Journal of Plant Biology, March 2005, 48(1) : 128-135

Reproductive Morphology of *Megaleranthis saniculifolia* Ohwi (Ranunculaceae) and Its Systematic Implications

Min Young Jang[1] and Kweon Heo[2]*
[1]The Gangwon Area Headquarter of the National Agricultural Cooperative Federation, Chuncheon 200-043, Korea
[2]Division of Applied Plant Sciences, Kangwon National University, Chuncheon 200-701, Korea

To confirm the taxonomic treatment of *Megaleranthis saniculifolia* Ohwi, an endemic genus and species in Korea, we compared its reproductive morphological characteristics with those of *Trollius* and other genera within the Ranunculaceae. Although its external morphology might suggest that *Megaleranthis* differs from *Trollius, Calathodes*, and etc., we found no distinctly different features in this genus. Likewise, previous studies of their pollen structures, chromosome data, and petal morphology have indicated no differences between *Megaleranthis* and *Trollius*. In fact, related genera share similar characteristics, such as a tetrasporangia anther, glandular tapetum, simultaneous cytokinesis, an anatropous and bitegmic ovule, embryo sac formation of the *Polygonum* type, exarillate and copious albuminous seed, and several apocarps. Although the unique feature of having both tenuinucellate and crassinucellate ovules simultaneously may initially seem particular to *Megaleranthis*, it is present in other genera of the same family. Therefore, based on this evidence of reproductive morphology and other information, we suggest that *M. saniculifolia* is closely related to *Trollius*, and should be included within that genus, i.e., as *T. chosenensis* Ohwi. Nevertheless, we have tentatively placed *Megaleranthis* within its own monotypic and endemic genus until definitive data become available.

Keywords: embryology, *Megaleranthis*, morphology, Ranunculaceae, systematics, *Trollius*

Megaleranthis saniculifolia Ohwi is a monotypic and endemic species in Korea (Lee, 1969; Lee, 1980). It is well characterized as an herbaceous plant with well-distributed vessels, triaperturate pollen, and apocarpous flowers (Ohwi, 1935). First collected by Ohwi at Unbon-Mudemi, Mt. Jiri, it was described as a new genus and new species (Ohwi, 1935). However, two years later its scientific name was changed to *Trollius chosenensis* by Ohwi (Ohwi, 1937). Morphologically, this plant is similar to *Trollius* or *Eranthis*, but is distinguished by several characteristics. Whereas *Trollius* has several cauline leaves and yellow or purplish sepals, *Megaleranthis* possesses an involucre under its single flower, as well as white sepals (Tamura, 1995). Furthermore, *Eranthis* has spherical, tuberous roots, while those of *Megaleranthis* are fibrous. Palynological data have revealed that the pollen structure of *M. saniculifolia* is very similar to *Trollius* (Kim and Lee, 1987). Lee (1990) has asserted that *Megaleranthis* be included in the genus *Trollius*, as was first indicated by Ohwi (1937).

Because of those previous reports, the taxonomic treatment of *Megaleranthis* continues to be controversial among researchers. Here, we present a full description of the reproductive features from *M. saniculifolia*. We also discuss whether this species should be treated separately from *Trollius* or else included in that genus based on its various, heretofore unexamined, reproductive characteristics. Such data have often provided good evidence for otherwise unknown relationships at various taxonomic levels (Tobe, 1989).

