

The morphology and anatomy of the achene in certain species of sub-family Ranunculoideae (Ranunculaceae) with special reference to the achene vasculature

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The achene in 17 species of the sub-family Ranunculoideae (Ranunculaceae) was morphologically and anatomically studied to show the variation patterns of these two parameters. The surface of the achene and the appearance of the style whether normal and plumose or in the form of a beak were found to be delimitate to the taxa studied. SEM investigation of the pericarp showed that six types of surface sculpture were recorded and the reticulate type was sub-divided into six sub-types. Stress was laid upon the achene vasculature for suggesting a phylogenetic status for the studied taxa. It was concluded that less advanced taxa are those having increased number of vascular bundles (8-12 bundles). Intermediate taxa are those with three vascular bundles; one dorsal and two distinct ventral bundles. More advanced taxa are those with one dorsal and two fused ventral bundles.

Key words: Achene anatomy, Achene morphology, Ranunculoideae (Ranunculaceae).

Introduction

The Ralian line of evolution is one of two accepted concepts from which different angiospermic taxa had arisen. The second concept is the Dillenian - Rosalian line (Kubitzki, 1969 & Bate - Smith, 1972).

Several authors (Hallier, 1912; Bessey, 1915; Lawrence, 1963; Takhtajan, 1969) agreed upon the primitive nature of the Ranunculaceae and this led to the consideration of this family as an ancestral stock to many other taxa. Stebbins (1974) and Heywood (1993), divided the Ranunculaceae into two sub-families, viz. Helleboroideae and Ranunculoideae. The Helleboroideae is characterized mainly by more than one - ovuled carpel and the follicle fruit; whereas the Ranunculoideae is characterized by the single - ovuled carpel and the fruit is achene.

A considerable attention has long been paid to the vegetative characters of the Ranunculaceae. The floral peculiarities as well have also attracted the attention towards the

study of the floral morphology, the floral vascularization and the fruit and the seed characteristics. All these parameters aimed at interpretation to the structure and nature of the floral organs and to trace the evolutionary pathways of the character states. Tobe (1979 & 1980) carried out several studies on *Clematis* which dealt with the vascular anatomy of the inflorescence axis (1979), the vascular anatomy of the calyx region in the four - sepal flowers (1980 a), the vascular anatomy of the androecial and the gynoecial regions in the receptacle (1980 b) and the anatomy of the inflorescence in the eight - sepal flowers (1980 c).

White *et al.* (1990), studied the floral limitation and development in *Aquilegia*, while Mc Lewin and Mathew (1996), studied the structure of Hellebore flowers with reference to the petaloid sepals as well as to the petaloid nectaries.

As regards the achene and/or the seed, Corner (1976) studied the seed structure of ten genera of Ranunculaceae.

Berestetskaya (1985), presented detailed descriptions of the seed coat of six *Aconitum* species. Halfon - Meiri *et al.* (1987), studied the history of seeds and achene of *Ranunculus asiaticus* infected with *Alternaria* species. Trzaski (1999), investigated the xylem distribution in both the developing and mature achenes in *Ranunculus* species, and suggested that this issue could be useful as a taxonomic criterion.

In the present work, we are investigating the morphological as well as the anatomical characters of both the pericarp and the seed coat in some taxa of sub-family Ranunculoideae for an accurate delimitation and also for a speculation to the evolutionary status of the studied taxa.

Materials and Methods

Mature achenes of two native and 15 foreign species were investigated^{*.} The classification of taxa was cited after Heywood (1993), and table 1 shows the collection data .

Five to ten achenes were investigated to determine the shape and the latter was drawn at bench level by the aid of stereomicroscope M5.

For SEM investigation the achenes were mounted on brass - stubs and then coated with gold paladium in sputter coating unit. The scanning was carried out by a Joel JSM 100 SEM at an accelerating voltage of 30 Kv. at Faculty of Science, Ain Shams University. The terminology of surface sculpture was adopted after Stearn (1978).

For the anatomical investigations, the achenes were fixed in F.A.A., then thoroughly washed with water, dehydrated in series of ethyl alcohol and finally embedded in Paraffin wax according to Johansen (1940). Drawings were made at bench level by the aid of Leitz Cammera Lucida.

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Table 1: Classification and collection data of the studied taxa of the Ranunculoideae (the classification was cited from Heywood, 1993)

Sub-family	Tribe	Taxa
Ranunculoideae	Ranunculeae	<i>Adonis dentata</i> • Del., <i>Ranunculus acris</i> L., <i>R. constantinopolitanus</i> (DC.) D'Urv., <i>R. illyricus</i> Poir., <i>R. lanuginosus</i> L., <i>R. marginatus</i> • D'Urv., <i>R. sceleratus</i> L., <i>Thalictrum aquilegifolium</i> L., <i>T. flavum</i> L., <i>T. foetidum</i> L., <i>T. minus</i> L., <i>T. morisoni</i> C.C. Gmel
	Anemoneae	<i>Anemone narcissiflora</i> L., <i>Pulsatilla pratensis</i> L., <i>P. vulgaris</i> Mill.
	Clematideae	<i>Clematis integrifolia</i> L., <i>C. tangutica</i> Korsh.

Results and discussion

A- General characters of the achene (Table 2, plate 1).

Table 2 shows the cumulative morphological characters of the achene as clarified by LM and SEM. As regards the achene shape and texture Table 2 and plate I show that both characters are consistent at the generic level (*Pulsatilla*) or varying among the species of the same genus (*Ranunculus*).

The style in the studied species shows various aspects. It is either normal and plumose (species nos. 3, 4, 5 & 6) or in the form of a beak. In the latter case, the beak is either straight (species no. 8, 9, 12, 13, 14, 16 & 17) or hooked (species nos. 1, 2, 7, 10, 11 & 15).

In the Ranunculaceae, Bailey (1949) stated that the style characters can be used to distinguish between the different taxa. Earlier, Rendle (1925) stated that the long feathery style on the achene, recorded in this work in *Clematis* and *Pulsatilla*, aids in the fruit dispersal by wind; whereas the hooked style, as in *Adonis dentata*, *Anemone narcissiflora*, *Ranunculus acris*, *R. lanuginosus*, *R. marginatus* and *Thalictrum foetidum*, aids in the distribution by animals.

