Cullumia</i>	15(7)	8(1)
<i>Cuspidia</i>	1(1)	1
<i>Didelta</i>	2(1)	2
<i>Heterorhachis</i>	2	1
Total	98(31)	67(9)

## Results and Discussion

### Description of morphological characters

#### *Achene*

Achene shape was recorded reliably for mature filled achenes. The mature achene shape was turbinate (e.g., *Berkheya zeyheri* (Harvey 1865: 496) Oliver & Hiern (1877: 429)), obovoid-turbinate (e.g., *B. montana* J.M.Wood & M.S.Evans in Medley Wood (1897: 351)), cylindrical-turbinate (e.g., *B. heterophylla* (Thunberg 1800: 141) Hoffman (1910: 314)), attenuate-turbinate (e.g., *B. cirsiifolia* (Candolle 1838: 519) Roessler (1959: 250)), cylindrical-obovoid to cylindrical-ellipsoid (*Cullumia* spp.), pyriform (e.g., *B. cuneata* (Thunberg 1793: 105) Willdenow (1803: 2270)), or obovoid-unguiculate (*Didelta spinosa* (Linnaeus 1782: 384) Aiton (1789: 256)).

In many taxa ribs (which coincide with vascular traces) were conspicuous on the achene surface. In certain taxa, notably *Cullumia* species, ribs were not obvious on the achene surface. In taxa with a dense covering of twin hairs on the achene surface, the presence of ribs was not always discernible.

In *Cullumia* species, a ring of pale, fleshy tissue was observable at the base of freshly collected achenes. In achenes from older herbarium specimens, this tissue is desiccated and inconspicuous and the achene base appears to have a minute stalk. This fleshy tissue may represent an elaiosome, which refers to an external tissue rich in lipids, proteins and sugars that is attractive to ants (Bennett & Krebs 1987). Elaiosome-bearing diaspores are inferred to be dispersed by ants (myrmecochory), which is recorded in about 20% of plant species in the Cape Floristic Region (Bond & Slingsby 1983) and 17% of all angiosperm families worldwide, including the Asteraceae (Lengyel *et al.* 2010). Ants take elaiosome-bearing diaspores to their nest, where the elaiosome is consumed and the remaining part of the seed is discarded. Further studies of *Cullumia* achenes are needed to elucidate the biochemical composition of the tissue and its attractiveness to ants. In certain *Berkheya* species, e.g., *B. chamaepeuce* (Moore in Schinz 1904: 1024) Roessler (1959: 141) and *B. schinzii* O.Hoffm. in Schinz (1894: 213), a narrow ring of bare, seemingly non-cellular tissue, from which no twin hairs arise, was present at the base of the achene. This tissue might be comparable to a carpopodium, but further examination of freshly collected achenes is needed to clarify the nature of this tissue.

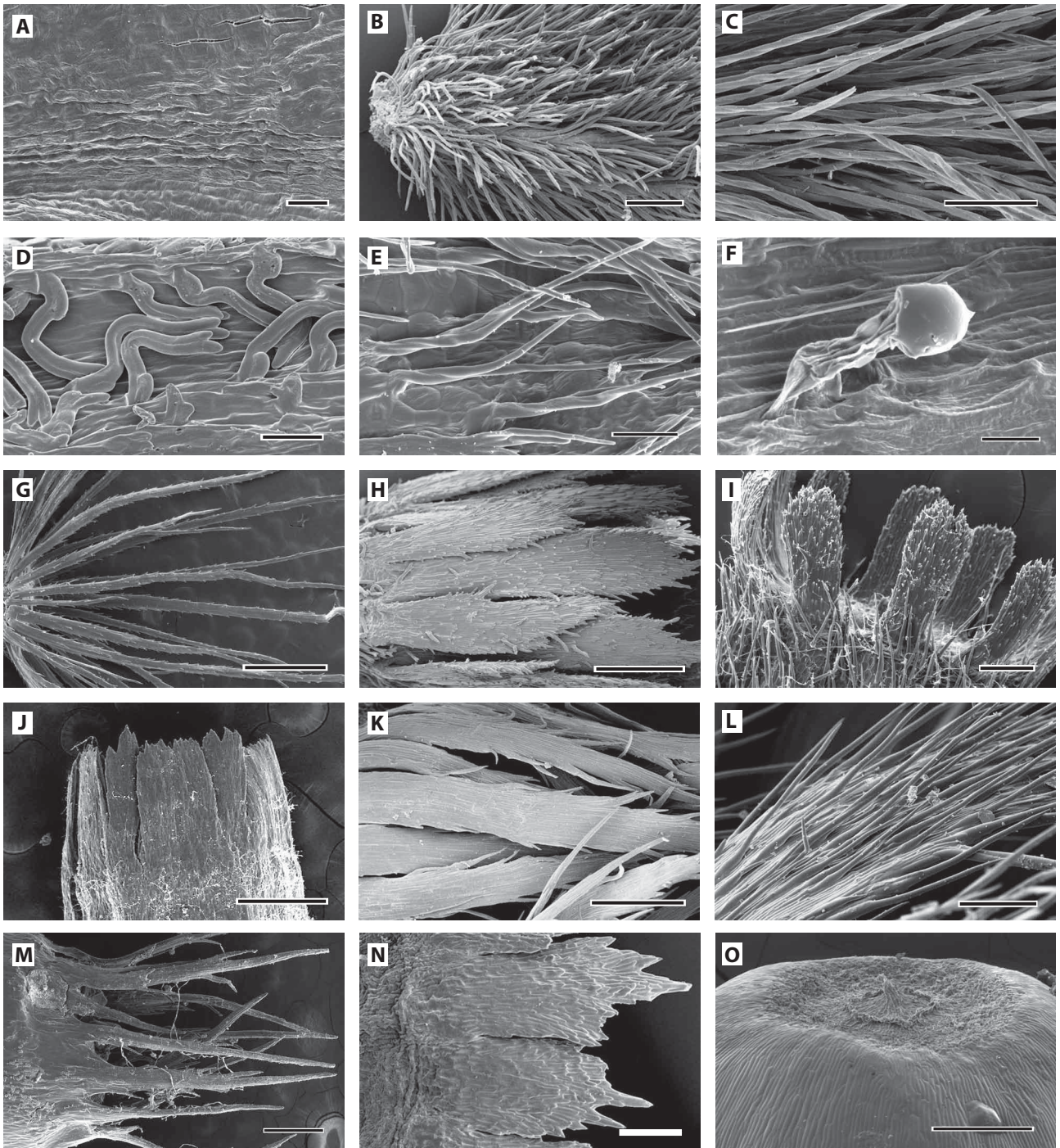
Differences in the sculpturing patterns on the achene surface, based on the external morphology of the epidermal cells, have been noted previously (Phaliso 2013). In the present study, due to unavailability of mature achenes, only semi-mature achenes were observed for some taxa. The degree of achene maturity may affect the external morphology of the epidermal cells. In addition, for taxa with a dense covering of twin hairs on the achene surface, removal of the twin hairs by physical or chemical means without potentially causing artefacts or damage to the epidermal cells could not be guaranteed. Consequently, sculpturing pattern on the achene surface was not recorded in the present study.

### *Trichomes*

In some taxa, the achene surface was completely glabrous, e.g. *Berkheya cirsiifolia* (Fig. 1A), *B. ferox* O.Hoffm. in Schinz (1894: 214) (Fig. 3B), and *Cullumia reticulata* (Linnaeus 1753: 170) Greuter *et al.* (2005: 155) (Fig. 3F). In the remaining taxa three trichome types were observed on the achene surface. The most common trichome type was the twin hair or ‘Zwillingshaare’ (Hess 1938; Herman 2001; Fig. 1B–E), which consists of two parallel, elongate terminal cells, sometimes of unequal length and often separated at their apices, and an extremely short basal cell. The basal cell may be swollen (Fig. 1D) or not visibly swollen relative to the terminal cells. In many species, the terminal cells are spirally twisted (Fig. 1C, E). Biseriate glandular hairs about 30 µm long, comprising a short stalk and a swollen terminal cell, were observed on the achenes of four species, namely *Berkheya latifolia* J.M.Wood & M.S.Evans in Medley Wood (1897: 351), *B. speciosa* (Candolle 1838: 518) O.Hoffm. in Zahlbruckner (1910: 314) (Fig. 1F), *Cuspidia cernua* (Linnaeus 1782: 382) Burt (1948: 316) and *Heterorhachis aculeata* (Burman 1768: 23) Roessler (1959: 313). Achenial glandular hairs are also reported in *Heterorhachis hystrix* Manning *et al.* (2010: 188). The possibility that glandular hairs are present on the ovary of additional taxa but are lost during achene development was not examined and cannot be discounted. Uniseriate multicellular trichomes, similar to those observed in *Arctotheca calendula* (Linnaeus 1753: 922) Levyns (1942: 284) in the Arctotidinae (McKenzie *et al.* 2005), were observed on the surface of *Cuspidia cernua* achenes.

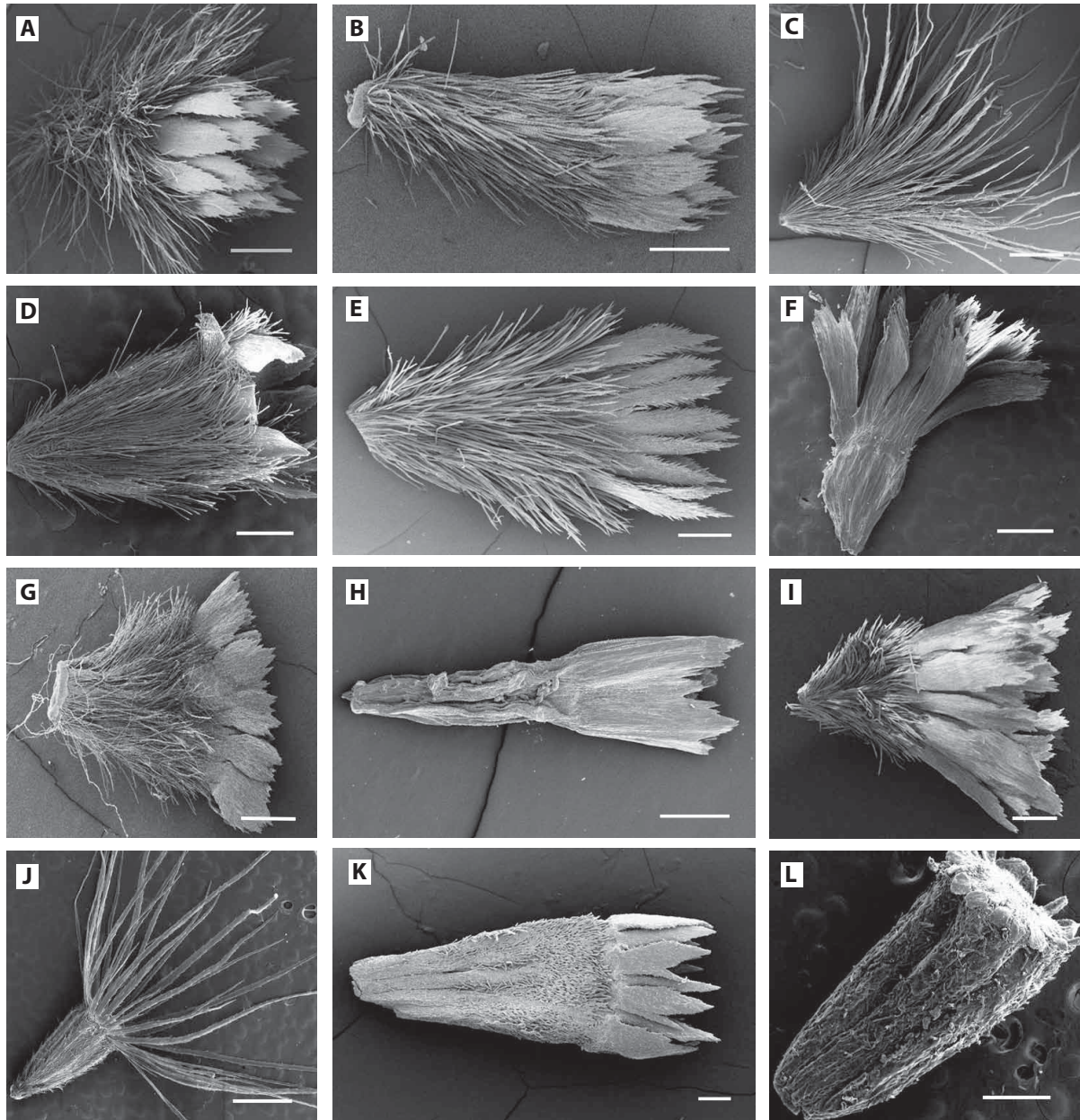
Twin hairs and uniseriate multicellular trichomes (when present) are most commonly distributed over the entire achene surface, but may be concentrated in the apical portion of the achene, e.g. *Berkheya annectens* Harvey (1865: 509) (Fig. 2K), or at the base of the achene as in



