

COMPARATIVE ANATOMY OF *MYOSOTON AQUATICUM* AND *STELLARIA MEDIA* AND ITS SYSTEMATIC SIGNIFICANCE

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Abstract

To provide evidence insight into the phylogenetic position of the *Myosoton* Monch., the anatomy of *Myosoton aquaticum* and *Stellaria media* were comparatively observed by light microscopy and scanning electron microscope (SEM). The anatomy of *M. aquaticum* and *St. media* is that (1) Root is composed of uniseriate epidermis, parenchyma cortex, endodermis, pericycle and central cylinder, and primary xylem of root is tetrarch. (2) Stem epidermis is arranged in closeness and parallel, and has distinct shuttle-shape tuber. (3) Leaf is dorsiventral mesophyll with palisade layer and spongy parenchyma. (4) Anomocytic type of stomatas are found in the epidermis of the leaf, stem and sepal. (5) Pollen is single and spheroidal. A number of germ pores are completely sunken, papillate and polypantoporate. (6) Crassuliod type of seeds have simple papillae and no strophiole is observed. Seed coat is rounded shape minute secondary sculpturing with an outer surface formed by foveolate epidermal walls. (7) From the globular floral primordium develop to complete bisexual flower, the process of floral initiation is nearly the same. The result supports that: the higher similarity in organ anatomy and floral ontogeny of *M. aquaticum* and *St. media*, justifies it is appropriate that *Myosoton aquaticum* belongs to *Stellaria*.

Key words: *Myosoton aquaticum*; *Stellaria media*; Anatomy; Floral ontogeny; systematic.

Introduction

According to China of flora, *Myosoton* Monch. is a monotypic genus of Caryophyllaceae (Wu & Raven, 2002). It is mainly distributed in temperate Asia and Europe. *Myosoton aquaticum*, giant chickweed, is used in traditional Chinese herbaceous medicine, as wild vegetable in seedling, and also affects crop's growth throughout the farmland.

The circumscription of giant chickweed has long been controversial. Linnaeus (1753) placed giant chickweed into the genera *Cerastium*, on basis of the number of stamens and carpels; *Stellaria* with ten stamens and three carpels, and *Cerastium* with ten stamens and five carpels. Scopoli (1772) transferred disposition the species from *Cerastium* to *Stellaria*, as expressing its natural affinity and morphological characteristics investigation, and named it *Stellaria aquaticum*. Moench (1794) placed the species in single genera *Myosoton*, and named it *Myosoton aquaticum*, because of it is the characteristic that peduncular foliar axillary terminal, petal profound bifid, calyces duplex longitudinal, capsule pendulous. In the subsequent century, taxonomist argued about giant chickweed's taxonomic status in accordance with the Scopoli's or Moench's view (Forbes & Hemsley 1886; Schaffner, 1927; Cronquist, 1988). At present, giant chickweed is placed in single genera *Myosoton* in APGIII, Flora of Europe and Flora of India. However, giant chickweed is placed in genera *Stellaria* in Flora of Japan (Ohwi & Kitagawa, 1983) and Flora of Taiwan (Ying, 1996). That is admits of dispute about giant chickweed's taxonomic status all over the world.

Features of vegetative organ, reproductive organ and floral initiation are key for researching the origin and evolution of angiosperms (Endress & Stumpf, 1991; Sun *et al.*, 1998), and can be put the date to assist systematic

