

**SYSTEMATIC IMPLICATIONS OF ACHENE CHARACTERISTICS IN GENERA
CENTAUREA L., CYANUS MILL., PSEHELLUS CASS. AND
RHAPONTICOIDES VAILL. (ASTERACEAE)**

MEHMET BONA¹

Department of Botany, Faculty of Science, Istanbul University, Istanbul, Turkey

Keywords: Achene; Asteraceae; Scanning Electron Microscopy; Taxonomy.

Abstract

This study examines the exomorphic achene characteristics of 23 taxa belonging to *Centaurea*, *Cyanus*, *Psephellus* and *Rhaponticoides* in Asteraceae using light microscope and scanning electron microscope (SEM). The exomorphic characteristics studied are shape, size, colour and surface pattern of achene, and hair, length and colour of pappus. The results of the present investigation showed that achene size of the studied taxa is 3–7 × 1–4 mm. Pappus length is 1–14 mm, deciduous for three taxa and inner row is differentiated from the outer, 1–3 mm long, for 10 taxa. SEM studies showed eight different types of achene surface patterns for the studied taxa: Glebulate, glebulate-ruminate, reticulate, ribbed, ruminate, smooth, smooth-glebulate and undulate. Achene characteristics are useful for both intrageneric and intraspecific classification of the studied taxa.

Introduction

Morphological and anatomical studies on fruit and seed structure play an important role in systematics (Kumar *et al.*, 2012). Microstructural details of the seed and fruit coat enable the distinguishing of taxa or the discovery of their affinities. This is especially useful for families in which the identification of particular taxa is complicated (Kumar *et al.*, 2012; Bona, 2013; Piwowarczyk *et al.*, 2014). Furthermore, observations of micromorphological features can also provide us with information about developmental strategies, adaptation to different environmental conditions and evolutionary tendencies within related groups of plants (Kreitschitz and Vallès, 2007; Moazzeni *et al.*, 2010).

The family Asteraceae is one of the largest angiosperm families and comprises about 1,300 genera and 2,500 species distributed over three subfamilies and 17 tribes (Ayad *et al.*, 2012). The genus *Centaurea* L. *s.l.* is one of the largest genera in the family Asteraceae. Among the challenging taxonomic problems persisting in the Asteraceae is the delimitation of the genus *Centaurea* (Bancheva and Raimondo, 2013; Ranjbar *et al.*, 2013). *Centaurea* has recently been divided into four genera, namely *Centaurea*, *Rhaponticoides*, *Psephellus* and *Cyanus* (Wagenitz and Hellwig, 2000; Greuter, 2003a, b). *Cyanus*, however, is not widely accepted by different authors (Susanna and Garcia-Jacas, 2007).

Though previous studies support the use of achene surface patterns as diagnostic characters at species and subspecies levels for the genus *Centaurea s.l.*, understanding the importance of these characters at generic and subgeneric levels requires further studies (Uysal *et al.*, 2005; Çelik *et al.*, 2005a, b; Aksoy *et al.*, 2010; Okay and Demir, 2010; Shabestari *et al.*, 2013; Bona, 2014; Candan *et al.*, 2015). With this in mind, this study examines the exomorphic achene characters of 23 taxa belonging to the genera *Centaurea*, *Cyanus*, *Psephellus* and *Rhaponticoides* in Asteraceae by using light microscope (LM) and scanning electron microscope (SEM).

¹Email: mehmetbona@gmail.com

Materials and Methods

The materials of this study are the mature achenes of 23 Asteraceae taxa belonging to the genera *Centaurea*, *Cyanus*, *Psephellus* and *Rhaponticoides*. The collected specimens were kept at the Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Botany Herbarium (ISTE). The exomorphic characteristics of the achene are achene colour, size, shape and surface pattern, and pappus colour, hair, and length (Table 1). All these characteristics are described, illustrated and compared. Up to 50 (at least 10) mature achenes for each taxon were measured and observed under the light microscope. During scanning electron microscopy, two mature achenes were selected and mounted onto stubs with double-sided adhesive tape, and were then coated with gold. The achene surfaces were examined from the lateral sides. For each sample, photographs of testa were taken using the JEOL JSM-5600 at a magnification of 22×–50×, 1000×, and 3000×. The terminology of achene characteristics in this work is based on the descriptions used by Barthlott (1981), Stearn (1992), Koul *et al.* (2000), and Bojňanský and Fargašová (2007).

