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THE PHYLOGENETIC SIGNIFICANCE OF FRUIT STRUCTURES IN RANUNCULACEAE OF CHINA

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Abstract

The external and internal structures of fruits from 95 taxa representing 27 Ranunculaceae genera of China were studied. The results show that Ranunculaceae could be divided into 4 groups based on the fruit types, epidermal surface, vascular bundle, mesocarp cell, and endocarp cell structures: Group 1: follicle or achene, branching or branching and anastomosing vascular bundles, mesocarp parenchyma, and endocarp with one layer of lignified cells (including *Aconitum* and other genera); Group 2: achene, vascular bundle branching, mesocarp lignified, endocarp with one layer of irregular and partly lignified cells (*Thalictrum* only); Group 3: achene, endocarp with multilayered thick-walled cells (including *Adonis*, *Batrachium* and *Ranunculus*); Group 4: achene, two non-branching vascular bundles, and endocarp with one layer of fibers (including *Anemone*, *Clematis* and *Pulsatilla*). This study show that the fruit structures of Ranunculaceae could provide morphological and anatomical evidences for molecular phylogeny.

Key words: Fruit, Structure, Systematics, Ranunculaceae.

Introduction

Ranunculaceae is a basal group of eudicots (Anon., 2009), about 60 genera and 2500 species in the world (Tamura, 1993; Takhtajan, 2009), and 38 genera and 921 species in China (Wang *et al*., 2001). In the family, many taxa are famous ornamental plants (e.g. *Clematis patens* and *Trollius macropetalus*) and some taxa have important medicinal values (e.g. *Aconitum* and *Delphinium*) (Hazrat *et al*., 2007, 2013; Zhang *et al*., 2009; Wang *et al*., 2010; Wu, 2011; Hao *et al*., 2013; Wen *et al*., 2013).

There are several taxonomic revisions of Ranunculaceae (Hutchinson, 1923; Wang & Wang, 1979, Wang *et al*. 1980, 2001). Hutchinson (1923) divided Ranunculaceae into two subfamilies based on the fruit characters, Helleboroideae (ovaries with more than 1 ovule; fruits follicular or baccate) and Ranunculoideae (ovaries with 1 ovule; fruit a bunch of dry achenes, very rarely baccate). After Hutchinson (1923) other researchers also focused on fruit features in their studies. Ranunculaceae was divided into 3 subfamilies, Helleboroideae, Ranunculoideae and Thalictroideae by Wang & Wang (1979) and Wang *et al*. (1980, 2001). Whereas it was divided in to 5 subfamilies Helleboroideae (follicle, berry or capsule), Hydrastidoideae (berry), Isopyroideae (follicle), Ranunculoideae (achene, with or without petals) and Thalictroideae (achene, without petals) by Tamura (1993). Takhtajan (2009) emphasized on the pollen grain features of Ranunculaceae and divided the family into seven subfamilies, Aconitoideae, Cimicifugoideae, Coptidoideae and Trollioideae were added except three subfamilies (Helleboroideae, Ranunculoideae, Thalictroideae) mentioned. Recently molecular studies resulted in some important new insights about Ranunculaceae (Jensen *et al*., 1995; Wang *et al*., 2009). In the new system five subfamilies were recognized, Coptidoideae, Glaucidioideae, Hydrastidoideae, Ranunculoideae and Thalictroideae. Coptidoideae and Thalictroideae as the natural groups, but Aconitoideae, Cimicifugoideae, Helleboroideae and Trollioideae (or Adonidoideae) were transferred into Ranunculoideae (Jensen *et al*., 1995; Takhtajan, 2009; Wang *et al*., 2009).

The critical question to be addressed in this study is following. Do fruit anatomy characters give reliable information on the main groups of the family? Tang *et al*. (2008) described the carpel fusion pattern of Ranunculaceae fruits that could provide morphological evidences for the molecular systematics. However the fruit microstructures were usually overlooked in most of the studies (Hutchinson, 1923; Wang & Wang, 1979, Wang *et al*., 1980, 2001; Tamura, 1993; Takhtajan, 2009). The present study thus aims to explore the morphological and anatomical characteristics of fruits in Chinese Ranunculaceae, especially the pericarp structures in detail and to identify structural characters that support the findings of molecular phylogeny.

Materials and Methods

The external and internal structure of mature fruits from 95 taxa representing 27 Ranunculaceae genera of China were studied carefully in this study. Sample names and voucher information are provided in Table 1.

Morphological and anatomical study: Fruit trichomes of each sample were first photographed, and then the fruits were rehydrated for further examination. The exocarps were carefully removed to observe and photographed the vascular bundles, endocarp cells and crystal structures. Additional fruits were placed in FAA (formalin–acetic acid–alcohol) for a minimum of 24 h and then prepared for sectioning using the glycol methacrylate (GMA) method (Feder & O'Brien, 1968), a minimum of 24 h was used for the first two infiltrations in GMA and a minimum of five days for the third infiltration. A Leica Ultracut R microtome was used to prepare transverse sections about 2 μm in thickness. Sections were stained using the periodic acid–Schiff/toluidine blue method (Feder & O'Brien, 1968) and photographed using an Olympus BX53 microscope and an Olympus DP26 digital camera. Some fruits showing the vascular bundles were drawn in Photoshop CS 2.0 on the computer.

Table 1. Taxa of Ranunculaceae in China examined for fruit structures, together with voucher specimens details and locality.

Species	Voucher specimens	Locality
Aconitum barbatum Per.	Han 622 (IFP)	Heilongjiang
A. barbatum var. *puberulum* Ledeb.	Liu 5351 (IFP)	Hebei
A. carmichaeli var. *truppelianum* (Ulbr.) W.T. Wang & Hsiao	Wang 2945 (IFP)	Liaoning
A.coreanum (H. Lév) Rapaics	Cheng 91 (HANU)	Heilongjiang
A. excelsum Rchb.	Medicinal investigation group 2649 (IFP)	Neimenggu
A. fischeri var. *arcuatum* (Maxim.) Regel	Wang 1779 (IFP)	Neimenggu
A. jaluense Kom.	Wang 133 (IFP)	Liaoning
A. kirinense Nakai	Wang 436 (IFP)	Liaoning
A.kusnezoffii Rchb.	Liu 1335 (IFP)	Heilongjiang
A. macrorhynchum Turcz.	Zhang 1120 (IFP)	Jilin
A. monanthum Nakai	Li 827 (IFP)	Jilin
A. sczukinii Turcz.	Liu *et al*. 287 (HANU)	Heilongjiang
A. umbrosum Kom.	Fu 3472 (IFP)	Heilongjiang
A. villosum var. *amurense* (Nakai) S.H. Li & Y.H. Huang	Yanbian group two 580 (IFP)	Jilin
A. volubile var. *pubescens* Regel	Wang 1425 (IFP)	Liaoning
Adonis amurensis Regel & Radde	Cheng 14 (HANU)	Heilongjiang
Anemone amurensis (Korsh.) Kom.	Cheng 13 (HANU)	Heilongjiang
A. dichotoma L.	Cheng 75 (HANU)	Heilongjiang
A. raddeana Regel	Cheng 16 (HANU)	Heilongjiang
A. rivularis var. *flore-minore* Maxim.	Li 4451 (IFP)	Neimenggu
A. silvestris L.	Cui 1217 (IFP)	Heilongjiang
Aquilegia oxysepala f. *pallidiflora* (Nakai) Kitag.	Li 1244 (IFP)	Liaoning
A. oxysepala Trautv. & C.A. Mey.	Cheng 71 (HANU)	Heilongjiang
A. parviflora Ledeb.	Han 661 (IFP)	Heilongjiang
A. viridiflora Pall.	Liu *et al*. 138 (HANU)	Heilongjiang
A. yabeana Kitag.	Li 4443 (IFP)	Neimenggu
Batrachium foeniculaceum (Gilib.) V. I. Krecz.	Cheng 76 (HANU)	Heilongjiang
B. kauffmanii (Clerc) Ovcz.	Yan 482 (IFP)	Jilin
Beesia calthifolia (Maxim.) Ulbr.	Bijiang group 1599 (KUN)	Yunnan
Caltha natans Pall.	Cheng 89 (HANU)	Heilongjiang
C. palustris var. *sibirica* Regel	Cheng 33 (HANU)	Heilongjiang
C. palustris var. *membranacea* Turcz.	Okubo (IFP)	Liaoning
Cimicifuga dahurica (Turcz.) Maxim.	Wang 3213(IFP)	Liaoning
C. simplex Wormsk.	Liu *et al*. 286 (HANU)	Heilongjiang
Clematis apiifolia var. *obtusidentata* Rehd. & Wils.	Chen 15355 (IFP)	Guangxi
C. brevicaudata DC.	Cheng 94 (HANU)	Heilongjiang
C. fusca Turcz.	Cheng 73 (HANU)	Heilongjiang
C. heracleifolia DC.	Liu *et al*. 285 (HANU)	Heilongjiang
C. hexapetala Pall.	Liu *et al*. 269 (HANU)	Heilongjiang
C. intricata Bunge	Wang 3022 (IFP)	Liaoning
C. koreana Kom.	Noda 248 (IFP)	Jilin
C. macropetala Ledeb.	Yanbian group one 208 (IFP)	Jilin
C. patens Morr. & Decne.	Liu *et al*. 263 (HANU)	Heilongjiang
C. serratifolia Rehd.	Cheng 137 (HANU)	Heilongjiang
C. sibirica (L.) Mill.	Li 7342 (IFP)	Heilongjiang
C. terniflora var. *mandshurica* (Rupr.) Ohwi	Liu *et al*. 268 (HANU)	Heilongjiang
Consolida ajacis (L.) Schur	Anon s. n. (KUN)	Yunnan

Table 1. (Cont'd.).