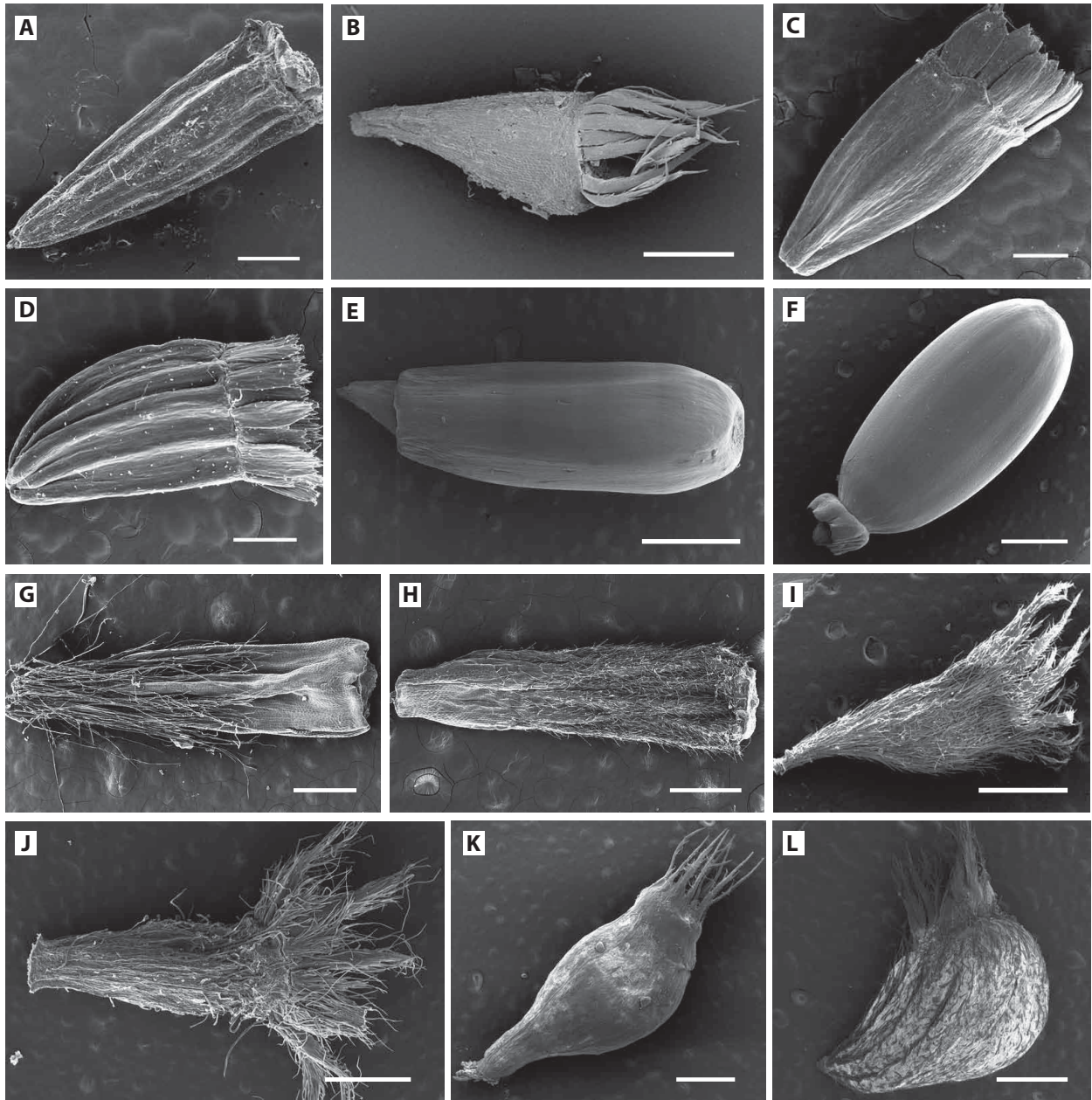


**Figure 1.** Achene and pappus morphological features. **A.** Achene surface glabrous (*Berkheya cirsifolia*). **B.** Dense twin hairs (*Berkheya francisci*). **C.** Twisted twin hairs (*Berkheya carlinopsis*). **D.** Swollen basal cell of twin hair (*Berkheya pinnatifida* subsp. *pinnatifida*). **E.** Twin hair (*Cullumia aculeata*). **F.** Glandular hairs (*Berkheya speciosa* subsp. *speciosa*). **G.** Linear-subulate pappus scales (*Berkheya umbellata*). **H.** Lanceolate pappus scales (*Berkheya pinnatifida* subsp. *pinnatifida*). **I.** Oblong pappus scales (*Berkheya bipinnatifida* subsp. *bipinnatifida*). **J.** Dimorphic, uniseriate pappus scales (*Berkheya robusta*). **K.** Spinescent pappus scales (*Berkheya ferox*). **L.** Fimbriate-spinescent pappus scales (*Berkheya chamaepeuce*). **M.** Partially connate, spinescent pappus scales (*Didelta carnosia* var. *tomentosa*). **N.** Partially connate pappus scales (*Berkheya cirsifolia*). **O.** Pappus absent (*Cullumia decurrens*). Scale bars: A, K, M, 300  $\mu$ m; B, H, O, 200  $\mu$ m; C, E, 30  $\mu$ m; D, L, N, 100  $\mu$ m; F, 20  $\mu$ m; G, 1 mm; I, J, 500  $\mu$ m.





**Figure 2.** Achenes of selected species of *Berkheya*. **A.** *Berkheya fruticosa*. **B.** *Berkheya spinosissima*. **C.** *Berkheya canescens*. **D.** *Berkheya cruciata*. **E.** *Berkheya herbacea*. **F.** *Berkheya speciosa* subsp. *speciosa*. **G.** *Berkheya purpurea*. **H.** *Berkheya carduoides*. **I.** *Berkheya echinacea* subsp. *echinacea*. **J.** *Berkheya umbellata*. **K.** *Berkheya annectens*. **L.** *Berkheya heterophylla* var. *radiata*. Scale bars: A, B, D, E–H, L, 500  $\mu$ m; C, J, 1 mm; I, 300  $\mu$ m; K, 200  $\mu$ m.



**Figure 3.** Achenes of selected species of *Berkheya*, *Cullumia*, *Cuspidia*, *Didelta* and *Heterorhachis*. **A.** *Berkheya cardopatifolia*. **B.** *Berkheya ferox* var. *ferox*. **C.** *Berkheya acanthopoda*. **D.** *Berkheya maritima*. **E.** *Cullumia decurrens*. **F.** *Cullumia reticulata* subsp. *reticulata*. **G.** *Cullumia patula* subsp. *uncinata* (immature). **H.** *Cullumia aculeata* var. *aculeata* (immature). **I.** *Cuspidia cernua*. **J.** *Heterorhachis aculeata*. **K.** *Didelta carnosia* var. *tomentosa*. **L.** *Didelta spinosa*. Scale bars: A, C, D, G, H, 500  $\mu$ m; B, E, F, I, J, K, 1 mm.

*Cullumia patula* subsp. *uncinata* Roessler (1959: 300) (Fig. 3G). In some taxa, the twin hairs occurred predominantly between the ribs, although owing to variability this was not recorded separately. The density of twin hairs ranged from a dense covering to a sparse covering on the achene surface.

The length of twin hairs relative to the achene length exhibited a continuum among taxa from individual twin hairs greatly exceeding the achene in length (to ca. 4 mm long in *Berkheya canescens* Candolle (1838: 507) [Fig. 2C] and *B. schinzii*) to extremely reduced twin hairs as short as 10 µm in length (*Berkheya robusta* Bohnen ex Roessler (1959: 260)).

### *Pappus*

A pappus was present in all taxa, except all but one *Cullumia* species. In all pappose taxa, the pappus consisted of a single or multiple series of scales at the apical end of the achene (Fig. 1G–N). Close examination of the insertion of the scales on the achene was required to determine the number of series reliably. In most taxa one or two series were present, but the pappus of *Berkheya canescens* was sometimes indeterminate (here termed ‘pluriseriate’) in part because of the high number of scales present in this species. In some taxa the scales were partially connate only at the base, e.g. *Didelta carnosus* (Linnaeus 1782: 384) Aiton (1789: 256) (Fig. 1M) and *B. cirsiifolia* (Fig. 1N), and in occasional taxa the scales were completely connate so that discrimination of individual scales was subjective or impossible, e.g. *Berkheya carduoides*. The scales were either morphologically uniform (monomorphic) or showed obvious dimorphism between the scale series, e.g. alternate broad and narrow scales on an achene, such as in *Berkheya robusta* (Fig. 1J) and *Cuspidia cernua* (Fig. 3I).

The scale shape ranged from linear-subulate (Fig. 1G) to lanceolate (Fig. 1H), ovate, oblong (Fig. 1I), obovate or suborbicular. The lateral margin of the scale was entire, serrate (±regularly dentate; Fig. 1G, H), spinescent (deeply serrated with spine-like protrusions; Fig. 1K) or fimbriate-spinescent (with long cilia-like protrusions; Fig. 1L). The apex shape ranged from truncate to acuminate. The apical margin was entire, erose (irregularly indented; Fig. 1I, J), serrate, crenate (with rounded teeth), lacerate (deeply and irregularly serrated; Fig. 1N), spinescent or fimbriate. In some taxa the scale apex was truncate and either erose, serrate or lacerate (here collectively termed ‘praemorse’; Fig. 1J).