Results

The results of the present investigation showed that the achene size of the studied taxa is 3–7 × 1–4 mm. The pappus length is changing from 1 to 14 mm, deciduous for three taxa and the inner row is differentiated (1–3 mm long) for 10 taxa. Testa cells of all the studied taxa appear regularly arranged and elongated-parallel with the seed surface. The results of the studied taxa are distinguished below.

1. *Centaurea antiochia* Boiss. var. *antiochia*: Achene straw-coloured, oblong, 5–6 × 2–3 mm, pappus scabrous, 5–6 mm long, inner row differentiated from outer row, c. 2 mm long. Seed surface pattern smooth. Cell boundaries thin and cell centres at ± equal levels with the boundaries (Fig. 1a–c).
2. *C. arifolia* Boiss.: Achene dark brown, oblong, 4–5 × 3 mm, pappus scabrous, 7–8 mm long, inner row differentiated from outer row, c. 1 mm. Seed surface pattern glebulate-ruminate. Cell boundaries very thin and cell centres raised above boundaries (Fig. 1d–f).
3. *C. carduiiformis* DC. subsp. *carduiiformis* var. *thrinciifolia* (DC.) Wagenitz: Achene blackish green, oblong, 5–6 × 3 mm, pappus dark silver, scabrous, 8–9 mm long, inner row differentiated from outer row, c. 2 mm long. Seed surface pattern undulate. Cell boundaries thin and cell centres at ± equal levels with the boundaries. Testa cells are apparently imbricate; this condition shows itself as waved layers on the edge of the achene (Fig. 1g–i).
4. *C. cassia* Boiss.: Achene black, oblong, 3 × 1.4–1.5 mm, pappus white, scabrous, c. 1 mm long. Seed surface pattern ruminate. Cell boundaries very thin and cell centres raised above the boundaries (Fig. 1j–l).
5. *C. cheirolopha* (Fenzl) Wagenitz: Achene light brown-straw-coloured, oblong, 4–5 × 2 mm, pappus absent or very short, scabrous, c. 1 mm long. Seed surface pattern glebulate. The cell boundaries are broad and appear raised above the cell centres (Fig. 1m–o).
6. *C. drabifolia* subsp. *cappadocica* (DC.) Wagenitz: Achene greyish, oblong-elliptic, 4–5 × 2 mm, pappus white-straw-coloured, plumose, 6–7 mm long, deciduous. Seed surface pattern glebulate-ruminate. The cell boundaries are thin and have a smooth structure and the cell centres are placed at ± equal levels with the boundaries (Fig. 1p–s).

Table 1. Pappus and achene characteristics of the studied taxa.

Taxa	Pappus				Achene				
	Colour	D/P	Hair	Length	Length (inner)	Colour	Shape	Size	Surface
<i>Centaurea antiiochia</i> var. <i>antiiochia</i>	Straw	P	Sc	5-6	2	Straw	Oblong	5-6 × 2-3	Smooth
<i>C. arifolia</i>	Purple to dark brown	P	Sc	7-8	1	Dark brown	Oblong	4-5 × 3	Glebulate-ruminate
<i>C. carduiiformis</i> subsp. <i>carduiiformis</i> var. <i>thriniciifolia</i>	Dark silver	P	Sc	8-9	2	Blackish green	Oblong	5-6 × 3	Undulate
<i>C. cassia</i>	White	P	Sc	1	N	Black	Oblong	3 × 1.5	Ruminate
<i>C. cheirololpha</i>	Straw	P	Sc	1	N	Light brown to straw	Oblong	4-5 × 2	Glebulate
<i>C. drabifolia</i> subsp. <i>cappadocica</i>	White-straw	D	Pl	6-7	N	Greyish	Oblong-elliptic	4 × 5 × 2	Glebulate-ruminate
<i>C. drabifolia</i> subsp. <i>floccosa</i>	Straw	P	Pl	8-10	N	Yellow to green	Oblong	4-5 × 2	Smooth
<i>C. foliosa</i>	Dark purple	P	Sc	5-6	N	Dark silver to brown	Oblong	5 × 3	Glebulate-ruminate
<i>C. lycopifolia</i>	Straw	P	Sc	1	N	Dark green to brown	Oblong	4-6 × 2	Glebulate-ruminate
<i>C. pseudoscabiosa</i> subsp. <i>araratica</i>	Silver brown	P	Sc	6	2	Dark brown	Oblong	5-6 × 3	Reticulate
<i>C. ptosimopappa</i>	Straw to purple	D	Sc	5-6	N	Black	Oblong	5-6 × 2.0-2.5	Glebulate-ruminate

Table 1. Contd.