B. Achene sculpture (Table 2, Plate II)

SEM investigation shows that six types of sculpture are recorded. These are reticulate, verrucate, scleariform, tuberculate, papillate and ruminant (Plate II).

1- The reticulate type comprises the following six subtypes:

sub-type i- Reticulate - foveolate with few remote hairs, recorded in *Clematis* species (Figs. 3 & 4).

• Native species

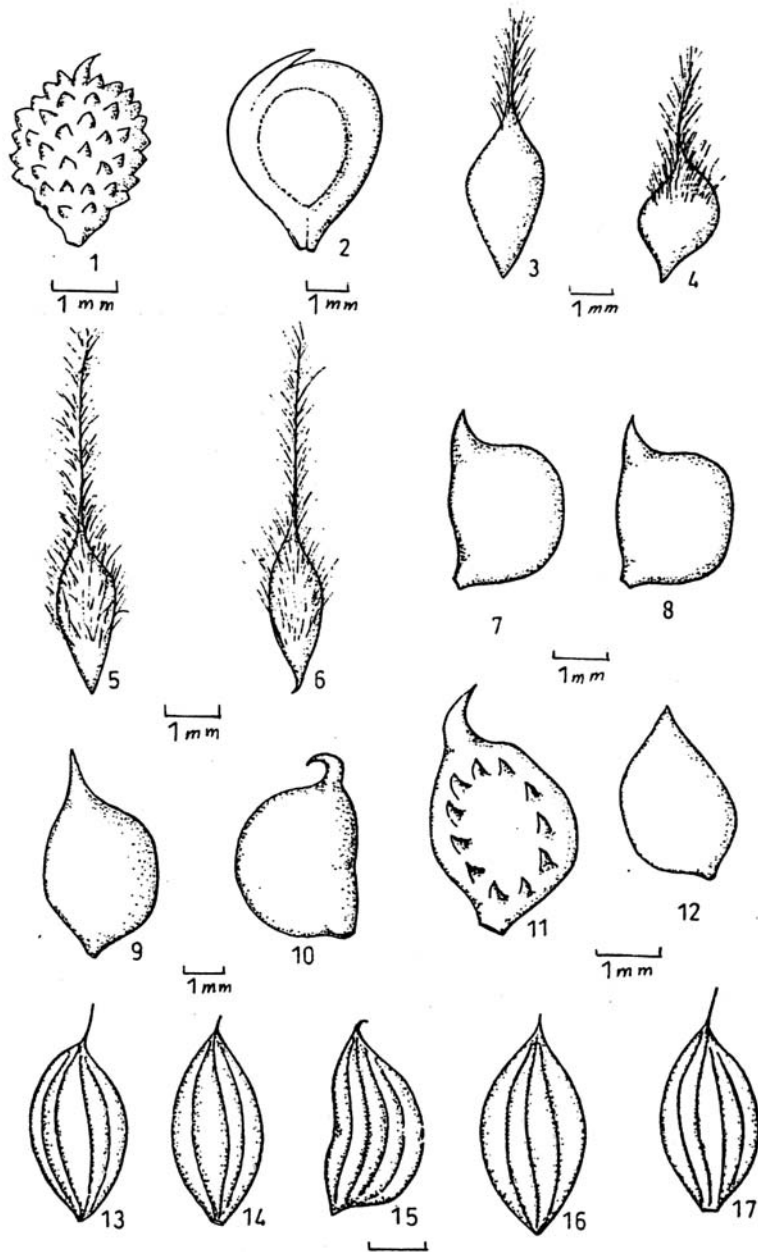


Plate I. Achene morphology (side view)

Fig. 1. *Adonis dentata*, Fig. 2. *Anemone narcissiflora*, Fig. 3. *Clematis integrifolia*, Fig. 4. *C. tangutica*, Fig. 5. *Pulsatilla pratensis*, Fig. 6. *P. vulgaris*, Fig. 7. *Ranunculus acris*, Fig. 8. *R. canstantinopolitanus*, Fig. 9. *R. illyricus*, Fig. 10. *R. lanuginosus*, Fig. 11. *R. marginatus*, Fig. 12. *R. sceleratus*, Fig. 13. *Thalictrum aquilegifolium*, Fig. 14. *T. flavum glaucum*, Fig. 15. *T. foetidum*, Fig. 16. *T. minus*, Fig. 17. *T. miorisoni morisoni*.

Morphology and anatomy of the achene

Table (2) Morphological characters of the Achene of the studied species of Ranunculoideae (LM & SEM)

Taxa	Attributes	LM						SEM of pericarp
		Shape (outline)	Surface	Normal plumose	Style		RLA	
					Striight	Beak Hooked		
<i>Adonis dentata</i>	± rounded - obovate	Tubercled	-	-	+	1:4	Verrucate	
<i>Anemone narcissiflora</i>	Flattened - obovate	Glabrous	-	-	+	1:5	Sc;arofpr,	
<i>Clematis integriflora</i>	Irr. elliptic	Glabrous	+	-	-	1:2	R. foveolate	
<i>C. tangutica.</i>	Irr. elliptic	Plumose	+	-	-	1:1	R. foveolate	
<i>Pulsatilla pratensis</i>	Irr. elliptic	Plumose	+	-	-	2:1	R. hairy	
<i>P. vulgaris</i>	Irr. elliptic	Plumose	+	-	-	2:1	R. hairy	
<i>Ranunculus acris</i>	± Irr. squared	Glabrous	-	+	+	1:5	Tuberculate	
<i>R.</i>	± Irr. squared	Glabrous	-	+	-	1:8	Tuberculate	
<i>constantinopolitanus</i>	± Ovate	Glabrous	-	+	-	1:5	Tuberculate	
<i>R. illyricus.</i>	± Irr. rectangle	Glabrous	-	-	+	1:5	R- pentagonal	
<i>R. lanuginosus.</i>	± Ovate	Prickly	-	-	+	1:3	R. papillate	
<i>R. marginatus.</i>	± Ovate	Glabrous	-	+	-	1:5	R. pentagonal	
<i>R. sceleratus.</i>	± Elliptic	Ribbed	-	+	-	1:5	Sclariform	
<i>Thalictrum</i>	± Elliptic	Ribbed	-	+	-	1:10	R. rectangular	
<i>aquilegfolium.</i>	± Elliptic	Ribbed	-	-	+	1:10	Papillate	
<i>T. flavum.</i>	± Elliptic	Ribbed	-	+	-	1:10	Ruminante	
<i>T. foetidum</i>	± Elliptic	Ribbed	-	+	-	1:5	Irr. R.	
<i>T. minus.</i>	± Elliptic	Ribbed	-	+	-			
<i>T. morisoni</i>								

