Pollen morphology of the genus *Aruncus* L. (Rosaceae)

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개승마속(*Aruncus* L., 장미과)의 화분형태학적 연구

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ABSTRACT: Pollen morphological characteristics of 7 taxa of the genus *Aruncus* L. (Rosaceae) were examined using scanning electron microscopy (SEM) to evaluate the taxonomic implication within the genus. *Aruncus* pollen grains were monad, tri-colporate with granular membranes, small in size ($P = 8.33\text{--}12.57\ \mu\text{m}$, $E = 8.93\text{--}14.40\ \mu\text{m}$), amb is sub-circular to circular, suboblate to prolate-spheroidal in shape ($P/E = 0.68\text{--}1.70$). The sexine ornamentation in all studied taxa was basically striate but existence of perforation (striate-perforate: *A. dioicus*, *A. dioicus* var. *acuminatus*, *A. dioicus* var. *astilboides*, *A. dioicus* var. *pubescens*, *A. dioicus* var. *vulgaris*; striate-pstilate: *A. dioicus* var. *aethusifolius*, *A. gombalamus*) and the widths of muri and grooves varied according to the taxa. In particular, relatively short, and sharply crested muri being a distinct feature of *A. dioicus* var. *acuminatus*. As a result, the various combination of each pollen characteristics could be useful to identify the some taxa of the genus *Aruncus*.

Keywords: *Aruncus*, Rosaceae, SEM, Pollen morphology, Sexine ornamentation, Principal Components Analysis (PCA)

적 요: 개승마속 내 7분류군이 갖는 화분형태학적 형질변이의 분류학적 유용성을 알아보기 위하여 주사전 자현미경을 사용하여 자세히 관찰하였다. 본 속의 화분은 단립으로 크기는 소립($P = 8.33\text{--}12.57\ \mu\text{m}$, $E = 8.93\text{--}14.40\ \mu\text{m}$)이며, 극측면에서는 아원형에서 원형의 형태를, 적도면에서는 아단구형에서 약장구형에 걸친 형태를 나타내고($P/E = 0.68\text{--}1.70$), 과립상 돌기를 지닌 3공구형의 발아구를 갖는 것으로 확인되었다. 화분의 표면무늬는 기본적으로 유선상의 특징을 지니고 있으나, 천공의 유무, 유선과 유선 사이의 너비 등에서는 차이를 보여주었다. 특히, *A. dioicus* var. *acuminatus*는 속내의 다른 분류군에 비해서 상대적으로 짧고 높게 솟아있는 유선을 갖는 특징으로 구분됨을 확인하였다. 화분학적 형질을 종합한 결과, 개승마속의 다양한 화분학적 변이의 조합은 속 내 일부 분류군을 구별하는데 유용한 것으로 나타났다.

주요어: 개승마속, 장미과, 주사전자현미경, 화분형태, 화분표면무늬, 주성분분석

Introduction

The genus *Aruncus* L. (tribe Spiraeae DC., subfamily Spiraeoideae Agardh., family Rosaceae Juss.) contains 10 taxa (2 species, 5 varieties, 3 formae) distributed throughout the

temperate regions of Northern Hemisphere (Tutin, 1964; Ikeda, 1995). Consistently belonging to the subfamily Spiraeoideae, *Aruncus* is a well-defined genus, characterized by herbaceous habit, follicle without bristles, and absence of epicalyx (Tutin, 1964; Ikeda, 1995; Potter et al., 2007). Taxa within *Aruncus* have been classified according to external morphological characters such as leaf shape; panicle density; and length of the petals, style, and follicle (Hara, 1955; Mellichamp, 1976). However, according to the Hara (1955)'s concept, it is better to treat *Aruncus* as a monotypic genus due to the high degree of

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variability in these characters (Hara, 1955; Mellichamp, 1976; Ikeda, 1995). He designated *A. dioicus* (Walter) Fernald as a type species of this genus, however, according to the most recently accepted classification, 5 varieties and 3 formae are recognized within this species, and *A. gombalanus* (Hand.-Mazz.) Hand.-Mazz. has been accepted as another distinct species within the genus (The Plant List, 2013, version 1.1., Published on the Internet; <http://www.theplantlist.org/>). In fact, within the genus *Aruncus*, morphological diagnostic characteristics are ambiguous between and within taxa.