Taxa	Pappus				Achene				
	Colour	D/P	Hair	Length	Length (inner)	Colour	Shape	Size	Surface
<i>C. virgata</i>	White	D	Sc	2	N	Brown to grey	Oblong	3-4 x 1-2	Glebate-ruminate
<i>Cyanus bourgaei</i>	Straw	P	Sc	1	N	Straw to brown	Oblong	5 x 2	Smooth
<i>Cy. cheiranthifolius</i> subsp. <i>purpurascens</i>	Straw	P	Sc	2	N	Straw to dark green	Oblong	6-7 x 3	Smooth-glebate
<i>Cy. depressus</i>	Straw	P	Sc	5-6	2	Dark green	Oblong	5-6 x 2-2.5	Smooth
<i>Cy. segetum</i>	Straw to red	P	Sc	2	N	Dark green	Narrowly oblong	3 x 1	Smooth-glebate
<i>Psephellus appendicigerus</i>	Straw	P	Pl	13-14	N	Straw	Narrowly ovate	6-7 x 3	Ribbed
<i>P. bornmuelleri</i>	Purple	P	Sc	8	3	Yellow	Lanceolate	6-7 x 3	Smooth
<i>P. brevifimbriatus</i>	Straw to brown	P	Sc	7	3	Straw	Oblong	6 x 3	Undulate
<i>P. micromifer</i>	Straw	P	Sc	4	1	Straw to greyish	Narrowly ovate	6 x 3	Smooth
<i>P. pulcherrimus</i>	Straw	P	Pl	10-12	N	Dark green to brown	Lanceolate	5-6 x 2	Ribbed
<i>P. pyrrholepharus</i>	Straw to yellow	P	Sc	2-4	1	Straw to green	Narrowly oblong	5-7 x 2	Ruminate
<i>Rhaponticoides wagenitziana</i>	Brown	P	Sc	7-8	2	Dark brown	Broadly oblong	6-7 x 4	Reticulate

Note: D, deciduous; N, not different; P, persistent; Pl, plumose; Sc, scabrous.