-, Absent; +, Present; Irr., Irregular; R, Reticulate; RLA, Relative Length to the Achene

- sub-type ii-* Reticulate - papillate in which the papillae arise from the periclinal walls. This type is recorded in *Ranunculus marginatus* (Fig. 11).
- sub-type iii-* Reticulate with rather dense hairs and the anticlinal walls are more or less rectangular and elevated; recorded in *Pulsatilla* species (Figs. 5 & 6).
- sub-type iv-* Rectangular - reticulate; recorded in *Thalictrum flavum* (Fig. 14).
- sub-type v-* Reticulate with more or less pentagonal cells; recorded in *Ranunculus lanuginosus* and *R. sceleratus* (Figs. 10 & 12).
- sub-type vi-* Irregularly - reticulate; recorded in *Thalictrum morisoni* (Fig. 17).
- 2- The verrucate type: in which the verrucae arise from the outer periclinal walls of the exocarp. This type is recorded in *Adonis dentata* (Fig. 1).
 - 3- The scalariform type: recorded in *Anemone narcissiflora* (Fig.2), due to fine projections, and in *Thalictrum aquilegifolium* (Fig. 13), due to remnants of the exocarp cells after being obliterated (See Plate III, Fig. 2.b).
 - 4- The tuberculate type: Where the tuberculae arise from the swollen cells of the mesocarp. This type is recorded in *Ranunculus acris*, *R. constantinopolitanus* and *R. illyricus* (Figs. 7, 8 & 9); Plate III, Fig. 7 b & c).
 - 5- The papillate type: where the papillae arise from the elevations of the periclinal walls. This type is recorded in *Thalictrum foetidum* (Fig. 15).
 - 6- The ruminant type: recorded in *Thalictrum minus* (Fig. 16).

C. Anatomical structure of the pericarp and testa (Table 3 & Plate III)

I- Number and description of the pericarp layers:

Generally, the pericarp is differentiated into three layers; exocarp, mesocarp and endocarp.

- 1) Exocarp: The exocarp cells have the following appearance:
 - a- Persistent and in the form of one layer of thick-walled cells; recorded in species nos. 1, 3 & 7-17. The exocarp cells have the following aspects:
 - i- Radially elongated and papillate; recorded in *Adonis dentata* (Fig 1 a-c).
 - ii- More or less cubic in species nos. 3 & 7-17. In the species nos. 7,8 & 9, where some cells of the mesocarp become swollen (Fig. 7 b&c & 9 b&c), the overtopping cells of the exocarp become tangentially elongated.
 - b- Partially persistent; some of its cells remain intact and some other cells are transformed into hair-like appendages as recorded in *Clematis tangutica* and *Pulsatilla* species (Figs. 3 b&c and 4 b&c).
 - c- Absent altogether and replaced by a hyaline layer resulting from the obliteration of its cells as recorded in *Anemone narcissiflora* (Fig. 2 b&c).
- 2) Mesocarp: It is formed of thin walled parenchymatous cells. It is either multi-layered in species nos. 1-4 & 9-17, two-four layers in *Pulsatilla* species (Fig. 5 b&c) or one layer in *Ranunculus acris* and *R. constantinopolitanus* (Fig. 7 b&c).