study. A study about the vegetative organ anatomy of plant was conducted in order to supply systematics date on Caryophyllaceae (Taia & Ismael, 1994; Schweingruber 2007), such as *Minuartia* (Gucel, 2013; Sahar & Sadeq, 2014), *Silene* (Sahreen *et al.*, 2010) and *Saponariakotschyana* (Ataslar, 2004). Based on the leaf length, number of flowers per peduncle, calyx length, and other morphological traits, numerical taxonomic analysis was observed on *Stellaria* and other genus of Caryophyllaceae (Prentice, 1984, Emery & Chinnappa 1994, Chen *et al.*, 2012). Reproductive organ was also hot research point in Caryophyllaceae systematic. Seed micromorphology for classification has been used in systematic studies on Caryophyllaceae, for instance, *Arenaria* (Wyatt, 1984), *Silene* (Greuter 1995, Hong *et al.*, 1999), *Minuartia* (Celebioglu *et al.*, 1983), *Moehringia* (Kapil *et al.*, 1980, Hind, 1988, Minuto *et al.*, 2006), *Gypsophila* (Elham *et al.*, 2011), *Stellaria* (Mahdavi *et al.*, 2012) and Caryophyllaceae species from Turkey (Yildiz, 2002). Pollen morphology of Caryophyllaceae also is reported by Vishnu & Gupta (1964), Ghazanfer (1984), Eisawi (1989), Kaplan (2008) and Ataslar (2009). Thomson (1942) at earliest completely described the floral morphology of Caryophyllaceae. Several studies of floral traits of the Caryophyllaceae have been published, and examples can be found such as floral ontogeny (Lister 1883, Warren & Elizabeth, 2000), floral vascular bundles (Saunders 1935; Li *et al.*, 2013).

In our preliminary work, we compared the floral vascular bundles of *M. aquaticum* and *St. media*, and concluded that *M. aquaticum* belongs to *Stellaria*, based on the high similarity in vascular anatomy (Li *et al.*, 2013). In current study, we comparatively narrated organ anatomy and floral ontogeny of *M. aquaticum* and *St. media* with the hope of clarifying the unclear position of *M. aquaticum* within the family.

Materials and Methods

M. aquaticum and *St. media* were continuously collected from school garden in Guizhou University, Guizhou province, China, at February-April 2015.

Cross sections were taken from the middle part of fresh vegetative organ and were fixed immediately in FAA (5 parts formalin: 5 parts acetic acid: 90 parts 50% alcohol) for at least 48 hours (Kadriye & Canan, 2015). Then dehydrated in an alcohol, embedded in paraffin, sectioned at a thickness of 10µm cross-sections by rotary microtome, stained by 1% safranin and 0.5% methylene blue and embedded in Canadian Balm. Figures of cross-sections were drawn by using Olympus BX51.

The maturation stage of pollen and seed were nature dehydrated. The different developmental stage of inflorescences were fixed in PBS buffer. Just before dissection, the flower buds were transferred to 95% alcohol through a series of concentrations of alcohol. Bracts and larger floral organs were removed from each piece under a dissection microscope (Liu *et al.*, 2014). Appropriate dissection was followed by further dehydration through an alcohol-isoamyl acetate series and critical point drying with CO₂. The seed and inflorescences were then mounted on aluminum stubs and coated with gold-palladium before they were observed under the Hitachi S-3400SEM.

Results

Root: The root of *M. aquaticum* and *St. media* are composed of periderm, epidermis, cortex, endodermis, pericycle and central cylinder (Fig. 1a, Fig. 2a). At outer most layer is periderm, then the layer is

uniseriate epidermis. Then the cortex make up of parenchyma cells. The endodermis is situated beneath the cortex, which performs the functions of sustaining and defense of the central cylinder and enable to transit water. Internally unstratified pericycle formed small cells, with thin, pecto-cellulosic walls. On the internal face of the pericycle is located the central cylinder, that consists of vascular bundles and pith. There are two areas: one part of area is protoxylem and metaxylem that the xylem conducting fascicles, another part of area is protophloem and metaphloem that phloem conducting fascicles. The primary xylem of the root is tetrarch (Fig. 1b, Fig. 2b). The medular rays are placed between the xylem conducting fascicles and the phloemic ones. The duct in protoxylem is spiral vessel or ranged vessel, and small lumen. The duct in metaxylem is reticulate vessel or pitted vessel, and big lumen (Fig. 1c d, Fig. 2c d).