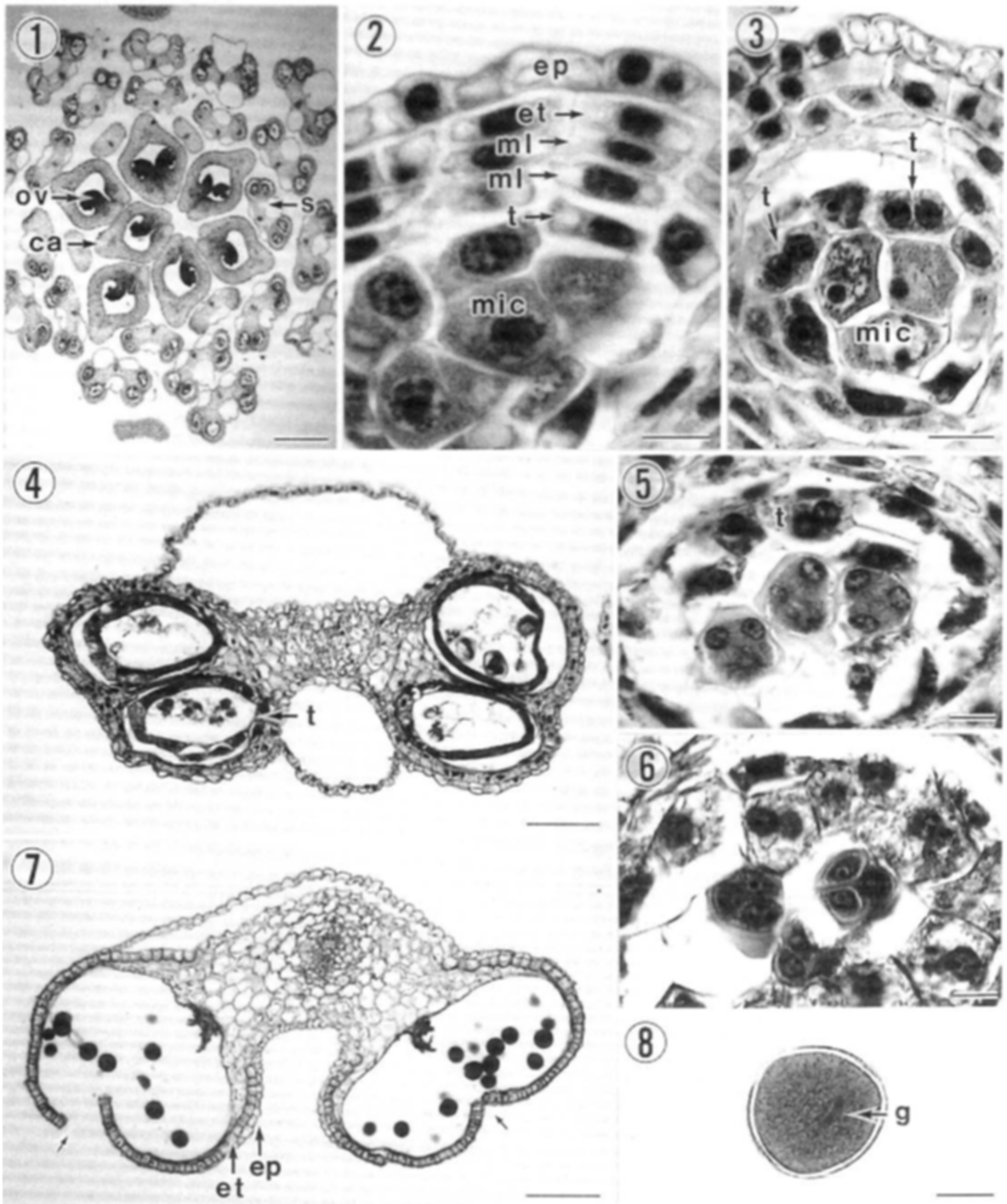
MATERIALS AND METHODS

Buds, flowers, and fruits of *M. saniculifolia* were gathered by the authors at Mt. Taegi, Gangwon Province, Korea, from 1999 to 2002. Collection data and representative samples of the developmental stages used in this study are vouched in Kangwon National University Herbarium (KWNU).