Morphology and anatomy of the achene

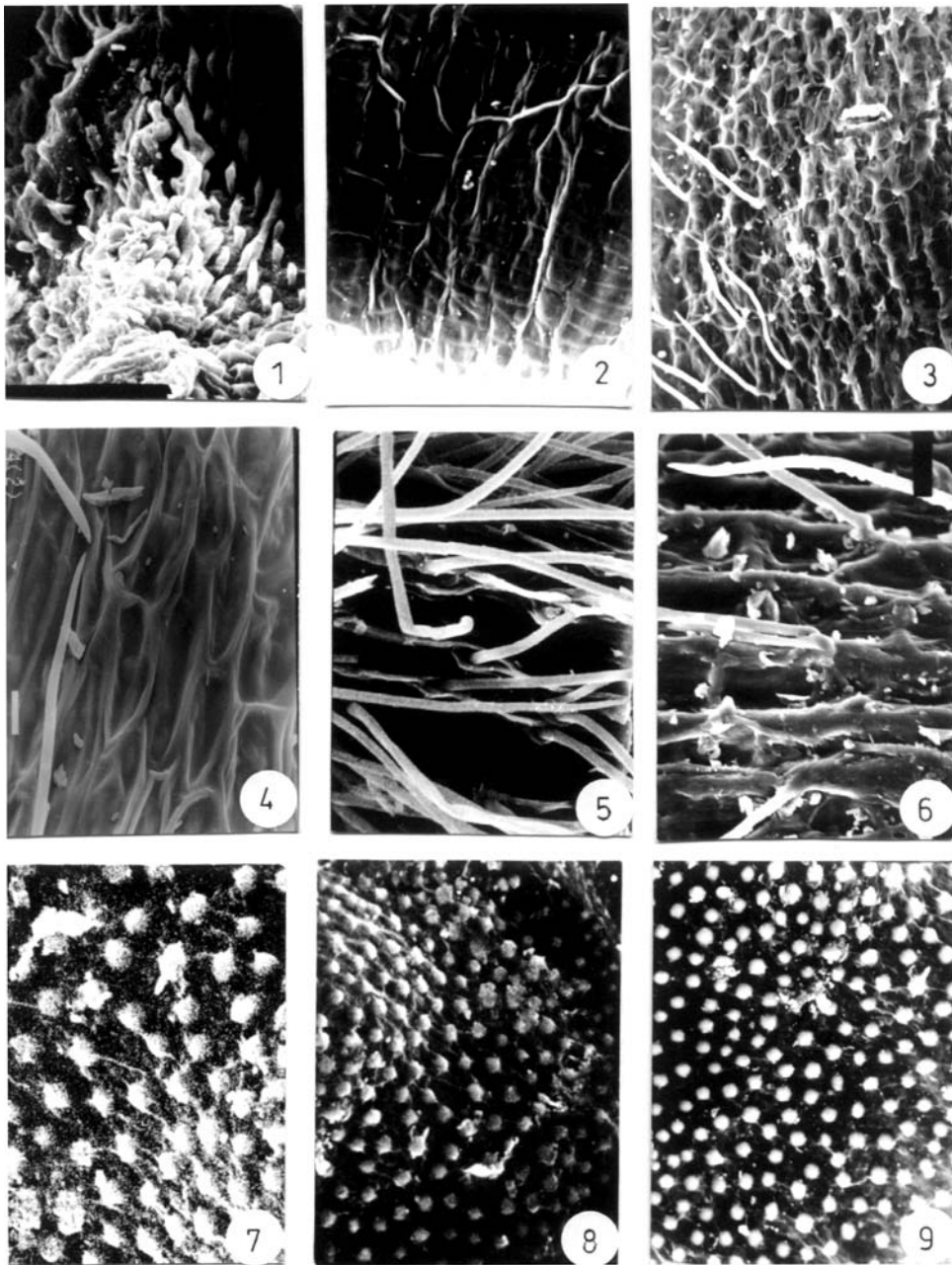


Plate II. Achene sculpture

Fig. 1. *Adonis dentata* X200, Fig. 2. *Anemone narcissiflora* X200, Fig. 3. *Clematis integrifolia* X200, Fig. 4. *C. tangutica* X1200, Fig. 5. *Pulsatilla pratensis* X200, Fig. 6. *P. vulgaris* X200, Fig. 7. *Ranunculus acris* X200, Fig. 8. *R. constantinopolitanus* X300, Fig. 9. *R. illyricus* X500.

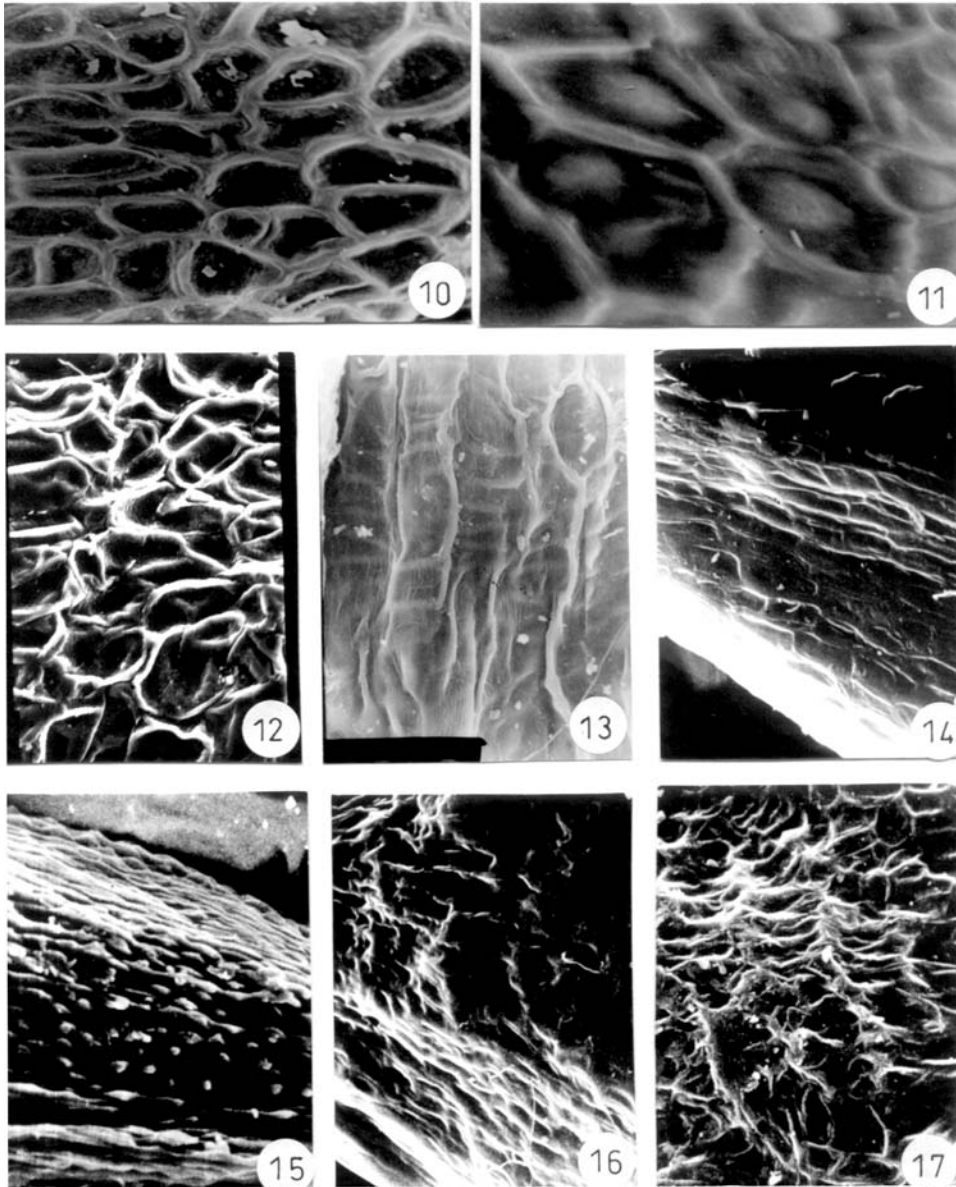


Plate II. Achene sculpture (cont.)

