The phylogenetic potential of orbicules in angiosperms

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(Received 21 November 2017; Revised 26 January 2018; Accepted 21 March 2018)

ABSTRACT: The distribution of orbicules was investigated for eleven taxa of six genera in Lamiaceae and four taxa of three genera in Verbenaceae using scanning electron microscopy. A literature survey to evaluate the phylogenetic potential of the orbicules and their possible correlations with tapetum types was also conducted. The orbicules are consistently absent in all investigated taxa of Lamiaceae, while small orbicules of an average size of less than 1 μm are densely distributed in Verbenaceae. In fact, orbicules appear consistently in 123 of 150 angiosperm families when investigated in at least one species. Thus, the distribution patterns of orbicules could be a useful diagnostic character in angiosperms. In addition, orbicules occur in 84% taxa of the secretory tapetum type, while they are commonly absent in the amoeboid tapetum type (ca. 80%). The presence of orbicules may be correlated with the secretory tapetum type. However, the study of orbicules is restricted in 150 families and the tapetum type within these families can be applied for 92 families out of a total of 416 angiosperm families. Thus, further investigation of orbicules is necessary in extended taxa to address the questions pertaining to orbicules.

Keywords: orbicules, scanning electron microscopy, tapetum type, angiosperms

Pollen morphological traits have been considered as useful diagnostic characters, since they often provide important clues to identify plant species and infer their evolutionary history. Recently, using electron microscope the micromorphological features on pollen wall reflect its phylogenetic potential (Moon et al., 2008a). The pollen exine consists of sporopollenin, a complex and highly resistant biopolymer which protect pollen grains from mechanical damage or degradations, thus pollen grains remain well preserved in fossils (Erdtman, 1960).

Orbicules (or Ubisch body) are small, granular structures that are found in mature anthers as a layer of tiny particles lining in the inner locule wall and are composed of sporopollenin, like the pollen exine (El-Ghazaly, 1999; Huysmans et al., 2000; Galati, 2003). Orbicules were observed for the first time by a Russian scholar Rosanoff (1865). Since then, much attention has been paid to these structures and palynological studies have been intensively conducted, coupled with endothecium structure, pollen development and sporopollenin synthesis (Huysmans et al., 1998, reference therein). Recent studies address that the distribution, size and

shape of orbicules has phylogenetic significance, so orbicules are drawing keen attention in that they could be the characters to reflect the phylogenetic correlation of angiosperms, as well as pollen morphological features (Moon et al., 2008a; Huysmans et al., 2010; Verstraete et al., 2011; Song et al., 2016).

Orbicules develop simultaneously with the pollen grains and are acellular tiny particles that generally observed on the innermost tangential and/or radial walls of secretory tapetum type (=parietal or glandular tapetum type) (Kosmath, 1927; Ubisch, 1927). Secretory tapetum type is a basic tapetum that can be found in angiosperms, along with amoeboid tapetum type (=plasmodial tapetum type) whose cell wall is disrupted and cytoplasm is released to anther locule. Universally, the secretory type occurs in the existing basal angiosperms, the majority of fossil plant taxa and angiosperm families, thus being regarded as more plesiomorphic type than amoeboid type (Furness and Rudall, 2001). In this context, orbicule development could be a plesiomorphic condition of taxa (Huysmans et al., 2010; Verstraete et al., 2011). However, since orbicules are observed in the taxa with the amoeboid tapetum

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type such as *Abutilon pictum* (Gillies ex Hook.) Walp., *Acacia conferta*, Benth. *Beta vulgaris* L., *Butomus umbellatus* L., *Canscora alata* (Roth) Wall., and *Gentiana acaulis* L., their correlation with the tapetum type remains unclear (Lombardo and Carraro, 1976; Fernando and Cass, 1994; Huysmans et al., 1998; Strittmatter and Galati, 2000; Vinckier and Smets, 2003; Furness, 2008; Taylor et al., 2008; Verstraete et al., 2014).

Orbicules are readily observable in mature anther and inner locule wall by scanning electron microscopy (SEM). Usually they are smaller than 1 μm , but orbicules with a diameter up to 15 μm are reported in *Quararibea* Aubl. (Nilsson and Robyns, 1974). Generally, orbicules are spherical with a smooth surface (psilate), but diverse shapes such as granulate, echinate, or microperforate are also found depending on taxa. It is noteworthy that in some species the sculpturing pattern of orbicules are similar with sexine ornamentation (Rowley et al., 1959; Hesse, 1986; Huysmans et al., 2000). Recently, intensive studies have been conducted to investigate the presence of orbicules and their morphological diversity, along with the pollen-morphology research (Moon et al., 2008b; Song et al., 2016, 2017a, 2017b). Nevertheless, there are very few data on orbicule research in Korea, except for some studies on orbicule development

associated with the pollen development (Jeong, 2009). In angiosperm as a whole the data on orbicules are rather restricted.

Thus, here I would add new data of orbicules in taxa of Lamiaceae and Verbenaceae to evaluate its phylogenetic potential. In addition, the literature survey is also conducted to check possible correlation between orbicule existence and tapetum type.