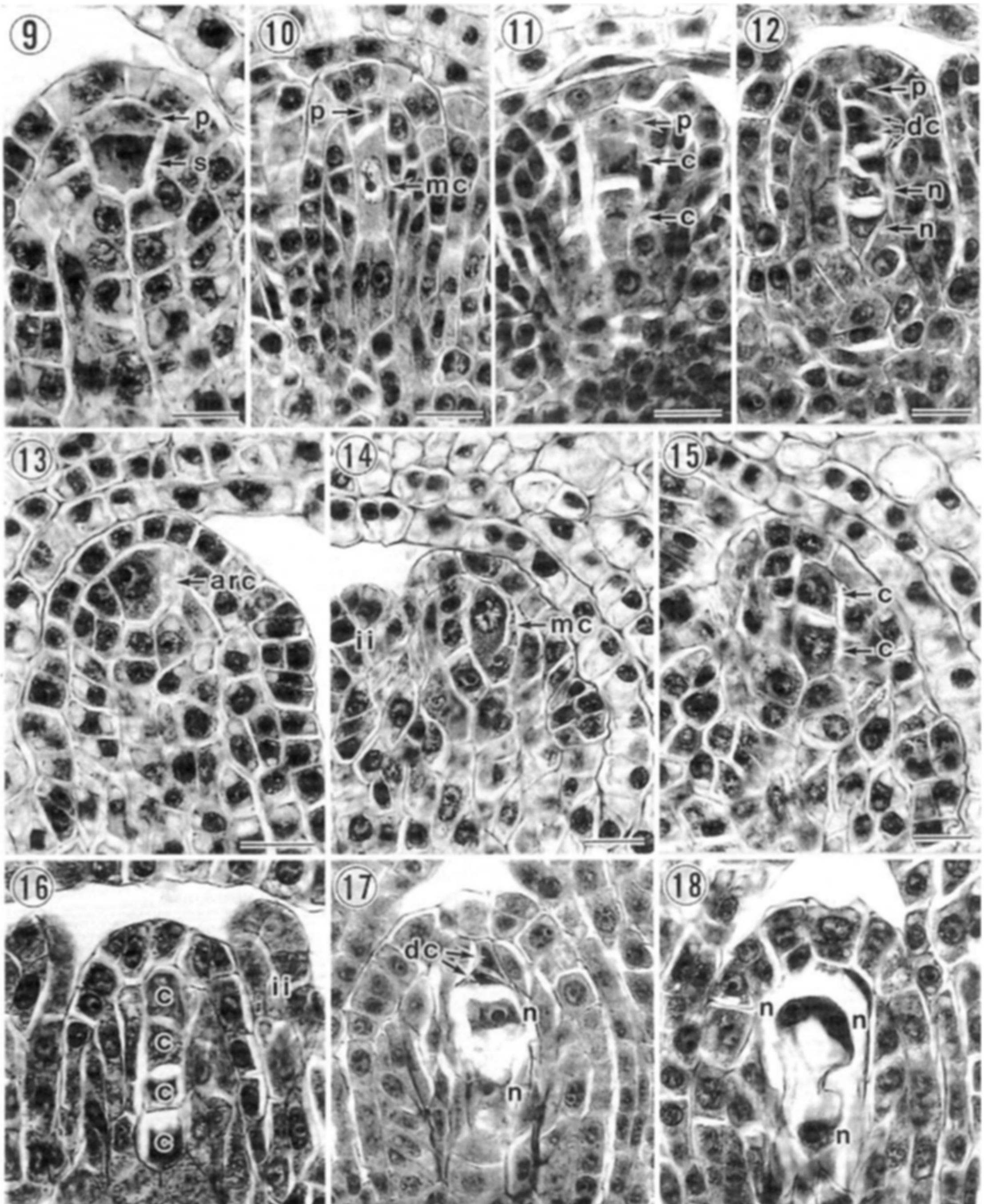
All plant materials were fixed in F.A.A. (5 : 5 : 90, formalin : acetic acid : 50% ethanol), and dehydrated through a t-butyl alcohol series. The samples were then embedded in Paraplast (melting point of 56 to 58°C). Structures of the anther, ovule, seed, and seed coat were observed from 6 to 8 μm thick embedded sections cut with a rotary microtome. Permanent slides were made and stained with Heidenhain's