One way to examine ambiguous taxonomic relationships is to study a relatively conservative characteristic such as pollen morphology (Hebda and Chinnappa, 1990b). Pollen morphology is diverse, and is widely accepted to reflect phylogenetic relationships (Lee et al., 2011). It has been used to discriminate genera and/or species, and in the Rosaceae, sexine ornamentation is a particularly informative feature (Reitsma, 1966; Thakur and Thakur, 1970; Hebda et al., 1988a, b; Hebda and Chinnappa, 1990a, b; Polyakova and Gataulina, 2008; Chung et al., 2010; Wrońska-Pilarek and Jagodziński, 2011; Shi et al., 2013). Hebda and Chinnappa (1994) classified rosaceous pollen types using sexine ornamentation patterns and described the pollen grain of *Aruncus dioicus* (Walter) Fernald as a striate with crested muri and perforation. Although moderate variation in pollen size were found between *A.*

dioicus ($13.57 \times 14.10 \mu\text{m}$, Hebda et al., 1988b) and *A. gombalanus* (Walter) Fernald ($16.93 \times 16.60 \mu\text{m}$, Zhou et al., 1999), detail pollen features of *A. gombalanus* were not observed by using SEM. To obtain additional clues and resolve the obscure interspecific delimitation within this genus, further morphological study of *Aruncus* pollen is necessary.

Here, we document detailed pollen morphology within the genus *Aruncus* based on SEM, to explore the potential taxonomic implications of pollen characters. We also identify the palynological characters that might be useful for taxa identification.

Materials and Methods

The present study was conducted on the 7 taxa (2 species, 5 varieties) of *Aruncus*. Most materials were taken from herbarium specimens from the following herbaria: F, KB, KHUS, NY, PE, TI and some plants were collected from natural populations in Korea. Specimen information for the taxa examined is summarized in the Table 1.

Pollen grains were prepared by critical point drying method. For morphological observation of pollen grains, dried flowers were first rehydrated with Agepon® (Agfa Gevaert, Leverkusen, Germany; Agepon wetting agent: distilled water, 1 : 200) before dehydration to recover their original shape. The anthers were picked from flowers, and dehydrated through

Table 1. Voucher specimens of the genus *Aruncus* that were examined in the present study.

Taxa	Voucher information	
<i>Aruncus dioicus</i> L. var. <i>dioicus</i> (Walter) Fernald	Korea, Seoul, Achasan Mt., 20 May 2014, <i>Song and Ok 1800001</i> (KHUS)	*
	Korea, Gyeonggi-do, 20 May 2014, <i>Song and Ok 050520</i> (KHUS)	
	Korea, Gyeongsangnam-do, Namdeogyusan Mt., 17 Jun. 2006, <i>Sun VP7400015156</i> (KB)	
<i>A. dioicus</i> L. var. <i>acuminatus</i> (Douglas ex Hook.) H.Hara	USA, Washington, 22 Jul. 1930, Aug. 1976, without collector's name (NY)	*
	USA, Oregon, Lincoln Co., 11 Aug. 2007, <i>Halse 7342</i> (F)	
<i>A. dioicus</i> L. var. <i>aethusifolius</i> (H.L.v.) H.Hara	Korea, Jeju Isl., Hallasan Mt., 09 Jul. 2012, <i>Choi and Bum VP 0000422303</i> (KB)	*
	Korea, Jeju Isl., Hallasan Mt., 15 Jul. 2014, <i>Song and Ok 1800003</i> (KHUS)	
	Korea, Jeju Isl., Hallasan Mt., 15 Jul. 2014, <i>Song and Ok 010714</i> (KHUS)	
<i>A. dioicus</i> L. var. <i>astilboides</i> (Maxim.) H.Hara	Japan, Hon-Shu, Hayachinesan Mt., Prox. Rikuchu, 21 Jul. 1913, without collector's name <i>M14-02(8)</i> (TI)	*
	Japan, Hon-Shu, Hayachinesan Mt., Prox. Rikuchu, 6 Aug. 1913, without collector's name <i>M14-02(10)</i> (TI)	
<i>A. dioicus</i> L. var. <i>pubescens</i> (Rydb.) Fernald	USA, St. Louis Co., 4 Jun 1926, Aug., 1976, without collector's name <i>1612</i> (F)	*
<i>A. dioicus</i> L. var. <i>vulgaris</i> (Maxim.) H.Hara	USA, California, Siskiyou county, 20 Aug. 1959, <i>Francia Chisaki, Helen K. Sharsmith s.n.</i> (NY)	*
	USA, Washington, 23 Jul. 1893, <i>Sandberg and Leiberger 561</i> (F)	
<i>A. gombalanus</i> (Hand.-Mazz.) Hand.-Mazz.	China, Mekong-Salween Divide, Sila, 5 Aug. 1938, <i>T. T. Yu 00561083</i> (PE)	*