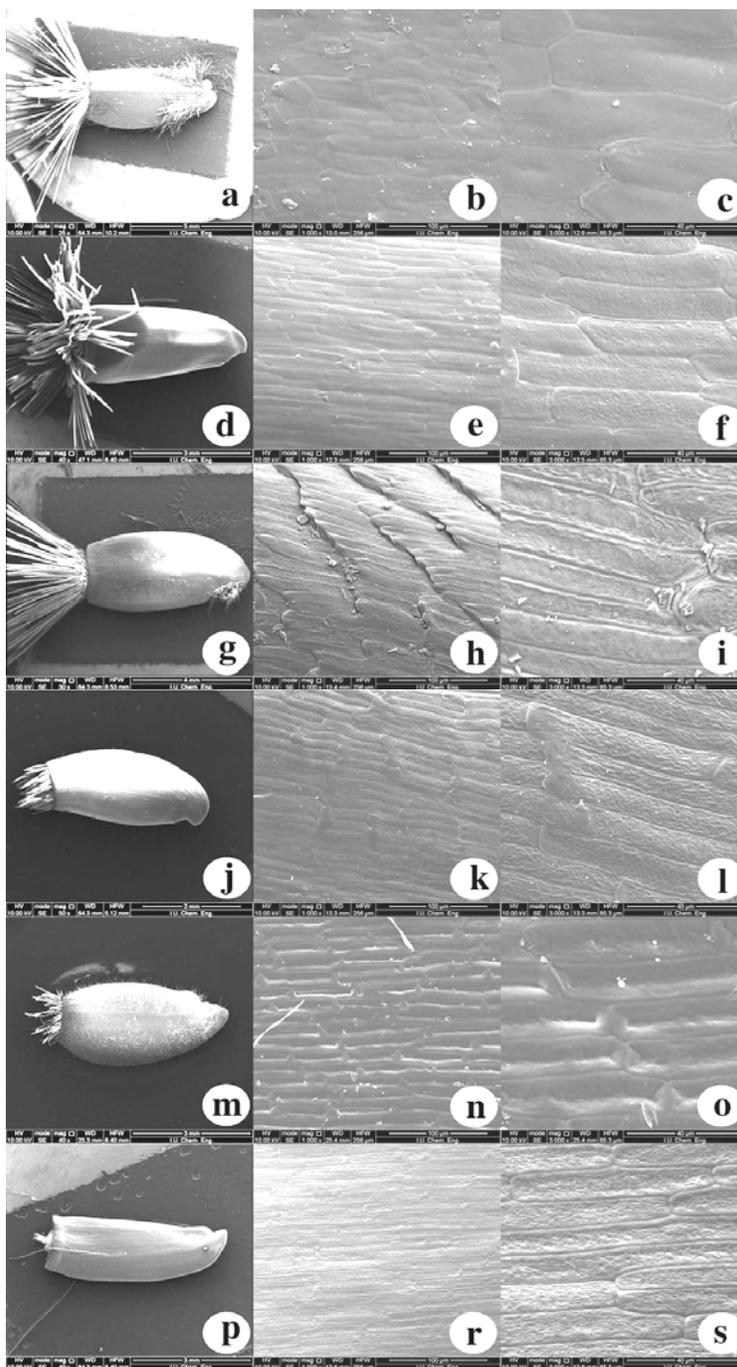


Fig. 1. SEM micrographs of achenes of *Centaurea antiochia* var. *antiochia* (a-c); *C. arifolia* (d-f); *C. carduiiformis* subsp. *carduiiformis* var. *thrinciifolia* (g-i); *C. cassia* (j-l), *C. cheirolopha* (m-o); *C. drabifolia* subsp. *cappadocica* (p-s).

7. *C. drabifolia* subsp. *floccosa* (Boiss.) Wagenitz & Greuter: Achene yellow-green, oblong, 4–5 × 2 mm, pappus straw-coloured, plumose, 8–10 mm long. Seed surface pattern smooth. Cell boundaries are thin and the boundaries appear raised above the cell centres (Fig. 2a–c).
8. *C. foliosa* Boiss. & Kotschy ex Boiss.: Achene dark silver-brown, oblong, c. 5 × 3 mm, pappus dark purple, scabrous, 5–6 mm long. Seed surface pattern glebulate-ruminate. Cell boundaries are thin and smooth and appear raised above the cell centres (Fig. 2d–f).
9. *C. lycopifolia* Boiss. & Kotschy ex Boiss.: Achene dark green-brown, oblong, 4–6 × 2 mm, pappus straw-coloured, scabrous, c.1 mm long. Seed surface pattern glebulate-ruminate. Cell boundaries are very thin and the cell centres are raised above the boundaries (Fig. 2g–i).
10. *C. pseudoscabiosa* subsp. *araratica* (Azn.) Wagenitz: Achene dark brown, oblong, 5–6 × 3 mm, pappus silvery brown, scabrous, 6 mm long, inner row differentiated from outer row, c. 2 mm long. Seed surface pattern reticulate. Cell boundaries are broad and have a smooth structure and the boundaries seem distinctly raised above the cell centres (Fig. 2j–l).
11. *C. ptosimopappa* Hayek: Achene straw-coloured to green when young and blackish when mature, oblong, 5–6 × 2.0–2.5 mm, pappus straw-coloured or sometimes purplish, scabrous, 5–6 mm long, deciduous. Seed surface pattern glebulate-ruminate. Cell boundaries are broad and have a smooth structure and the boundaries seem distinctly raised above the cell centres (Fig. 2m–o).
12. *C. virgata* Lam.: Achene brown-grey, oblong, 3–4 × 1–2 mm, pappus white, scabrous, c. 2 mm long, deciduous. Seed surface pattern glebulate-ruminate. Cell boundaries are thin and smooth and appear raised above the cell centres (Fig. 2p–s).
13. *Cyanus bourgaei* (Boiss.)Wagenitz & Greuter: Achene straw-coloured to brown, oblong, c. 5 × 2 mm, pappus straw-coloured, scabrous, c.1 mm long. Seed surface pattern smooth. Cell boundaries are thin and the cell centers are ± equal with the boundaries. Testa cells are sulcate at the centres of the cells (Fig. 3a–c).
14. *Cy. cheiranthifolius* (Willd.) Soják subsp. *purpurascens* (DC.) Wagenitz: Achene straw-coloured to dark green, oblong, 6–7 × 3 mm, pappus straw-coloured, scabrous, c. 2 mm long. Seed surface pattern smooth-glebulate. The cell boundaries are thin and the cell centres are ± equal with the boundaries (Fig. 3d–f).
15. *Cy. depressus* (M. Bieb.) Soják: Achene dark green, oblong, 5–6 × 2–2.5 mm, pappus straw-coloured, scabrous, 5–6 mm long, inner row differentiated from outer row, c. 2 mm long. Seed surface pattern smooth. Cell boundaries are very thin and the cell centres are raised above the boundaries (Fig. 3g–i).
16. *Cy. segetum* Hill: Achene dark green, narrowly oblong, c. 3 × 1 mm, pappus straw-coloured to red, scabrous, c. 2 mm long. Seed surface pattern smooth-glebulate. The cell boundaries are thin and have a smooth structure and the cell centres are ± equal with the boundaries (Fig. 3j–l).