*Corresponding author; fax +82-33-244-6410
e-mail laurus@kangwon.ac.kr

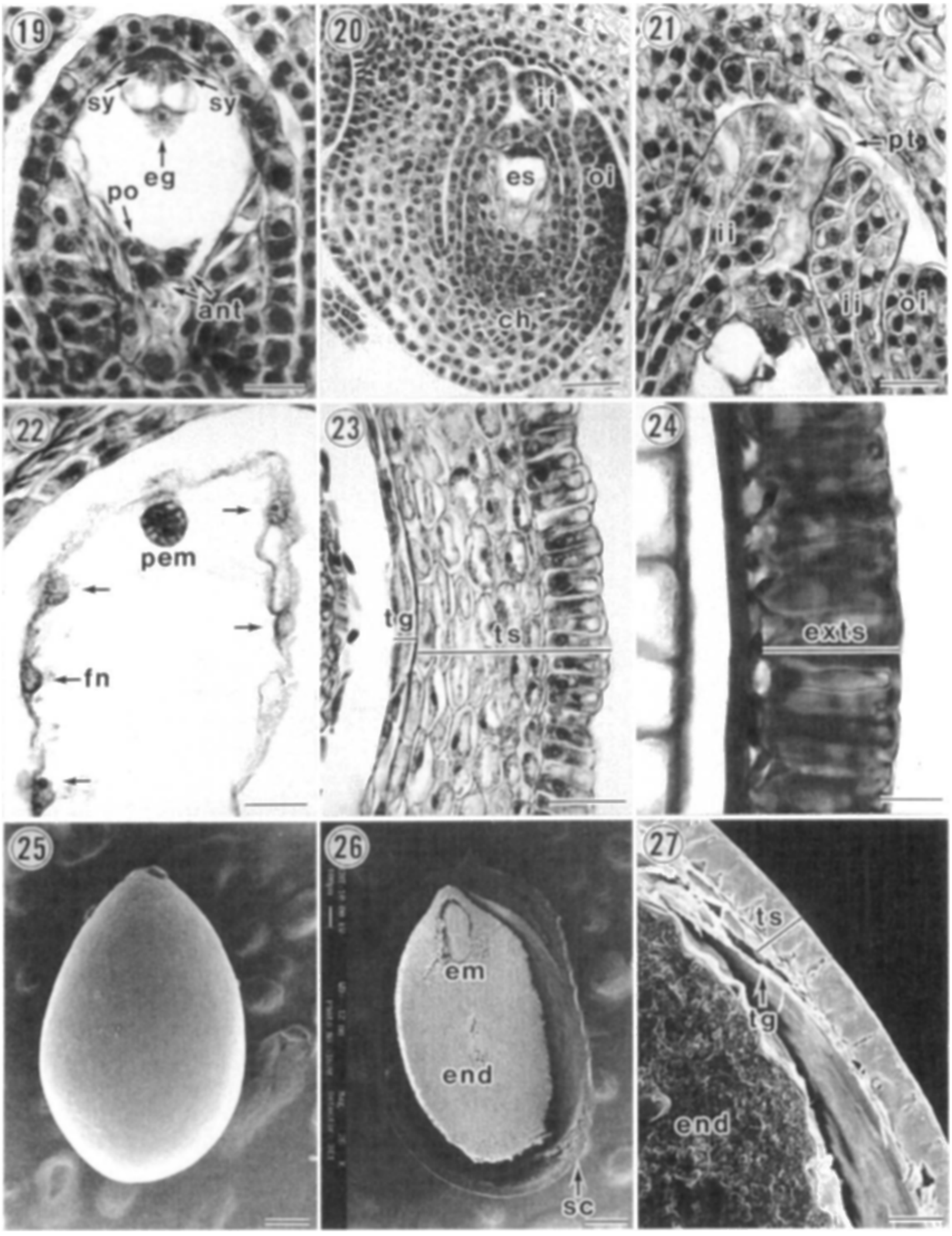
Hematoxylin, Safranin O, and Fast Green FCF. They were then mounted with Entellan. Ovules, pollen grains, and seeds were examined under a BX 50 microscope (Olympus, Japan). A scanning electron microscope (SEM) was also used to observe the entire shape and surface of the seed and seed coat. To do so, seeds were dehydrated through an acetone series and dried to the critical point dryer. After being coated with gold, they were observed with an S3500 SEM (Hitachi, Japan). Terminology to describe the



Figures 1-8. Development of microspores and anthers in *Megaleranthis*. **1.** Transverse section (TS) of flower showing several carpels and anthers. **2.** Young anther showing wall formation. **3.** Two nuclei tapetal cells. **4.** Tetrasporangia anther with glandular tapetum. **5.** Simultaneous cytokinesis in meiosis. **6.** Tetrahedral microspores. **7.** Longitudinal slit dehiscing anther. **8.** Two-celled mature pollen grain with generative cell stained by acetocarmine. ca, carpel; ep, anther epidermis; et, endothecium; g, generative cell; mic, microspore mother cell; ml, middle layer; ov, ovule; s, stamen; t, tapetum. Scale bars equal to 1 mm in **1**; 10 μm in **2**, **3**, **5**, **6**, and **8**; and 200 μm in **4** and **7**.



Figures 9-18. Development of nucellus and embryo sac in *Megaleranthis*. **9.** Longitudinal section (LS) of young ovule with periclinally divided archesporium cell. **10.** Megaspore mother cell with parietal cell, i.e., crassinucellate ovule. **11.** LS of ovule with dyad of megaspores. **12.** Crassinucellate ovule with linear tetrad of megaspores; chalazal functioning megaspore is at two-nucleate stage. **13.** LS of young ovule with one archesporium cell. **14.** Megaspore mother cell without parietal cells, i.e., tenuinucellate ovule. **15.** Tenuinucellate ovule with dyad of megaspores. **16.** Tenuinucellate ovule with linear tetrad of megaspores. **17.** Chalazal megaspore is functioning and two-nucleate. **18.** Four-nucleate embryo sac, one-nucleate is seen at next section. arc, archesporium cell; c, cells of megaspore; dc, degenerated cell; ii, inner integument; mc, megaspore mother cell; n, nucleus of embryo sac; p, parietal cell; s, sporogeneous cell. Scale bars equal to 10 μm.