Materials and Methods

To investigate the distribution and characteristics of orbicules two families were selected based on previous studies (Moon et al., 2008a, 2008b, 2008c). Orbicules of 15 taxa were observed using scanning electron microscopy (SEM); 11 taxa of 6 genera in Lamiaceae and 4 taxa of 3 genera in Verbenaceae. The literature search encompassed the data screened until 2012 (Verstraete et al., 2014) and 19 additional papers published until September 2017 (5,154 taxa of 58 genera in 12 families; Appendix 1). Based on the results of this study, I attempted to evaluate the orbicule distribution in angiosperms and its possible correlation with tapetum type.

For SEM observation of orbicules, fully matured anthers

Table 1. The orbicule existence data of present study and their size variation with voucher information.

+, orbicule presence; –, absence.

Taxon	Orbicules	Size (μm) Min–max (mean±SD)	Voucher information
Lamiales			
Lamiaceae			
<i>Agastache rugosa</i> (Fisch. & C.A.Mey.) Kuntze	–	–	Korea, 3 Sep 2012, <i>Moon et al. s.n.</i> , KHUS
<i>Elsholtzia angustifolia</i> (Loes.) Kitag.	–	–	Korea, 4 Oct 2002, <i>Moon et al. s.n.</i> , KHUS
<i>E. ciliata</i> (Thunb.) Hyl.	–	–	Korea, 10 Oct 2003, <i>Moon et al. s.n.</i> , KHUS
<i>E. minima</i> Nakai	–	–	Korea, 25 Sep 2003, <i>Moon et al. s.n.</i> , KHUS
<i>E. splendens</i> Nakai ex F.Maek.	–	–	Korea, 30 Sep 2002, <i>Moon et al. s.n.</i> , KHUS
<i>Isodon japonicus</i> (Burm.f.) H.Hara	–	–	Korea, 13 Sep 2012, <i>Moon et al. s.n.</i> , KHUS
<i>Lycopus lucidus</i> Turcz. ex Benth.	–	–	Korea, 17 Aug 1999, <i>Hong and Moon s.n.</i> , KHUS
<i>L. lucidus</i> var. <i>hirtus</i> Regel	–	–	Korea, 9 Aug 2010, <i>Kim and Chae s.n.</i> , KHUS
<i>L. maackianus</i> (Maxim. ex Herder) Makino	–	–	Korea, 13 Sep 2001, <i>Moon s.n.</i> , KHUS
<i>Mosla dianthera</i> (Buch.-Ham. ex Roxb.) Maxim.	–	–	Korea, 13 Sep 2012, <i>Moon et al. s.n.</i> , KHUS
<i>Teucrium japonicum</i> Houtt.	–	–	Korea, 31 Jul 2012, <i>Lee et al. s.n.</i> , KB
Verbenaceae			
<i>Duranta erecta</i> L.	+	0.71–1.11 (0.89 ± 0.11)	Indonesia, 8 Jul 2005, without collector 64, KH
<i>Phyla canescens</i> (Kunth) Greene	+	0.61–0.78 (0.69 ± 0.04)	Korea, 14 Jul 2005, <i>Paik et al. s.n.</i> , KH
<i>Verbena bonariensis</i> L.	+	0.57–0.87 (0.71 ± 0.14)	Korea, 9 Aug 2001, <i>Park s.n.</i> , KH
<i>V. officinalis</i> L.	+	0.61–0.88 (0.74 ± 0.09)	Korea, 31 Jul 2001, <i>Im 038498</i> , KH

were collected from herbarium specimen (Table 1). Dried anthers were rehydrated for at least one day in a commercially available wetting agent Agepon or Photo Flo (1:200 in distilled water). Following the dehydration procedures by a graded ethanol series (30, 50, 70, 90, and 100%) twice with 10 min incubation for each step, the completely dehydrated anthers were critical point dried (SPI-13200J-AB; SPI Supplies, West Chester, PA, USA) using CO₂ gas to optimally conserve their natural size and shape. Dried anthers were fixed to aluminum stubs with double adhesive carbon tape for observing the inner locule wall. Stubs were sputter coated with platinum (ion-sputter coater; JFC-1100; JEOL, Tokyo, Japan). Orbicules were observed using a SEM (JEOL JSM-6360) or a Field Emission Scanning Electron Microscope (FE-SEM, S-4700; Hitachi, Tokyo, Japan) at a working distance of 10–15 mm and an accelerating voltage of 5–10 kV. Once orbicules were observed, the size measurements of at least 30 orbicules were ascertained using a microscope with Macnification 2.0 (Orbicule, Leuven, Belgium). The results are presented in Table 1, together with orbicule distribution and voucher specimen information.

The scientific literatures under “orbicules or Ubisch body” in the title or text were screened for information of orbicules from 2012 to September 2017, aiming to investigate the possible correlations between distribution of orbicules and tapetum type in angiosperms. Additional 19 papers (including previously omitted papers; Rao, 1954 and Nilsson and Robyns, 1974) were combined to the data published by Verstraete et al. (2014: papers published until 2012) and the results of this study (Table 1, Appendix 1). The occurrence data of orbicules were re-arranged based on APG IV system with 416 families of 64 orders (Angiosperm Phylogeny Group, 2016; Table 2). In addition, the tapetum type in taxa with orbicule data was investigated to evaluate the relationship between orbicules and tapetum type (Table 3).