Fig. 10. *R. lanuginosus* X500, Fig. 11. *R. marginatus* X1000, Fig. 12. *R. sceleratus* X800, Fig. 13. *Thalicttrum aquilegifolium* X300, Fig. 14. *T. flavum glaucum* X300, Fig. 15. *T. foetidum* X300, Fig. 16. *T. minus* X300, Fig. 17. *T. miorisoni morisoni* X200.

- 3) Endocarp: In all the studied taxa (except *Thalictrum* species), the endocarp consists of highly lignified sclerenchymatous cells. It is either multi-layered in *Ranunculus acris*, *R. constantinopolitanus* and *R. marginatus* (Figs. 7 b&c and 11 b&c) two - three layers in *Adonis dentata*, *Ranunculus illyricus*, *R. lanuginosus* and *R. scelerotus* (Fig. 1 b&c, 10 b&c and 12 b&c) or one layer of radially elongated cells in *Anemone narcissiflora*, *Clematis* species and *Pulsatilla* species (Figs. 2 b&c, 3 b&c and 5 b&c). In *Thalictrum* species, the endocarp consists of one layer of radially elongated cells. Both the inner perinclinal and radial walls are thick, whereas the outer periclinal walls are thin (Fig. 13 d).

II- Vasculature of the pericarp (Table 3, Plate III):

Phylogenetically, the carpel has resulted from a vegetative leaf by folding of its margins and their fusion to enclose the locule and the ovule (s). Fahn (1969), stated that this origin of the carpel is the most accepted view and therefore it is homologous with the leaf. Fraser (1937), stated that the vascular supply of the carpel is fundamentally the same as that of the leaf.

Normally, a vegetative dicotyledonous leaf is supplied with three main vascular bundles diverging from the axial stele, viz. one mid-vein and two lateral bundles (Pandey, 1993). The achene arising from such a carpel will have also the same vascular supply, where the mid-vein is termed the dorsal bundle and the two laterals are termed the ventral bundles, and this will represent the most primitive type of vasculature. However, deviations from this basic type may occur.

In the present work the following vascularization cases of the achene are recorded:

- 1- The achene is supplied with increased number of vascular bundles (8-12 bundles) as recorded in *Thalictrum* species (Plate III, Figs. 13-17).
- 2- The achene is supplied with one dorsal and two distinct ventrals in *Ranunculus* species (Plate III, Figs. 7-12).
- 3- The achene is supplied with one dorsal and two fused ventrals in all the remainder taxa (Plate III, Figs. 1-6).

However, ramification of either the dorsal bundle or the ventral bundles does occur. In all *Ranunculus* species as well as in *Adonis dentata* and *Anemone narcissiflora*, the dorsal bundle ramifies in the body of the achene to give two-four minor bundles (Figs. 1a, 2a, & 7a-11a). Also the two ventral bundles ramify to give two additional minor bundles and this is recorded in *Ranunculus marginatus* (Fig. 11a). In *Thalictrum* species (case 1; Plate III, Figs. 13a-17a) the pericarp is found to contain a relatively large number of bundles almost of the same size. Here it could not be ruled out whether this increased number of bundles has arisen from either the dorsal bundle or the ventral bundles. To determine this, further ontogenetic study should be carried out.

Table (3): Anatomical structure of the pericarp and testa of the studied species of Ranunculoideae

Taxa	Attributes				Vasculature				Testa			
	Exocarp		Mesocarp	Endocarp	Dorsal No.	Bundles	Ventral No.	Bundles	No of Layers	Appearance	Thickness	Vasculature
	Shape	Thickning	No. of layers	No. of layers								
1- <i>Adonis dentata</i>	Papillate	k	Multi	2-3	1	4	1	-	1	Tan.	k	-
2- <i>Anemone narcissiflora</i>	*	*	Multi	1	1	3	1	-	1	Ten	K	-
3- <i>Clematis integriflora</i>	Cubic	*	Multi	1	1	3	1	-	1	Tan	K	-
4- <i>C. tangutica</i>	*	**	2	1	1	-	1	-	1	Tan	k	-
5- <i>Pulsatilla pratensis</i>	Cubic	outer N, inner k	1-2	1	1	-	1	-	1	Tan	k	-
6- <i>P. vulgaris</i>	Cubic	outer N, inner k	1-2	1	1	-	1	-	1	Tan	k	-
7- <i>Ranunculus acris</i>	Cubic	k	1	Multi	1	2	2	-	1	Tan	k	-
8- <i>R. constantinopolitanus</i>	Cubic	k	1	Multi	1	2	2	-	1	Tan	k	-
9- <i>R. illyricus</i>	Cubic	k	1	2-3	1	2	2	-	1	Tan	k	-
10- <i>R. lanuginosus</i>	Cubic	k	Multi	2-3	1	2	2	-	1	Tan	k	-
11- <i>R. marginatus</i>	Cubic	k	Multi	Multi	1	2	2	2	2	Tan	outer N inner k	-
12- <i>R. sceleratus</i>	Cubic	k	Multi	2-3	1	-	2	-	1	Tan	k	-
13- <i>Thalictrum aquilegifolium</i>	Cubic	k	Multi	1	•	•	•	•	2	Rad	k	+
14- <i>T. flavum</i>	Cubic	k	Multi	1	•	•	•	•	2	Rad	k	+
15- <i>T. foetidum</i>	Cubic	k	Multi	1	•	•	•	•	2	Rad	k	+
16- <i>T. minus</i>	Cubic	k	Multi	1	•	•	•	•	2	Rad	k	+
17- <i>T. morisoni</i>	Cubic	k	Multi	1	•	•	•	•	2	Rad	k	+

-, Absent; +, Present; k, Thick; Multi, Multi-layered; N, Thin; Rad., Radially elongated; Ram., Ramification; Tan., Tangentially elongated; * the exocarp is obliterated; ** the exocarp is disintegrated and the remaining walls extending into hair like structures; •, increased number of bundles (see text)