Species	Voucher specimens	Locality
Coptis chinensis Franch.	Anon 319 (KUN)	Sichuan
C. teeta Wall.	Qingzang group 74355 (HITBC)	Yunnan
Delphinium cheilanthum Fisch. Ex DC.	Palonov 246 (IFP)	Neimenggu
D. grandiflorum L.	Liu *et al*. 227 (HANU)	Heilongjiang
D. korshinskyanum Nevski	Han 730 (IFP)	Heilongjiang
D. maackianum Regel	Cheng 128 (HANU)	Heilongjiang
Dichocarpum. adiantifolium (Hk. f. & Thoms.) W.T. Wang & Hsiao	Yunnan northeast group 445 (KUN)	Yunnan
D. hypoglaucum W. T. Wang & Hsiao	Anon 6111 (HITBC)	Yunnan
Enemion raddeanum Regel	Liu *et al*. 444 (HANU)	Heilongjiang
Eranthis stellata Maxim.	Liu *et al*. 126 (HANU)	Heilongjiang
Halerpestes cymbalaria (Pursh) Greene	Cheng 78 (HANU)	Heilongjiang
H. ruthenica (Jacq.) Ovcz.	Lin 127 (IFP)	Neimenggu
Helleborus thibetanus Franch.	Wang 4172 (KUN)	Gansu
Isopyrum manshuricum Kom.	Li 7053 (IFP)	Liaoning
Leptopyrum fumarioides (L.) Rchb.	Liu *et al*. 236 (HANU)	Heilongjiang
Paraquilegia microphylla (Royle) Drumm. & Hutch.	Boufford 42174 (KUN)	Yunnan
Pulsatilla cernua (Thunb.) Bercht. & J. Presl	Cheng 39 (HANU)	Heilongjiang
P. chinensis (Bunge) Regel	Liu *et al*. 238 (HANU)	Heilongjiang
P. dahurica (Fisch.) Spreng.	Cheng 64 (HANU)	Heilongjiang
P. patens var. *multifida* (Pritz.) S. H. Li & Y. H. Huang	Fu 2185 (IFP)	Neimenggu
P. sukaczevii Juz.	Zhao 838 (IFP)	Neimenggu
Ranunculus amurensis Kom.	Zhang 1602 (IFP)	Heilongjiang
R. chinensis Bunge	Cheng 47 (HANU)	Heilongjiang
R. cuneifolius Maxim.	Investigation group 188 (IFP)	Liaoning
R. franchetii H. Boissieu	Cheng 25 (HANU)	Heilongjiang
R. japonicus Thunb.	Cheng 50 (HANU)	Heilongjiang
R. microphyllus Hand.-Mazz.	Sato 7752 (IFP)	Jilin
R. monophyllus Ovcz.	Wang 1824 (IFP)	Heilongjiang
R. natans C. A. Mey.	Li 7764 (IFP)	Heilongjiang
R. pulchellus C. A. Mey.	Fu 2549 (IFP)	Neimenggu
R. radicans C. A. Mey.	Liu 6058 (IFP)	Heilongjiang
R. repens L.	Cheng 49 (HANU)	Heilongjiang
R. sceleratus L.	Liu 5834 (IFP)	Jilin
Semiaquilegia adoxoides (DC.) Makino	Qiao 1110 (KUN)	Shanxi
Souliea vaginata (Maxim.) Franch.	Qingzang group 2181 (KUN)	Yunnan
Thalictrum aquilegifolium var. *sibiricum* Regel & Tiling	Liu *et al*. 264 (HANU)	Heilongjiang
T. baicalense Turcz.	Liu *et al*. 255 (HANU)	Heilongjiang
T. filamentosum Maxim.	Li 3205 (IFP)	Heilongjiang
T. foetidum L.	Ding 1885 (IFP)	Heilongjiang
T. przewalskii Maxim.	Fang 918 (IFP)	Hebei
T. simplex var. *glandulosum* W. T. Wang	Liu *et al*. 261 (HANU)	Heilongjiang
T. sparsiflorum Turcz. ex Fisch. & C. A. Mey.	Wang 2351 (IFP)	Jilin
T. squarrosum Steph.	Skvortsov 809 (IFP)	Heilongjiang
T. tuberiferum Maxim.	Wang 2720 (IFP)	Jilin
Trollius chinensis Bunge	Fang 981 (IFP)	Hebei
T. ledebourii Rchb.	Zhao 1601 (IFP)	Neimenggu
T. macropetalus Fr. Schmidt	Liu *et al*. 242 (HANU)	Heilongjiang
Urophysa henryi (Oliv.) Ulbr.	Wulingshan group 3448 (KUN)	Guizhou

Clustering analyses: All fruit anatomical characters (as listed in Table 2) were considered, but 16 qualitative characters were used for clustering analyses (listed in Table 3). Some flower and vegetative organ characteristics (6 qualitative characters) from Wang & Wang (1979) and Wang *et al*. (1980) were also used. All characters were treated as unordered and unweighted. All the analyses were performed using NTSYS-pc Version 2.10e (Rohlf, 2000). Cluster analysis using UPGMA (unweighted pair group method with arithmetic average).

Results

Variation in fruit types, trichomes and structures was characterized and described for each of the 95 taxa of Ranunculaceae sampled (Figs. 1, 2). The following characters were examined: trichome type, fruit epidermal cells, mesocarp, endocarp, vascular bundles, and crystals. A summary of these characters is provided in Table 2.

Fruit and trichome type: There are two types of fruits in Ranunculaceae taxa studied. Most species are with follicles (e.g. *Aconitum coreanum* - Fig. 1A) and a few species achenes (e.g. *Ranunculus chinensis* - Fig. 1B). Two types of unicellular trichomes occur on the fruits, non-glandular hairs (Fig. 1C, D) and glandular hairs with rich inclusions and their surface are with mucus (Fig. 1E-G). The former are curly (e.g. *Aconitum coreanum* - Fig. 1C) or straight (e.g. *A. sczukinii* - Fig. 1D), and the latter obovate (e.g. *Caltha* and *Trollius* - Fig. 1E), bottle shaped (e.g. *Aquilegia* and *Consolida* - Fig. 1F) or papillary (e.g. *Leptopyrum fumarioides* - Fig. 1G). One species usually has one kind of trichome, but a few species have both types of trichomes (e.g. *Consolida ajacis* - Fig. 1F).

Exocarp: Exocarp is composed of one layer of cells which are more or less square (e.g. *Aquilegia oxysepala* - Fig. 2C) or elliptic (e.g. *Thalictrum simplex* var. *glandulosum* - Fig. 2M). The outer walls of exocarp cells may be thick (e.g. *Aquilegia oxysepala* - Fig. 2C) or thin (e.g. *Caltha natans* - Fig. 2E). The tannins occur in the exocarps in some species (e.g. *Clematis patens* - Fig. 2G).

Mesocarp: The mesocarp may consist of one (e.g. *Ranunculus japonicas* - Fig. 2K) or a few layers of cells (e.g. *Thalictrum baicalense* - Fig. 2L). The cells are uaually parenchyma (e.g. *Batrachium foeniculaceum* - Fig. 2D), but lignified in *Thalictrum* and *Leptopyrum* (Fig. 2I). The intercellular space is usually obvious (e.g. *Batrachium*, *Caltha*, *Eranthis* - Fig. 2D, E, H) but not obvious in some cases (e.g. *Clematis* - Fig. 2G). The calcium oxalate crystals are present in the mesocarp close to the endocarp in some species of *Ranunculus* (e.g. *R. japonicas* - Fig. 2N). The three types of vascular bundles are present viz. branching and anastomosing (e.g. *Aconitum carmichaeli* var. *truppelianum* - Fig. 1H), branching (e.g. *Trollius macropetalus* - Fig. 1I), and non-branching (e.g. *Ranunculus chinensis* - Fig. 1J). Fibers occur in the mesocarp close the vascular bundles in the taxa studied and they may be next to the phloem (e.g. *Thalictrum simplex* var. *glandulosum* - Fig. 2M), or around the vascular bundles (e.g. *Aquilegia oxysepala* - Fig. 2C). A few species have no fibers or fibers are very rare close to the vascular bundles (e.g. *Caltha palustris* var. *sibirica* - Fig. 2F).