Results and Discussion

Orbicules

Rosanoff (1865) observed minute granules of sporopollenin, called orbicules or Ubisch bodies, in *Mimosa* taxa of Fabaceae. In 1962, a palynologist Rowley introduced the term “Ubisch bodies” commemorating the contribution of Gerta von Ubisch (1882–1965) who published a list of taxa with or without orbicules, but currently, the term “orbicules” has been used more generally and widely (Huysman et al., 1998; Verstraete et al., 2014). Orbicules (or Ubisch body) are small particles of sporopollenin that are found in mature anthers as a layer of tiny particles lining the inner locule wall that surrounds

developing pollen grains. Usually orbicules are tiny, spherical granules with individually independent shapes. Based on these features of orbicules, this paper suggests their Korean term as ‘So-gu-sang-che (소구상체)’ (Yamada, 1987; Chung and Kuo, 2005; Moon, 2008).

Orbicule characteristics

Usually orbicules are observable when the pollen develops in the secretory tapetum, and fully matured orbicules are present in anther and inner locule wall. The existence of orbicules can be easily identified in mature anther and inner locule wall (Fig. 1A, B). If orbicules are absent, the surface of the inner locule wall is smooth and in the presence of orbicules, lots of orbicules are evenly distributed on the surface of the inner locule wall (Fig. 1C–H). Previous studies indicate that orbicules are most variable in size, shape and surface sculpturing of taxa. The surface sculpturing of orbicules is correlated with sexine ornamentation of the pollen (Rowley et al., 1959; Hesse, 1986; Huysmans et al., 2000).

The results of this study show that the orbicules are consistently absent in 11 taxa of Lamiaceae and small, spherical orbicules (the average size of less than 1 µm) are densely distributed throughout the anther and inner locule wall in 4 taxa of Verbenaceae (Fig. 1E–H, Table 1). In Lamiaceae, orbicules are present in some taxa of Chloantheae (Raj and El-Ghazaly, 1987), but they are absent in Mentheae, *Galeopsis* L., *Scutellaria* L., and *Stachys* Tourn. ex. L. (Moon et al., 2008a, 2008b, 2008c; Verstraete et al., 2014). In fact, orbicule distribution was intensively studied in Mentheae which showed consistently absence of orbicules in all studied taxa (150 species from 61 genera; Moon et al., 2008a, 2008b, 2008c). In the present study, orbicules are absent in *Elsholtzia* Willd. and *Mosla* (Benth.) Buch.-Ham. ex Maxim. of the tribe Elsholtzieae and *Isodon* (Benth.) Spach of the tribe Ocimeae. These results suggest that absence of orbicules could be a putative synapomorphic condition in Nepetoideae which consists of three tribes Elsholtzieae, Ocimeae and Mentheae (Moon, 2008).

In Verbenaceae, orbicules are present in all studied taxa with similar shape and size that observed in *Junellia thymifolia* (Lag.) Moldenke (Moon, 2008). The orbicules of studied taxa are spherical with a psilate surface and the average size is less than 1 µm. However *Duranta erecta* L. possessed comparatively larger orbicules with over than 1 µm in diameter and *Phyla canescens* (Kunth) Greene has a relatively small orbicules. In addition, two species of *Verbena* have similar orbicules in diameter (Table 1). According to the phylogenetic analysis within the family with respect to the size variation of orbicules, smaller orbicules occurred in advanced groups (Marx

et al., 2010). This result would run counter to Rubiaceae showing the relatively small orbicules as a plesiomorphic character (Verstraete et al., 2011). Therefore, to elucidate the phylogenetic potential of orbicules, further study is necessary to investigate the occurrence of orbicules and their characters in the entire family.

Systematic implication of orbicules and their

relationship to tapetum type

In the present study, the additional orbicule data (11 orders, 14 families, 67 genera, 169 taxa) updated the previous data from Verstraete et al. (2014) and this dataset was interpreted with most recent angiosperm phylogeny (Angiosperm Phylogeny Group, 2016). Most findings from this study support the previous studies like distribution patterns of orbicules at

Fig. 1. Scanning electron microscopy micrographs of anther and inner locule wall. **A, B.** Fully matured open anther. **A.** *Elsholtzia ciliata*. **B.** *Verbena bonariensis*. **C, D.** Inner locule wall without orbicules. **C.** *Agastache rugose*. **D.** *Elsholtzia angustifolia*. **E–H.** Inner locule wall with orbicules. **E.** *Verbena bonariensis*. **F.** *Verbena officinalis*. **G.** *Duranta erecta*. **H.** *Phyla canescens*.

Table 2. Occurrence patterns of orbicules based on APG IV (2016).