Morphology and anatomy of the achene

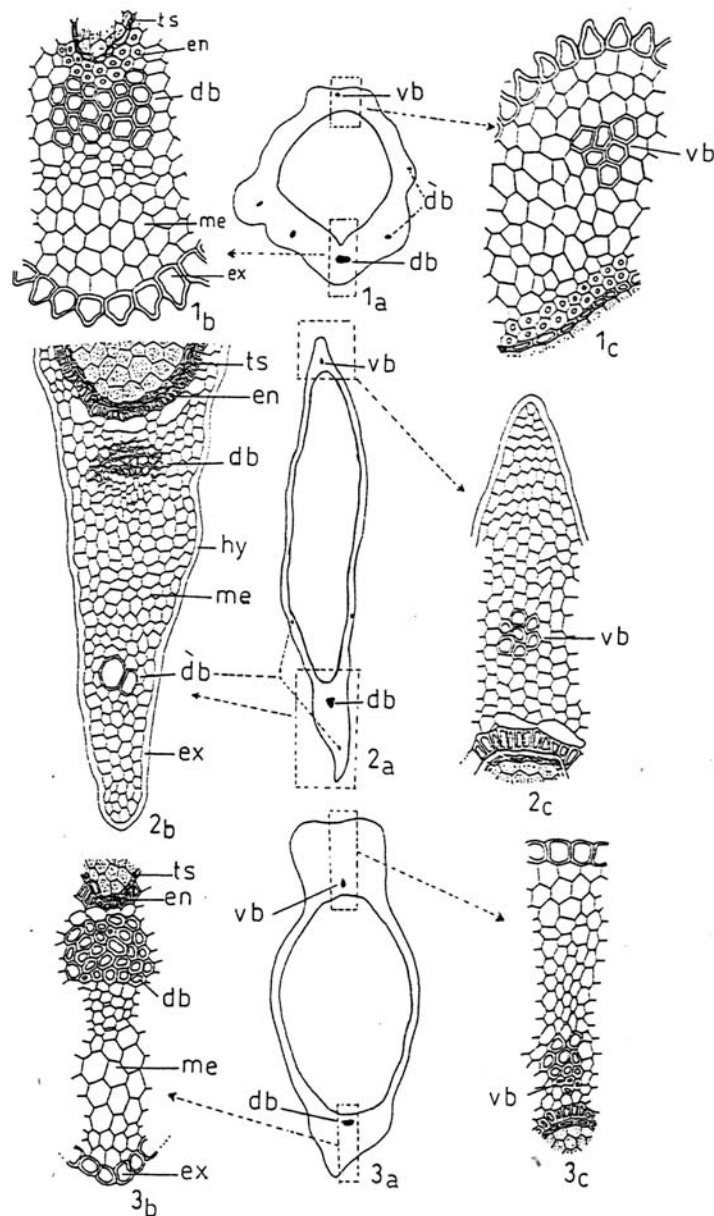


Plate III. Anatomy of the pericarp and testa (Figs. 1-3)

Fig. 1. *Adonis dentata*, Fig. 2. *Anemone narcissiflora*, Fig. 3. *Clematis integrifolia*.

a, diagram of the achene to show the distribution of the vascular bundles; b, sector to show the dorsal bundle and anatomy of the pericarp and testa; c, the same as b to show the ventral bundles (s); db, dorsal bundle; d'b, a branch of the dorsal bundle; en, endocarp; ex, exocarp; hy, hyaline layer; me, mesocarp; ts, testa; vb, ventral bundle (s).

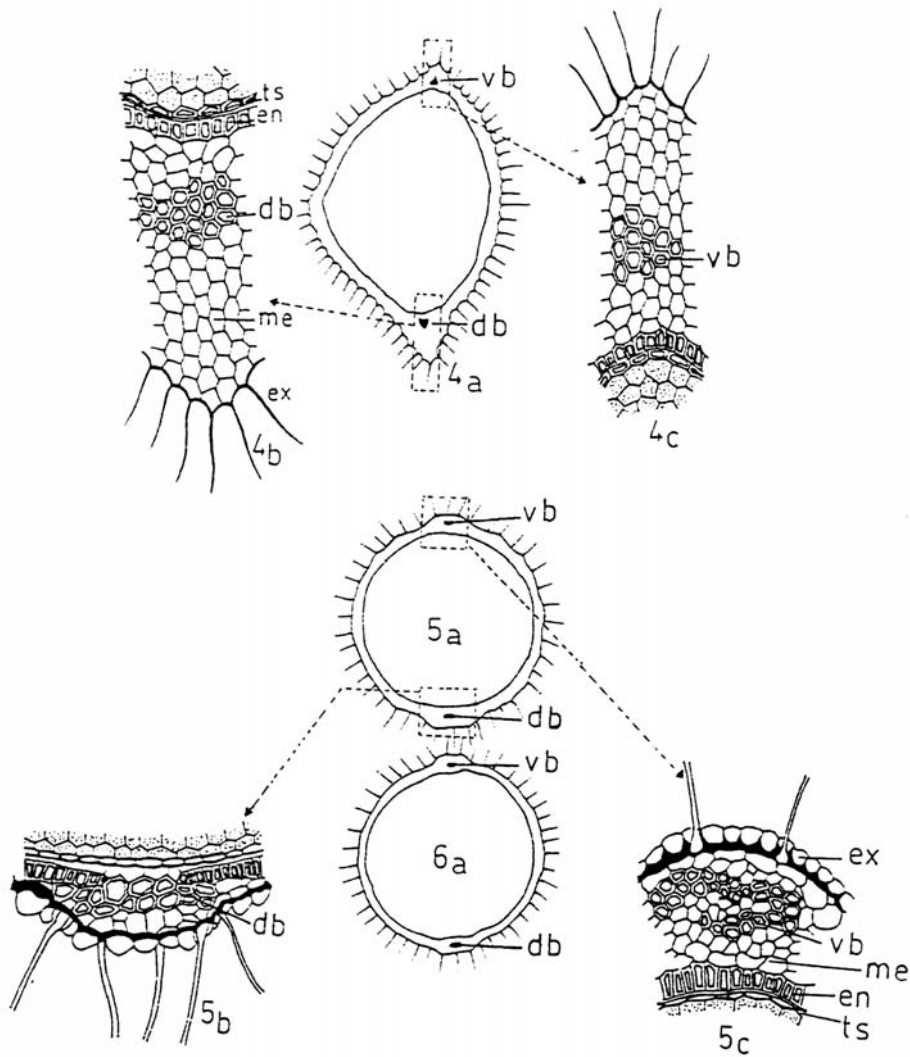


Plate III (cont.). Anatomy of the pericarp and testa (Figs. 4-6)

Fig. 4. *C. tangutica*, Fig. 5. *Pulsatilla pratensis*, Fig. 6. *P. vulgaris*.

a, diagram of the achene to show the distribution of the vascular bundles; b, sector to show the dorsal bundle and anatomy of the pericarp and testa; c, the same as b to show the ventral bundles (s); db, dorsal bundle; en, endocarp; ex, exocarp; me, mesocarp; ts, testa; vb, ventral bundle (s).