On the outer surface of the pappus scale, cell apices projected to varying degrees (termed papillate; Fig. 1H, I) or did not project (smooth; Fig. 1N). In some species the projections were short, rounded and papilla-like, whereas in some species the projections were longer, acute and spine-like.

The total number of scales per achene was highly variable among taxa and often within a taxon. In many taxa the number of scales per achene varied within a limited range, usually centred around 10, 20 or 30. Overall, the number of individual scales per achene (excluding taxa with completely connate scales) ranged from 6–9 (partially connate) in *Berkheya mackeenii* (Harvey 1865: 494) Roessler (1959: 261) to 40–50 in *B. canescens*. In his taxonomic descriptions Roessler (1959) cited the predominant pappus scale number per achene (e.g. ‘10 + 10’, ‘cr. 20’) and rarely indicated the range of variation within a taxon.

### **Intraspecific variation in achene and pappus morphology**

Acknowledging that sample sizes per taxon were small in the present study (achenes were examined from mostly <10 specimens per taxon), intraspecific variation was limited and predominantly observed in achene pubescence, number of pappus scales per achene, and indentation of the pappus scale margin, was observed (see Table 2). Pappus scale shape commonly varied on an individual achene, but in comparison showed limited variation between specimens within a taxon. Achene shape was normally consistent within a taxon. However, slight curvature of the achene from the outermost disc florets within in a capitulum was observed in some *Berkheya* species and was especially marked in *Didelta spinosa*.

The small sample sizes per taxon in the present study strongly reflects the limited accessibility of mature achenes from herbarium vouchers, which have been predominantly collected at anthesis and lack capitula containing mature achenes. Additional sampling is required to determine if the characteristics described for each taxon are representative of the entire taxon and to better sample the entire range of intraspecific variation. While fresh collections were made for many taxa during the course of this study, given the wide geographic distribution of the *Berkheya* clade in sub-Saharan Africa, and the enormous climatic and ecological diversity under which different species grow, collecting fresh mature achenes from multiple populations of each taxon is not a simple undertaking.

**Table 2.** Achene and pappus morphological characters for the ‘*Berkheya* clade’ taxa studied. ‘Group’ refers to species groupings discriminated in this study exclusively on achene and pappus features (see Results and Discussion for brief descriptions of each grouping).

Taxon	Group	I	II	III	IV	V	VI	VII	VII I	I X	X	XI	XII	XII I	XI V	XV	XV I	XV II	XVIII	XI X	XX	XX I
<b><i>Berkheya</i></b>																						
<b>Series <i>Armatae</i></b>																						
<i>B. armata</i>	I	0	1	1	0	1	0	0	1	0	1	1	0	1	2,3	2	0	1	20–25	0	1,3	1,2
<i>B. francisci</i>	I	0	1	1	0	1	0	0	1	0	1	0	0	0,1	1,2	2,4	0	1	20–22	0	1	0
<i>B. herbacea</i>	I	0	1	1	0	1	0	0	1	0	1	1	0	1	2,3	2	0	1	20–30	0	1,3	1
<i>B. macrocephala</i>	I	0	1	1	0	1	0	0	1	0	1	1	0	1	2,3	2	0	2	24	0	3	2
<i>B. rosulata</i>	I	2	1	2	0	1	0	0,1	1	0	1	0	0	1	2	2	0	2	20	0	3	0,1
<b>Series <i>Cruciatae</i></b>																						
<i>B. cruciata</i>	IV	0	1	2	1	1	0	0	1	0	1	1	0	0,1	1	1,2	0,1	1	10–18	0	0,1	0
<b>Series <i>Decurrentes</i></b>																						
<i>B. acanthopoda</i>	VIII	1	0	0	NA	NA	NA	NA	1	0	1	0	1	0	0	4	0	1	10 (5+5)	1	0,1	0
<i>B. cf. caffra</i>	VIII	3	0	0	NA	NA	NA	NA	1	0	1	0	1	0	0,1	4	1	1	10(5+5)	1	1	0
<i>B. cirsifolia</i>	VIII	3	0	0	NA	NA	NA	NA	1	0	1	1	0	0	0,1, 2	1	0,1	1	16–21	0	0,1, 3	0
<i>B. decurrens</i>	VIII	1,3	0	0	NA	NA	NA	NA	1	0	1	0	1	0	0	1,4	0	1	10 (5+5)	1	0,1	0
<i>B. discolor</i>	VIII	3	0,1	0,2	0	0	1	1	1	0	1	0	1	0	0	1,4	0,1	1	10 (5+5)	1	1	0
<i>B. griquana</i>	VIII	3	0,1	0	NA	NA	NA	NA	1	0	1	0	1	1	0	1	0	1	10 (5+5)	1	1	0
<i>B. latifolia</i>	VIII	3	0,2	0	NA	NA	NA	NA	1	0	1	0	1	0	1	1	0	1	10(5+5)–13	1	0	0
<i>B. sp. cf. latifolia</i>	VIII	3	0	0	NA	NA	NA	NA	1	0	1	0	1	0	1	2,4	0	1	17–22	1	1	0
<i>B. mackenii</i>	VIII	3	0	0	NA	NA	NA	NA	1	0	1	0	1	0	0,1	1,4	0,1	1	6–9, 10 (5+5)	0,1	1	0
<i>B. maritima</i>	VIIIA	3	0	0	NA	NA	NA	NA	1	0	1	0	1	1	1	1	0	2	10	1	1	0
<i>B. montana</i>	VIIIA	1	0	0	NA	NA	NA	NA	1	0	1	0	0	0	0,2	1,4	0	1	10 (5+5)	1	1	0
<i>B. onopordifolia glabra</i>	VIII	3	0	0	NA	NA	NA	NA	1	0	1	0	1	0	0,2	1	1	1	8	1	NA	0
<i>B. onopordifolia onopordifolia</i>	VIII	3	0	0	NA	NA	NA	NA	1	0	1	0	1	0	0,2	1	1	1	12–14	1	NA	0
<i>B. pauciflora</i>	VIII	3	1	2	0	0	1	1	1	0	1	0	0	0	1,2	1,4	0	2	20–21	0	1,3	0
<i>B. radula</i>	VIII	3	0	0	NA	NA	NA	NA	1	0	1	0	0	0	0	1,2	0,1	1	10 (5+5)–13	1	1	0
<i>B. robusta</i>	VIIIA	3	0,1	2	0	0	0	1	0	0	1	0	1	1	0	1	1	1	10(5+5)	1	1	0

																			-14			
<i>B. sphaerocephala</i>	VIII	3	0	0	NA	NA	NA	NA	1	0	1	0	1	0	2	1	1	1	10-13	0	1,3	0
<b>Series Fruticosae</b>																						
<i>B. angustifolia</i>	II	0,1	1	1	0	1	0	0	1	0	1	1	0	2	3	5	0	2	16-23	0	3	1
<i>B. barbata</i>	II	0,1	1	1	0	1	0	0	1	0	1	0	0	2	3	5	0	2	20 (10+10)	0	1,3	0
<i>B. canescens</i>	IIA	0,1	1	1	0	1	0	1	1	0	1	0	0	1	2	2	0	2,3	40-50	0	4	2
<i>B. carlinopsis magalismontana</i>	II	0,1	1	1	0	1	0	0	1	0	1	0	0	1	0,1(2)	4,5	0	2	18-21	0	1	0
<i>B. chamaepeuce</i>	II	0,1	1	1	1	1	0	0	1	0	1	0	0	0	2	5	0	2	20-21	0	1,3, 4, 5	0
<i>B. coriacea</i>	II	0,1	1	1	0	1	0	0	1	0	1	1	0	1	2	1,4	0	2	18-24	0	1,3	1
<i>B. cuneata</i>	IX	4	1	1,2	0	1	0	0,1	1	0	1	1	0	2	3	0,2	0	1	16-20	0	3,4	0
<i>B. fruticosa</i>	II	0,1	1	1	0	1	0	0	1	0	1	1	0	0,1	2,3	1,2	0	2	20 (10 + 10)	1	3,5	1
<i>B. schinzii</i>	II	2	1	1	0	1	0	0	1	0	1	1	0	1	2,3	1,4	0	2	18-20	1	1,3	0
<i>B. spinosa</i>	IX	4	0,1	1	0	1	0	0	1	0	1	1	0	1	2,3	0,2	0	2	18-20	0	4	2
<i>B. spinosissima spinosissima</i>	II	1,6	1	2	0	1	0	1	1	0	1	1	0	2,3	3	1	0	2	20 (10+10)	1	3,4	0
<b>Series Rigidae</b>																						
<i>B. annectens</i>	VIIA	2	1	2	0	0	1	0	1	0	1	1	0	1	2	0,2	0	1	18-20	0	1,3	0
<i>B. bipinnatifida</i>	VIIIB	3	1	2	0	1	0	1	1	0	1	1	0	0	0,1	1,2	0	1	16-20	0	0,1	0
<i>B. buphthalmoides</i>	VII	2	1	2	0	0	1	0,1	1	0	1	1	0	0	0,1	0,1	0	1	20	0	0,1	0
<i>B. cardopatifolia</i>	VIIIB	3	1	2	0	0	1	1	1	0	1	1	1	0	0	2	0	1	13-20	0	1	0
<i>B. carlinifolia</i>	VII	2	1	2	0	0,1	0,1	0,1	1	0	1	0,1	0	0,1	0,1, 2	1,2	0	1,2	14-22	0	1	0
<i>B. eriobasis</i>	VIIA	2	1	2	0	1	1,1	1	1	0	1	0,1	0	0,1	1,2	0,1, 2	0	1	14-21	0	1,3	0
<i>B. ferox</i>	IX	4	0	0	NA	NA	NA	NA	1	0	1	0	0	1,2	3	2	0	1	20	0	4	0,1
<i>B. glabrata</i>	VII	2	1	2	0	1	0	0	1	0	1	1	0	0	1	1,2	0	2	19-20	0	0,1	0
<i>B. heterophylla heterophylla</i>	VII	2	1	2	0	1	0	0,1	1	0	1	1	0	0,1	0,1	1	0	1	17-20	0	1	0
<i>B. heterophylla radiata</i>	VII	2	0,1	2	0	0,1	0,1	0,1	1	0	1	0,1	0	0	0,1, 2	1,2	0	1	16-22	0	0,1	0
<i>B. jardineana</i>	VII	2	1	2	0	0	0	0	1	0	1	0	0	1	1	2	0	2	19-22	0	0,1	0
<i>B. onobromoides</i>	VII	2	1	2	0	1	1	1	1	0	1	1	1	1	0	2	0	1	20-21	0	0,1, 5	0
<i>B. pinnatifida pinnatifida</i>	VIIA	2	1	2	0	1	0,1	0,1	1	0	1	1	0	1	2	1,2	0	2	16-24	0	1,3, 5	0