Herbarium acronyms follow Index Herbariorum (Thiers, 2015); Asterisks indicate the used materials for figures and principal component analysis.

an acetone series (50%, 70%, 90%, 100%). The completely dehydrated anthers were critical-point-dried by using CP-dryer (SPI-13200J-AB, SPI Supplies, USA) and mounted on an aluminium stub with double adhesive tapes. The stubs were coated with gold using an ion-sputtering device (Cressington Sputter Coater 108, Ted Pella Inc., USA). The pollen grains were observed using a field emission scanning electron microscope (FE-SEM; JSM-7600F, JEOL, Japan) at an accelerating voltage of 10 kV with working distance 8–10 mm.

For each taxa, polar axis (P), equatorial diameter (E), equatorial diameter in polar view (EDPV), diameter between the apices of two ectocolpi (AC), polar area index (PAI), width of muri (WM) and width of grooves (WG), WM/WG ratio (M/G) of sexine ornamentation were measured on SEM images using Macnification software (version 2.0, Orbicule Inc, Leuven, Belgium). In particular, WM and WG were measured

on the middle area of striation to exclude junction of striation.

Principal component analysis (PCA) was performed to verify whether the quantitative pollen data allowed the grouping of taxa. The first three principal components (PCs) with eigenvalues greater than 1 were represented. The results were presented in a two-dimensional plot of the first and second principal components. All analyses were done with statistical software R version 3.2.2 (R Development Core Team., 2015). We conducted an ANOVA to determine interspecific differences of principal component scores among the 7 taxa.

Results and Discussion

The pollen morphological characteristics are described below with regard to the following pollen features: size (P, E), shape (P/E), aperture number, equatorial diameter in polar view

Table 2. Pollen morphological characteristics of taxa within *Aruncus*.