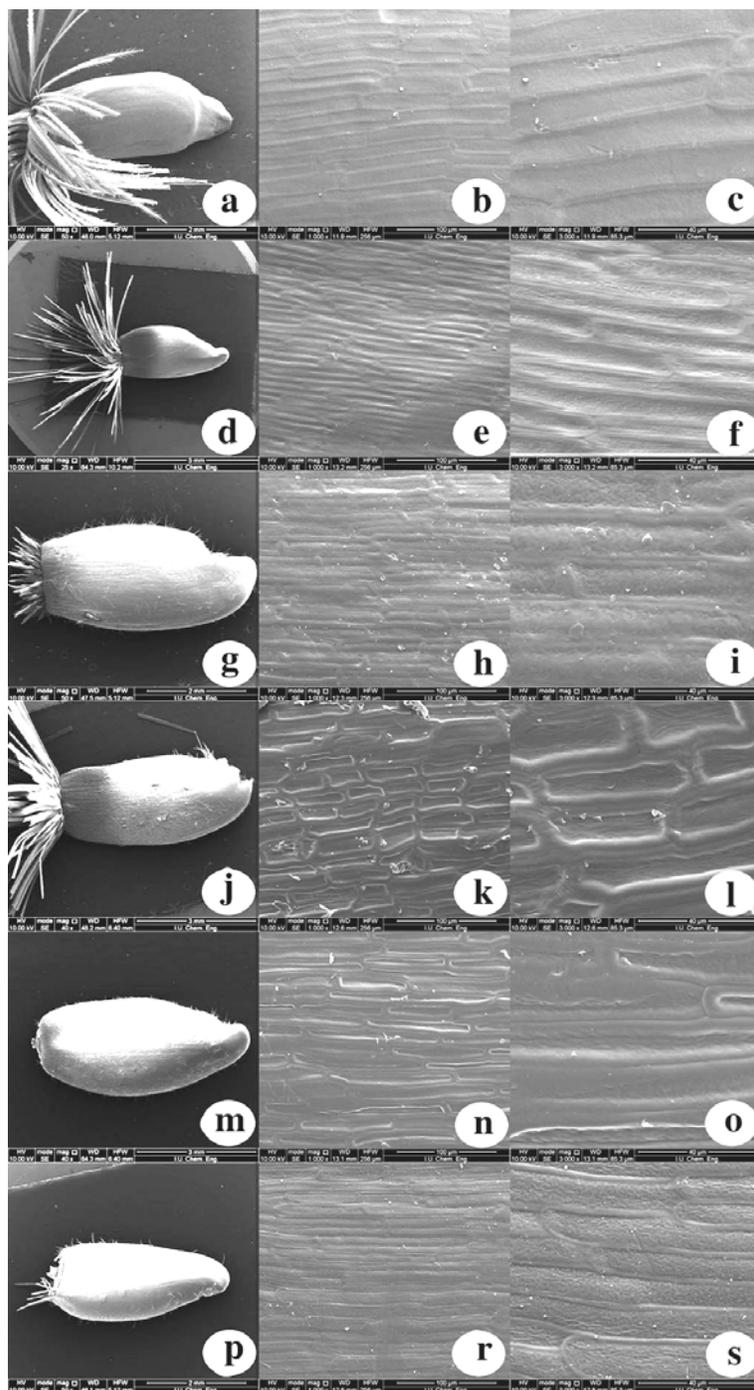


Fig. 2. SEM micrographs of achenes of *Centaurea drabifolia* subsp. *floccosa* (a-c); *C. foliosa* (d-f); *C. lycopifolia* (g-i); *C. pseudoscabiosa* subsp. *araratica* (j-l); *C. ptosimopappa* (m-o); *C. virgata* (p-s).