APG IV order	Orbicle occurrence pattern			Family information	
	Present	Mixed	Absent	Nonstudied	Total
Amborellales	1	0	0	0	1
Nymphaeales	2	0	0	1	3
Austrobaileyales	2	0	0	1	3
Chloranthales	1	0	0	0	1
Canellales	0	0	1	1	2
Piperales	3	0	0	0	3
Laurales	2	1	1	3	7
Magnoliales	3	1	1	1	6
Acorales	0	0	0	1	1
Alismatales	2	0	1	11	14
Petrosaviales	0	0	0	1	1
Dioscoreales	0	2	0	1	3
Pandanales	2	0	3	0	5
Liliales	4	0	0	6	10
Asparagales	6	0	1	7	14
Arecales	0	0	0	2	2
Poales	5	0	0	9	14
Commelinales	0	0	2	3	5
Zingiberales	0	0	3	5	8
Ceratophyllales	0	0	0	1	1
Ranunculales	3	2	0	2	7
Proteales	2	0	1	1	4
Trochodendrales	0	0	0	1	1
Buxales	0	0	0	1	1
Gunnerales	0	0	0	2	2
Dilleniales	0	0	0	1	1
Saxifragales	3	0	0	12	15
Vitales	0	0	1	0	1
Zygophyllales	0	0	0	2	2
Celastrales	0	0	0	2	2
Oxalidales	1	0	0	6	7
Malpighiales	4	4	0	28	36
Fabales	1	1	0	2	4
Rosales	2	2	1	4	9
Cucurbitales	1	0	1	6	8
Fagales	2	0	0	5	7
Geraniales	0	1	1	0	2
Myrtales	1	0	1	6	8

Table 2. Continued.

APG IV order	Orbicle occurrence pattern			Family information	
	Present	Mixed	Absent	Nonstudied	Total
Crossosomatales	0	0	0	7	7
Picramniales	0	0	0	1	1
Sapindales	2	0	0	7	9
Huerteales	0	0	0	4	4
Malvales	1	1	0	8	10
Brassicales	3	0	5	9	17
Berberidopsidales	1	0	0	1	2
Santalales	0	0	0	7	7
Caryophyllales	6	2	1	29	38
Cornales	4	1	1	1	7
Ericales	5	1	1	15	22
Icacinales	0	0	0	2	2
Metteniusales	0	0	0	1	1
Garryales	0	0	0	2	2
Solanales	1	1	1	2	5
Vahliales	0	0	0	1	1
Gentianales	2	3	0	0	5
Lamiales	7	3	2	12	24
Boraginales	1	0	0	0	1
Aquifoliales	0	1	0	4	5
Asterales	0	0	3	8	11
Escalloniales	0	0	0	2	2
Bruniales	1	0	0	1	2
Paracryphiales	0	0	0	1	1
Dipsacales	1	0	1	0	2
Apiales	1	0	0	6	7
Total number	89	27	34	266	416

Data synchronized with Fig. 2 and numbers indicates number of families. Mixed means the family included both taxa with presence or absence of orbicules.

order level. The occurrence of orbicules is ascertained in Eupteleaceae and Ochnaceae at the family level for the first time (Appendix 1). Family Rosaceae was reported as a consistent occurrence group of orbicules (Verstraete et al., 2014) but recent studies showed the distribution patterns are varying according to the taxa with consistent trend at generic level (Song et al., 2016, 2017a, 2017b).

According to the APG IV system, all angiosperms split into 64 orders and 416 families (Angiosperm Phylogeny Group,

Fig. 2. Modified phylogeny of APG IV from Cole et al. (2017) with proportion data of orbicule occurrence at family level of each order (based on present data and Verstraete et al., 2014; Table 2). Different colors: orange, family with orbicules; blue, family without orbicules; green, family both with and without orbicules; grey, no data available.

2016). Of the 416 families, the number of orbicules investigated for at least one species accounted for about 64% of total 150 families, of which 266 families remained uninvestigated (Fig. 2, Table 2). Orbicules observed in the 150 families are extremely restricted to specific taxa, so the systemic significance of orbicules has yet to be fully elucidated due to insufficient data. Notwithstanding this, the consistent tendency about presence or absence of orbicules at family level counts in 123 families, and in 27 families only, the presence and absence of orbicules are both observed between taxa. Among these 27 families, the consistency of orbicules existence appears in 139 of total 147 genera at generic level, while the presence of orbicules differs from the following genera only: *Aletris* L. (Nartheciaceae), *Coptosapelta* Korth. (Rubiaceae), *Dioscorea* Plum. ex. L. (Dioscoreaceae), *Gentiana* Tourn. ex. L. (Gentianaceae), *Ilex* L. (Aquifoliaceae), *Monodora* Dunal. (Annonaceae), *Passiflora* L. (Passifloraceae), and *Ziziphus* Mill. (Rhamnaceae). The distribution pattern of orbicules may have a systematically consistent tendency (Santos and Mariath, 1999; Schols et al., 2001, 2003; García et al., 2002; Vinckier and Smets, 2003; Verellen et al., 2004; Furness, 2008, 2011; Merckx et al., 2008; Huysmans et al., 2010; Gotelli et al., 2016).

Generally, orbicule development has been reported from ANITA group (the earliest diverged grade), the most primitive taxa, even within pteridophytes, gymnosperms and angiosperms. The occurrence of orbicules is believed to reflect the plesiomorphy of taxa. Thus orbicules are suggested as having a correlation with the primitive, secretory tapetum type (Huysmans et al., 1998). The consistent tendency was reported in Rubiaceae (322 taxa of 163 genera) and Annonaceae (46 taxa of 29 genera) which are extensively studied groups in orbicule distribution of angiosperms. The presence of orbicules in both families is common in the basal group and restricted to the secretory tapetum type (Huysmans et al., 2010; Verstraete et al., 2011). However, orbicule distribution is ascertained in the taxa with amoeboid tapetum type, such as *Abutilon pictum* and *Modiolastrum malviflorum* (Griseb.) K. Schum. of Malvaceae, and *Canscora alata*, *Gentiana acaulis*, and *Swertia perennis* L. of Gentianaceae (Lombardo and Carraro, 1976; Strittmatter and Galati, 2000; Vinckier and Smets, 2003; Galati et al., 2007). The occurrence of orbicules is also observed from the following extremely restricted taxa, with invasive tapetum type as the intermediate form of amoeboid and secretory types: *Aechmea diclamydea* Baker (Bromeliaceae), *Quercus robur* L. (Fagaceae), *Modiolastrum malvifolium* (Griseb.) K. Schum. (Malvaceae) and *Vinca rosea* L. (Apocynaceae). Thus, more diversified approaches are needed to investigate the correlation between tapetum type and

Table 3. Comparison of number of taxa between orbicule occurrences and tapetum type.