Taxon*	n	Size		P/E	Shape				EDPV	AC	PAI
		P (μm)	E (μm)		SO	OS	S	PS			
<i>A. dioicus</i> var. <i>dioicus</i>	30	10.46±0.70 (9.29–11.90)	12.23±0.77 (10.54–13.44)	0.86±0.03 (0.80–0.92)	++	+	-	-	11.78±0.37 (10.99–12.47)	4.06±1.51 (3.26–4.48)	0.32±0.03
	10	10.76±0.66 (9.25–12.30)	12.40±0.51 (11.48–13.29)	0.87±0.04 (0.81–0.95)	++	+	-	-	11.90±0.49 (10.97–12.83)	3.80±0.31 (3.04–4.40)	0.32±0.03
	10	10.56±0.74 (9.25–11.60)	12.29±0.58 (11.48–13.29)	0.87±0.03 (0.81–0.93)	++	+	-	-	12.55±0.59 (11.34–13.84)	4.03±0.30 (3.33–4.56)	0.32±0.03
<i>A. dioicus</i> var. <i>acuminatus</i>	30	10.12±0.55 (9.23–11.52)	10.46±0.50 (9.64–11.43)	0.97±0.04 (0.86–1.05)	-	++	+	+	10.13±0.43 (9.26–11.13)	3.19±1.52 (2.11–4.25)	0.29±0.05
	10	9.89±0.55 (8.97–10.97)	10.44±0.58 (9.24–11.66)	0.95±0.05 (0.87–1.08)	+	++	-	+	9.88±0.37 (9.37–10.50)	2.94±0.36 (2.32–3.72)	0.30±0.04
<i>A. dioicus</i> var. <i>aethusifolius</i>	30	11.60±0.41 (10.58–12.57)	13.18±0.69 (12.15–14.40)	0.88±0.04 (0.81–0.96)	+	++	-	-	12.75±0.40 (11.63–13.42)	3.66±1.79 (2.45–4.24)	0.26±0.04
	10	11.41±0.48 (10.62–12.12)	12.97±0.79 (11.66–14.26)	0.88±0.04 (0.79–0.96)	+	++	-	-	13.42±0.82 (11.83–14.37)	3.94±0.39 (3.49–4.66)	0.29±0.03
	10	11.32±0.61 (10.21–12.30)	12.80±0.68 (11.70–13.96)	0.88±0.05 (0.78–0.98)	+	+	-	-	13.45±0.79 (12.17–15.33)	4.07±0.42 (3.16–4.93)	0.30±0.03
<i>A. dioicus</i> var. <i>astilboides</i>	30	10.84±0.70 (9.99–12.39)	11.80±0.70 (10.64–13.65)	0.92±0.05 (0.84–1.05)	+	++	+	+	11.43±0.50 (10.10–12.38)	4.01±0.49 (2.98–5.18)	0.35±0.04
	10	10.74±0.76 (9.73–12.22)	11.47±0.76 (10.44–13.01)	0.94±0.06 (0.81–1.06)	+	++	-	+	11.04±1.07 (9.20–12.85)	3.61±0.43 (2.29–4.28)	0.33±0.05
<i>A. dioicus</i> var. <i>pubescens</i>	30	9.88±0.86 (8.33–12.34)	10.63±0.75 (8.93–12.72)	0.93±0.06 (0.84–1.06)	+	++	-	+	10.04±0.65 (8.40–11.56)	2.86±0.53 (1.00–3.85)	0.29±0.04
<i>A. dioicus</i> var. <i>vulgaris</i>	30	10.06±0.61 (8.90–11.45)	11.08±0.61 (9.99–12.46)	0.91±0.05 (0.84–1.02)	+	++	-	+	10.77±0.66 (9.43–11.94)	3.80±0.55 (2.00–4.66)	0.36±0.03
	10	10.20±0.68 (8.79–11.30)	11.18±0.77 (9.58–13.38)	0.91±0.05 (0.83–1.00)	+	++	+	-	11.20±0.84 (9.8–13.17)	3.95±0.64 (2.86–5.23)	0.35±0.06
<i>A. gombalanus</i>	30	10.74±0.63 (9.74–11.92)	11.42±0.69 (9.84–12.75)	0.94±0.06 (0.84–1.10)	+	++	+	+	10.61±0.62 (9.78–12.44)	3.57±1.13 (2.49–4.44)	0.32±0.05

Numbers refer to mean±standard deviation (minimum–maximum); Mean±standard deviation only in PAI; Polar axis (P); Equatorial diameter (E); Suboblate (SO); Oblate spheroidal (OS); Spheroidal (S); Prolate-spheroidal (PS); Absent (-); Present (+); Dominant (++); Equatorial diameter in polar view (EDPV); Diameter between the apices of two ectocolpi (AC); Polar area indices (PAI), *Order of taxa was consistent with Table 1.

Fig. 1. SEM micrographs of *Aruncus* pollen grains (with striate-perforate sexine ornamentation). A–C, *Aruncus dioicus* var. *dioicus*; D–F, *A. dioicus* var. *acuminatus*; G–I, *A. dioicus* var. *astilboides*; J–L, *A. dioicus* var. *pubescens*; M–O, *A. dioicus* var. *vulgaris*; A, D, G, J, M, Polar view of pollen grains; B, E, H, K, N, Equatorial view of pollen grains; C, F, I, L, O Sexine ornamentation at mesocolpium.

Fig. 2. SEM micrographs of *Aruncus* pollen grains (with striate–psilate sexine ornamentation). A–C, *A. dioicus* var. *aethusifolius*; D–F, *A. gombalanus*; A, D, Polar view of pollen grains; B, E, Equatorial view of pollen grains; C, F, Sexine ornamentation at mesocolpium.

(EDPV), diameter between the apices of two ectocolpi (AC), polar area index (PAI), and sexine ornamentation. The representative pollen characters are summarized in Tables 2–3 and illustrated in Figures 1–2.