Stem: The stem of *M. aquaticum* and *St. media* revealed a uniseriate epidermis covered by a cuticle with uniseriate trichomes. Epidermis cells were arranged closeness and parallel, and have distinct shuttle-shape tuber. Anticlinal wall is waving undular and sunk groove. Internally to the epidermis lies the cortex, which composes 5 to 6 layers of cortical parenchyma cells. Follow-up of it is a circular collateral vascular bundles surrounding the cortex (Fig. 3a, Fig. 4a). The secondary vascular bundles tissue is formed with an outer phloem layer and an innermost xylem produced by the vascular cambium formed. In the innermost, the pith consists of layers of parenchyma cells. (Fig. 3b, Fig. 4b) The duct in protoxylem is spiral vessel or ranged vessel, and small lumen. The duct in metaxylem is reticulate vessel or pitted vessel, and big lumen (Fig. 3c d, Fig. 4c d).

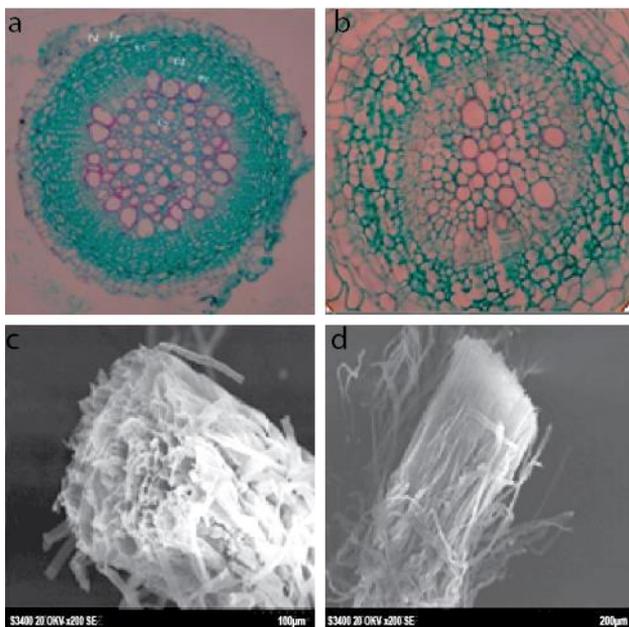


Fig. 1. Anatomy of *M. aquaticum* root; a,b cross section of *M. aquaticum* root in light microscopy, a showing root structure integrity (×200), b showing primary xylem (×400); c,d cross section of *St. media* root in SEM, c showing root duct partly (bar=100µm), d showing root duct periphery (bar=200µm); Cr, cortex; Cy, central cylinder; Ep, epidermis; Ed, endodermis; Pd, periderm; Pe, pericycle

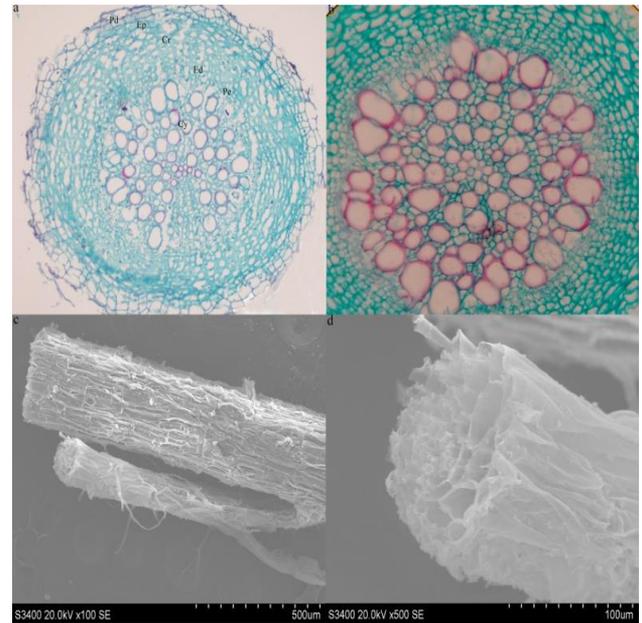


Fig. 2. Anatomy of *St. media* root; a,b cross section of *St. media* root in light microscopy, a showing root structure integrity (×200), b showing primary xylem (×400); c,d cross section of *St. media* root in SEM, c showing root duct periphery (bar=500µm), d showing root duct partly (bar=100µm); Cr, cortex; Cy, central cylinder; Ep, epidermis; Ed, endodermis; Pd, periderm; Pe, pericycle.