Endocarp: The endocarp consists of one layer (e.g. *Thalictrum simplex* var. *glandulosum* - Fig. 2M) or many layers of cells (e.g. *Adonis*, *Batrachium* and *Ranunculus* - Fig. 2A, D, K), which may be parenchymatous (e.g. *Caltha natans* - Fig. 2E), partly lignified (the outer tangential walls are not or slightly lignified but the others obviously lignified, e.g. *Aquilegia oxysepala* - Fig. 2C), completely lignified (e.g. *Eranthis stellata* - Fig. 2H), fibers which may be evenly lignified (e.g. *Clematis patens* - Fig. 2G) or not (e.g. some species of *Anemone* - Fig. 2B), and sclereids (e.g. *Adonis amurensis* - Fig. 2A). The endocarp cells are completely lignified (e.g. *Ranunculus japonicas* - Fig. 2K) or somewhat parenchyma close to the ventral and dorsal sutures (e.g. *Pulsatilla* - Fig. 2J). These endocarp cells are usually rectangular (e.g. *Delphinium maackianum* - Fig. 2O) and some irregular in shapes (e.g. *Helleborus thibetanus* - Fig. 2P). In longitudinal view, the long axis of the endocarp cells are perpendicular (e.g. *Eranthis stellat* - Fig. 2H) or parallel (e.g. *Clematis patens* - Fig. 2G) to the fruits. However, in *Ranunculus* (Fig. 2K) and *Batrachium* (Fig. 2D), the long axis of the innermost layer endocarp cells are perpendicular to the fruits and the others are parallel to the fruits. Anomocytic stomata were observed on the endocarps of *Consolida* and *Delphinium* (e.g. *D. maackianum* - Fig. 2Q).

Clustering analysis: The 22 morphological characters of fruits, flowers and vegetative organs were applied (encoded details are shown in Table 3) for cluster analysis and UPGMA dendrogram (Fig. 3). The four groups were obtained at 0.66 similarity coefficient.

Discussion

Our results clearly indicate that most of the fruit features, such as the distribution of fibers around the vascular bundles and the types of endocarp cells, can be used as taxonomic evidences to distinguish the subfamilies and genera. Ranunculaceae could be divided into 4 groups based on the fruit characters studied.

Group 1: Follicles (except *Halerpestes* with achenes). The mesocarp is not lignified (except *Leptopyrum*) and lignification occurs in the endocarp. The vascular bundles are branching or branching and anastomosing. This group includes most genera of the family studied (including *Aconitum*, *Aquilegia*, *Beesia*, *Caltha*, *Cimicifuga*, *Consolida*, *Coptis*, *Dichocarpum*, *Delphinium*, *Enemion*, *Eranthis*, *Halerpestes*, *Helleborus*, *Leptopyrum*, *Isopyrum*, *Paraquilegia*, *Semiaquilegia*, *Souliea*, *Trollius* and *Urophysa*). Hutchinson (1923) placed all these genera (except *Halerpestes*) in the subfamily Helleboroideae according to the follicle and ovaries with more than one ovule.

Table 2. Summary of taxonomically important characters of fruit structures in the family Ranunculaceae of China.

Species	Fruit type	Trichome type	Vascular bundle in longitudinal view	Number of vascular bundles in transversal section	Fiber close to vascular bundle	Endocarp cell layer	Endocarp cell shape	Long axis direction of endocarp cell *	Endocarp cell type
Aconitum barbatum	follicle	non-glandular hair	branching and anastomosing	9-11	close phloem	1	rectangle	perpendicular	partly lignified cell
A. barbatum var. *puberulum*	follicle	non-glandular hair	branching and anastomosing	9-13	close phloem	1	rectangle	perpendicular	partly lignified cell
A. carmichaeli var. *truppelianum*	follicle	absent	branching and anastomosing	9-10	close phloem	1	rectangle	perpendicular	partly lignified cell
A. coreanum	follicle	non-glandular hair	branching and anastomosing	13-23	close phloem	1	rectangle	perpendicular	completely lignified cell
A. excelsum	follicle	absent	branching and anastomosing	7-12	close phloem	1	rectangle	perpendicular	partly lignified cell
A. fischeri var. *arcuatum*	follicle	non-glandular hair	branching and anastomosing	11-14	close phloem	1	rectangle	perpendicular	partly lignified cell
A. jaluense	follicle	non-glandular hair	branching and anastomosing	9-11	close phloem	1	rectangle	perpendicular	partly lignified cell
A. kirinense	follicle	absent	branching and anastomosing	9-12	close phloem	1	rectangle	perpendicular	partly lignified cell
A. kusnezoffii	follicle	absent	branching and anastomosing	8-9	close phloem	1	rectangle	perpendicular	partly lignified cell
A. macrorhynchum	follicle	non-glandular hair	branching and anastomosing	6-8	close phloem	1	rectangle	perpendicular	partly lignified cell
A. monanthum	follicle	absent	branching and anastomosing	8-11	close phloem	1	rectangle	perpendicular	completely lignified cell
A. sczukinii	follicle	non-glandular hair	branching and anastomosing	13-17	unobserved	1	rectangle	perpendicular	unobserved
A. umbrosum	follicle	absent	branching and anastomosing	8-10	unobserved	1	rectangle	perpendicular	partly lignified cell
A. villosum var. *amurense*	follicle	non-glandular hair	branching and anastomosing	7-9	close phloem	1	rectangle	perpendicular	completely lignified cell
A. volubile var. *pubescens*	follicle	absent	branching and anastomosing	8-10	close phloem	1	rectangle	perpendicular	completely lignified cell
Adonis amurensis	achene	non-glandular hair	branching	8-10	close phloem	6-8	fusiform	parallel	sclereid, fiber
Anemone amurensis	achene	non-glandular hair	non-branching	2	close phloem	1	fusiform	parallel	fiber
A. dichotoma	achene	absent	non-branching	2	close phloem	5-8	fusiform	parallel	fiber
A. raddeana	achene	non-glandular hair	non-branching	2	close phloem	1	fusiform	parallel	fiber
A. rivularis var. *flore-minore*	achene	absent	non-branching	2	unobserved	1	fusiform	parallel	unobserved
A. silvestris	achene	non-glandular hair	non-branching	2	close phloem	1	fusiform	parallel	fiber
Aquilegia oxysepala f. *pallidiflora*	follicle	bottle-shaped glandular hair	branching	14-17	around vascular bundle	1	rectangle	perpendicular	partly lignified cell
A. oxysepala	follicle	bottle-shaped glandular hair	branching	13-18	around vascular bundle	1	rectangle	perpendicular	partly lignified cell
A. parviflora	follicle	bottle-shaped glandular hair	branching	15-20	around vascular bundle	1	rectangle	perpendicular	partly lignified cell

Table 2. (Cont'd.).