Pollen grains are monad and mostly small in size ($P = 8.33\text{--}12.57\text{ }\mu\text{m}$, $E = 8.93\text{--}14.40\text{ }\mu\text{m}$; Table 2). *A. dioicus* var. *pubescens* having the smallest pollen grains, of all taxa investigated (a mean value of $P \times E$ was $9.88 \times 10.63\text{ }\mu\text{m}$), and *A. dioicus* var. *aethusifolius* having the largest ($11.60 \times 13.18\text{ }\mu\text{m}$). The shape of the pollen grains is sub-circular to circular, or rarely semi-angular in polar view, and ranges from suboblate to prolate-spheroidal in equatorial view ($P/E = 0.68\text{--}1.70$; Figs. 1, 2, Table 2). Most taxa have predominantly oblate-spheroidal pollen grains. The exception is *A. dioicus* var. *dioicus*, which has mostly sub-oblate pollen grains. All pollen grains studied were tri-colporate, the simple colpi are distributed symmetrically, elongated, and narrowed toward the poles with granular aperture membranes (Figs. 1, 2; Table 2). The apocolpium was rather narrow, with lower PAI value ranging from 0.26 to 0.36 (Table 2). *A. dioicus* var. *aethusifolius* had the lowest value (0.26), and *A. dioicus* var. *vulgaris* had the highest (0.36).

The pollen grains examined in this study were consistently smaller than those examined in previous *Aruncus* pollen studies. In a previous study of *A. dioicus*, the average size of pollen grains was $13.57 \times 14.10\text{ }\mu\text{m}$ (Hebda et al., 1988a); for *A. gombalanus*, the previously described average was $16.93 \times 16.60\text{ }\mu\text{m}$ (Zhou et al., 1999). The present results for

the same taxa were $10.46 \times 12.23\text{ }\mu\text{m}$ and $10.74 \times 11.42\text{ }\mu\text{m}$, respectively. All previous studies are based on acetolysed material, whereas the present study treated using the critical point drying process. The use of different preparation techniques is known to affect the size and shape of pollen grains, with grains that are treated using critical point drying always being smaller than acetolysed grains (Reitsma, 1969, Moon et al., 2008). Because the acetolysis method can destroy the colpus membranes, the size and shape of the pollen grains is modified, whereas the critical point drying maintains these natural features (Demissew and Harley, 1992).

Sexine ornamentation of the pollen grains was consistently striate, consisting of muri and grooves with or without microperforations on the grooves. The width of striae varied, with the largest values of muri and grooves observed in *A. dioicus* and *A. dioicus* var. *astilboides* pollen grains, respectively (Table 3). The striate-microperforate pattern can be subdivided into two patterns based on the widths of muri and grooves (Fig. 1). A pattern with wider muri than grooves was found in *A. dioicus* var. *pubescens* (Fig. 1J–L) and *A. dioicus* var. *vulgaris* (Fig. 1M–O), whereas *A. dioicus* var. *dioicus* (Fig. 1A–C), *A. dioicus* var. *acuminatus* (Fig. 1D–F), and *A. dioicus* var. *astilboides* (Fig. 1G–I) possess wider grooves than muri. The striate-psilate pattern can also be subdivided into two patterns based on relative widths of muri and grooves (Fig. 2). *A. dioicus* var. *aethusifolius* has wider muri than grooves (Fig. 2A–C), whereas *A. gombalanus* has

muri and grooves of a similar width (Fig. 2D–F). More specifically, the direction and length of the striae (muri and grooves) patterns in the mesocolpium are variable among the *Aruncus* taxa. The muri are prominently formed in *A. dioicus* var. *dioicus*, *A. dioicus* var. *acuminatus*, *A. dioicus* var. *astilboides*, and *A. gombalanus*, with relatively short, and sharply crested muri being a distinct feature of *A. dioicus* var. *acuminatus*. Striae tend to be parallel with the colpus, but often displayed a looped shape on or near the poles and/or mesocolpium in most taxa. However, the muri of *A. dioicus* var. *astilboides* are branched, anastomosed, wiggly and parallel to aperture at mesocolpium (Fig. 1I).