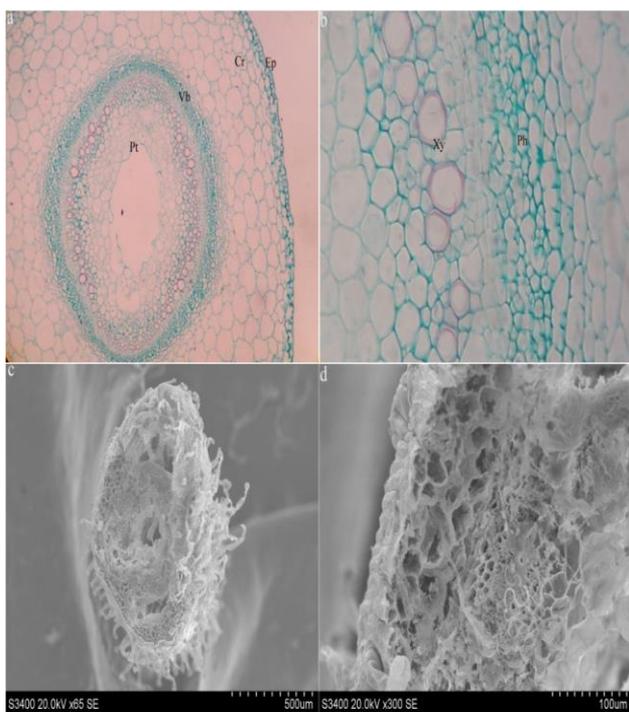


Fig. 3. Anatomy of *M. aquaticum* stem; a,b cross section of *St. media* stem in light microscopy, a showing stem structure integrity ($\times 100$), b showing vascular bundles ring ($\times 200$); c d cross section of *M. aquaticum* stem in SEM, c showing stem duct integrity (bar=500um), d showing stem duct partly (bar=100um); Ep, epidermis; Vb, vascular bundles; Cr, cortex; Pt, pith; Ph, phloem; Xy, xylem.

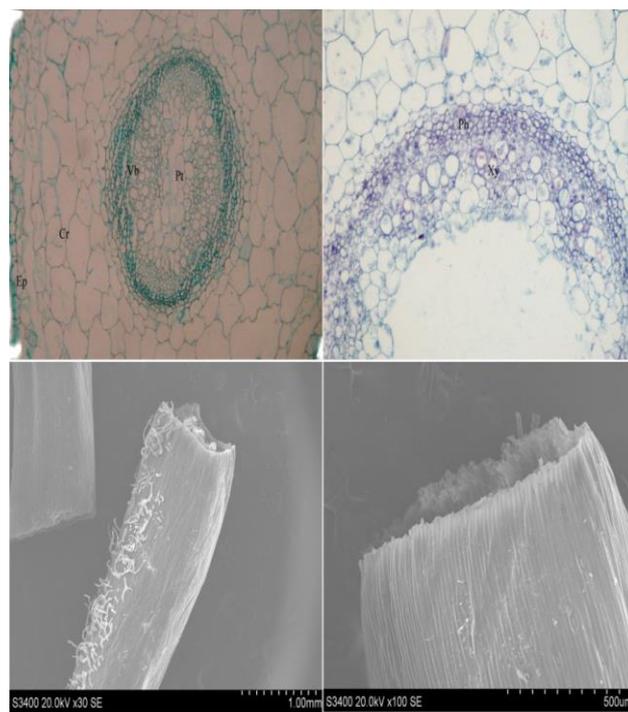


Fig. 4. Anatomy of *St. media* stem; a,b cross section of *St. media* stem in light microscopy, a showing stem structure integrity ($\times 100$), b showing vascular bundles ring ($\times 200$); c d cross section of *St. media* stem in SEM, showing duct in stem, c showing stem duct periphery (bar=1mm), d showing stem duct partly (bar=500um); Ep, epidermis; Vb, vascular bundles; Cr, cortex; Pt, pith; Ph, phloem; Xy, xylem.