<i>B. pinnatifida ingrata</i>	VIIA	2	0	0	NA	NA	NA	NA	1	0	1	0	0	1	2	1,2	0	2	20	0	0,1, 3	0
<i>B. pinnatifida stobaeoides</i>	VIIA	2	0	0	NA	NA	NA	NA	1	0	1	0	0	1	2,3	2	0	2	18–20	0	3	0
<i>B. rigida</i>	VII	2	1	2	0	0	0,1	0,1	1	0	1	0	0	0	0,1	1	0	1	13–20	0	1	0
<b>Series Speciosae</b>																						
<i>B. carduoides</i>	VIII	3	0,1	0,2	0	0	1	1	1	0	1	0	1	0	0	1,4	1	0,1	8–14	0	NA	0
<i>B. echinacea</i>	III	0	1	1,2	1	1	0	0	1	0	1	1	0	1,2	0	1,4	0	2	20	0	0,1	0
<i>B. purpurea</i>	VIII	1	1	1	0	1	0	1	1	0	1	0	0	0	1,2	1,2	0	2	16–20	0	0,1	0
<i>B. rhapontica aristosa aristosa</i>	III	0	1	2	1	1	1	0	1	0	1	0	0,1	1	1,2	2,4	0	2	14–20	0	1,3, 5	0
<i>B. rhapontica rhapontica</i>	III	0	1	2	1	1	0	1	1	0	1	0	0	0	1	1,2	0	2	20	0	0,1	0
<i>B. setifera</i>	IIIA	0	1	2	1	0	0	0	1	0	1	0	0	1	3	2	0	2	30–36	1	4	2
<i>B. speciosa</i>	V	0	1,2	2	0	0	0	1	1	0	1	0	1	1	0,1, 2	1,2	0,1	2	14–20	0	0	1,2
<i>B. umbellata</i>	IIIA	0	1	2	0	0	0	1	1	0	1	1	0	1	3	2	0	2	29–40	0	4	2
<b>Series Subulatae</b>																						
<i>B. coddii</i>	VI	2	1	2	0	0	0	0,1	1	0	1	0	0	0,1	1	1,2, 3	0	2	17–20	0	0,1	2
<i>B. densifolia</i>	VI	2	1	2	0	1	0	0	1	0	1	0	0	0	0,1	1,2	0	1	11–15	0	0, 1, 2	0
<i>B. subulata wilmsiana</i>	VI	2	1	1	0	1	0	0	1	0	1	1	0	1	1	1,4	0	1	18–20	0	0	1
<i>B. zeyheri rehmannii</i>	VI	0	1	2	0	0	0	1	1	0	1	1	0	1,2	0,1	1,4	0	2	20	0	0	2
<i>B. zeyheri zeyheri</i>	VI	0	1	2	0	0	0	1	1	0	1	1	0	1,2	0,1	1,4	0	2	20	0	0	2
<b>Cullumia</b>																						
<i>Cullumia aculeata</i>	XIVA	5	1	2	0	1	0	1	0	1	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>Cullumia bisulca</i>	XIV	5	0	0	NA	NA	NA	NA	0	1	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>Cullumia decurrens</i>	XIV	5	0	0	NA	NA	NA	NA	0	1	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>Cullumia patula patula</i>	XIV	5	0	0	NA	NA	NA	NA	0	1	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>Cullumia patula uncinata</i>	XIVB	5	1	2	0	1	2	0	0	1	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>Cullumia reticulata</i>	XIV	5	0	0	NA	NA	NA	NA	0	1	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

<i>Cullumia</i> sp.	XIV	5	0	0	NA	NA	NA	NA	0	1	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>Cullumia sulcata</i>	XIV	5	0	0	NA	NA	NA	NA	0	1	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<b><i>Cuspidia</i></b>																						
<i>C. cernua</i>	X	4	2,3	2	NA	NA	0	0	1	0	1	1	0	2,3	3	1,5,6	0	2	20 (10+10)	1	3,4	0
<b><i>Didelta</i></b>																						
<i>Didelta carnosa</i>	XII	4	0	NA	NA	NA	NA	NA	0	0	1	0	0	2	3	5	1	1	10–11	0	3	0
<i>Didelta spinosa</i>	XIII	6	1	2	1	1	0	1	1	0	1	0	0	2	2,3	5	0	1	14–20	0	3	0
<b><i>Heterorhachis</i></b>																						
<i>H. aculeata</i>	XI	2	1,2	2	0	1	0	1	1	0	1	1,2	0	3	2,3	5,6	1	2	14–20	1	3,4	1

### Key to characters

**Achene:** I, Achene shape: 0, turbinate; 1, obovoid-turbinate; 2, cylindrical-turbinate; 3, attenuate-turbinate; 4, pyriform; 5, cylindrical-obovoid to cylindrical-ellipsoid; 6, obovoid-unguiculate. II, Hairs on achene surface: 0, glabrous; 1, twin hairs present; 2, glandular hairs present; 3, uniseriate multiseptate trichomes present. III, Twin hair/uniseriate hair length: 0, hairs absent; 1, approximately half or longer than the cypsela length; 2, less than half the cypsela length. IV, Twin hair basal cell morphology: 0, basal cell not swollen; 1, basal cell swollen. V, Terminal cell morphology: 0, straight; 1, twisted. VI, Twin hair/uniseriate hair distribution: 0, distributed over entire cypsela surface; 1, mainly apical; 2, basal only. VII, Twin hair density: 0, dense; 1, sparse. VIII, Ribs: 0, absent; 1, present. IX, Elaiosomes: 0, absent; 1, present. **Pappus:** X, Pappus: 0, absent; 1, present. XI, Scale surface papillate: 0, absent; 1, present; 2, glandular. XII, Scale apex praemorse: 0, absent; 1, present. XIII, Scale lateral margins: 0, entire; 1, serrate; 2, spinescent; 3, fimbriate. XIV, Scale apex shape: 0, truncate; 1, obtuse; 2, acute; 3, acuminate. XV, Scale apex margin: 0, entire; 1, erose; 2, serrate; 3, crenate; 4, lacerate; 5, spinescent; 6, fimbriate. XVI, Scale fusion: 0, scales free; 1, scales at least fused at base; 2, coronate. XVII, Number of scale series: 1, uniseriate; 2, biseriate; 3, pluriseriate. XVIII, Total number of scales. XIX, Pappus dimorphism: 0, absent; 1, present. XX, Pappus scale shape: 0, obovate; 1, oblong; 2, suborbicular; 3, lanceolate; 4, linear-subulate; 5, ovate. XXI, Pappus scale length relative to achene: 0, shorter; 1,  $\pm$ equal; 2, longer.

Of the taxa for which samples from >10 specimens were examined, some variation in achene pubescence and pappus scale number per achene was observed in *Berkheya carlinifolia*, *B. heterophylla* and *B. speciosa* subsp. *speciosa* (see Table 2). Both *B. carlinifolia* and *B. heterophylla* are morphologically variable species complexes that are currently under investigation (PO Karis unpublished data). The variation in achene and pappus morphology in these two species might be concomitant with variability in other vegetative and reproductive organs and thus might be informative to assist with delimitation of morphology-defined entities within both complexes.

With the above caveats in mind, the following discussion relates what is presently known about the achene and pappus morphology of the studied taxa to the prevailing generic concepts in the *Berkheya* clade and Roessler's (1959) infrageneric classification of *Berkheya*.

### **Achene and pappus morphology in relation to the current taxonomic classification**

#### *Generic circumscription*

With regard to the current generic circumscription in the *Berkheya* clade, only segregation of *Cullumia* is supported by achene morphological characters considered in isolation from other morphological data. *Cullumia* possesses an achene type unique in the clade, namely a cylindrical-obovoid or cylindrical-ellipsoid, usually glabrous achene with inconspicuous ribs (Fig. 3E, F), and with an alleged occurrence of elaiosomes. *Cullumia* might further be diagnosed as epappose (Fig. 1O), except for the presence of minute scales observed in some exemplars of *C. aculeata* (Houttuyn 1776: 158) Roessler (1959: 291) (Karis 2006). As molecular phylogenetic reconstructions indicate that *Cullumia* is embedded within a paraphyletic *Berkheya* (Funk & Chan 2008), the *Cullumia* achene type can be interpreted as an evolutionary novelty with a single origin within *Berkheya* s.str.

Two *Cullumia* taxa stand apart from the remaining taxa in the genus in producing achenes bearing twin hairs on the surface as reported by Roessler (1959). *Cullumia aculeata* achenes have a moderate or dense covering of short (<0.5 mm long) twin hairs on the achene surface except in the basal portion (Fig. 1E, 3H). In *Cullumia patula* subsp. *uncinata* deciduous twin hairs (ca. 2 mm long) about half the achene length are attached only in the basal portion of the achene (Fig. 3G). In both *Cullumia* species, especially *C. patula* subsp. *uncinata*, the terminal cells of the twin hairs differ considerably in length.

Although other morphological characters are held to support the generic segregation of *Cuspidia*, *Didelta* and *Heterorhachis* (Karis 2006), the achene and pappus of the species presently classified in these genera show few morphological features that are unique within the *Berkheya* clade. In *Didelta spinosa* the lanceolate, fimbriate-spinescent pappus scales (Fig. 3L) are similar to those of *Berkheya cuneata* and *B. ferox*. The present study revealed similarities in the achene and pappus morphology of *Cuspidia cernua* (Fig. 3I) with those of *Didelta carnososa*, *Berkheya cuneata*, *B. ferox* and *B. spinosa* (Linnaeus 1782: 381) Druce (1917: 609), which has not been noted previously. In these species the achene is pyriform in shape, with the basal, constricted portion more elongated in *Cuspidia cernua* (Fig. 3I) and *Didelta carnososa* (Fig. 3K). The pappus scales are variable in shape and length but are spinescent to differing degrees. The presence of glandular trichomes on the pappus scale surface of *Cuspidia cernua* is a feature shared with the two *Heterorhachis* species in the *Berkheya* clade (Karis 2006; Manning *et al.* 2010; this study). The presence of glandular trichomes on the achene of *Heterorhachis* species (this study; Manning *et al.* 2010) is shared with *Berkheya latifolia*, *B. speciosa* and *Cuspidia cernua*, and is indicated to be homoplasious based on the phylogenetic reconstructions of Phaliso (2013). However, the spinescent-fimbriate pappus scales of *Heterorhachis* species are unusual and have their closest resemblance in the spinescent-fimbriate scales of *Berkheya chamaepeuce* (termed ‘fimbriate’ by Roessler 1959).

#### *Roessler's Berkheya series*

Roessler (1959) divided *Berkheya* into eight infrageneric series. The achene and pappus features used, in conjunction with other morphological features, by Roessler to characterize these series are listed in Table 3. Observations in the present study indicate that achene and pappus morphology partially supports these groupings. However, Roessler used a combination of vegetative and reproductive morphological characters to distinguish each series. Consequently, it is not unexpected that certain series are indicated to be unnatural groupings based solely on achene and pappus features.