Morphology and anatomy of the achene

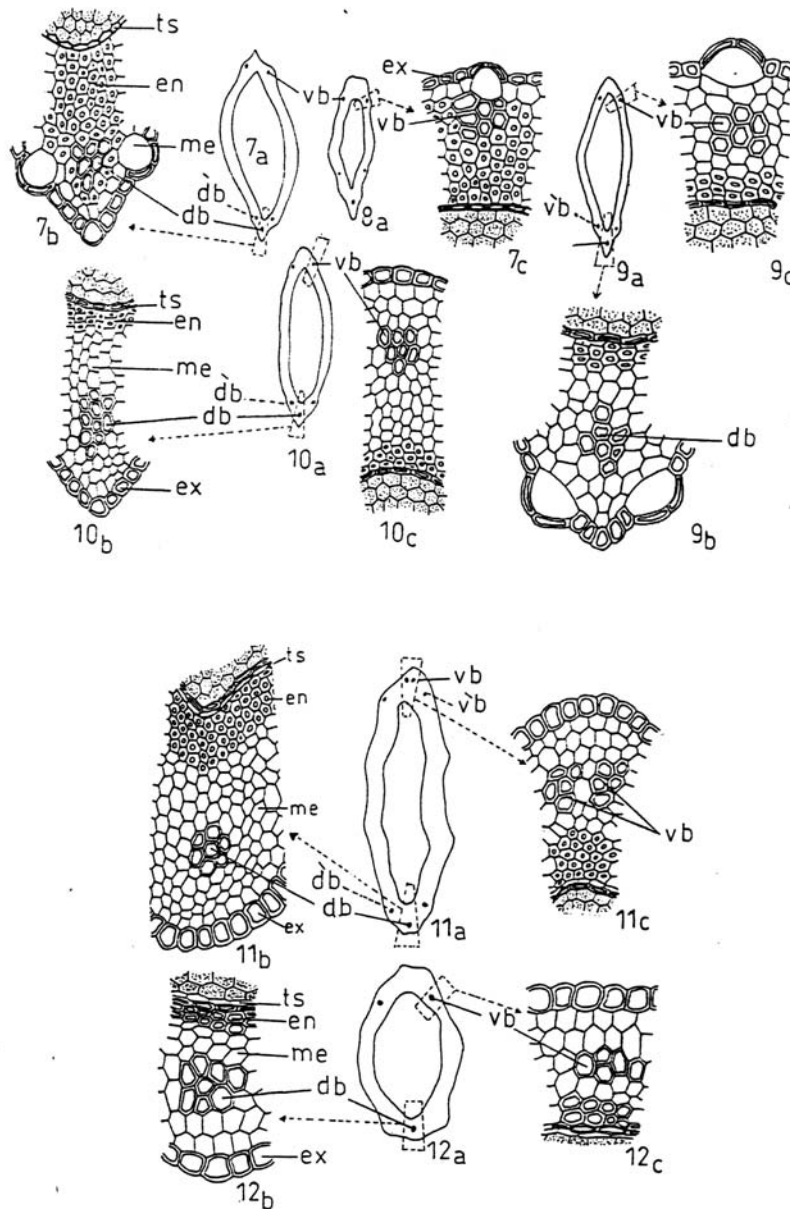


Plate III (cont.). Anatomy of the pericarp and testa (Figs. 7-12)

Fig. 7. *Ranunculus acris*, Fig. 8. *R. constantinopolitanus*, Fig. 9. *R. illyricus*, Fig. 10. *R. lanuginosus*, Fig. 11. *R. marginatus*, Fig. 12. *R. sceleratus*.

a, diagram of the achene to show the distribution of the vascular bundles; b, sector to show the dorsal bundle and anatomy of the pericarp and testa; c, the same as b to show the ventral bundles (s); db, dorsal bundle; d'b, a branch of the dorsal bundle; en, endocarp; ex, exocarp; me, mesocarp; ts, testa; vb, ventral bundle (s); v'b, a branch of the ventral bundle.