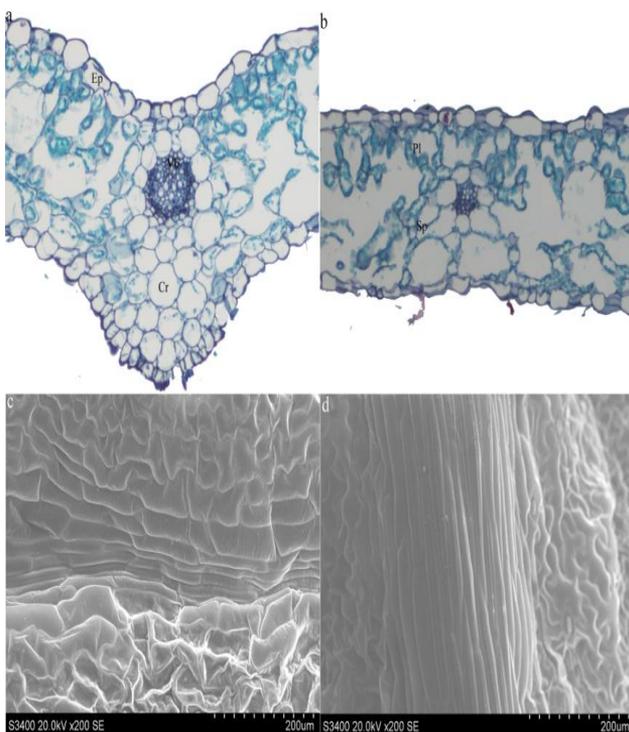


Fig. 5. Anatomy of *M. aquaticum* leaf; a,b cross section of *M. aquaticum* leaf in light microscopy ($\times 200$), a showing main vein, b showing mesophyll cells; c, cross section of leaf above epidermis in SEM (bar=200um), showing vein in epidermis; d cross section of leaf lower epidermis in SEM (bar=200um), showing vein in epidermis; Ep, epidermis; Vb, vascular bundles; Cr, cortex; Pl, palisade layer; Sp, spongy parenchyma.