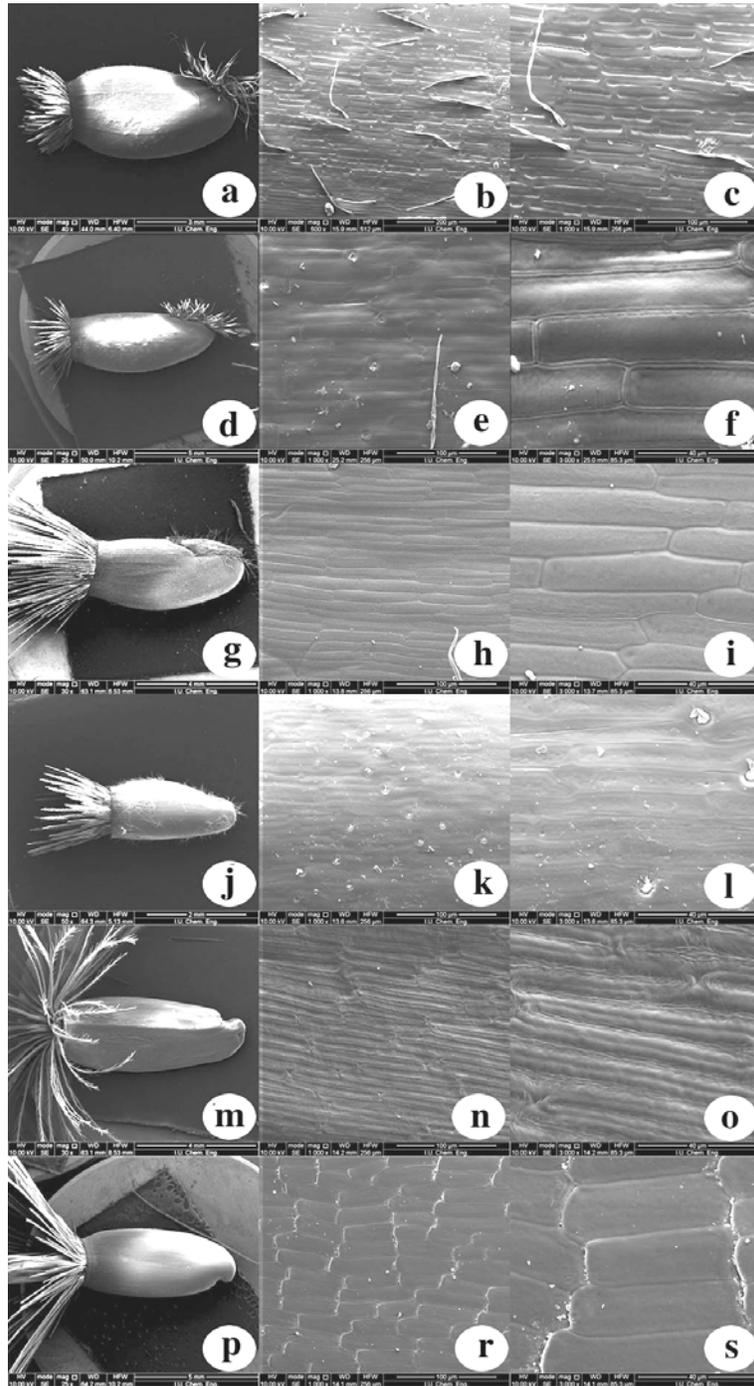


Fig. 3. SEM micrographs of achenes of *Cyanus bourgaei* (a-c); *Cy. cheiranthifolius* subsp. *purpurascens* (d-f); *Cy. depressus* (g-i); *Cy. segetum* (j-l); *Psephellus appendicigerus* (m-o); *P. bornmuelleri* (p-s).

17. *Psephellus appendicigerus* (K. Koch) Wagenitz: Achene is straw-coloured, narrowly ovate, 6–7 × 3 mm, pappus light straw-coloured, plumose, 13–14 mm long. Seed surface pattern ribbed. The cell boundaries are broad and have smooth structure and appear raised above the cell centres. Cell centres are sulcate (Fig. 3m–o).

18. *P. bornmuelleri* (Hausskn. ex Bornm.) Wagenitz: Achene yellowish, lanceolate, 6–7 × 3 mm, pappus purple, scabrous, 8 mm long, and its inner row is differentiated from the outer row, c. 3 mm long. Seed surface pattern smooth. The cell boundaries are thin and the cell centres are ± equal with the boundaries. Testa cells are apparently imbricate; this condition shows itself as waved layers (Fig. 3p–s).

19. *P. brevifimbriatus* (Hub.-Mor.) Wagenitz: Achene straw-coloured, oblong, c. 6 × 3 mm, pappus straw-coloured to light brown, scabrous, 7 mm long, and its inner row is differentiated from the outer row, c. 3 mm long. Seed surface pattern undulate. The cell boundaries are thin and the cell centres are ± equal with the boundaries. Cell centres are slightly sulcate (Fig. 4a–c).