Species	Fruit type	Trichome type	Vascular bundle in longitudinal view	Number of vascular bundles in transversal section	Fiber close to vascular bundle	Endocarp cell layer	Endocarp cell shape	Long axis direction of endocarp cell *	Endocarp cell type
A. viridiflora	follicle	bottle-shaped glandular hair	branching	15-18	around vascular bundle	1	rectangle	perpendicular	partly lignified cell
A. yabeana	follicle	bottle-shaped glandular hair	branching	15-18	around vascular bundle	1	rectangle	perpendicular	partly lignified cell
Batrachium foeniculaceum	achene	non-glandular hair	non-branching	2	absent	3-4	fusiform	parallel, perpendicular	fiber
B. kauffmanii	achene	absent	non-branching	2	absent	3-4	fusiform	parallel, perpendicular	fiber
Beesia calthifolia	follicle	non-glandular hair	branching	7-9	around vascular bundle	1	rectangle	perpendicular	completely lignified cell
Caltha natans	follicle	absent	branching	4-5	absent	1	rectangle	perpendicular	parenchyma cell
C. palustris var. *sibirica*	follicle	obovate glandular hair	branching	5-7	absent	1	rectangle	perpendicular	partly lignified cell, parenchyma cell
C. palustris var. *membranacea*	follicle	obovate glandular hair	branching	5-7	absent	1	rectangle	perpendicular	partly lignified cell
Cimicifuga dahurica	follicle	non-glandular hair	branching	6-8	close phloem	1	rectangle	perpendicular	completely lignified cell
C. simplex	follicle	non-glandular hair	branching	7-9	close phloem	1	rectangle	perpendicular	completely lignified cell
Clematis apiifolia var. *obtusidentata*	achene	non-glandular hair	non-branching	2	close phloem	1	fusiform	parallel	fiber, parenchyma cell **
C. brevicaudata	achene	non-glandular hair	non-branching	2	close phloem	1	fusiform	parallel	fiber, parenchyma cell **
C. fusca	achene	non-glandular hair	non-branching	2	close phloem	1	fusiform	parallel	fiber, parenchyma cell **
C. heracleifolia	achene	non-glandular hair	non-branching	2	close phloem	1	fusiform	parallel	fiber, parenchyma cell **
C. hexapetala	achene	non-glandular hair	non-branching	2	close phloem	1	fusiform	parallel	fiber, parenchyma cell**
C. intricata	achene	non-glandular hair	non-branching	2	close phloem	1	fusiform	parallel	unobserved
C. koreana	achene	non-glandular hair	non-branching	2	close phloem	1	fusiform	parallel	fiber, parenchyma cell **
C. macropetala	achene	non-glandular hair	non-branching	2	close phloem	1	fusiform	parallel	unobserved
C. patens	achene	non-glandular hair	non-branching	2	close phloem	1	fusiform	parallel	fiber
C. serratifolia	achene	non-glandular hair	non-branching	2	close phloem	1	fusiform	parallel	fiber, parenchyma cell **
C. sibirica	achene	non-glandular hair	non-branching	2	close phloem	1	fusiform	parallel	fiber, parenchyma cell **
C. terniflora var. *mandshurica*	achene	non-glandular hair	non-branching	2	close phloem	1	fusiform	parallel	fiber, parenchyma cell **
Consolida ajacis	follicle	non-glandular hair, bottle-shaped glandular hair	branching and anastomosing	15-20	absent	1	rectangle	perpendicular	partly lignified cell
Coptis chinensis	follicle	non-glandular hair	branching	6-7	close phloem	1	rectangle	perpendicular	partly lignified cell

Table 2. (Cont'd.).

Species	Fruit type	Trichome type	Vascular bundle in longitudinal view	Number of vascular bundles in transversal section	Fiber close to vascular bundle	Endocarp cell layer	Endocarp cell shape	Long axis direction of endocarp cell *	Endocarp cell type
C. teeta	follicle	non-glandular hair	branching	5	close phloem	1	rectangle	perpendicular	partly lignified cell
Delphinium cheilanthum	follicle	non-glandular hair	branching and anastomosing	9-12	close phloem	1	rectangle	perpendicular	partly lignified cell
D. grandiflorum	follicle	non-glandular hair	branching and anastomosing	18-24	close phloem	1	rectangle	perpendicular	partly lignified cell
D. korshinskyanum	follicle	absent	branching and anastomosing	14-17	close phloem	1	rectangle	perpendicular	completely lignified cell
D. maackianum	follicle	absent	branching and anastomosing	20-24	close phloem	1	rectangle	perpendicular	partly lignified cell
Dichocarpum adiantifolium	follicle	absent	branching	9-10	around vascular bundle	1	polygon	absent	partly lignified cell
D. hypoglaucum	follicle	absent	branching	8-12	around vascular bundle	1	polygon	absent	partly lignified cell
Enemion raddeanum	follicle	absent	branching	11-15	close phloem	1	rectangle	perpendicular	partly lignified cell
Eranthis stellata	follicle	bottle-shaped glandular hair	branching	5-7	around vascular bundle	1	rectangle	perpendicular	completely lignified cell
Halerpestes cymbalaria	achene	absent	branching	9-11	absent	1	irregular-shape	parallel	completely lignified cell
H. ruthenica	achene	absent	branching	10-16	absent	1	irregular-shape	parallel	completely lignified cell
Helleborus thibetanus	follicle	absent	branching	9-13	around vascular bundle	1	irregular-shape	perpendicular	completely lignified cell
Isopyrum manshuricum	follicle	absent	branching	8-11	close phloem	1	rectangle	perpendicular	partly lignified cell
Leptopyrum fumarioides	follicle	papillary glandular hair	branching and anastomosing	13-20	close phloem	1	rectangle	perpendicular	partly lignified cell
Paraquilegia microphylla	follicle	papillary glandular hair	branching	10-12	close phloem	1	rectangle	perpendicular	partly lignified cell
Pulsatilla cernua	achene	non-glandular hair	non-branching	2	close phloem	1	fusiform	parallel	fiber, parenchyma cell **
P. chinensis	achene	non-glandular hair	non-branching	2	close phloem	1	fusiform	parallel	fiber, parenchyma cell **
P. dahurica	achene	non-glandular hair	non-branching	2	close phloem	1	fusiform	parallel	fiber, parenchyma cell **
P. patens var. *multifida*	achene	non-glandular hair	non-branching	2	close phloem	1	fusiform	parallel	fiber, parenchyma cell **
P. sukaczevii	achene	non-glandular hair	non-branching	2	close phloem	1	fusiform	parallel	fiber, parenchyma cell **
Ranunculus amurensis	achene	non-glandular hair	non-branching	3	absent	5-6	fusiform	parallel, perpendicular	fiber
R. chinensis	achene	absent	non-branching	5	absent	4-7	fusiform	parallel, perpendicular	fiber
R. cuneifolius	achene	absent	non-branching	5	absent	5-7	fusiform	parallel, perpendicular	fiber

Table 2. (Cont'd.).

Species	Fruit type	Trichome type	Vascular bundle in longitudinal view	Number of vascular bundles in transversal section	Fiber close to vascular bundle	Endocarp cell layer	Endocarp cell shape	Long axis direction of endocarp cell *	Endocarp cell type
R. franchetii	achene	non-glandular hair	non-branching	3	absent	5-7	fusiform	parallel, perpendicular	fiber
R. japonicus	achene	absent	non-branching	5	absent	5-6	fusiform	parallel, perpendicular	fiber
R. microphyllus	achene	absent	unobserved	unobserved	unobserved	2-3	fusiform	parallel, perpendicular	fiber
R. monophyllus	achene	non-glandular hair	non-branching	3	absent	unobserved	fusiform	parallel, perpendicular	fiber
R. natans	achene	absent	unobserved	unobserved	unobserved	2-4	fusiform	parallel, perpendicular	fiber
R. pulchellus	achene	absent	non-branching	3	absent	2-3	fusiform	parallel, perpendicular	fiber
R. radicans	achene	absent	unobserved	unobserved	unobserved	2-3	fusiform	parallel, perpendicular	fiber
R. repens	achene	absent	non-branching	5	absent	6-8	fusiform	parallel, perpendicular	fiber
R. sceleratus	achene	absent	unobserved	unobserved	unobserved	3-4	fusiform	parallel, perpendicular	fiber
Semiaquilegia adoxoides	follicle	absent	branching	10-15	close phloem	1	rectangle	perpendicular	partly lignified cell
Souliea vaginata	follicle	absent	branching and anastomosing	12-18	around vascular bundle	1	irregular-shape	perpendicular	partly lignified cell
Thalictrum aquilegifolium var. *sibiricum*	achene	absent	branching	4	close phloem	1	irregular-shape	parallel	partly lignified cell
T. baicalense	achene	absent	branching	10-13	close phloem	1	irregular-shape	parallel	completely
T. filamentosum	achene	absent	branching	10	close phloem	1	irregular-shape	parallel	partly lignified cell
T. foetidum	achene	non-glandular hair, papillary glandular hair	branching	8	close phloem	1	irregular-shape	parallel	partly lignified cell
T. przewalskii	achene	absent	branching	8-10	close phloem	1	irregular-shape	parallel	partly lignified cell
T. simplex var. glandulosum	achene	papillary glandular hair	branching	10	close phloem	1	irregular-shape	parallel	partly lignified cell
T. sparsiflorum	achene	unobserved	branching	10-11	close phloem	1	irregular-shape	parallel	partly lignified cell
T. squarrosum	achene	absent	branching	13-15	close phloem	1	irregular-shape	parallel	partly lignified cell
T. tuberiferum	achene	absent	branching	8	close phloem	1	irregular-shape	parallel	partly lignified cell
Trollius chinensis	follicle	obovate glandular hair	branching	6-8	close phloem	1	rectangle	perpendicular	partly lignified cell
T. ledebourii	follicle	obovate glandular hair	branching	5-7	around vascular bundle	1	rectangle	perpendicular	partly lignified cell
T. macropetalus	follicle	obovate glandular hair	branching	5-8	around vascular bundle	1	rectangle	perpendicular	partly lignified cell
Urophysa henryi	follicle	non-glandular hair	branching	14-17	around vascular bundle	1	rectangle	perpendicular	partly lignified cell

* In longitudinal view, the long axis of the endocarp cells are perpendicular or parallel to the fruits.

** The endocarp cells close to the ventral suture and dorsal suture are parenchyma.

Table 3. The coding of characters and character states* for genera of Ranunculaceae [The characteristics 17-22 were quoted from Wang and Wang (1979) and Wang *et al*. (1980)].