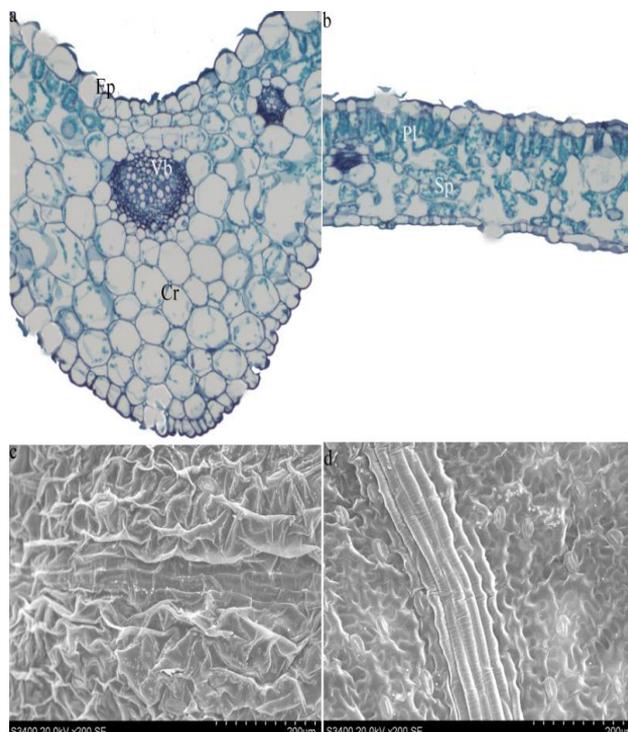


Fig. 6. Anatomy of *St. media* leaf; a,b cross section of *St. media* leaf in light microscopy ($\times 200$), a showing main vein, b showing mesophyll cells; c, cross section of leaf above epidermis in SEM (bar=200um), showing vein in epidermis; d cross section of leaf lower epidermis in SEM (bar=200um), showing vein in epidermis; Ep, epidermis; Vb, vascular bundles; Cr, cortex; Pl, palisade layer; Sp, spongy parenchyma.

Leaf: The leaves of *M. aquaticum* and *St. media* have dorsiventral mesophyll with a palisade layer immediately beneath the adaxial epidermis and spongy parenchyma immediately beneath the palisade layer (Fig. 5b, Fig. 6b). Cross-section of the midrib region of leaf displayed a uniseriate epidermis below a vascular system composed of xylem on the adaxial side and phloem on the abaxial side, and a lot of fundamental parenchyma at its around (Fig. 5a, Fig. 6a). In midrib sclerenchymas and collenchymas are not found. The vein is stripe structure, and the epidermis cells are anomocytic (Fig. 5c d, Fig. 6c d). It's similar that anatomical structure of *M. aquaticum* and *St. media* leaves without the thickness of palisade and spongy parenchyma

Stomata: The stomata of *M. aquaticum* and *St. media* belong to anomocytic type (ranunculaceous type) (Fig.7). Stomata guard cells are surrounded by several cells that are the same morphology of epidermal cells. The stomata are distributed randomly above and lower the epidermis(Fig.7).There are no difference between the polar and equatorial diameters across the position analyzed except stomatal density. The stomata of *M. aquaticum* and *St. media* are observed on the epidermis of the stem and sepal at first (Fig.8). Stem stomatas belong to diacytic type, sepal stomatas belong to anomocytic type.

Pollen: The pollen grains of *M. aquaticum* and *St. media* are single, spheroidal, apolar and radially symmetrical(Fig.9a b). A number of germ pores are found in the pollen coat. The pores are completely sunken, papillate and polyaperturate (Fig.9c d).The pollen extine sculptures obviously spinulose to papillate and sparsely perforate around pores (Fig.9c d).There are just difference between pores in diameter and circular.

Seed: The seed of *M. aquaticum* and *St. media* show a dark brown and dull surface when mature. Seed shape can be referred to as the crassuloid type (Fig.10a b). Seed coat cells are a cell of rounded shape minute secondary sculpturing, showing 10-15 teeth on each side with irregular or in some case V-shape contours resembling a

jigsaw puzzle, and with an outer surface formed by foveolate epidermal walls (Fig. 10c d). Simple papillae are found on outer surface. The dorsal ridge are recognized as complete ridge according to longer and can almost cover the whole dorsal margin (Fig. 10e f). No strophiole are found on the surface seed. It's the distinction that is their seed size.

Floral ontogeny: *M. aquaticum* and *St. media* flowers terminal or axillary. Bracts leaf like, margin glandular hairy. Pedicel 1-2cm, slender, densely glandular hairy. Sepals 5, ovate-lanceolate, 4-5mm. Petals 5, white, deeply bifid, lobes lanceolate, 3-3.5mm. Stamens 10, stamens shorter than petals. Ovary 1-loculed, ovules numerous. Styles linear. Capsule ovoid, dehiscing nearly to 1/2 with 5 shortly bifid valves.