Figures 19-27. Development of ovules and seeds in *Megaleranthis*. **19.** Longitudinal section (LS) of mature embryo sac. **20.** Nearly mature anatropous ovule showing micropyle formed by inner integument alone. **21.** LS of fertilized ovule showing path of pollen tube. **22.** LS of young seed showing globular proembryo and nuclear endosperm. **23.** Transverse section (TS) of young seed coat. **24.** TS of mature seed coat. **25.** Scanning electron microscopy (SEM) photograph of mature seed. **26.** SEM photograph of seed LS showing small embryo and copious endosperm. **27.** SEM photograph of LS of mature seed coat. ant, antipodal cells; ch, chalaza; eg, egg cell; em, embryo; end, endosperm; es, embryo sac; exts, exotesta; fn, free nucleus; ii, inner integument; oi, outer integument; pem, proembryo; po, polar nuclei; pt, pollen tube; sc, seed coat; sy, synergid; tg, tegmen; ts, testa. Scale bars equal to 10 μm in **19**, **21** and **22**; 20 μm in **23** and **24**; 50 μm in **20** and **27**; and 200 μm in **25** and **26**.

seed and seed coat morphology followed that proposed by Corner (1976) and Schmid (1986).

RESULTS

Anthers and Microspores

Anthers from *M. saniculifolia* are tetrasporangiate (Fig. 4); their mature walls comprise five cell layers, i.e., an epidermis, an endothecium, two middle layers, and a tapetum (Fig. 2). This confirms the anther wall formation to be of the basic type (see Davis, 1966; p.10). During maturation, the epidermal cells enlarge while those of the middle layers degenerate (Fig. 3). The tapetum is glandular (Fig. 4), and its cells are 2-nucleate (Fig. 3 and 5). At maturity, the epidermis is nearly persistent and the endothecium develops fibrous thickenings (Fig. 7). Meiosis in the microspore mother cell is accompanied by simultaneous cytokinesis (Fig. 5); the resultant microspore tetrads are tetrahedral (Fig. 6). Anther dehiscence occurs by longitudinal slits, with each slit common to two microsporangia of a theca (Fig. 7). Pollen grains are two-celled when they are shed (Fig. 8).

Ovule, Nucellus, and Megagametophyte

M. saniculifolia flowers have several apocarpous carpels (Fig. 1), with each having six to eight ovules arranged in two rows along the ventral suture, i.e., a marginal placentation (Fig. 1). The ovules are anatropous (Fig. 1 to 20), and either crassinucellate (Fig. 9 to 12) or tenuinucellate (Fig. 13 to 18). The single archesporium differentiates beneath the apical epidermal layer of the nucellus (Fig. 13). In crassinucellate ovules, the archesporial cell cuts off a primary parietal cell and a sporogenous cell (Fig. 9). Usually, the primary parietal cell undergoes further periclinal divisions to form two or three parietal layers. The sporogenous cell functions as the megaspore mother cell (Fig. 10). In succession, that megaspore mother cell then undergoes meiosis to produce a dyad (Fig. 11) and a linear tetrad of megaspores (Fig. 12).

In tenuinucellate ovules, the hypodermal archesporial cell develops directly into a megaspore mother cell (Fig. 14), which later divides and forms a dyad (Fig. 15) and linear tetrad (Fig. 16). The megaspore on the chalazal side functions in the megaspore tetrad (Fig. 12 and 16). A functional megaspore then develops into a two- (Fig. 12 and 17), four- (Fig. 18), and eight-nucleate organized embryo sac (Fig. 19). Therefore, this mode of embryo sac formation is of the *Polygonum* type. The organized embryo sac is ellipsoid in shape and positioned at the upper region of the nucellus (Fig. 19 and 20). Its three antipodal cells are persistent until the time of fertilization, but degenerate soon thereafter. During megasporogenesis and megagametogenesis, the apical epidermal cells of the nucellus are divided periclinally in two layers to form a nucellar cap (Fig. 18). Neither a hypostase nor an obturator is formed during ovule development.

Integuments

The ovule is bitegmic, having an inner and an outer integument (Fig. 20). From its beginning, the inner integument is always two to three cells thick, while the outer integument is initially two to three cells thick, but later becomes five to nine cells thick. The inner integument forms the micropyle alone (Fig. 20 and 21). No endothelium is formed.