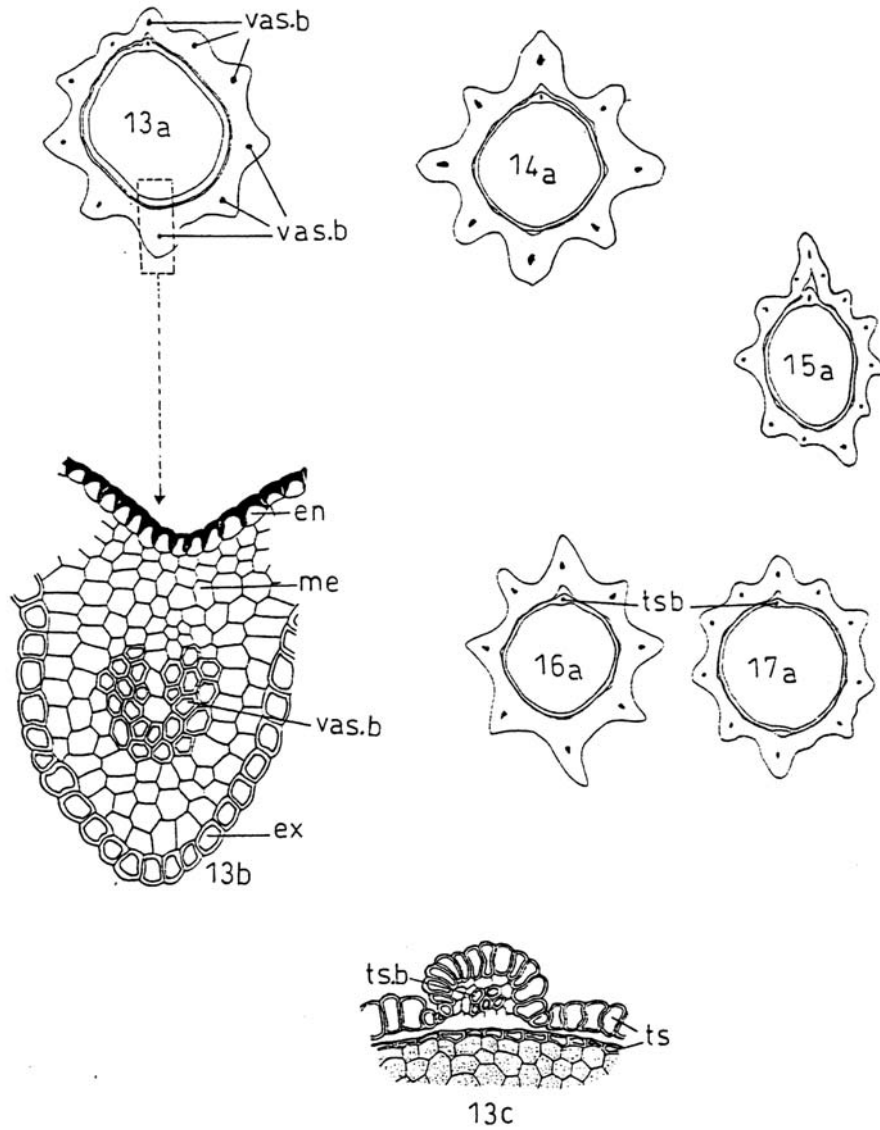


Plate III (cont.). Anatomy of the pericarp and testa (Figs. 13-17)

Fig. 13. *Thalictrum aquilegifolium*, Fig. 14. *T. flavum*, Fig. 15. *T. foetidum*, Fig. 16. *T. minus*, Fig. 17. *T. miorisoni*.

a, diagram of the achene to show the distribution of the vascular bundles ; b, the number of bundles are not decided ; c, showing the testal bundle.

en, endocarp; ex, exocarp; me, mesocarp; ts, testa; ts.b, testal bundle. a, diagram of the achene to show the distribution of the vascular bundles en, endocarp; ex, exocarp; me, mesocarp; ts, testa; ts.b, testal bundle.

D. Seed coat anatomy (Table 3, Plate III):

The seed coat, (*sensu lato*), is formed of an outer and an inner tegmens (Corner, 1976). Deviations from this general structure may occur where one of the two tegmens disappears. Corner (op.cit.) did not decide which of the two tegmens becomes crushed. In the present work the subsequent structures of the seed coat are recorded:

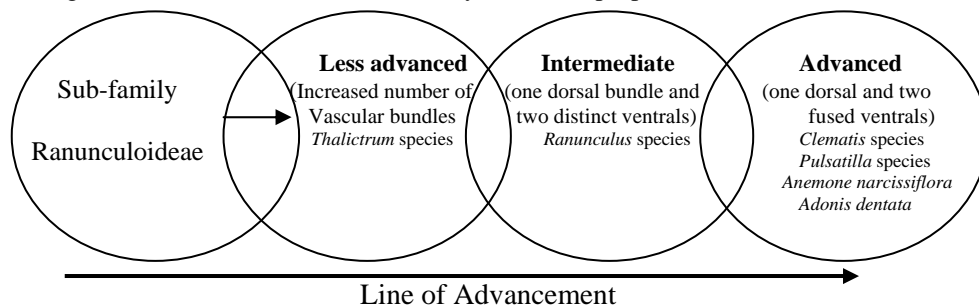
- 1- In *Adonis dentata*, *Anemone narcissiflora*, *Clematis* species, *Pulsatilla* species and *Ranunculus* species (except *R. marginatus*), the seed coat is formed of one layer of tangentially elongated, thick-walled cells.
- 2- In *Ranunculus marginatus*, the seed coat is formed of two layers of tangentially elongated cells; the outer layer is formed of thin - walled cells and the inner layer of thick - walled cells.
- 3- In *Thalictrum* species, the seed coat is formed of two layers of either radially or tangentially elongated thick - walled cells.

Concerning the seed coat vasculature, Fahn (1969) stated that relatively well developed vascular bundles may be found in the testa of certain plants while in others they can hardly be distinguished.

In the present study, a vascular bundle in the seed coat is recorded only in *Thalictrum* species; in the remainder studied taxa such a bundle is absent.

Perhaps the organ vascularization is the most indispensable feature that determines both the organ nature and its phylogenetic status. Several documented cases had been reported to occur in many families (Puri, 1951). In accordance we here, are adopting the vascularization of the achene for suggesting an evolutionary status for the studied taxa of the Ranunculoideae.

According to the evolutionary dicta set earlier by Bessey (1915) reduction in number and the phenomenon of fusion (*sensu lato*) are two of the dicta that indicate the phylogenetic status. An organ exhibiting one or both of them will show an advance. Relying on these two criteria an achene with the full number of vascular bundles just as the vegetative leaf from which it originated, viz. one median and two laterals is considered to be less advanced than an achene with fewer number of bundles. Similarly an achene with two distinct ventral bundles is less advanced than that where these bundles are fused. Taking this in consideration an evolutionary scheme is proposed for the studied taxa.



In this scheme the taxa are arranged from less advanced to more advanced in the following sequence:

- 1- Less advanced taxa are *Thalictrum* species where the pericarp contains an increased number of bundles. An additional evidence to this status is the seed vasculature. In these species the seeds were found to be vascularized, whereas all the seeds of other taxa are devoid of the vascular supply.
- 2- Intermediate taxa are *Ranunculus* species where the number of bundles is the basic number viz. one dorsal and two ventrals; the latter are distinct.
- 3- The more advanced taxa are *Adonis dentata*, *Anemone narcissiflora*, *Clematis* species and *Pulsatilla* species where the dorsal bundle is present and the two ventral bundles are fused.

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