Genera	Characters																					
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
Aconitum	0	1	0	0	0	1	0	1	0	1	0	1	0	0	0	0	0	1	0	0	0	0
Adonis	1	1	0	0	0	0	0	1	1	1	0	2	1	0	0	1	0	0	0	0	0	0
Anemone	1	1	0	0	0	2	1	1	0	1	0	2	1	0	0	1	0	0	0	1	0	2
Aquilegia	0	0	1	1	0	0	0	2	0	1	0	1	0	0	0	0	0	0	0	0	1	0
Batrachium	1	1	0	0	0	2	1	0	1	1	1	1	1	0	0	1	0	0	0	0	0	0
Beesia	0	1	0	0	0	0	0	2	0	1	0	1	1	0	0	0	0	0	0	1	0	2
Caltha	0	0	1	2	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0
Cimicifuga	0	1	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	0	0	1	1	0
Clematis	1	1	0	0	0	2	1	1	0	1	0	2	1	0	1	1	1	0	0	1	0	1
Consolida	0	1	1	1	0	1	0	0	0	1	0	1	0	1	0	0	0	1	0	0	0	0
Coptis	0	1	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	1	0	0	2
Delphinium	0	1	0	0	0	1	0	1	0	1	0	1	0	1	0	0	0	1	0	0	1	0
Dichocarpum	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Enemion	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	1	0	0
Eranthis	0	0	1	1	0	0	0	2	0	1	0	1	1	0	0	0	0	0	0	0	0	2
Halerpestes	1	0	0	0	0	0	0	0	0	2	0	2	1	0	0	0	0	0	0	0	0	2
Helleborus	0	0	0	0	0	0	0	2	0	2	0	1	1	0	0	0	0	0	0	0	0	0
Isopyrum	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0
Leptopyrum	0	0	1	3	1	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0
Paraquilegia	0	0	1	3	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0
Pulsatilla	1	1	0	0	0	2	1	1	0	1	0	2	1	0	1	1	0	0	0	1	1	2
Ranunculus	1	1	0	0	0	2	2	0	1	1	1	1	1	0	0	1	0	0	0	0	0	0
Semiaquilegia	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	0
Souliea	0	0	0	0	0	1	0	2	0	2	0	1	0	0	0	0	0	0	0	0	0	0
Thalictrum	1	1	1	3	1	0	0	1	0	2	0	2	0	0	0	0	0	0	0	1	0	0
Trollius	0	0	1	2	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0
Urophysa	0	1	0	0	0	0	0	2	0	1	0	1	0	0	0	0	0	0	0	0	1	2

*1. Fruit type: follicle(0); achene(1). 2. Non-glandular hair: absent(0); present(1). 3. Glandular hair: absent(0); present(1). 4. The shape of glandular hair: absent(0); bottle shape(1); obovate(2); papillary(3). 5. Lignified mesocarp: absent(0); present(1). 6. Pericarp vascular bundles: branching(0); branching and anastomosing(1); non-branching(2). 7. The number of vascular bundle: indefinite(0); two(1); above two(2). 8. The distribution of vascular bundle fiber: absent(0); near phloem(1); around vascular bundles(2). 9. Endocarp cell layer: monolayer(0); multilayer(1). 10. Endocarp cell shape: polygonal(0); rectangle or fusiform(1); irregular(2). 11. The long axis directions of endocarp cells: same(0); different(1). 12. The long axis direction of innermost endocarp cells: no long axis(0); vertical to the longitudinal axis of fruit(1); parallel to the longitudinal axis of fruit(2). 13. Lignified ways of endocarp cell: partly lignified(0); completely lignified(1). 14. Endocarp stoma: absent(0); present(1). 15. Endocarp cell type: lignified cell(0); the endocarp cells which near the ventral suture and dorsal suture are parenchyma, and the others are lignified(1). 16. Endocarp fiber: absent(0); present(1). 17. Plant type: herb(0); liana(1). 18. Flower symmetrical pattern: radial symmetry(0); bilateral symmetry(1). 19. Long carpophore: absent(0); present(1). 20. Petal: present(0); absent(1). 21. Staminodium: absent(0); present(1). 22. Leaves growth manner: alternation(0); opposition(1); all basal(2).

However, in some other classification systems they were placed in different subfamilies based on the external morphological characteristics of leaves, flowers and fruits (Wang & Wang, 1979, Wang *et al*., 1980, 2001; Tamura, 1993; Takhtajan, 2009). Many phylogenetic studies (Jensen *et al*., 1995; Ro *et al*., 1997; Wang *et al*., 2009) based on molecular sequence data have demonstrated that these genera belonged to three subfamilies, Coptidoideae (*Coptis*), Ranunculoideae (*Aconitum*, *Beesia*, *Caltha*, *Cimicifuga*, *Consolida*, *Coptis*, *Delphinium*, *Eranthis*, *Halerpestes*, *Helleborus*, *Souliea* and *Trollius*) and Thalictroideae (*Aquilegia*, *Dichocarpum*, *Enemion*, *Isopyrum*, *Leptopyrum*, *Paraquilegia*, *Semiaquilegia* and *Urophysa*). In this study we divided the genera into five sub-groups based on the trichome types, the endocarp cells shape and the distribution of fibers around vascular bundles at 0.77 similarity coefficient. Sub-group 1 and 2 are almost matches with the findings of molecular phylogenetics studies (Jensen *et al*., 1995; Wang *et al*., 2009).

Sub-group 1: Follicles. The endocarp cells are rectangle and anomocytic stomata were observed in the endocarp (the endocarp of *Aconitum* is not with stomata) (including *Aconitum*, *Consolida* and *Delphinium*). In the previous classification systems the three genera were placed in the tribe *Delphineae* (Wang & Wang, 1979; Tamura, 1993; Takhtajan, 2009). The results were supported by the molecular phylogeny (Jensen *et al*., 1995; Wang *et al*., 2009).

Sub-group 2: Follicles are with glandular hairs or hairless. The endocarp cells are rectangle (including *Aquilegia*, *Caltha*, *Enemion*, *Eranthis*, *Leptopyrum*, *Isopyrum*, *Paraquilegia*, *Semiaquilegia* and *Trollius*). Some previous researchers suggested that *Caltha* had no close relationship with *Trollius* (Song *et al*., 2007; Takhtajan, 2009; Wang *et al*., 2009; Cai *et al*., 2010). However, our study shows that both genera are similar in fruit structures, for example, obovate glandular hairs, branching vascular bundles, rectangular endocarp cells. The chromosome types and vessel elements are also similar in both genera (Chen & Li, 1991; Yang, 2002). Six genera of the sub-group 2 (*Aquilegia*, *Enemion*, *Leptopyrum*, *Isopyrum*, *Paraquilegia* and *Semiaquilegia*) were placed in the tribe *Isopyreae* in molecular systematics (Jensen *et al*., 1995; Wang *et al*., 2009). Fruit structures showed in our study provide the anatomical evidences for the molecular phylogeny.