Fertilization, Endosperm, and Embryo

Fertilization is porogamous (Fig. 21), and endosperm formation is of the nuclear type (Fig. 22). In mature seed, the embryo is small, with two cotyledons, and is enclosed by copious endosperm (Fig. 26).

Seed and Seed Coat

Each follicle contains many seeds, which are dispersed upon dehiscence of that structure. The mature seeds are broadly ellipsoid without any appendages, such as an aril or a wing (Fig. 25). They are also dark and small, i.e., 2 mm long and 1 mm wide (Fig. 25 and 26). Young seed coats are formed by two to three cell-layered tegmen and seven to eight cell-layered testa (Fig. 23). As the seed develops, all cells of the tegmen are crushed. The mature seed coat comprises a palisadal exotesta, a crushed few cell-layered mesotesta, and the endotesta (Fig. 24 and 27). Exotesta cells are tanniniferous, and radially elongate to form palisadal tissue. Therefore, this type of seed coat is considered exotestal.

DISCUSSION

Summary of the Reproductive Morphology of *M. saniculifolia*

The reproductive morphological features of *M. sani-*

Table 1. Comparisons of *Megaleranthis* with *Trollius* and other Helleboreae in reproductive morphology.

Characters	*Megaleranthis*	*Trollius*[1)]	Other Helleboreae[2)]
Anthers and microspores			
No. of sporangia	4	4	4
Mode of wall formation	Basic	NA	NA
Anther epidermis	Persistent	NA	Persistent
Thickness of anther wall	5 Cell-layers	NA	5 or 6 Cell-layers
Endothecium	Fibrous	NA	Fibrous
Middle layers	Crushed	NA	Crushed
Tapetum	Glandular	NA	Glandular
No. of nuclei in tapetal cell	2	NA	2 or 4
Cytokinesis in meiosis	Simultaneous	NA	Simultaneous
Shape of microspore tetrads	Tetrahedral	NA	Tetrahedral, decussate
Mature pollen	2-Celled	2-Celled	2- or 3-Celled
Ovule, nucellus, and megagametophyte			
Ovule orientation	Anatropous	Anatropous	Anatropous, rarely orthotropous
Nature of nucellus	Crassi- or tenuinucellate	Crassinucellate	Crassi- or tenuinucellate
No. of archesporial cells	1	NA	1- or multi-celled
Mode of embryo sac formation	*Polygonum*	*Allium, Polygonum*	*Polygonum*
Antipodal cells	Enlarge, persistent	Enlarge, persistent	Enlarge, persistent
Nucellar cap	Formed	Formed	Formed
Hypostase	Not formed	NA	Rarely formed
Obturator	Not formed	NA	NA
Integuments			
No. of integuments	2	2	1 or 2
Vasculature in integuments	Absent	Absent	Absent
Micropyle formation	By inner integument	By inner integument	By inner integument
Endothelium	Not formed	Not formed	Not formed
Fertilization, endosperm, and embryo			
Path of pollen tube	Porogamous	NA	NA
Mode of endosperm formation	Nuclear	NA	Nuclear
Type of embryogeny	NA	NA	Onagrad, solanad
Mature embryo	Small, straight	Small, straight	Small, straight
Seed and seed coat			
Appendages	Absent	Absent	Absent
Endosperm in mature seed	Copious	Copious	Copious
Cells of exotesta	Lignified, palisadal	Lignified, palisadal	Cuboid or palisadal
Cells of mesotesta	Crushed	Crushed	Crushed
Cells of endotesta	Crushed	Crushed	Crushed
Cells of tegmen	Crushed	Crushed	Crushed
Type of seed coat	Exotestal	Exotestal	Exotestal
References			
	Present study	Ly TB (1961) Bhandari & Kapil (1964) Corner (1976) Johri et al. (1992) Lee (1990)	Kapil & Jalan (1962) Jalan (1968) Corner (1976) Johri et al. (1992)

[1)] Data from descriptions and drawings indicated in articles.

[2)] Data of Helleboreae are based on *Caltha*, *Calathodes*, *Eranthis*, and *Helleborus* of tribe Helleboreae sense Tamura (1993).

culifolia are summarized here and in Table 1.

Anther tetrasporangiate; anther wall prior to maturation five cell-layers; mode of wall formation is of basic type; endothecium fibrous; tapetum glandular, and its cells two-nucleate. Cytokinesis in the microspore mother cell simultaneous and microspore tetrads tetrahedral; pollen grains two-celled when shed.