Tapetum type	Orbicules present	Orbicule absent
Secretory	240	45
Amoeboid	7	29
Invasive	4	6
Unclear	8	8
No data	891	531
Total	1,150	619

Data based on present data in Appendix 1 and Verstraete et al. (2014).

orbicules existence (El-Ghazaly and Nilsson, 1991; Galati 2003; Rowley and Gabarayeva, 2004; Sajo et al., 2005; Galati et al., 2007). As a result, orbicules are present in 240 of 285 taxa with the secretory tapetum type and orbicules are absent in 29 of 36 taxa with the amoeboid tapetum type, indicating a clear tendency between orbicule development and tapetum type (Table 3).

Orbicules-related studies were conducted in about 36% of total angiosperms at family level (accounts for less than 5% of all taxa at species level) and among the taxa whose orbicules were investigated, tapetum type-identified taxa account for about 18% of total taxa. Under such circumstances, further comparative study with expanded taxa is preferentially required to elucidate the systematic significance and evolutionary tendency on the distribution and characteristics of orbicules as well as their relationships with tapetum type.

According to all of the orbicule characteristics studied up to this date, it can be concluded that the presence of orbicules reflects the primitive characteristics of taxa and the occurrence of orbicules represents the consistent characteristics within specific taxa at family level, thereby orbicule distribution might have predictive value in systematics. In particular, since orbicule development is generally common in secretory tapetum and orbicules develops in amoeboid tapetum in an extremely restricted manner, further study is warranted to clarify the correlation between tapetum type and orbicules.

Acknowledgments

I am grateful to Prof. Dr. Suk-Pyo Hong and members of the Lab. of Plant Systematics at Kyung Hee University for their various help. I thank the directors of the following herbaria for permitting the examination of specimens, either through loans or during visits: KB, KH, KHUS. I also thank the editor

in chief Prof. Dr. Young-Dong Kim and two anonymous reviewers for their constructive comments, which helped me to improve this paper. This research was supported by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education (NRF-2016R1D1A1B04934156).

Conflict of Interest

The author declare that there is no conflict of interest.

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소구상체(Orbicules)의 계통분류학적 검토

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적 요: Orbicule(소구상체)의 분포를 확인하기 위하여 꿀풀과에 속하는 6속 11분류군과 마편초과 3속 4분류군의 약벽을 주사전자현미경(scanning electron microscope)을 이용하여 관찰하였으며, 문헌조사를 통해 현화 식물 내 소구상체의 분포가 갖는 계통분류학적 유용성 및 융단조직 유형과의 관련성에 대해 검토하였다. 그 결과 꿀풀과 분류군들에서는 모두 소구상체가 관찰되지 않았고, 마편초과 분류군들에서는 평균 1 µm 이하, 구형의 소구상체들이 조밀하게 분포하는 것을 살펴볼 수 있었다. 이와 같은 과 수준에서 일관성 있는 소구상체의 분포 특징은 현화식물 전체 orbicules에 대해 조사된 150과 중 123과와 일치하는 것으로 소구상체의 분포 유형 자체가 분류학적 예견적 가치를 지니는 것으로 나타났다. 또한, 융단조직 유형이 확인된 분류군들 중 분비형 융단조직을 갖는 분류군의 약 84%에서 소구상체가 나타나고, 아메바형 융단조직을 갖는 분류군의 약 80%은 소구상체가 나타나지 않는 것으로 조사되어 소구상체의 발달이 융단조직 유형과 밀접한 관련이 있는 것으로 조사되었다. 하지만, 현재까지 수행된 소구상체 연구는 현화식물 전체 416과들 중 150과에 제한되어 있고, 이들 분류군들 중 융단조직 유형은 92과에서만 알려져 있어 소구상체의 기능 및 융단조직 유형과의 유연관계를 규명하기 위해서는 보다 광범위한 분류군을 대상으로 한 연구가 필요한 것으로 나타났다.

주요어: 소구상체, SEM, 융단조직 유형, 현화식물

Appendix 1. Updated orbicule existence data and tapetum type based on APG IV (2016).