The *M. aquaticum* and *St. media* floral primordium are globular, which locates in the axis of the bract (Fig. 11a, 12a). As the flower primordium becomes larger, five sepal primordia are initiated in a 2/5spiral sequence (Fig. 11b, 12b). Between the two bracts, the abaxial sepal primordium develops first. Then in inner first sepal primordium, the second and third sepal primordium initiate in succession (Fig.11c,12c). The fourth sepal primordium locate between the first and second. The fifth sepal primordium develops between the second and third. The initiation of the petal primordia is sequential following the development of the sepals (Fig.11d,12d). The petal primordia alternate with the sepal in the outer axis. The stamens primordia lags behind the petal primordia. After the petals initiate and grow, gradually widening and elongating, the stamens begin development (Fig.11e f,12e f). Following petal and stamen primordia initiation, the gynoecium develops as a global primordium in the floral apex (Fig.11g, 12g). The floral apex becomes gradually of sunken and three transverse slits are visible adaxially of the bulges (Fig.11g h, 12g h). The three bulges are three carpel margins, later in development the carpel margins abut the margins of the other carpel. With the carpel deep development, the style start to grow (Fig.11i j k, 12i j k). At topmost of style, the stigma is closed and linear (Fig.11 l, 12 l). The *M. aquaticum* and *St. media* flower grow some time, develop to complete bisexual flower.

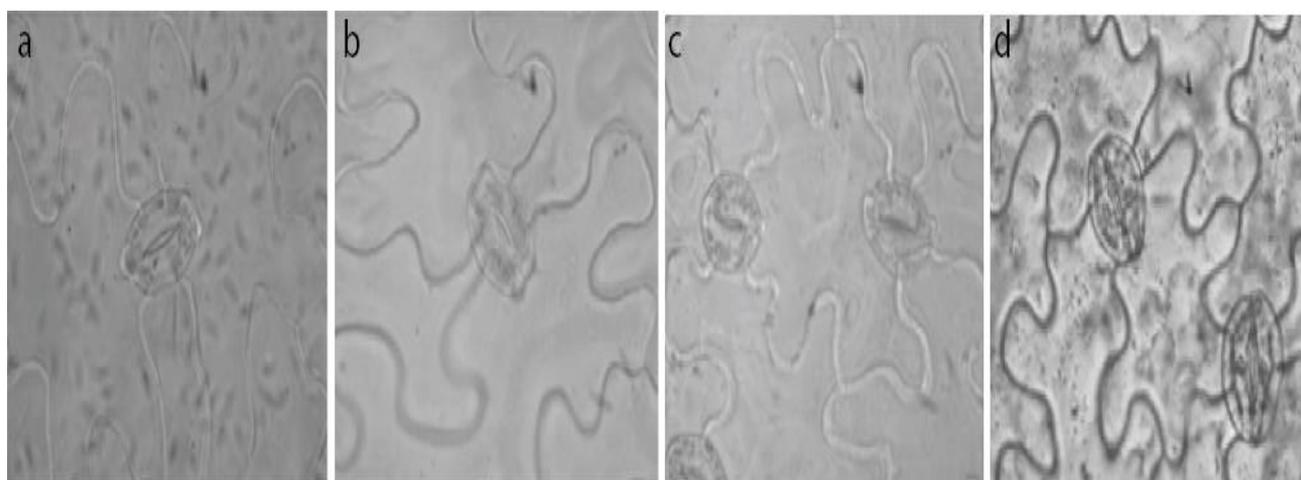


Fig. 7. The leaf stomata of *M. aquaticum* and *St. media* ($\times 400$); a the above epidermis of *M. aquaticum*; b the above epidermis of *St. media*; c the lower epidermis of *M. aquaticum*; d the lower epidermis of *St. media*.