Ovules anatropous, and either tenuinucellate or crassinucellate; one archesporial cell differentiates into megaspore mother cell; megaspore tetrads linear; chalazal megaspore functional, developing into an eight-nucleate *Polygonum* type embryo sac; antipodal cells more or less persistent; two to three cell-layered nucellar cap formed; neither hypostase nor obturator formed.

Ovule bitegmic; both inner and outer integuments of dermal origin, and initially two to three cell-layered; inner integument remaining two to three cell-layered, outer integument later multiplicatively thickening from five to nine cell-layered in young seed stage. Micropyle formed by inner integument alone.

Fertilization porogamous; endosperm formation nuclear type; embryogenesis not conformed; proembryo has short suspensor.

Seeds small, exarillate and with copious endosperm; dicotyledonous, symmetrical, straight, minute embryo formed. Mature seed surface smooth; seed coat is exotestal and tanniniferous.

Comparison and Systematic Implications of *M. saniculifolia*

When comparing *Megaleranthis* to other genera in the tribe Helleboreae, we found no notable differences among their reproductive morphologies (Table 1). First, with regard to *Trollius*, *Megaleranthis* has the same features across several characteristics. However, *Megaleranthis* has megagametophyte development of the *Polygonum* type. In contrast, embryo sac formation in *Trollius* has two patterns within the same species, i.e., the *Allium* and the *Polygonum* types (Bhandari and Kapil, 1964). Except for this characteristic, *Megaleranthis* is very similar to other genera of Helleboreae. Such reproductive morphological diversity and a lack of uniformity apparently are quite typical among members of the Ranunculaceae (Johri et al., 1992). In fact, data from a chromosome study have shown *Megaleranthis* to be of R-type and with $x=8$, thereby making it a more primitive member of the family (Lee and Yeau, 1985). Interestingly, *Trollius* also has the same chromosome number ($x=8$) uniformly within the genus (Tamura, 1995).

All pollen grains are tricolpate and spheroic among *Megaleranthis, Trollius, Eranthis*, and *Helleborus*. However, within the pollen surface, *Megaleranthis* and *Trollius* have a striate type of structural element, whereas those of *Helleborus* and *Eranthis* possess reticulate and microechinate types, respectively (Kim and Lee, 1987; Tamura, 1993). The pollen wall structures of *Trollius, Eranthis*, and *Helleborus* comprise the tectum, columella, and foot layer of the ectexine, but are not formed in the endexine (Joan and Skvarla, 1979). Some of these same palynological features have been observed in *Megaleranthis* (unpublished data), which supports the belief that *Megaleranthis* is closely related to *Trollius*, as noted by Kim and Lee (1987). Furthermore, Lee (1990) has proposed that *Megaleranthis* be included within *Trollius* and that it be correctly named *T. chosenensis* Ohwi. Finally, *Megaleranthis* has been shown to be very similar to *T. albiflorus*, a species that is distributed in North America, mainly from Montana to Colorado in the Rocky Mountains. Its sepal color is also white, just as is found in *T. afghanicus*, *Calathodes*, and *Megaleranthis* (Kadota, 1996).

Yoo et al. (1999) have adopted three types to describe variations in cauline leaf morphology: normal, elongated, or branched. This third stem type is exhibited in *Calathodes*, *Trollius*, and *Megaleranthis*. In addition to the presence of petals, this characteristic is evidence that *Megaleranthis* and *Trollius* are closely related. Nevertheless, Tamura (1966) has stated that *Megaleranthis* is not related phylogenetically to *Trollius* but to *Calathodes* because of the resemblance of their follicles. However, *Calathodes* also differs from *Megaleranthis* because petals are absent there (Tamura, 1995). One unique feature of *Megaleranthis* is that a single cauline leaf is located close to the flower (Tamura, 1995). Despite that previous observation, Yoo et al. (1999) have now found branched cauline leaves in some populations of *Megaleranthis* as well as *Trollius*.

As mentioned above, *Megaleranthis* is very similar to *Trollius* with respect to their reproductive morphology, striate pollen wall architecture, chromosomal make-up, and external characteristics, such as cauline and sessile leaves, the presence of petals, and (sometimes) common sepal color. Ro et al. (1999) have also suggested that *Megaleranthis* and *Trollius* be integrated into the same genus, based on 26S rDNA sequence data. Within a neighbor joining tree, *Megaleranthis* is part of a monophyletic group with *Trollius* and *Adonis* (Ro et al., 1999), although this does not coincide with the classification by Tamura (1995). Historically, *Adonis* has been included in the Ranunculoideae because of its achene fruit type, while *Megaleranthis* and *Trollius*, with their follicle fruits, have been considered members of Helleboroideae.