*Berkheya series Fruticosae* contains 13 species, including the type for the genus, *B. fruticosa*. Eleven species were sampled in the present study (e.g. Fig. 2A–C). The majority of these species had turbinate or obovoid-turbinate achenes with a dense covering of twin hairs over the entire surface. The pappus scales were biseriate (sometimes pluriseriate in *B. canescens*), usually

**Table 3.** Comparison of achene and pappus morphological features emphasized by Roessler (1959) to delimit series in *Berkheya*. Note that Roessler also emphasized other vegetative and reproductive morphological features to distinguish the series.

Characteristic	Fruticosae	Angustae	Cruciatae	Armatae	Speciosae	Rigidiae	Subulatae	Decurrentes
<b>Achene</b>								
Hair covering (when present)	Pilose-sericeous	Pilose-sericeous	Pilose-sericeous	Pilose-sericeous	Pilose-sericeous	Pilose-sericeous	Pilose-sericeous	Pilose
Hair length	ca. 1–4 mm	0.5 mm	0.5–1 mm	0.5–3 mm	To ca. 0.5 mm	To ca. 0.2 mm	0.2–1 mm	
Hair density	Dense	Dense	Dense	Dense	Dense, sparse, subglabrous or glabrous	Dense, sparse, subglabrous or glabrous	Dense or sparse	Glabrous or sparse
<b>Pappus</b>								
No. of series	Subbiseriate or pluriseriate	Uniseriate	Uniseriate	Subbiseriate	Subbiseriate or pluriseriate	Subbiseriate or uniseriate	Subbiseriate or $\pm$ uniseriate	Uniseriate
No. of scales	ca. 10 + 10 or 40-50	ca. 20	ca. 10–15	ca. 10 + 10	ca. 10 + 10	ca. 20	10 + 10 or 10–16	ca. 10 or up to 20
Scale shape	Lanceolate, oblong, linear-subulate, linear-setiform	Linear-oblong	Oblong or obovate-lanceolate	Lanceolate or oblong	Obovate, oblong, lanceolate, linear-setiform	Subrotund, ovate, oblong or linear-lanceolate	Obovate or lanceolate	Oblong
Scale apex	$\pm$ Acute	Acute	Acute or subobtuse	Acute	Acute, subacute or obtuse	Obtuse or acute	Obtuse or acute	Obtuse or subacute
Scale fusion	free	free	free or connate	free	Free	free	free	Free or connate
Scale length	1–7 mm	ca. 1 mm	0.5–1 mm	0.5–5 mm	ca. 1–8 mm	0.2–3 mm	0.8–6 mm	0.2–2 mm

lanceolate, with a serrate margin and an acute or obtuse apex. In some species the pappus scales may be linear-subulate (*B. canescens*, *B. chamaepeuce* and *B. spinosissima* (Thunberg 1793: 108) Willdenow (1803: 2270)). The scales were somewhat cryptically dimorphic in *B. fruticosa*, *B. schinzii* and *B. spinosissima*, and monomorphic in the remaining species. Considerable variation in pappus scale number, length, shape and margin serration was evident among the species. *Berkheya cuneata* and *B. spinosa* fundamentally differed in producing pyriform-shaped achenes. Thus, with the exception of *B. cuneata* and *B. spinosa*, the sampled species in series *Fruticosae* produced broadly similar achenes but showed considerable diversity in pappus morphology.

*Berkheya cruciata* (Houttuyn 1779a: 21) Willdenow (1803: 2276) was placed in the monotypic *Berkheya* series *Cruciatae* by Roessler (1959). The achene and pappus of *B. cruciata* (Fig. 2D) do not exhibit a unique character state or combination of character states that support its distinction in its own series. The achene was turbinate with a dense covering of twin hairs, shorter than the achene, over the entire surface. The pappus scales were free, uniseriate, 10–18, oblong or obovate, with an erose or serrate apex. The pappus scales were markedly shorter than the achene, but exhibited no distinctive character states not observed in other species. In addition to achene and pappus characteristics, Roessler (1959) used receptacle alveole features, growth form, leaf characters and radiate capitula to support segregation of series *Cruciatae*.

*Berkheya* series *Armatae* contains six species, of which five were sampled in the current study (e.g. Fig. 3E). The achenes were similar to those of the preceding series in being obovoid-turbinate with a dense covering of twin hairs, shorter or longer than the achene, distributed over the entire surface. The pappus scales were free, uniseriate or biseriate, 20–30, monomorphic, oblong or lanceolate. The scale margins were usually serrate (rarely entire) and the apex was usually acute to acuminate (sometimes obtuse). The pappus scale length relative to the achene length varied considerably between species. The pappus scale outer surface was papillate in *B. armata* (Vahl 1793: 39) Druce (1917: 608), *B. herbacea* and *B. macrocephala* Medley Wood (1907: 50), but smooth in *B. francisci* Bolus (1906: 396) and *B. rosulata* Roessler (1959: 163). Thus the achenes of the sampled species are broadly similar in morphology with comparatively limited variation in pappus morphology.

Nine species were classified in *Berkheya* series *Speciosae* by Roessler (1959) and *B. pannosa* Hilliard & Burt (1975: 77) was described subsequently (Hilliard & Burt 1975). Considerable variation in achene and pappus morphology was evident among the six species sampled in the



present study (e.g. Fig. 3F–J). The achene was turbinate in most species, but was cylindrical-turbinate in *B. carduoides* and obovoid-turbinate in *B. purpurea* (Candolle 1838: 518) Masters (1872: 1262). In most species twin hairs were distributed over the entire achene surface. However, in *B. speciosa* twin hairs were absent or, when present, extremely sparse and extremely reduced in size, and short glandular trichomes were usually present on the achene surface. In *B. rhapontica* subsp. *aristosa* (Candolle 1838: 518) Roessler (1959: 170) var. *aristosa* the twin hairs were only present in the apical portion of the achene. The twin hairs were usually less than half the achene length, but were longer in *B. purpurea* and some samples of *B. echinacea* (Harvey 1865: 495) O.Hoffm. ex Burt Davy in Burt Davy & Pott-Leendertz (1912: 121). The twin hair density and achene surface sculpturing varied considerably among the sampled species. The pappus scales were free, biseriate and monomorphic. The pappus of *B. setifera* Candolle (1838: 507) and *B. umbellata* Candolle (1838: 507) comprised 29–40 linear-subulate scales; in the remaining species the pappus consisted of 14–20, usually obovate or oblong scales (but sometimes lanceolate or ovate in *B. rhapontica* subsp. *aristosa* var. *aristosa*). The pappus showed considerable variation in margin serration and apex shape among the sampled species. The pappus scale outer surface was sparsely papillate in *B. echinacea* and *B. umbellata*, and smooth in the remaining species. Achene and pappus morphology suggested that *B. carduoides* shared a closer affinity with species in series *Decurrentes*, on account of having a cylindrical-turbinate, usually glabrous achene with a much shorter, variably connate (sometimes entirely coronate) pappus. Thus, in terms of achene and pappus morphology, series *Speciosae* was indicated to be among the most variable series and as circumscribed by Roessler (1959) may be an unnatural assemblage of species.

Roessler (1959) recognised 14 species in *Berkheya* series *Rigidae*, and subsequently three additional species have been described (Manning *et al.* 2010; Manning & Goldblatt 2012; Bergh & Helme 2014). Among the 13 species sampled (e.g. Figs. 2K, L, 3A, B), the achenes were obovoid-turbinate or turbinate, glabrous or with a sparse to dense covering of twin hairs shorter than achene, over the entire achene surface or mainly apical, and achene surface swollen/colliculate, sunken or reticulate. The pappus scales were much shorter than achene, 13–24, free, monomorphic, oblong, obovate or lanceolate, outer surface rugose and papillate or not, margin entire, erose or serrate, apex obtuse, truncate or acute.

Three subgroupings were discernible among species classified in *Berkheya* series *Rigidae*. In *B. carlinifolia* (Candolle 1838: 516) Roessler (1959: 203), *B. heterophylla*, *B. jardineana* Manning & Goldblatt (2012: 57), *B. onobromoides* (Candolle 1838: 516) O.Hoffm. & Muschl. in Zahlbruckner (1910: 319), and *B. rigida* (Thunberg 1800: 141) Ewart *et al.* (1909: 20), the achenes were obovoid-turbinate, with short pappus scales were oblong or obovate, with an obtuse or truncate apex, and much shorter than the achene (0.2–1.5 mm long). The placement of the distinctive *B. jardineana* in series *Rigidae* by Manning & Goldblatt (2010) is supported by achene and pappus morphology. The achenes of *B. bipinnatifida* (Harvey 1865: 499) Roessler (1959: 212) and *B. cardopatifolia* (Candolle 1838: 514) Roessler (1959: 215) were similar but differed in being distinctly turbinate and in the former species the pappus was uniseriate with obvious gaps between the individual scales. In *B. annectens*, *B. bupthalmoides* (Candolle 1838: 517) Schlechter (1897: 343), *B. eriobasis* (Candolle 1838: 516) Roessler (1959: 205), *B. glabrata* (Thunberg 1800: 141) Fourcade (1941: 22) and *B. pinnatifida* (Thunberg 1800: 141) Thell. in Probst (1928: 77), the pappus scales were slightly longer (0.3–2 mm long vide Roessler 1959), but still shorter than the achene, and differed in overall shape and apex shape. In *B. annectens* and *B. pinnatifida* subsp. *pinnatifida* the pappus scales were lanceolate with acute apices and the scale length within a series on an individual achene was variable, and the twin hairs were concentrated in the apical half of the achene (especially in *B. annectens*). The achenes were glabrous in *B. pinnatifida* subsp. *ingrata* (Bolus 1907: 346) Roessler (1959: 210) and *stobaeoides* (Harvey 1865: 505) Roessler (1959: 209). In *B. bupthalmoides*, *B. eriobasis* and *B. glabrata* the scales were obovate or oblong with an obtuse apex and  $\pm$ uniform in length, and the twin hairs were distributed over the entire achene surface.

On the basis of achene and pappus morphology, *Berkheya ferox* var. *ferox* (Fig. 3B) was indicated to be misplaced in series *Rigidae*. In this taxon, the achenes were pyriform with a uniseriate pappus of free monomorphic scales, which are linear-subulate with an acuminate apex and a serrate to spinescent margin (features shared with *B. cuneata* and *B. spinosa* in series *Fruticosae*). From the latter two species, *B. ferox* differed primarily in the achenes being glabrous and the pappus scale outer surface smooth. Roessler (1959) gave no indication of the reasons for his placement of *B. ferox* in series *Rigidae*. The similarity in achene morphology of *B. cuneata*, *B. ferox* and *B. spinosa* suggests that a reappraisal of their affinities might be warranted.

*Berkheya* series *Subulatae* contains 11 species, of which four species were examined in the present study. In these species the achene was turbinate or obovoid-turbinate, always with a sparse or dense covering of twin hairs over the entire surface. The pappus comprised monomorphic free scales, either uniseriate or biseriate, usually 17–20 (but 11–15 in *B. densifolia* Bohnen ex Roessler (1959: 229)). The scales were usually obovate, but were occasionally oblong or suborbicular. The apex was usually obtuse or truncate. The scale outer surface was smooth or papillate. The scale length varied from shorter to longer than the achene among different taxa. The scale margins were notably variable in the type of serration.