Taxon	O	T	Reference: orbicules	Reference: tapetum
Nymphaeales				
Nymphaeaceae				
<i>Nymphaea amazonum</i>	+	S	Zini et al., 2017	Zini et al., 2017
<i>Nymphaea gardneriana</i>	+	S	Zini et al., 2017	Zini et al., 2017
<i>Nymphaea prolifera</i>	+	S	Zini et al., 2017	Zini et al., 2017
Chloranthales				
Chloranthaceae				
<i>Hedyosmum translucium</i>	+	S	Doria et al., 2012	Doria et al., 2012
<i>Hedyosmum crenatum</i>	+	S	Doria et al., 2012	Doria et al., 2012
<i>Hedyosmum racemosum</i>	+	S	Doria et al., 2012	Doria et al., 2012
Asparagales				
Asphodelaceae (=Xanthorrhoeaceae)				
<i>Asphodelus aestivus</i>	+	S	Vardar et al., 2013	Vardar et al., 2013
Poales				
Poaceae				
<i>Briza subaristata</i>	+		Lovisolo and Galati, 2012	
<i>Chaetotropis imberbis</i>	+		Lovisolo and Galati, 2012	
<i>Deyeuxia viridiflavescens</i> var. <i>montevidensis</i>	+		Lovisolo and Galati, 2012	
<i>Distichlis laxiflora</i>	+		Lovisolo and Galati, 2012	
<i>Distichlis spicata</i>	+		Lovisolo and Galati, 2012	
<i>Eleusine tristachya</i>	+		Lovisolo and Galati, 2012	
<i>Glyceria multiflora</i>	+		Lovisolo and Galati, 2012	
<i>Melica brasiliana</i>	+		Lovisolo and Galati, 2012	
<i>Paspalidium geminatum</i> ex <i>P. paludivagum</i>	+		Lovisolo and Galati, 2012	
<i>Stenotaphrum secundatum</i>	+		Lovisolo and Galati, 2012	
Ranunculales				
Eupteleaceae				
<i>Euptelea pleiosperma</i>	+	S	Pérez-Gutiérrez et al., 2016	Pérez-Gutiérrez et al., 2016
<i>Euptelea polyandra</i>	+	S	Pérez-Gutiérrez et al., 2016	Pérez-Gutiérrez et al., 2016
Papaveraceae (incl. Fumariaceae, Pteridophyllaceae)				
<i>Pteridophyllum racemosum</i>	+	S	Pérez-Gutiérrez et al., 2016	Pérez-Gutiérrez et al., 2016
Oxalidales				
Oxalidaceae				
<i>Oxalis adenophylla</i>	+		López & Rosenfeldt, 2015	
<i>Oxalis cinerea</i>	+		López & Rosenfeldt, 2016	
<i>Oxalis compacta</i>	+		López & Rosenfeldt, 2016	
<i>Oxalis enneaphylla</i>	+		López & Rosenfeldt, 2015	
<i>Oxalis erythrorhiza</i>	+		López & Rosenfeldt, 2016	
<i>Oxalis famatinae</i>	+		López & Rosenfeldt, 2016	

Appendix 1. Continued.

Taxon	O	T	Reference: orbicules	Reference: tapetum
<i>Oxalis holosericea</i>	+		López & Rosenfeldt, 2016	
<i>Oxalis hypsophila</i>	+		López & Rosenfeldt, 2016	
<i>Oxalis laciniata</i>	+		López & Rosenfeldt, 2015	
<i>Oxalis loricata</i>	+		López & Rosenfeldt, 2015	
<i>Oxalis micrantha</i>	+		López & Rosenfeldt, 2016	
<i>Oxalis morronei</i>	+		López & Rosenfeldt, 2015	
<i>Oxalis muscoides</i>	+		López & Rosenfeldt, 2016	
<i>Oxalis nahuelhuapiensis</i>	+		López & Rosenfeldt, 2016	
<i>Oxalis pycnophylla</i>	+		López & Rosenfeldt, 2016	
<i>Oxalis squamata</i>	+		López & Rosenfeldt, 2016	
<i>Oxalis subacaulis</i>	+		López & Rosenfeldt, 2016	
<i>Oxalis valdiviensis</i>	+		López & Rosenfeldt, 2016	
Malpighiales				
Ochnaceae (incl. Medusagynaceae, Quiinaceae)				
<i>Medusagyne oppositifolia</i>	+	S	Furness, 2014	Furness, 2014
Rosales				
Rhamnaceae				
<i>Colletia paradoxa</i>	+	S	Gotelli et al., 2016	Gotelli et al., 2016
<i>Condalia buxifolia</i>	+	S	Gotelli et al., 2016	Gotelli et al., 2016
<i>Cryptandra tomentosa</i>	+	S	Gotelli et al., 2016	Gotelli et al., 2016
<i>Gouania ulmifolia</i>	-	S	Gotelli et al., 2016	Gotelli et al., 2016
<i>Hovenia dulcis</i>	+	S	Gotelli et al., 2016	Gotelli et al., 2016
<i>Kentrothamnus weddellianus</i>	+	S	Gotelli et al., 2016	Gotelli et al., 2016
<i>Retanilla patagonica</i>	+	S	Gotelli et al., 2016	Gotelli et al., 2016
<i>Scutia buxifolia</i>	-	S	Gotelli et al., 2016	Gotelli et al., 2016
<i>Siegfriedia darwinoides</i>	+	S	Gotelli et al., 2016	Gotelli et al., 2016
<i>Stenanthemum humile</i>	+	S	Gotelli et al., 2016	Gotelli et al., 2016
<i>Ziziphus jujuba</i>	-	S	Gotelli et al., 2016	Gotelli et al., 2016
<i>Ziziphus mistol</i>	+	S	Gotelli et al., 2016	Gotelli et al., 2016
<i>Ziziphus mucronata</i>	-	S	Gotelli et al., 2016	Gotelli et al., 2016
<i>Ziziphus spinossisima</i>	+	S	Gotelli et al., 2016	Gotelli et al., 2016
Rosaceae				
<i>Adenostoma fasciculatum</i> var. <i>fasciculatum</i>	+		Song et al., 2016	
<i>Adenostoma fasciculatum</i> var. <i>obtusifolium</i>	+		Song et al., 2016	
<i>Adenostoma sparsifolium</i>	+		Song et al., 2016	
<i>Chamaebatiaria millefolium</i>	+		Song et al., 2016	
<i>Gillenia trifoliata</i>	+		Song et al., 2016	
<i>Lyonothamnus floribundus</i> ssp. <i>floribundus</i>	+		Song et al., 2016	
<i>Lyonothamnus floribundus</i> ssp. <i>aspleniifolius</i>	+		Song et al., 2016	