The sexine ornamentation has provided useful characters for delimiting genera and species in Rosaceae (Ueda and Tomita, 1989; Ueda, 1992; Hebda and Chinnappa, 1994; Chung et al., 2010; Wrońska-Pilarek and Jagodziński, 2011; Shi et al., 2013). In the present study, considerable variation in sexine ornamentation was observed between the studied taxa. As shown in earlier studies, the striate-perforate pattern is a common feature in genus *Aruncus* (Hebda et al. 1988a). However, we observed additional sexine ornamentation patterns such as striate-psilate (Fig. 2). Moreover, detailed ornamentation characteristic, such as width of striae (muri and grooves), are informative within the genus *Aruncus*. For example, *A. dioicus* var. *acuminatus* has a unique sexine ornamentation pattern, with sharply crested, relatively short

length of muri. Such features could be applicable as identifying and diagnostic characters for the taxa within the genus *Aruncus*. Further, the fine variations in sexine ornamentation, such as the thickness or pattern of striae, may provide additional taxonomic significance among the taxa. Therefore, more detailed study of pollen wall stratification through transmission electronic microscope (TEM) is required. Such study may be helpful in elucidating the infrageneric relationships between *Aruncus* taxa.

Statistical methods such as PCA have been used to evaluate the systematic usefulness of pollen data (Akhila and Beevy, 2015; de Abreu et al., 2015; da Luz et al., 2015; Mezzonato-Pires et al., 2015). In the present study, PCA was used to explore the relationships between taxa. Three principle components (PCs), with eigenvalues larger than 1, explained 74.6% of the variation in the data (Figs. 3–4; Table 4). The PC1 explains 31.1% of the variance based on the pollen size (P, E), and diameter between the apices of two ectocolpi (AC). The PC2 accounts for 22.4% of the data variability mostly related to shape (P/E) and sexine ornamentation characteristic such as width of muri (WM) and WM/WG ratio (M/G). *A. dioicus* var. *dioicus*, *A. dioicus* var. *aethusifolius* and *A. dioicus* var. *astilboides* were grouped on the positive side of the PC1 axis based on their relatively larger pollen grains (Figs. 3, 4A). Four taxa (*A. dioicus* var. *acuminatus*, *A. dioicus* var. *aethusifolius*, *A. dioicus* var. *astilboides*, and *A. gombalanus*)

Table 3. The characteristics of sexine ornamentation within *Aruncus* taxa.

Taxon*	n	Width of		M/G	Perforate
		Muri	Grooves		
<i>A. dioicus</i> var. <i>dioicus</i>	30	0.25±0.04 (0.20–0.35)	0.33±0.07 (0.23–0.51)	-	+
	10	0.20±0.06 (0.14–0.36)	0.34±0.10 (0.24–0.59)	-	+
	10	0.27±0.06 (0.17–0.37)	0.39±0.06 (0.31–0.47)	-	+
<i>A. dioicus</i> var. <i>acuminatus</i>	30	0.12±0.03 (0.07–0.18)	0.31±0.09 (0.21–0.58)	-	+
	10	0.15±0.03 (0.10–0.21)	0.40±0.11 (0.20–0.61)	-	+
<i>A. dioicus</i> var. <i>aethusifolius</i>	30	0.21±0.04 (0.14–0.30)	0.13±0.03 (0.07–0.19)	+	-
	10	0.22±0.03 (0.16–0.28)	0.11±0.03 (0.06–0.17)	+	-
	10	0.25±0.04 (0.21–0.33)	0.12±0.03 (0.06–0.15)	+	-
<i>A. dioicus</i> var. <i>astilboides</i>	30	0.16±0.02 (0.12–0.20)	0.43±0.10 (0.26–0.65)	-	+
	10	0.23±0.04 (0.17–0.30)	0.46±0.10 (0.32–0.62)	-	+
<i>A. dioicus</i> var. <i>pubescens</i>	30	0.20±0.04 (0.12–0.27)	0.05±0.02 (0.03–0.09)	+	+
<i>A. dioicus</i> var. <i>vulgaris</i>	30	0.25±0.07 (0.14–0.41)	0.05±0.02 (0.03–0.09)	+	+
	10	0.26±0.04 (0.16–0.33)	0.06±0.02 (0.03–0.14)	+	+
<i>A. gombalanus</i>	30	0.25±0.05 (0.18–0.39)	0.28±0.06 (0.19–0.42)	±	-

Numbers refer to mean±standard deviation (minimum–maximum) μm; Muri (M); Grooves (G); Width of muri/Width of grooves (M/G) <1: -, >1: +, ≥ 1: ±; Absent (-); Present (+), *Order of taxa was consistent with Table 1.

with similar shape, narrow muri (small WM value), and a small M/G ratio were positioned on the positive side of the PC2 axis; others were grouped on negative (Figs. 3, 4B). Also, the equatorial diameter in polar view (EDPV), Polar area indices (PAI), and width of grooves (WG) related variables included in PC3. Two taxa: *A. dioicus* var. *aethusifolius*, *A. dioicus* var. *pubescens* were distributed on the positive side of PC3 (Figs. 3, 4C).