20. *P. mucronifer* (DC.) Wagenitz: Achene straw-coloured to greyish, narrowly ovate, c. 6 × 3 mm, pappus straw-coloured, scabrous, c. 4 mm long, and its inner row differentiated from the outer row, c. 1 mm long. Seed surface pattern smooth. The cell boundaries are thin and have smooth structure and the centres of the cells are placed at ± equal levels with the boundaries (Fig. 4d–f).

21. *P. pulcherrimus* (Willd.) Wagenitz: Achene dark green to brown, lanceolate, 5–6 × 2 mm, pappus straw-coloured, plumose, 10–12 mm long. Seed surface pattern ribbed. The cell boundaries are very thin and the centres of the cells are raised above the boundaries. Cell centers are ribbed (Fig. 4g–i).

22. *P. pyrrhoblepharus* (Boiss.) Wagenitz: Achene straw-coloured to green, narrowly oblong, 5–7 × 2 mm, pappus straw-coloured to yellow, scabrous, 2–4 mm long, and its inner row different, c. 1 mm long. Seed surface pattern ruminant. The cell boundaries are broad and have smooth structure and the boundaries appear raised above the cell centres (Fig. 4j–l).

23. *Rhaponticoides wagenitziana* (Bancheva & Kit Tan) M.V. Agab. & Greuter: Achene dark brown above, yellow at base, broadly oblong, 6–7 × 4 mm, pappus brown, scabrous, 7–8 mm long, inner row differentiated from outer row, c. 2 mm long. Seed surface pattern reticulate. Cell boundaries are distinctly broad and appear seem distinctly raised above the cell centres. Achenes have waved layers which are independent from cells and cell boundaries (Fig. 4m–o).

Discussion

Rhaponticoides wagenitziana is the only species of the studied taxa which belongs to genus *Rhaponticoides*. The achenes are dark brown above, yellow at base and have waved layers which are independent from the cells and cell boundaries. These two characteristics seem specific to *R. wagenitziana*. More work is required to confirm the use of characteristics for delimitation of the genus *Rhaponticoides*.

Psephellus coruhensis, *P. turcicus* and *P. psephelloides* have reticulate seed surface patterns (Duran and Hamzaoglu, 2005; Duran *et al.*, 2009). Our study shows that pappus length, colour and achene colour characteristics show differences at the specific level. Additionally, the achene