Appendix 1. Continued.

Taxon	O	T	Reference: orbicules	Reference: tapetum
<i>Sorbaria arborea</i> var. <i>arborea</i>	+		Song et al., 2016	
<i>Sorbaria arborea</i> var. <i>glabrata</i>	+		Song et al., 2016	
<i>Sorbaria arborea</i> var. <i>subtomentosa</i>	+		Song et al., 2016	
<i>Sorbaria grandiflora</i>	+		Song et al., 2016	
<i>Sorbaria kirilowii</i>	+		Song et al., 2016	
<i>Sorbaria rhoifolia</i>	+		Song et al., 2016	
<i>Sorbaria sorbifolia</i> var. <i>sorbifolia</i>	+		Song et al., 2016	
<i>Sorbaria sorbifolia</i> var. <i>stellipila</i>	+		Song et al., 2016	
<i>Sorbaria sorbifolia</i> f. <i>incerta</i>	+		Song et al., 2016	
<i>Sorbaria tomentosa</i> var. <i>tomentosa</i>	+		Song et al., 2016	
<i>Sorbaria tomentosa</i> var. <i>angustifolia</i>	+		Song et al., 2016	
<i>Spiraeanthus schrenkianus</i>	+		Song et al., 2016	
<i>Neillia affinis</i> var. <i>affinis</i>	+		Song et al., 2017a	
<i>Neillia affinis</i> var. <i>longisepala</i>	+		Song et al., 2017a	
<i>Neillia affinis</i> var. <i>pauciflora</i>	+		Song et al., 2017a	
<i>Neillia affinis</i> var. <i>polygyna</i>	+		Song et al., 2017a	
<i>Neillia gracilis</i>	+		Song et al., 2017a	
<i>Neillia rubiflora</i>	+		Song et al., 2017a	
<i>Neillia serratisepala</i>	+		Song et al., 2017a	
<i>Neillia sinensis</i> var. <i>sinensis</i>	+		Song et al., 2017a	
<i>Neillia sinensis</i> var. <i>hypomalaca</i>	+		Song et al., 2017a	
<i>Neillia sparsiflora</i>	+		Song et al., 2017a	
<i>Neillia thibetica</i> var. <i>thibetica</i>	+		Song et al., 2017a	
<i>Neillia thibetica</i> var. <i>lobata</i>	+		Song et al., 2017a	
<i>Neillia thyrsiflora</i>	+		Song et al., 2017a	
<i>Neillia uekii</i>	+		Song et al., 2017a	
<i>Physocarpus alternans</i>	+		Song et al., 2017a	
<i>Physocarpus amurensis</i>	+		Song et al., 2017a	
<i>Physocarpus capitatus</i>	+		Song et al., 2017a	
<i>Physocarpus insularis</i>	-		Song et al., 2017a	
<i>Physocarpus malvaceus</i>	+		Song et al., 2017a	
<i>Physocarpus monogynus</i>	+		Song et al., 2017a	
<i>Physocarpus opulifolius</i>	+		Song et al., 2017a	
<i>Spiraea chamaedryfolia</i>	-		Song et al., 2017a	
<i>Spiraea pseudocrenata</i>	-		Song et al., 2017a	
<i>Spiraea pubescens</i>	-		Song et al., 2017a	
<i>Stephanandra chinensis</i>	+		Song et al., 2017a	
<i>Stephanandra incisa</i> var. <i>incisa</i>	+		Song et al., 2017a	
<i>Stephanandra incisa</i> var. <i>quadrifissa</i>	+		Song et al., 2017a	
<i>Stephanandra tanakae</i>	+		Song et al., 2017a	

Appendix 1. Continued.