In the PCA of the pollen characters, PC1 best explained the variability of pollen size while the sexine ornamentation features were explained by PC2 and PC3 (Table 4). All differences between the taxa were significant. Thus, the pollen morphological characteristic should be useful for identification of taxa in the genus *Aruncus*. Although the scattered plot of *A. dioicus* var. *dioicus* was partially overlapped by that of another species *A. gombalanus*, the infraspecies of *A. dioicus* were separated into two groups. One group was overlapped by *A. dioicus*, and the other was distinguished from *A. dioicus*. Therefore, to clarify the relationship between *Aruncus* taxa,

more detailed taxonomical study is needed.

A. dioicus var. *aethusifolius*, endemic to Korea, is differentiated by deeply incised leaflet. Its distribution is restricted to Hallasan Mt. on Jeju Is. Although Nakai designated *A. aethusifolius* as an independent species within the genus in 1912, Hara (1955) recognized it as a variety of *A. dioicus* (Hara, 1955). According to the present study, *A. dioicus* var. *dioicus* and *A. dioicus* var. *aethusifolius* showed considerable distance in palynological characteristics such as pollen size and sexine ornamentation. In particular, sexine ornamentation was striate-perforate in *A. dioicus* and all of its infrageneric taxa except *A. dioicus* var. *aethusifolius*, which instead exhibited a striate-psilate pattern like *A. gombalanus*. This suggest that palynological characteristics could provide evidence to aid in taxonomical delimitation of this taxon. To confirm the taxonomical rank of *A. dioicus* var. *aethusifolius*, further study is necessary.

In summary, this study provides additional pollen morphological data for the genus *Aruncus*. The pollen grains

Fig. 3. Scatter plot of the principal component analysis for pollen morphological characteristics in *Aruncus* taxa. The first two principal components were plotted and each color represents a different taxa; Polar axis (P); Equatorial diameter (E); P/E ratio (P/E); Equatorial diameter in polar view (EDPV); Apocolpus side (AS); Polar area indexes (PAI); Width of muri (WM) and width of grooves (WG); WM/WG ratio (M/G); The arrows are displayed for variables.

Fig. 4. Comparison of the three component scores of pollen characteristics among the taxa. Dots in the middle and error bars denote the average and standard error of component scores, respectively. All differences were significant.

Table 4. Variable loadings of pollen morphological characters of *Auruncus* for three principal components. The first three PCs with eigenvalue larger than one were represented here. Each percentage in parenthesis indicates the amount of variation explained by each PC, and the components that were loaded most highly for each variable are in bold.

Variables	PC1 (31.1%)	PC2 (22.4%)	PC3 (21.2%)
P	0.39734909	0.18989358	0.21148638
E	0.53729035	-0.03983447	0.2384708
P/E	-0.29225047	0.30372253	-0.07963313
EDPV	0.30045671	-0.34309789	-0.48952192
AC	0.49634425	-0.03779548	0.16760828
PAI	0.04758188	-0.34603765	-0.59747827
WM	0.11037634	-0.45284322	0.16012831
WG	0.22042085	0.39749682	-0.41290375
M/G	-0.26145415	-0.5178309	0.26688695

Polar axis (P); Equatorial diameter (E); P/E ratio (P/E); Equatorial diameter in polar view (EDPV); Diameter between the apices of two ectocolpi (AC); Polar area indices (PAI); Width of muri (WM); Width of grooves (WG); WM/WG ratio (M/G)

are small, tri-colporate, and sub-oblate to prolate-spheroidal in shape. They show striate sexine ornamentation, but there is significant variation in the shape and thickness of striae between taxa. Although further study of *Aruncus* is necessary to clarify the taxonomic delimitation of its taxa, in this study, palynological characteristics of *Aruncus* did illustrate some of the variation within and between the studied taxa.

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