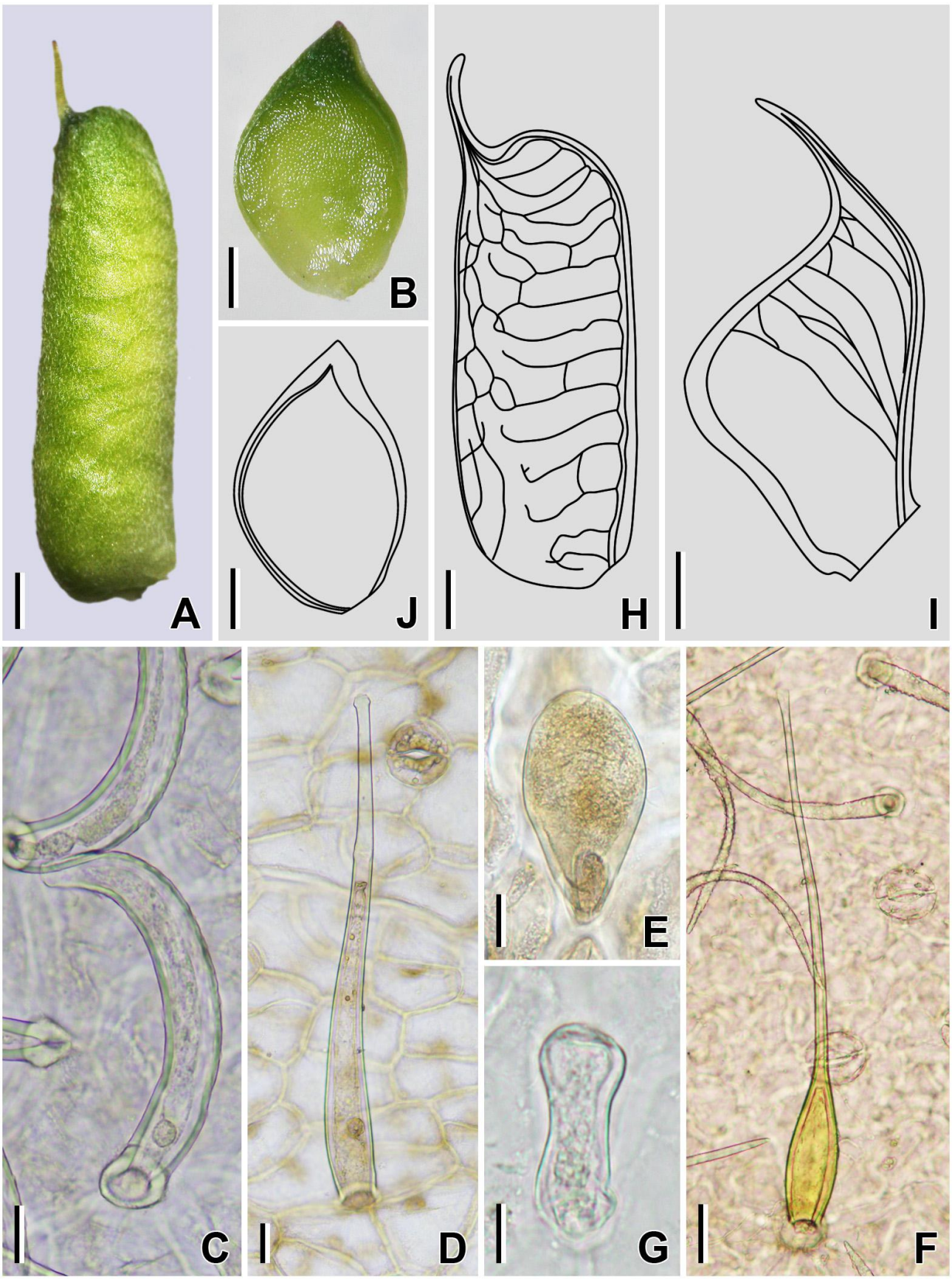

Fig. 1. Fruit morphology of Ranunculaceae showing their follicle (A), achene (B), non-glandular hair (C, D), glandular hair (E-G), branching and anastomosing vascular bundles (H), branching vascular bundles (I), non-branching vascular bundles (J). (A, C) *Aconitum coreanum*. (B, J) *Ranunculus chinensis*. (D) *Aconitum sczukinii*. (E) *Caltha palustris* var. *sibirica*. (F) *Consolida ajacis*. (G) *Leptopyrum fumarioides*. (H) *Aconitum carmichaeli* var. *truppelianum*. (I) *Trollius macropetalus*. Scale bars = 2 mm in A, H, I; 1 mm in B, J; 20 μm in C-E; 40 μm in F; 10 μm in G.

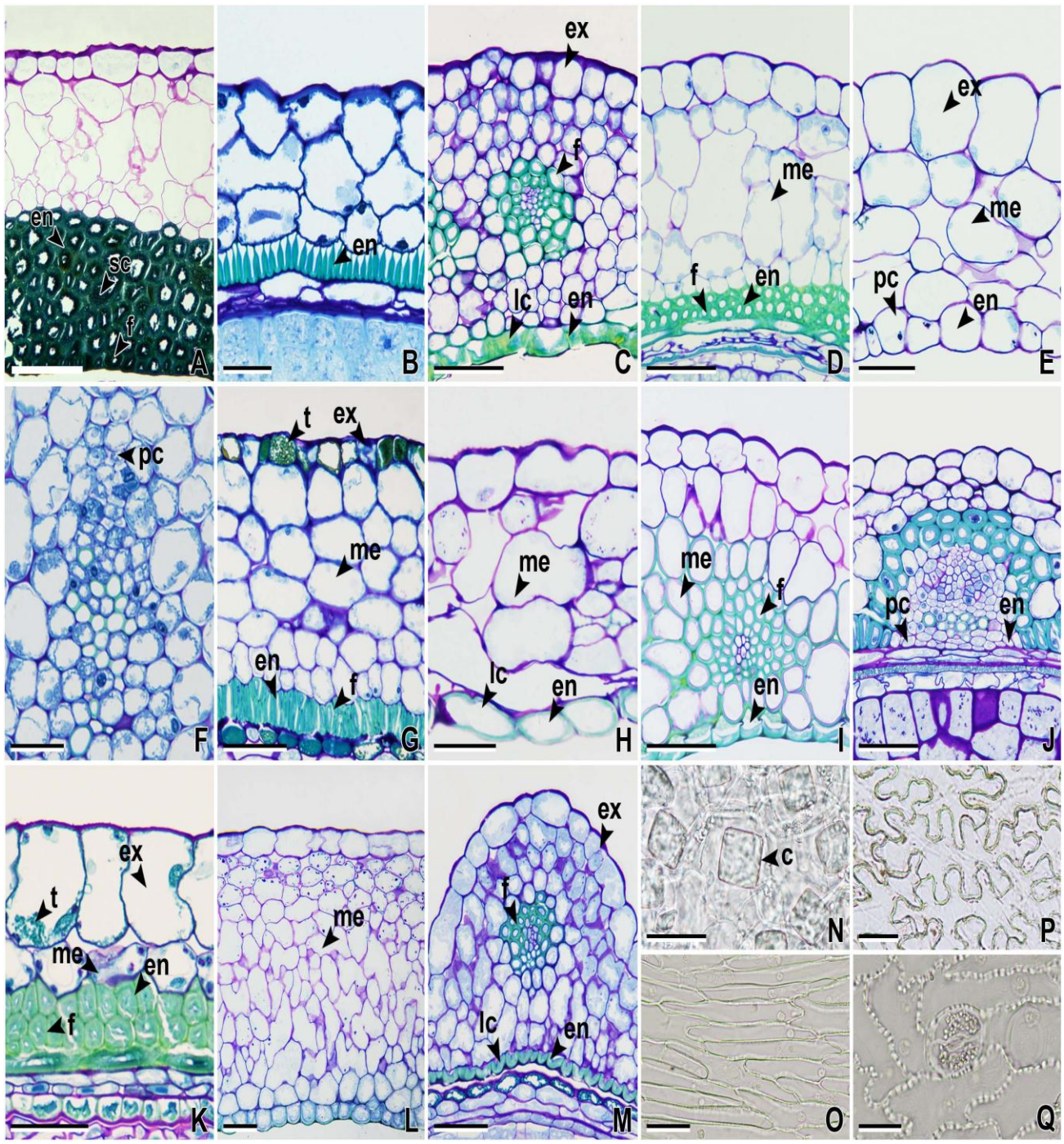

Fig. 2. Fruit structures of Ranunculaceae showing pericarp structures (A-M), crystals in mesocarp (N), endocarp cells (O, P), endocarp stoma (Q). (A) *Adonis amurensis*. (B) *Anemone raddeana*. (C) *Aquilegia oxysepala*. (D) *Batrachium foeniculaceum*. (E) *Caltha natans*. (F) *C. palustris* var. *sibirica*. (G) *Clematis patens*. (H) *Eranthis stellata*. (I) *Leptopyrum fumarioides*. (J) *Pulsatilla cernua*. (K, N) *Ranunculus japonicus*. (L) *Thalictrum baicalense*. (M) *T. simplex* var. *glandulosum*. (O, Q) *Delphinium maackianum*. (P) *Helleborus thibetanus*; c = crystal, en = endocarp, ex = exocarp, f = fiber, lc = lignified cell, me = mesocarp, pc = parenchyma cell, sc = stone cell, t = tannin. Scale bars = 100 µm in A, C, G, L; 50 µm in B-F, H-K, M-Q.

Sub-group 3 and 4. Sub-group 3: Follicles are with non-glandular hairs. The mesocarp is composed of parenchymatous cells and the endocarp cells are rectangle (including *Beesia*, *Cimicifuga*, *Coptis* and *Urophysa*). Sub-group 4. Follicles are smooth. The vascular bundles are surrounded by fibers. The endocarp cells are irregular or polygonal (including *Dichocarpum*, *Helleborus* and *Souliea*). The both sub-groups were not recognized in the classification systems (Wang & Wang, 1979; Tamura, 1993; Takhtajan, 2009; Wang *et al*., 2009), for example, Wang & Wang (1979) divided these genera in four tribes, Cimicifugeae (*Beesia*, *Cimicifuga* and *Souliea*), Coptideae (*Coptis*), Helleboreae (*Helleborus*) and Isopyreae (*Dichocarpum*, *Urophysa*) according to the types of chromosome and leaves. However, the internal structure characteristics of fruit were not considered in the previous studies (Wang & Wang, 1979; Tamura, 1993; Takhtajan, 2009; Wang *et al*., 2009).