Roessler (1959) accepted 17 species in *Berkheya* series *Decurrentes*, and subsequently *B. pauciflora* Roessler (1960: 519) and *B. griquana* Hilliard & Burt (1985: 229) were described (Roessler 1960; Hilliard & Burt 1985). Achene and pappus morphology was investigated for 16 species (e.g. Fig. 3C, D). The achene shape of these species was either obovoid-turbinate (and often somewhat bilaterally flattened) (e.g. *B. acanthopoda* (Candolle 1838: 521) Roessler (1959: 255), *B. maritima* Medley Wood & Evans (1899: 253), *B. montana* and *B. robusta*) or cylindrical-turbinate (e.g. *B. cirsiifolia* and *B. sphaerocephala* (Candolle 1838: 518) Roessler (1959: 265)). The achene surface was consistently glabrous in most species, but rare short twin hairs (up to about 25 µm long) were present at the apical end of some achenes of *B. discolor* (Candolle 1838: 520) O.Hoffm. in Zahlbruckner (1910: 319), *B. griquana* and *B. robusta*. Extremely short and extremely sparse twin hairs were present, only between the ribs, on all achenes of *B. pauciflora* examined. Sparse short glandular hairs were observed on the surface of some *B. latifolia* achenes. The achene surface was usually reticulate or sunken, but in some species was swollen/colliculate. Ribs were observed in most species, although in some species ribs were only conspicuous in the basal portion of the achene. The pappus was always much shorter than the achene. The form of the pappus ranged from entirely free scales (e.g. *B. maritima*) to shortly connate at the base of the scales (e.g. *B. griquana* and *B. sphaerocephala*) to almost entirely connate (i.e. coronate; e.g. *B. carduoides*). The number of scales was diverse, most commonly 10 (e.g. *B. discolor* and *B. montana*), but rarely fewer (ca. 6–9 with partially connate scales in the Mpumalanga form of *B. mackenii*) or up to 16–22 (*B. cirsiifolia* and *B. pauciflora*). The species with 10 scales usually exhibited a strongly dimorphic pappus with alternate broad oblong and narrow lanceolate scales. The pappus was uniseriate in most taxa and only rarely biseriate (*B. maritima* and *B. pauciflora*). The scale apex was usually truncate or obtuse; taxa with dimorphic scales the apices were usually

truncate (broad scales) and acute (narrow scales). The apex margin was usually erose or lacerate, but occasionally serrate. The scale lateral margins were usually entire but occasionally serrate. Thus, collectively the taxa in series *Decurrentes* represented a coherent group with two achene types discernible and notable variation in pappus scale number and degree of fusion. As mentioned above, the present observations suggested that *B. carduoides* (placed in series *Speciosae* by Roessler) may have a closer affinity with series *Decurrentes*.

*Berkheya* series *Angustae* is monotypic and known only from the type collection of *B. angusta* Schlechter (1899: 219). Attempts to relocate the species in recent years have proved unsuccessful and other herbarium specimens identified as *B. angusta* have proved to be misidentified. It was not possible to extract an immature achene from the isotype lodged in GRA for examination in the present study. Roessler (1959), who provided greater detail than in the original description by Schlechter (1899), described the achene as being shortly and densely pilose, with a uniseriate pappus of about 20 scales, with each scale linear-oblong, acute, minutely denticulate and about 1 mm long. Achene and pappus morphology on its own provides weak support for segregation of series *Angustae*. In terms of vegetative morphology, *B. angusta* shows affinities with *B. barbata* (Linnaeus 1782: 382) Hutch. in Fourcade (1932: 86) and *B. angustifolia* (Houttuyn 1779b: 518) Merrill (1938: 369). Roessler's (1959) description of *B. angusta* suggests the former two species differ primarily in bearing opposite leaves and a biseriate pappus of lanceolate, slightly longer scales (2–4 mm and 1.5–3 mm, respectively). Roessler also noted morphological similarities between *B. angusta* and *Cullumia* species, but the achene and pappus of *Cullumia* species differ notably in morphology (e.g., in being epappose and glabrous or only sparsely hairy). Rediscovery of *B. angusta* is of utmost importance to provide fresh material with which to determine its affinities.

### **Phylogenetic interpretation of achene and pappus morphology in the *Berkheya* clade**

The most complete published molecular phylogenies currently available for the *Berkheya* clade are those of Funk & Chan (2008), which were derived from chloroplast and nuclear sequence data. Their study was focused on elucidating relationships within the entire subtribe Gorteriinae, hence taxonomic sampling of the *Berkheya* clade was limited (30 spp.). Therefore, only a preliminary interpretation of achene and pappus morphology in the *Berkheya* clade within a phylogenetic context is currently possible.

The phylogenies of Funk and Chan (2008) consistently place both *Didelta* species in a clade with *Berkheya spinosissima*. Phylogenetic analyses of the cpDNA data placed this clade sister to the *Gorteria* clade, whereas the ITS and combined data sets placed the clade sister to the remainder of the *Berkheya* clade. The achenes of these species differ considerably in shape, pubescence, and pappus morphology, which collectively do not provide support for a phylogenetic relationship. The most notable inconsistency is that in *B. spinosissima* the capitulum does not function as a diaspore and the mature achenes are released from the capitulum to achieve dispersal. Further sampling of relatives of *B. spinosissima* (*B. chamaepeuce* and *B. schinzii*) and additional phylogenetic analyses are needed to confirm the validity of this relationship and to resolve the clade's affinities within the Gorteriinae.

*Cuspidia cernua* and *Heterorhachis aculeata* were placed as monotypic lineages within the *Berkheya* clade by the cpDNA and ITS data in the study by Funk & Chan (2008). Both data sets implicated *C. cernua* to be an early divergence within the clade, whereas *H. aculeata* was suggested to be an early divergence in the cpDNA phylogeny but was embedded within the *Berkheya* clade in the ITS phylogeny. The achene and pappus of both species show unusual features, of which some, as discussed already, may be indicative of distant affinities with *Berkheya* species. Therefore the suggestion of a close relationship with *Berkheya* species is not unexpected with consideration of achene and pappus morphology.

The five sampled *Cullumia* species were embedded within *Berkheya* in the phylogenies of Funk & Chan (2008). However, the precise relationships of these taxa were not resolved. Chloroplast sequence data placed the *Cullumia* species in a clade with *Berkheya cruciata*, and sister to a clade consisting of *B. canescens*, *B. fruticosa* and *B. spinosa*. The ITS sequence data placed all of these taxa in an unresolved polytomy but indicated that *Cullumia patula* and *C. rigida* are more closely related to *B. cruciata* than to the other sampled *Cullumia* species. *Cullumia* species produce achenes that are morphologically unique within the *Berkheya* clade. In all *Cullumia* taxa the achene is cylindrical-obovoid or cylindrical-ellipsoid and epappose. The achene surface is glabrous in most taxa, except for *C. aculeata* (minutely pilose) and *C. patula* subsp. *uncinata* (with twin hairs about half the achene length and attached only in the basal portion of the achene). The phylogenetic association of *B. cruciata* with *Cullumia* species is not supported by achene and pappus morphology. In *B. cruciata* the achene is turbinate with a dense covering of twin hairs shorter than the achene distributed over the entire achene surface. In addition, a

uniseriate pappus consisting of 10–18 free (or sometimes connate; Roessler 1959) scales is present. The glabrous, epappose achene was emphasized to support the segregation of *Cullumia* (Aiton 1813). In addition, possession of an elaiosome may be autapomorphic to *Cullumia* species within the *Berkheya* clade. However, the existing phylogenetic evidence suggests that *Cullumia* should be subsumed into *Berkheya*. Given the presence of twin hairs on the achene surface, it would be interesting to examine whether *C. aculeata* and *C. patula* represent early divergences in the evolution of a *Cullumia* lineage from within *Berkheya*.

Species currently classified in *Berkheya* exhibit notable diversity in achene and pappus features, but interpretation in relation to species diversification and evolutionary history is premature. While Funk & Chan (2008) resolved at least two major clades among *Berkheya* spp., relationships among these taxa might alter and additional clades might be resolved with more complete taxonomic coverage. Additional genetic data might also suggest differing relationships. Therefore discussion of achene and pappus morphology among *Berkheya* species in an evolutionary context awaits further phylogenetic analyses with comprehensive sampling of *Berkheya* species.

Both the *Berkheya* and *Gorteria* clades within the subtribe Gorteriinae are notable for the evolution of the capitulum or capitular segments as a diaspore. Within the *Gorteria* clade, limited evolution in achene and pappus morphology is apparent among species of *Gorteria* and those species formerly placed in *Hirpicium* (Stångberg 2014; Stångberg & Anderberg 2014). In the *Berkheya* clade, taxa showing retention of the achene and dispersal of the capitulum in segments or as a single unit (*Berkheya cuneata*, Jan Vlok pers. comm.; *Cuspidia cernua* and *Didelta* spp.) show limited reduction in the size of the pappus scales, at least compared with other taxa in the *Berkheya* clade, suggesting that evolution of the capitulum or capitular segments as the diaspore may have been relatively recent evolutionary events. In these taxa the pappus scales show a tendency towards spinescence, which might be an autapomorphy conferring additional protection of the achene that promotes retention of the pappus. However, other taxa placed in the same subclade as *Didelta*, as well as *B. ferox*, show similarly or more spinescent pappus scales, so the trait may have pre-existed before evolution of the capitulum or capitular segments as the diaspore.

The subtribes Arctotidinae and Gorteriinae show interesting differences in diaspore dispersal strategies. Compared with the greater extent of achene evolution in Arctotidinae (McKenzie *et al.*



2005), achene morphology in the *Berkheya* clade, as well as the sister *Gorteria* clade (Stångberg 2014), appears to have been under weaker selective pressure and thus more limited achene evolution is evident. However, in both the *Berkheya* and *Gorteria* clades evolution of the entire capitulum or capitular segments as the diaspore, with retention of the individual achenes, has occurred independently. Such an evolutionary strategy is unknown among extant Arctotidinae. Geocarpy, which has evolved independently in three *Haplocarpha* species in the Arctotidinae (Barker 2005), is unknown in Gorteriinae. Development of wings on the achene, which characterises the achene of *Arctotis* Linnaeus (1753: 922) (McKenzie *et al.* 2005), is unknown elsewhere in the tribe Arctotideae.

### **Species groupings based on achene and pappus morphology**

In the absence of a well-resolved phylogeny with comprehensive taxonomic coverage, the following broad species groupings are distinguished based on observations in the present study. These groupings could be tested in future phylogenetic investigations of the *Berkheya* clade.