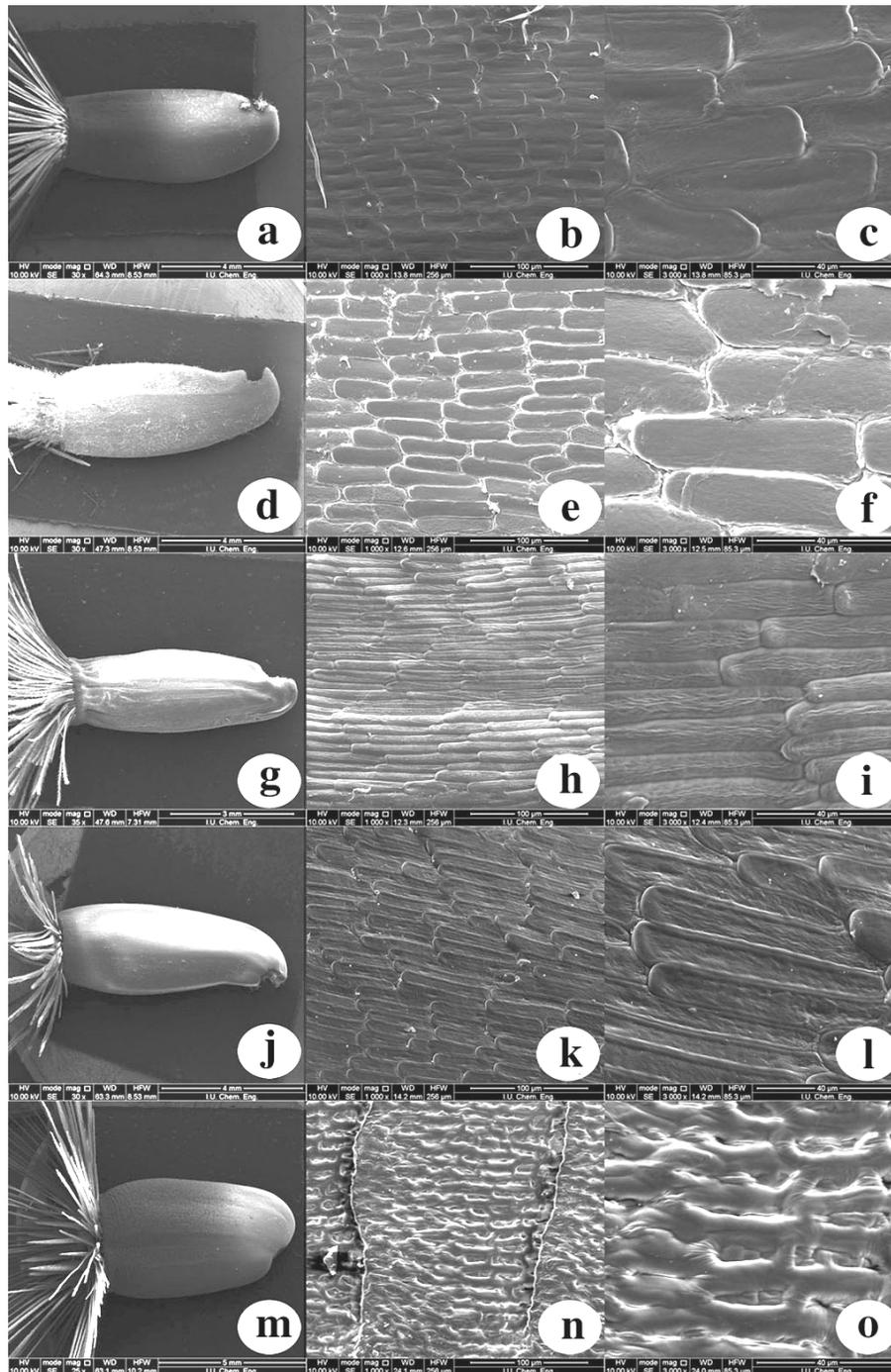


Fig. 4. SEM micrographs of achenes of *Psephellus brevifimbriatus* (a-c); *P. mucronifer* (d-f); *P. pulcherrimus* (g-i); *P. pyrrohlepharus* (j-l); *Rhaponticoides wagenitziana* (m-o).

surface pattern can be ribbed, smooth or undulate for the genus *Psephellus*. Because of the overlap, achene characteristics are not useful for determining generic limits in *Psephellus*. Despite that, achene characteristics provide strong support in the delimitation of the studied *Psephellus* taxa at the specific level. *P. appendicigerus* and *P. pulcherrimus* were placed into Section *Aetheopappus* (Wagenitz, 1975). These two taxa are easily separated from other *Psephellus* taxa by their ribbed achene surface pattern and their pappus, which do not have differentiated inner rows. Other studied *Psephellus* taxa belong to section *Psepheloideae* (Wagenitz, 1975) in which their inner pappus row is differentiated. These findings show that achene characteristics could be useful for sectional classification of the genus *Psephellus*.

There is no specific pappus length, pappus colour, achene size or colour for the genus *Cyanus*, but all the studied *Cyanus* taxa have smooth or smooth-glebulate achene surface pattern.

This study supports the use of achene surface patterns as diagnostic characters at both specific and infraspecific levels in the genus *Centaurea*. There are other achene characteristics, including achene length and colour, pappus length and colour, that might be helpful to distinguish more taxa. However, though achene characteristics provide strong support in the delimitation of the studied taxa at specific level, understanding the importance of these characteristics for intrageneric classification of the genus *Centaurea* requires further study. *Centaurea drabifolia* subsp. *cappadocica* and *C. drabifolia* subsp. *floccosa* are separated from each other based on stem length in the Flora of Turkey (Wagenitz, 1975). This study shows that achene characteristics are useful in the separation of these taxa, because pappus length, achene colour and achene surface patterns of these taxa are different from each other. The SEM study of achene surfaces showed that the cell wall in *C. kurdica* is thicker than that of *C. sclerolepis*, especially on the margins and could be a useful character in the delimitation of species (Uysal *et al.*, 2005).

In conclusion, the achene characteristics of 23 taxa belonging to the genera *Centaurea*, *Cyanus*, *Psephellus* and *Rhaponticoides* were evaluated in this study. Results support the use of achene characteristics as diagnostic characters for intrageneric and intraspecific classification of these taxa. Achene characteristics provide strong support for the delimitation of the studied taxa at specific level, but understanding the importance of these characteristics at generic and intrageneric levels needs further studies. Hopefully, this investigation will encourage additional studies about achene morphology of this complex family to further elucidate the complex taxonomy of the Asteraceae.

Acknowledgements

The author thanks the Scientific Investigation Project Coordinator of Istanbul University (project no: 42493) and Edinburgh Botanic Garden (Sibbald) Trust for their financial support. Thanks are also due to Dr. Sabina Knees for her help in improving the manuscript.

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(Manuscript received on 9 July 2015; revised on 3 November 2015)