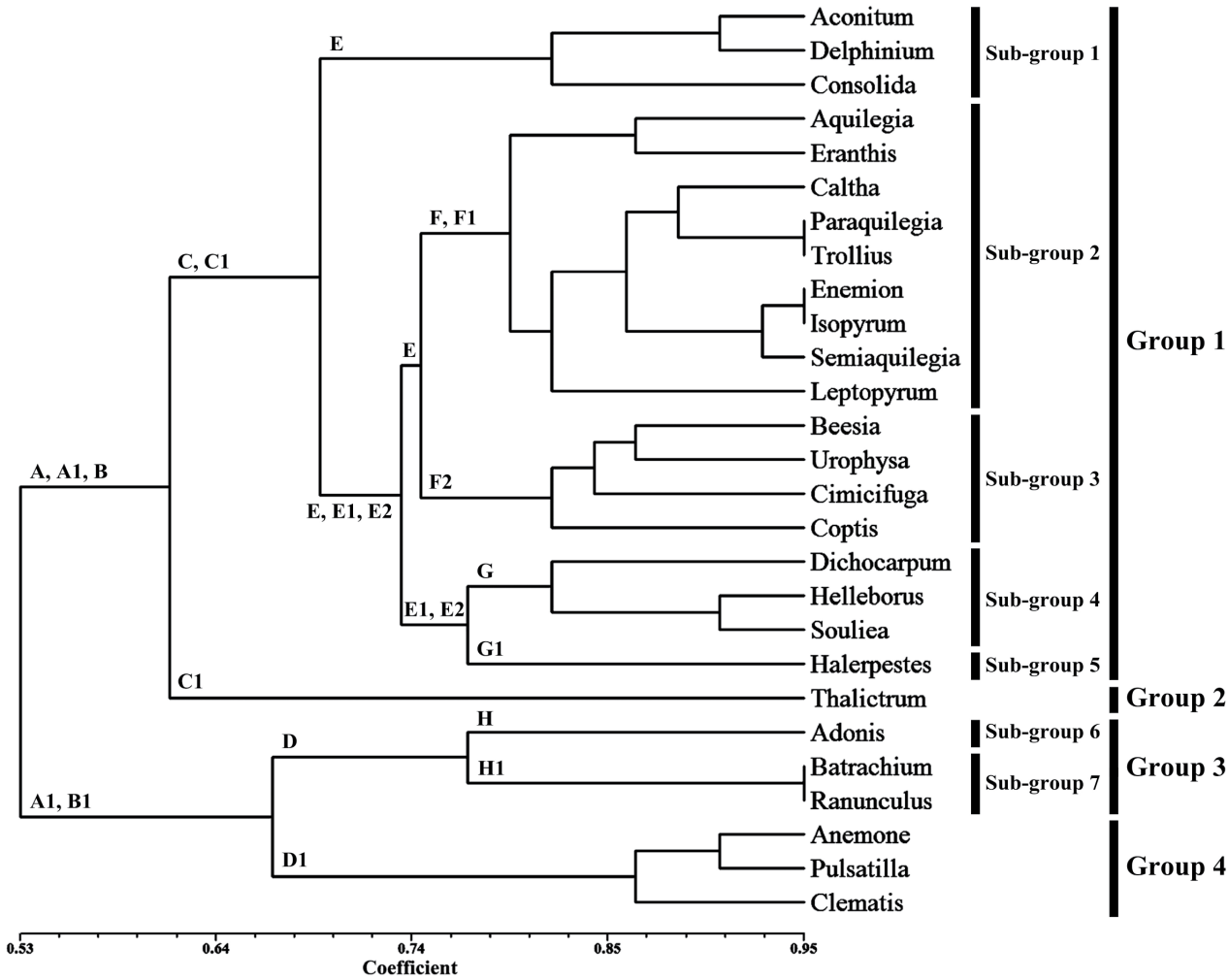

Fig. 3. UPGMA dendrogram based on fruit characteristics showing the relationships of the 27 genera in Ranunculaceae: (A) Follicle. (A1) Achene. (B) Lignified endocarp. (B1) Endocarp fibers. (C) Non-lignified mesocarp. (C1) Lignified mesocarp. (D) Endocarp consists of multilayer cells. (D1) Endocarp consists of one layer cells. (E) Endocarp cells are rectangle. (E1) Endocarp cells are irregular shape. (E2) Endocarp cells are polygon. (F) Hairless. (F1) Glandular hairs. (F2) Non-glandular hairs. (G) Vascular bundles surrounded by fibers. (G1) No fiber nearby the vascular bundles. (H) Branching vascular bundles. (H1) Non-branching vascular bundles.

Sub-group 5: Achenes are with branching vascular bundles. There is no fiber in the mesocarp close to the vascular bundles. The endocarp cells are irregular in shape (*Halerpestes* only). Zhao (2008) moved *Halerpestes* into *Ranunculus sensu lato*, but some studies (Wang *et al*., 1980, Tamura 1993, Takhtajan 2009, Wang *et al*., 2009) showed *Halerpestes*, *Batrachium*, *Ranunculus*, and some related genera should be placed in tribe Ranunculeae. Wang *et al*. (1980) also described the fruit type and the flower characteristics of *Halerpestes* which were similar to *Ranunculus* and *Batrachium*. However, in our study the endocarp thickness and cell types of *Halerpestes* differ from the other two genera. The former has branching vascular bundles, endocarp consists of one layer of irregularly lignified cells but the later two genera have non-branching vascular bundles and endocarp consists of multilayer fibers. More morphological work is necessary to determine if the three genera have close relationships.

Group 2: Achenes. The mesocarp is lignified with branching vascular bundles. The endocarp cells are irregular and partly lignified (*Thalictrum* only). Tamura (1993) suggested *Thalictrum* as a subfamily according to the genus has no petal, and has pendulous ovule and achenes with distinct longitudinal veins. Our research showed that the fruits of the genus share some characteristics with follicles, for example, branching vascular bundles and lignified endocarp, and these characteristics were not found in other taxa of Ranunculaceae studied, and therefore it is reasonable to suggest the *Thalictrum* should be placed under separate subfamily.

Group 3: Achene. The vascular bundles are branching or not. The endocarp consists of multilayered sclerenchyma (including *Adonis*, *Batrachium* and *Ranunculus*). In the molecular phylogenetic studies the three genera were placed in two tribes of the subfamily Ranunculoideae, the tribes Adonideae including *Adonis* and Ranunculeae including *Batrachium* and *Ranunculus* (Jensen *et al*., 1995; Wang *et al*., 2009). The study showed there are some differences among the three genera in the vascular bundle and endocarp cell structures. This group is divided into two sub-groups at 0.77 similarity coefficient.

Sub-group 6: The vascular bundles are branching. The endocarp consists of (multilayer) stone cells and (monolayer) fibers. In longitudinal view, the long axis of the innermost endocarp cells is parallel to fruits (*Adonis* only). The stone cells occurred in the *Adonis* were not found in any other taxa studied. The close relationship between *Trollius* and *Adonis* was also indicated by molecular phylogenetic studies (Ro *et al*., 1997; Ren *et al*., 2009; Wang *et al*., 2009; Cai *et al*., 2010). The two genera were suggested to be placed in different subfamilies (Tamura 1993, Wang & Wang 1979, Wang *et al*., 1980). Our study showed *Adonis* is different from *Trollius*. The latter has follicle with glandular hairs and the endocarp consists of single layer lignified cells. More morphological and molecular researches are necessary to confirm the relationship between the two genera.

Sub-group 7: The vascular bundles are not branching. The endocarp consists of multilayer fibers. In longitudinal view, the long axis of the innermost layer cells of endocarp is usually perpendicular to fruits (including *Ranunculus* and *Batrachium*). *Batrachium* was usually placed in *Ranunculus* (Gray, 1821; Tamura, 1993; Emadzade, 2010). Wang *et al*. (1980, 2001) demonstrated that *Batrachium* and *Ranunculus* differed in flower colors, leaf shapes and habitats. However, that the fruit structures of both genera are very similar, thus the present study supports the previous taxonomical treatments.

Group 4: Achenes are with two non-branching vascular bundles. The endocarp consists of one layer of fibers (including *Anemone*, *Clematis* and *Pulsatilla*). Tamura (1993) and Takhtajan (2009) pointed the three genera should be placed in one subfamily (Ranunculoideae) based on the fruit characters (e.g. achene has no obvious longitudinal veins), and molecular phylogenetics provide the evidence for the result (Johansson, 1995; Jensen *et al*., 1995; Ro *et al*., 1997; Cai *et al*., 2010). This group was considered to have a close relationship with Group 3, and both were placed in Ranunculoideae (Tamura, 1993; Wang *et al*., 2009). Hoot *et al*. (2012) pointed that *Pulsatilla* and *Anemone* have a close relationship with *Clematis*.