**GROUP I:** *Berkheya armata*, *B. francisci*, *B. herbacea*, *B. macrocephala*, *B. rosulata* — achenes turbinate or cylindric-turbinate, with dense covering of twin hairs over entire surface, shorter or longer than the achene; pappus uniseriate or biseriate, scales free, monomorphic, 20–30, shorter,  $\pm$ equal or longer than achene, oblong or lanceolate, lateral margin entire or serrate, apex acute or acuminate (sometimes obtuse), apex serrate (sometimes lacerate), outer surface smooth or papillate (Fig. 1B, 2E).

**GROUP II:** *Berkheya angustifolia*, *B. barbata*, *B. carlinopsis* subsp. *magalismontana*, *B. chamaepeuce*, *B. coriacea*, *B. fruticosa*, *B. schinzii*, *B. spinosissima* — achene turbinate or obovoid-turbinate, dense covering of twin hairs over the entire surface, twin hairs  $\pm$ equal or longer than achene; pappus biseriate, scales free, monomorphic or cryptically dimorphic, 16–24, lanceolate, oblong or ovate, scale length shorter or  $\pm$ equal to achene, lateral margin variable (entire, serrate, spinescent), apex variable (truncate, obtuse, acute, acuminate), apex margin variable (erose, serrate, lacerate, spinescent), outer surface smooth or papillate (Fig. 1D, L, 2A, B).

**SUBGROUP IIA:** *Berkheya canescens* – twin hairs sparse; pappus scales pluriseriate, ca. 40–50, linear-subulate, longer than achene (Fig. 2C).

GROUP III: *Berkheya echinacea*, *B. rhapontica* — achene turbinate, dense covering of twin hairs over the entire surface, twin hairs shorter than achene; pappus uniseriate or biseriate, scales free, monomorphic, 14–20, shape variable (obovate, oblong, lanceolate, ovate), scale length shorter than achene, lateral margin variable (entire, serrate, spinescent), apex variable (truncate, obtuse, acute), apex margin variable (erose, serrate, lacerate), outer surface smooth or papillate (Fig. 2I).

SUBGROUP IIIA: *Berkheya setifera*, *B. umbellata* — pappus biseriate, scales monomorphic or cryptically dimorphic, 29–40, linear-subulate, longer than achene, apex acuminate (Fig. 1G, 2J).

GROUP IV: *Berkheya cruciata* — achene turbinate, with dense covering of twin hairs over entire surface, twin hairs shorter than achene; pappus uniseriate, markedly shorter than achene, scales free or sometimes connate, monomorphic, 10–18, oblong or obovate, lateral margins entire or serrate, apex obtuse, apex margin erose or serrate, outer surface papillate (Fig. 2D).

GROUP V: *Berkheya speciosa* — achene turbinate, with glandular hairs and sparse twin hairs over entire surface, twin hairs much shorter than achene; pappus biseriate,  $\pm$ equal or longer than achene, scales free or shortly connate at base, monomorphic, 14–20, obovate, lateral margins serrate, apex truncate, obtuse or acute, apex margin erose or serrate, outer surface smooth (Fig. 1E, 2F).

GROUP VI: *Berkheya coddii*, *B. densifolia*, *B. subulata* var. *wilmsiana*, *B. zeyheri* — achene turbinate or cylindric-turbinate, with sparse or dense covering of twin hairs over entire surface, twin hairs shorter or longer than achene; pappus uniseriate or biseriate, shorter to longer than achene, scales free, monomorphic, 11–20, obovate, oblong or suborbicular, lateral margins entire, serrate or spinescent, apex truncate or obtuse, apex margin variable (erose, serrate, crenate, lacerate), outer surface smooth or papillate.

GROUP VII: *Berkheya buphthalmoides*, *B. carlinifolia*, *B. glabrata*, *B. heterophylla*, *B. jardineana*, *B. onobromoides*, *B. rigida* — achene cylindric-turbinate, with sparse or dense covering of twin hairs over entire surface or in apical portion of achene, twin hairs much shorter

than achene; pappus uniseriate or biseriate, shorter than achene, scales free, monomorphic, 13–22, obovate or oblong (sometimes ovate), lateral margin entire or serrate, apex obtuse or truncate, apex margin variable (entire, erose, serrate), outer surface smooth (sometimes papillate) (Fig. 2L).

SUBGROUP VIIA: *Berkheya annectens*, *B. eriobasis*, *B. pinnatifida* — pappus scales lanceolate or oblong (sometimes ovate), lateral margin serrate (sometimes entire), apex acute (sometimes obtuse or acuminate) (Fig. 1C, H, 2K).

SUBGROUP VIIB: *Berkheya bipinnatifida*, *B. cardopatifolia* — achene attenuate-turbinate (Fig. 1I, 3A).

GROUP VIII: *Berkheya acanthopoda*, *B. cf. caffra*, *B. carduoides*, *B. cirsiifolia*, *B. decurrens*, *B. discolor*, *B. griquana*, *B. latifolia*, *B. mackenii*, *B. multijuga*, *B. onopordifolia*, *B. pauciflora*, *B. radula*, *B. sphaerocephala* — achene attenuate-turbinate, glabrous or with sparse twin hairs in upper portion of achene (sometimes over entire surface), twin hairs minute, rarely with glandular hairs; pappus uniseriate or biseriate, scales free or connate at base (sometimes more than half of pappus length), monomorphic or dimorphic, 6–21, commonly 10 (5+5), shorter than achene, oblong or lanceolate, sometimes obovate, lateral margin entire or serrate, apex truncate, obtuse or acute, apex margin erose or lacerate (sometimes serrate), outer surface smooth (rarely papillate) (Fig. 1A, N, O, 2H, 3C).

SUBGROUP VIIIA: *Berkheya maritima*, *B. montana*, *B. purpurea*, *B. robusta* — achene obovoid-turbinate (Fig. 2G, 3D).

GROUP IX: *Berkheya cuneata*, *B. ferox*, *B. spinosa* — achene pyriform, glabrous or with sparse or dense covering of twin hairs over entire surface, twin hairs shorter or longer than achene; pappus uniseriate or biseriate, scales free, monomorphic, 16–20, shorter to longer than achene, lanceolate or linear-subulate, lateral margin serrate to spinescent, apex acute or acuminate, apex margin entire or serrate, outer surface smooth or papillate (Fig. 1K, 3B).

GROUP X: *Cuspidia cernua* — achene obovoid-pyriform, glandular and dense uniseriate multiseptate hairs over entire surface, multiseptate hairs shorter than achene; pappus biseriate, scales free, dimorphic, 20 (10+10), shorter than achene, lanceolate or linear-subulate, lateral

margin fimbriate-spinescent, apex acuminate, apex margin erose or fimbriate-spinescent, outer surface papillate (Fig. 3I).

GROUP XI: *Heterorhachis aculeata* — achene cylindrical-turbinate, glandular hairs and sparse twin hairs over entire surface, twin hairs shorter than achene; pappus biseriate, scales free, dimorphic, 14–20,  $\pm$ equal to achene length, lanceolate or linear-subulate, lateral margin fimbriate, apex acute or acuminate, apex margin spinescent-fimbriate, outer surface papillate and glandular (Fig. 1F, 3J).

GROUP XII: *Didelta carnosa* — achene pyriform, glabrous; pappus uniseriate, scales free, monomorphic, 10–11, shorter than achene, lanceolate, lateral margin spinescent, apex acuminate, apex margin spinescent, outer surface smooth (Fig. 1M, 3K).

GROUP XIII: *Didelta spinosa* — achene obovoid-unguiculate, sparse twin hairs over entire surface, twin hairs much shorter than achene; pappus uniseriate, scales free, monomorphic, 14–20, shorter than achene, lanceolate, lateral margin spinescent, apex acuminate or acute, apex margin spinescent, outer surface smooth (Fig. 3L).

GROUP XIV: *Cullumia bisulca*, *C. decurrens*, *C. patula* subsp. *patula*, *C. reticulata*, *C. sp.*, *C. sulcata* — achene cylindric-obovoid to cylindric-ellipsoid, glabrous, ribs inconspicuous; pappus absent (Fig. 3E, F).

SUBGROUP XIVA: *Cullumia aculeata* — sparse twin hairs over entire achene surface, twin hairs much shorter than achene (Fig. 3H).

SUBGROUP XIVB: *Cullumia patula* subsp. *uncinata* — dense deciduous twin hairs only in basal portion of achene, twin hairs about half achene length (Fig. 3G).

## **Conclusion**

The present study provides data on achene and pappus morphology for approximately two-thirds of the species of the *Berkheya* clade. Variation in achene shape, pubescence, and pappus form and micromorphology was recorded, which only partially supports the prevailing taxonomic classification at the generic and infrageneric levels. Segregation of *Cullumia* with

emphasis on achene characteristics and the absence of a pappus is not supported, however, by existing molecular data. Achene and pappus morphology generally supports Roessler's species groupings as series in *Berkheya* but with some notable exceptions. In particular, heterogeneity of series *Speciosae* and misplacement of a number of species in series *Decurrentes* and *Rigidae* are indicated. Detailed reappraisal of other morphological characters and reconstruction of molecular phylogenies with complete taxonomic coverage are required as part of the ongoing taxonomic reassessment of the *Berkheya* clade. Future reconstruction of taxonomically comprehensive phylogenies also will permit formulation of hypotheses for achene and pappus evolution in the *Berkheya* clade.

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**Appendix I.** Collection and voucher information for the specimens examined.

All vouchers are deposited in the Selmar Schonland Herbarium, Grahamstown, unless indicated otherwise. NBG, Compton Herbarium, Cape Town; PRE, National Herbarium, Pretoria.

\* Achene immature.

*Berkheya acanthopoda* (DC.) Roessler: N.P. Barker s.n., 2005; R.G. Strey 10807\*.

*B. angustifolia* (Houtt.) Merr.: H.H. Burrows 3955; E.C. Feyt s.n., 10 Apr 1991; R.J. McKenzie 3047; Taylor & Edwards 8846\*.

*B. annectens* Harv.: J.P.H. Acocks 13193\* (PRE); R.O. Peter 01 (PRE).

*B. armata* (Vahl) Druce: R.J. McKenzie 2568\*, 3145\*.

*B. barbata* (L.f.) Hutch.: R.J. McKenzie 2591\*, 3121\*; sine coll., 1899.

*B. bipinnatifida* subsp. *bipinnatifida*: R.J. McKenzie 1839\*.

*B. buphthalmoides* (DC.) Schltr.: A. Dieterlen 1280\* (PRE); H.G. Flanagan 1604\* (PRE); N. Phaliso 89\*; J.E. Victor 1445\* (PRE).

*B. cf. caffra* MacOwan: L. Mucina 200207/1.

*B. canescens* DC.: C. Evrard 8856 (PRE); L. Mucina 290806/32\*; R. Schlechter 11335\*.

*B. cardopatifolia* (DC.) Roessler: Clark, Barker, Ramdhani & Kelly 41\*; Clark & Pienaar 460\*; Clark, Pienaar & Daniels 672\*; Clark & Te Water Naude 365\*.

*B. carduoides* (Less.) Hutch.: N.P. Barker 1924\*; M. Koekemoer 4144 (PRE); R.J. McKenzie s.n., 5 Mar 2009.