Taxon	O	T	Reference: orbicules	Reference: tapetum
<i>Aruncus aethusifolius</i>	-		Song et al., 2017b	
<i>Aruncus dioicus</i> var. <i>dioicus</i>	-		Song et al., 2017b	
<i>Aruncus dioicus</i> var. <i>acuminatus</i>	-		Song et al., 2017b	
<i>Aruncus dioicus</i> var. <i>kamtschaticus</i>	-		Song et al., 2017b	
<i>Aruncus vulgaris</i>	-		Song et al., 2017b	
<i>Holodiscus discolor</i>	-		Song et al., 2017b	
<i>Holodiscus dumosus</i>	-		Song et al., 2017b	
<i>Holodiscus fissus</i>	-		Song et al., 2017b	
<i>Holodiscus microphyllus</i>	-		Song et al., 2017b	
<i>Kelseya uniflora</i>	-		Song et al., 2017b	
<i>Luetkea pectinata</i>	+		Song et al., 2017b	
<i>Pentactina rupicola</i>	-		Song et al., 2017b	
<i>Petrophytum caespitosum</i>	-		Song et al., 2017b	
<i>Sibiraea angustata</i>	+		Song et al., 2017b	
<i>Sibiraea croatica</i>	+		Song et al., 2017b	
<i>Sibiraea laevigata</i>	+		Song et al., 2017b	
<i>Spiraea salicifolia</i>	-		Song et al., 2017b	
<i>Spiraea fritschiana</i>	-		Song et al., 2017b	
<i>Spiraea japonica</i>	-		Song et al., 2017b	
<i>Spiraea microgyna</i>	-		Song et al., 2017b	
<i>Spiraea miyabei</i>	-		Song et al., 2017b	
<i>Spiraea stevenii</i>	-		Song et al., 2017b	
<i>Spiraea trichocarpa</i>	-		Song et al., 2017b	
<i>Spiraea blumei</i>	-		Song et al., 2017b	
<i>Spiraea cantoniensis</i>	-		Song et al., 2017b	
<i>Spiraea chamaedryfolia</i>	-		Song et al., 2017b	
<i>Spiraea chinensis</i>	-		Song et al., 2017b	
<i>Spiraea insularis</i>	-		Song et al., 2017b	
<i>Spiraea pseudo-crenata</i>	-		Song et al., 2017b	
<i>Spiraea pubescens</i>	-		Song et al., 2017b	
<i>Spiraea trilobata</i>	-		Song et al., 2017b	
<i>Xerospiraea hartwegiana</i>	+		Song et al., 2017b	
Malvales				
Malvaceae				
<i>Adansonia digitata</i>	-	S	Rao, 1954	Rao, 1954
<i>Bombax malabaricum</i>	-	S	Rao, 1954	Rao, 1954
<i>Corchorus</i>	-	S	Lattar et al., 2014	Lattar et al., 2014
<i>Corchorus argutus</i>	-	S	Lattar unpubl. data	Lattar unpubl. data
<i>Corchorus hirtus</i>	-	S	Lattar unpubl. data	Lattar unpubl. data
<i>Eriodendron anfractuosum</i>	-	S	Rao, 1954	Rao, 1954

Appendix 1. Continued.

Taxon	O	T	Reference: orbicules	Reference: tapetum
<i>Eriolaena candollei</i>	-	A	Tang et al., 2009	Tang et al., 2009
<i>Excentrodendron hsienmu</i>	-	S	Tang et al., 2006	Tang et al., 2006
<i>Heliocarpus</i>	+	S	Lattar et al., 2014	Lattar et al., 2014
<i>Heliocarpus popayanensis</i>	+	S	Lattar unpubl. data	Lattar unpubl. data
<i>Luehea</i> sp.	+	S	Lattar et al., 2014	Lattar et al., 2014
<i>Luehea candicans</i>	+	S	Lattar unpubl. data	Lattar unpubl. data
<i>Luehea divaricata</i>	+	S	Lattar et al., 2012	Lattar et al., 2012
<i>Ochroma lagopus</i>	-	S	Rao, 1954	Rao, 1954
<i>Pachira aquatica</i>	-	S	Rao, 1954	Rao, 1954
<i>Quararibea asymmetrica</i>	+		Nilsson & Robyns, 1974	
<i>Quararibea cordata</i>	+		Nilsson & Robyns, 1974	
<i>Quararibea lasiocalyx</i>	+		Nilsson & Robyns, 1974	
<i>Quararibea ochocalyx</i>	+		Nilsson & Robyns, 1974	
<i>Triumfetta</i>	+	S	Lattar et al., 2014	Lattar et al., 2014
<i>Triumfetta semitriloba</i>	+	S	Lattar unpubl. data	Lattar unpubl. data
Gentianales				
Rubiaceae				
<i>Cephalanthus glabratus</i> (Spreng.) K. Schum.	+	S	Verellen et al., 2007	Romero et al., 2017
Lamiales				
Lamiaceae				
<i>Agastache rugosa</i>	-		Present study	
<i>Elsholtzia angustifolia</i>	-		Present study	
<i>Elsholtzia ciliata</i>	-		Present study	
<i>Elsholtzia minima</i>	-		Present study	
<i>Elsholtzia splendens</i>	-		Present study	
<i>Isodon japonicus</i>	-		Present study	
<i>Lycopus lucidus</i> var. <i>hirtus</i>	-		Present study	
<i>Lycopus lucidus</i> var. <i>lucidus</i>	-		Present study	
<i>Lycopus maackianus</i>	-		Present study	
<i>Mosla dianthera</i>	-		Present study	
<i>Teucrium japonicum</i>	-		Present study	
Verbenaceae				
<i>Duranta erecta</i>	+		Present study	
<i>Phyla canescens</i>	+		Present study	
<i>Verbena bonariensis</i>	+		Present study	
<i>Verbena officinalis</i>	+		Present study	

Notes: O=orbicules; + = presence; - = absence, T=tapetum; A=amoeboid tapetum, S=secretory tapetum.