In conclusion, we suggest that *Megaleranthis* is closely related to *Trollius*, and should be classified within that genus based on their common reproductive morphologies and other available information. However, we will tentatively continue to place *Megaleranthis* within its own genus until further, more powerful phylogenetic evidence becomes available.

ACKNOWLEDGEMENT

This work was supported by a grant from the Korea Science and Engineering Foundation (Grant number R05-2001-000-00336) to K. Heo.

Received November 2, 2004; accepted January 18, 2005.

LITERATURE CITED

Bhandari NN, Kapil RN (1964) Studies in the family Ranunculaceae. VII. Two types of embryo sacs in *Trollius* L. Beitr Biol Pflanzen 40: 113-120

Corner EJH (1976) The Seeds of Dicotyledons. Vol 2. Cambridge University Press, Cambridge, pp 8-24

Davis GL (1966) Systematic Embryology of the Angiosperms. John Wiley & Sons, New York, pp 8-11

Jalan S (1968) Some further observations on the embryology of *Caltha palustris* L. Curr Sci 37: 621-623

Joan WN, Skvarla JJ (1979) Pollen morphology. The potential influence in higher orders systematics. Ann Missouri Bot Gard 66: 633-700

Johri BM, Ambegaokar KB, Srivastava PS (1992) Comparative Embryology of Angiosperms. Vol 1. Springer-Verlag, Berlin, pp 285-291

Kadota Y (1996) Ranunculaceae. Botanical World (Weekly Magazine of Asahi News Paper Co., Japan) 92: 226-234

Kapil RN, Jalan S (1962) Studies in the family Ranunculaceae. I. The embryology of *Caltha palustris* L. Plant embryology symposium, New Delhi, pp 205-214

Kim MY, Lee ST (1987) Palynotaxonomic relationship of *Megaleranthis saniculifolia* Ohwi to the relative species (Ranunculaceae). Kor J Plant Tax 17: 13-20

Lee ST (1990) On the taxonomic position of *Trollius chosenensis* Ohwi (Ranunculaceae). Kor J Plant Tax 20: 1-8

Lee TB (1980) Rare and endangered species in the area of Mt. Sorak. Bull Kwanak Arbor 3: 197-201

Lee WT (1969) A discussion on the Korean endemic genera. Kor J Plant Tax 1: 15-21

Lee YN, Yeau SH (1985) Taxonomic characters of *Megaleranthis saniculifolia* Ohwi (Ranunculaceae). Kor J Plant Tax 15: 127-131

Ly TB (1961) Embryogenie des Renonculacees, developpement de l'embryon chez le *Trollius europaeus* L. Compt Rend Hebd Seances Acad Sci 253: 2753-2755

Ohwi J (1935) *Megaleranthis*, genus novum Ranunculacearum. Acta Phytotax et Geobot 4: 130-131

Ohwi J (1937) Symbolae and Floram Asiae Orientalis 15. Acta Phytotax et Geobot 6: 151

Ro KE, Han HY, Lee ST (1999) Phylogenetic contributions of partial 26S rDNA sequences to the tribe Helleboreae (Ranunculaceae). Kor J Biol Sci 3: 9-15

Schmid R (1986) On Cornerian and other terminology of angiospermous and gymnospermous seed coats: Historical perspective and terminological recommendations. Taxon 35: 476-491

Tamura M (1966) Morphology, ecology and phylogeny of the Ranunculaceae. Sci Rep Osaka Univ 15: 13-35

Tamura M (1993) Ranunculaceae. *In* K Kubitzki, JG Rohwer, V Bittrich, eds, The Families and Genera of Vascular Plants. Vol 2. Springer-Verlag, Berlin, pp 563-583

Tamura M (1995) Die Naturlichen Pflanzenfamilien nebst ihren Gattungen und wichtigsten Arten, Band 17a IV, *In* P Hiepko, ed, Systematics: Ordnung Ranunculales, Family Ranunculaceae. Duncker und Humblot Verlag, Berlin, pp 225-247

Tobe H (1989) The embryology of angiosperms: Its broad application to the systematic and evolutionary study. Bot Mag (Tokyo) 102: 352-367

Yoo KO, Lee WT, Oh YJ (1999) External morphology and vegetation of *Megaleranthis saniculifolia* populations in four different habitats. Kor J Plant Res 12: 312-323