*B. carlinifolia* (DC.) Roessler: Clark and Midgley 302; Clark & O'Connor 358; Clark, Pienaar and Daniels 653; R.J. McKenzie 2762\*, 3043, 3139\*, 3140, 3142, 3143\*, 3153; L. Mucina 211006/29.

*B. carlinopsis* subsp. *magalismsontana* (Bolus) Roessler: R. Leendertz 3714\*; N. Phaliso 64\*, 66\*.

*B. chamaepeuce* (S.Moore) Roessler: J.P.H. Acocks 18011 (PRE); B. de Winter 3282 (PRE); H. Merxmüller and W. Giess 28130 (PRE).

*B. cirsiifolia* (DC.) Roessler: J. Fearon 09-005\*; Jacot-Guillarmod, Getliffe & Mzamane 245; R.A. Lubke 6220\*; R.J. McKenzie 973; N. Phaliso 88.

*B. coddii* Roessler: L.E. Codd 10338 (PRE).

*B. coriacea* Harv.: J. Muir 1462; R.J. McKenzie 3121.

*B. cruciata* (Houtt.) Willd. subsp. *cruciata*: J.P.H. Acocks 18291 (PRE); R.J. McKenzie 2600.

*B. cruciata* subsp. *subintegra* Roessler: H.C. Taylor 4714 (PRE).

*B. cuneata* (Thunb.) Willd.: J.J. Meyer 1759 (PRE).

*B. decurrens* (Thunb.) Willd.: E.E.A. Archibald 5042; P. Brown 00/05/10/PB/219; E. Brink 219; G.E. Gibbs Russell 3674\*; R.J. McKenzie 1189, 1838; A.A. Merti 1045\*; P.T. Robertshaw s.n., 20 Feb 1979\*.

*B. densifolia* Bohnen ex Roessler: R.J. McKenzie 2051\*.

*B. discolor* (DC.) O.Hoffm. & Muschl.: R.J. McKenzie 998; N. Phaliso 22, 28.

*B. echinacea* (Harv.) O.Hoffm. ex Burt Davy subsp. *echinacea*: N. Phaliso 63\*; H.J. Schlieben 9495\* (PRE).

*B. eriobasis* (DC.) Roessler: J.P.H. Acocks 17388\* (PRE); E.E. Galpin 11160\* (PRE); R.J. McKenzie 2772\*, 3016\*, 3019\*.

*B. ferox* O.Hoffm. var. *ferox*: L.E. Codd 5898 (PRE).

*B. francisci* Bolus: R.J. McKenzie 2599; E. Pienaar T707.

*B. fruticosa* (L.) Ehrh.: F.W., S.K. & R.W. Gess 95/96/220; S.K., F.W. & D.W. Gess 89/90/14, 89/90/68; Kolbe s.n., Sep 1917\*; R.J. McKenzie 1301\*, 2779\*.

*B. glabrata* (Thunb.) Fourcade: M. Koekemoer 2046 (PRE).

*B. griquana*: N. Phaliso 27.

*B. herbacea* (L.f.) Druce: R.J. McKenzie 1176.

*B. heterophylla* (Thunb.) O.Hoffm. var. *heterophylla*: K.Å. Dalstrand 2513; T. Dold s.n., 19 Oct 2011\*; P. Philipson 5572; S.C. Troughton 209.

*B. heterophylla* var. *radiata* (DC.) Roessler: E. Brink 220, 316; L. Britten s.n., 10 Nov 1919; F.R. Long 26; R.J. McKenzie 1578, 2559, 2895, 3001, 3002, 3028\*, 3031, 3033, 3040, 3041, 3112\*, 3136; S. Schonland 840, 3469, 3652; W. Tyson 56.

*B. jardineana* J.C.Manning & Goldblatt: R.J. McKenzie 3042.

*B. latifolia* J.M.Wood & M.S.Evans: K.E. Codd 7880 (PRE); G.B. Deall 1980 (PRE); M. Koekemoer 2223\* (PRE); S. Kurzweg K1103 (PRE).

*B. sp. cf. latifolia* J.M.Wood & M.S.Evans: N. Phaliso 56, 57.

*B. mackenii* (Harv.) Roessler: J. Medley Wood 5310 (PRE); R.J. McKenzie 2043 & N. Phaliso\*.

*B. macrocephala* J.M.Wood: O. West 1387\* (PRE).

- B. maritima* J.M.Wood & M.S.Evans: W. Tyson 2740.
- B. montana* J.M.Wood & M.S.Evans: J.Medley Wood 6958\*; N. Phaliso 34\*, 41.
- B. onobromoides* (DC.) O.Hoffm. & Muschl. var. *onobromoides*: J.P.H. Acocks 17358\* (PRE);  
M.M. Page s.n., x.1921\* (PRE);
- B. onopordifolia* var. *glabra* Bohnen ex Roessler: N. Phaliso 40.
- B. onopordifolia* (DC.) O.Hoffm. ex Burt Davy var. *onopordifolia*: N. Phaliso 83.
- B. pauciflora* Roessler: D. McMurtry 12633\* (PRE); N. Phaliso 65.
- B. pinnatifida* (Thunb.) Thell. subsp. *pinnatifida*: J.P.H. Acocks 1502\* (PRE); Baaschens and  
Scheepers 2\* (PRE); C.A. Smith 908\* (PRE).
- B. pinnatifida* subsp. *ingrata* (Bolos) Roessler: N. Phaliso 72.
- B. pinnatifida* subsp. *stobaeoides* (Harv.) Roessler: L. Henderson 787 (PRE); N. Phaliso 23.
- B. purpurea* (DC.) Masters: J. Fearon s.n. 4.iv.2009; D.J.B. Killick & J. Vahrmeijer 3793\*  
(PRE); M. Koekemoer 3448 (PRE); N. Phaliso 24, 90; J.P. Roux 1385 (PRE); R. Story 3735  
(PRE).
- B. radula* (Harv.) De Wild.: O.J. Hansen 3410 (PRE); M. Koekemoer 3090\*, 4169 (PRE); R.J.  
McKenzie 1151\*; J. Woollard 256 (PRE).
- B. rhapontica* subsp. *aristosa* (DC.) Roessler var. *aristosa*: R.J. McKenzie 2084\*, N. Phaliso 31\*.
- B. rhapontica* (DC.) Hutch. & Burt Davy subsp. *rhapontica*: V.A. Funk 12422 & M. Koekemoer  
(PRE).
- B. rigida* (Thunb.) Bolus & Wolley-Dod ex Ewart, Jean White & B.Rees: R.H. Compton 164,  
15399, 16659, 19025, 21852 (NBG); S.M. Johnson 9 (NBG); R.J. McKenzie 1805\*, 2501\*,  
2517\*; L. Mucina 281203/6.
- B. robusta* Bohnen ex Roessler: N. Phaliso 62; F.A. Rogers 5195 (PRE).
- B. rosulata* Roessler: M. Schmitz 6901 (PRE); Clark & Keevey 10.
- B. schinzii* O.Hoffm. ex Hutch.: F.W. & S.K. Gess 03/04/78, 05/06/75.
- B. setifera* DC.: N. Phaliso 37; F.A. Rogers s.n., 29.v.1905.
- B. speciosa* subsp. *lanceolata* Roessler: J.P.H. Acocks 11245\* (PRE); P.M. Burgoyne 966 (PRE);  
G. Germishuizen 3883 (PRE); R.G.M. Young 26596 (PRE).
- B. speciosa* (DC.) O.Hoffm. subsp. *speciosa*: J.P.H. Acocks 13079\*; C.L. Bredenkamp 1350\*; E.  
Cloete 1059\*; K.Å. Dahlstrand 1817\*; E.E. Galpin 14711\* (PRE); L. Mucina 010303/3\*; N.

Phaliso 32; H.G. Schweickert 826 (PRE); R. Story 4136\* (PRE); R.G. Strey 5946\* (PRE); T. Martin, L. De Wett and R.A. Lubke 17 (PRE); Watt and Brandwyk 1257 (PRE).

*B. sphaerocephala* (DC.) Roessler: N. Phaliso 26.

*B. spinosa* (L.f.) Druce: J.P.H. Acocks 14567\* (PRE); P. Bohnen 8512\*; H.H. Burrows 2933; M. Koekemoer 1457 (PRE); R. Marloth 13140\* (PRE); L.W. Powrie 889\*; B. Sachse 605\* (PRE).

*B. spinosissima* (Thunb.) Willd. subsp. *spinosissima*: F.W. & S.K. Gess 05/06/67.

*B. subulata* var. *wilmsiana* Roessler: R.J. McKenzie 2046 & N. Phaliso.

*B. umbellata* DC.: H.G. Flanagan 1134\*; L. Mucina 081103/2.

*B. zeyheri* subsp. *rehmannii* (Thell.) Roessler var. *rehmannii*: N. Phaliso 70.

*B. zeyheri* (Sond. & Harv.) Oliv. & Hiern subsp. *zeyheri*: N. Phaliso 67.

*Cullumia aculeata* (Houtt.) Roessler var. *aculeata*: R.J. McKenzie 2574\*.

*C. bisulca* (Thunb.) Less.: J.P.H. Acocks 23703 (PRE); M. Koekemoer 2604 (PRE).

*C. carlinoides* DC.: M. Koekemoer 3422 (PRE).

*C. decurrens* Less.: L. Hugo 2054 (PRE).

*C. patula* (Thunb.) Less. subsp. *patula*: H.G. Fourcade 2491\* (PRE).

*C. patula* subsp. *uncinata* Roessler: J.P.H. Acocks 20544 (PRE).

*C. reticulata* (L.) Greuter, M.V. Agab. & Wagenitz subsp. *reticulata*: L. Mucina 021106/05.

*C. sulcata* (Thunb.) Less. var. *sulcata*: P. Goldblatt 5909 (PRE).

*C. sp.*: R.J. McKenzie 3038 (Gouritzmond).

*Cuspidia cernua* subsp. *annua* (Less.) Roessler: E.E.A. Gledhill s.n., 18.xi.1983; R.J. McKenzie 2606\*; L. Mucina 261105/3\*.

*Didelta carnosa* (L.f.) Aiton var. *carnosa*: B. de Winter 3564\* (PRE); M. Koekemoer 2479\* (PRE); L. Mucina 150704/11\*; I. Örtendahl 167\* (PRE); L.M. Raitt 241\* (PRE); E. van Hoepen 1901\* (PRE).

*D. carnosa* var. *tomentosa* (Less.) Roessler: R. Marloth 4729\*; H. Stummer 1869.

*D. spinosa* (L.f.) Aiton: H.H. Burrows 2936\*; E.E. Galpin 9638 (PRE); G. Germishuizen 4536\* (PRE); M. Koekemoer 1936 and V.A. Funk\* (PRE); R.J. McKenzie s.n., 14.ix.2013; M.A. Pocock 705 (PRE); H.J.T. Venter 8146\* (PRE); I.A. Walters 43 (PRE).

*Heterorhachis aculeata* (Burm.f.) Roessler: R.J. McKenzie 2950\*, 3014; E.G.H. Oliver 3867\* (PRE).