Conclusions

Fruits are usually considered to have stable and higher taxonomic values (Liu *et al*., 2002). In the previous classification systems (Wang & Wang, 1979, Wang *et al*., 1980, 2001; Johansson *et al*., 1993, Johansson, 1995; Jensen *et al*., 1995; Tamura, 1993; Tatlidil *et al*., 2005; Perveen & Qaiser, 2006; Takhtajan, 2009; Wang *et al*., 2009), the fruit types and the external morphology, the characteristics of pollen grain were emphasized, but the fruit anatomy are usually overlooked. Therefore, some relationships between the taxa provided by the molecular researches had no anatomical evidence support. Our study showed that the endocarp structure characters differed in the two tribes Anemoneae and Ranunculeae, and both were having achenes. Fruit anatomical characters also support the placement of the taxa showed by DNA phylogeny of Ranunculaceae (Johansson *et al*., 1993, Johansson, 1995; Jensen *et al*., 1995; Wang *et al*., 2009). The present study also reinforces the notion that fruit morphology and anatomy, when examined in detail, can provide valuable additional insight into relationships within many groups of the family.

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References

Anonymous. 2009. Angiosperm Phylogeny Group III (APG III). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants. *Bot. J. Linn. Soc.*, 161(2): 105-121.

Cai, Y.F., S.W. Li, M. Chen, M.F. Jiang, Y. Liu, Y.F. Xie, Q. Sun, H.Z. Jiang, N.W. Yin, L. Wang, R. Zhang, C.L. Huang and K.R. Lei. 2010. Molecular phylogeny of Ranunculaceae based on *rbc*L sequences. *Biologia*, 65(6): 997-1003.

Chen, Y.Z. and Z.L. Li. 1991. Comparative anatomical studies on vessel elements of Chinese Ranunculaceae. *Acta Bot. Sin.*, 33(6): 459-464.

Emadzade, K., C. Lehnebach, P. Lockhart and E. Hörandl. 2010. A molecular phylogeny, morphology and classification of genera of Ranunculeae (Ranunculaceae). *Taxon*, 59(3): 809-828.

Feder, N. and T.P. O'Brien. 1968. Plant microtechnique: some principles and new methods. *Amer. J. Bot.*, 55(1): 123-142.

Gray, S.F. 1821. *A natural arrangement of British plants, vol. 2*. Academic Press, London.

Hao, D.C., X.J. Gu, P.G. Xiao, L.J. Xu and Y. Peng. 2013. Recent advances in the chemical and biological studies of *Aconitum* pharmaceutical resources. *J. Chin. Pharm. Sci.*, 22(3): 209-221.

Hazrat, A., J. Shah, M. Ali and I. Iqbal. 2007. Medicinal value of Ranunculaceae of Dir valley. *Pak. J. Bot.*, 39(4): 1037-1044.

Hazrat, A., M. Nisar and S. Zaman. 2013. Antibacterial activities of sixteen species of medicinal plants reported from Dir Kohistan valley KPK, Pakistan. *Pak. J. Bot.*, 45(4): 1369-1374.

Hoot, S.B., K.M. Meyer and J.C. Manning. 2012. Phylogeny and reclassification of Anemone (Ranunculaceae), with an emphasis on austral species. *Syst. Bot.*, 37(1): 139-152.

Hutchinson, J. 1923. Contributions towards a phylogenetic classification of flowering plants. I. *Bull. Misc. Inform. Roy. Bot. Gard. Kew*, 1923(2): 65-89.

Jensen, U., S.B. Hoot, J.T. Johansson and K. Kosuge. 1995. Systematics and phylogeny of the Ranunculaceae - a revised family concept on the basis of molecular data. *Plant Syst. Evol.*, Suppl. 9: 273-280.

Johansson, J.T. 1995. A revised chloroplast DNA phylogeny of the Ranunculaceae. *Plant Syst. Evol.*, Suppl. 9: 253-261.

Johansson, J.T. and R.K. Jansen. 1993. Chloroplast DNA variation and phylogeny of the Ranunculaceae. *Plant Syst. Evol.*, 187(1-4): 29-49.

Liu, M., B.E. Wyk and P.M. Tilney. 2002. The taxonomic value of fruit structure in the Chinese endemic genus Dickinsia (Apiaceae). *Nord. J. Bot.*, 22(5): 603-607.

Perveen, A. and M. Qaiser. 2006. Pollen flora of Pakistan-L. Ranunculaceae. *Pak. J. Bot.*, 38(3): 499-509.

Ren, Y., H.L. Chang, X.H. Tian, P. Song and P.K. Endress. 2009. Floral development in Adonideae (Ranunculaceae). *Flora*, 204(7): 506-517.

Ro, K.E., C.S. Keener and B.A. McPheron. 1997. Molecular phylogenetic study of the Ranunculaceae: utility of the nuclear 26s ribosomal DNA in inferring intrafamilial relationships. *Mol. Phylogenet. Evol.*, 8(2): 117-127.

Rohlf, F.J. 2000. *NTSYS-pc, numerical taxonomy and multivariate analysis system, version 2.10e*. Applied Biostatistics Inc, New York.

Song, P., X.H. Tian and Y. Ren. 2007. Floral morphogenesis of *Caltha* and *Trollius* (Ranunculaceae) and its systematic significance. *Acta Phytotax. Sin.*, 45(6): 769-782.

Takhtajan, A. 2009. *Flowering Plants*. (2nd Ed) Springer-Verlag New York Inc., New York.

Tamura, M. 1993. Ranunculaceae. In: *The Families and Genera of Vascular Plants, vol. 2*. (Eds.): Kubitzki, K., J.G. Rohwer and V. Bittrich. Springer-Verlag, Berlin, pp. 563-583.

Tang, X.H., X.H. Tian, Y. Ren and X.H. Zhang. 2008. Anatomic study of the Carpels in *Trollius* and *Adonis*. *Anhui Agri. Sci. Bull.*, 14(9): 44, 92.

Tatlidil, S., A. Bicakci, H. Malyer and K.H.C. Baser. 2005. Pollen morphology of *Thalictrum* L., species (Ranunculaceae) in Turkey. *Pak. J. Bot*., 37(2): 203-212.

Wang, C., Y.G. Wang, Q.D. Liang, W.Q. Rang and Y. Gao. 2010. Analysis of chemical composition in the combination of monkshood and pinellia by UPLC/Q-TOFMS with multivariate statistical analysis. *Acta Pharm. Sin.*, 45(10): 1301-1306.

Wang, W., A.M. Lu, Y. Ren, M.E. Endress and Z.D. Chen. 2009. Phylogeny and classification of Ranunculales: Evidence from four molecular loci and morphological data. *Perspect. Pl. Ecol. Evol. Syst.*, 11(2): 81-110.

Wang, W.T. and S.H. Wang. 1979. Ranunculaceae. In: Delectis Florae Reipublicae Popularis Sinicae Agendae Academiae Sinicae Edita (Eds.), *Flora Reipublicae Popularis Sinicae, vol. 27*. Science Press, Beijing, pp. 59-601.

Wang, W.T., D.Z. Fu, L.Q. Li, B. Bartholomew, A.R. Brach, B.E. Dutton, M.G. Gilbert, Y. Kadota, O.R. Robinson, M. Tamura, M.J. Warnock, G.H. Zhu and S.N. Ziman. 2001. Ranunculaceae. *In:* Wu, Z.Y. and P.H. Raven (Eds.), *Flora of China*. Science Press, Beijing, Missouri Botanical Garden Press, St. Louis, pp. 133-438.

Wang, W.T., L. Liou, S.H. Wang, M.C. Chang, C.T. Ting, P.P. Ling and M.Y. Fang. 1980. Ranunculaceae (2). In: *Flora Reipublicae Popularis Sinicae, vol. 28*. (Ed.): Wang, W.T. Science Press, Beijing, pp. 1-345.

Wen, R.Q., D.H. Li, X. Zhao, J.B. Wang, Y.L. Zhao, P. Zhang, Z.Y. Sun, D. Yan, X.H. Xiao, Y.Z. Ren, F. Li, J. Du and H.Y. Zhou. 2013. Rationality of the processing methods of Aconiti Lateralis Radix (Fuzi) based on chemical analysis. *Acta Pharm. Sin.*, 48(2): 286-290.

Wu, R. 2011. *Study on Invitro Culture of Ornamental Clematis*. Southwest Forestry University, Kunming.

Yang, Q.E. 2002. Cytology of the tribe Trollieae and of the tribe Cimicifugeae in the Ranunculaceae: a comparative study. *Acta Phytotax. Sin.*, 40(1): 52-65.

Zhang, C.J., C.M. Liu, T. Yun and H.Y. Li. 2009. Chemical composition and antimicrobial activities of the essential oil of *Aconitum tanguticum*. *J. Chin. Pharm. Sci.*, 18(3): 240-244.

Zhao, L. 2008. *Systematics study of Ranunculeae (Ranunculaceae)*. Shannxi Normal University, Xian.

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