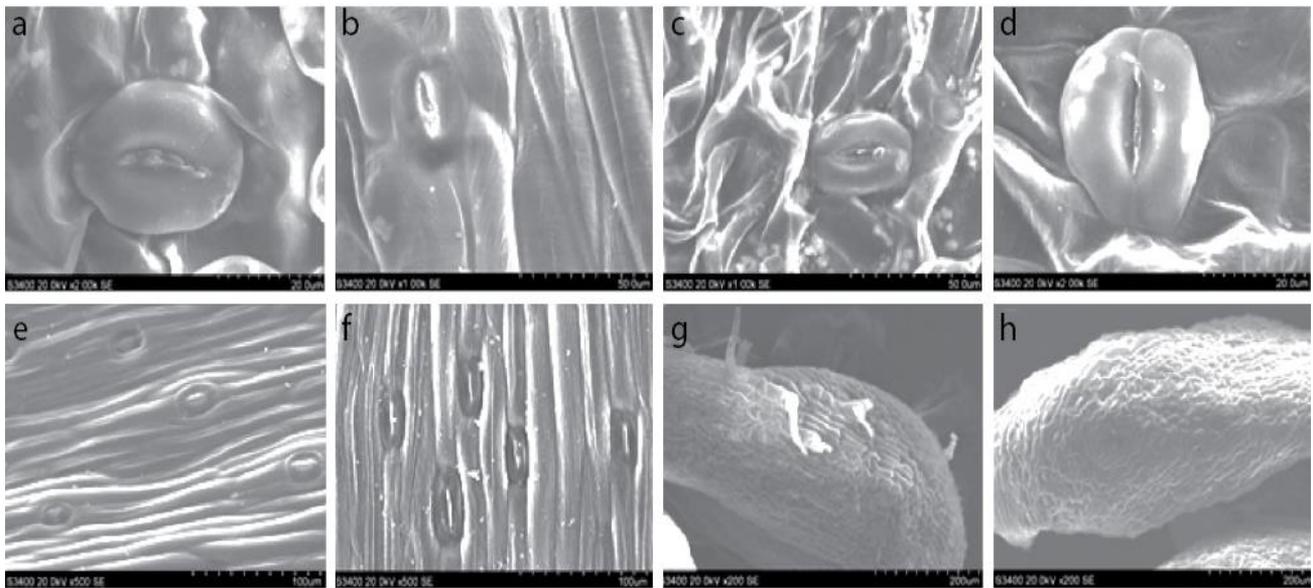


Fig.8. SEM photos of displace stomata of *M. aquaticum* and *St. media*, a in the above epidermis of *M. aquaticum* (bar=20um); b in the above epidermis of *St. media* (bar=50um); c in the lower epidermis of *M. aquaticum* (bar=50um); d in the lower epidermis of *St. media* (bar=20um); e in stem of *M. aquaticum* (bar=100um); f in stem of *St. media*(bar=100um); g in the sepal of *M. aquaticum* (bar=200um); h in the sepal of *St. media* (bar=200um).

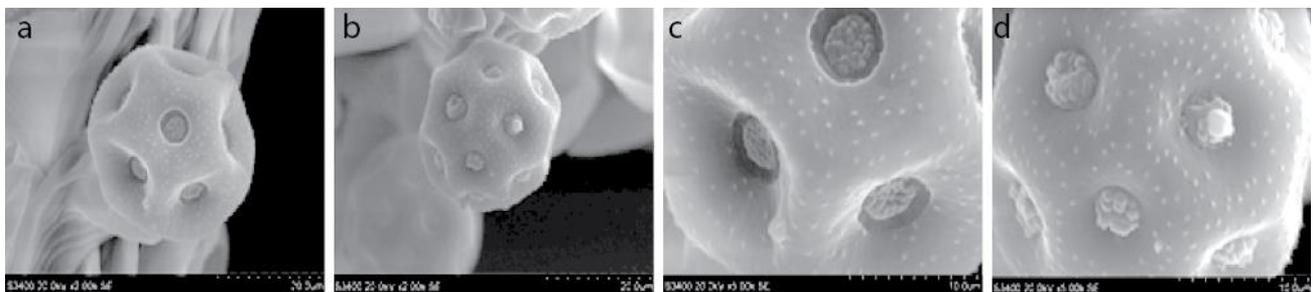


Fig. 9. SEM micrographs of pollen of *M. aquaticum* and *St. media*, a *M. aquaticum* pollen grain (bar=20um); b *St. media* pollen grain(bar=20um); c *M. aquaticum*, showing detail of exine sculpture at aperture(bar=10um); d *St. media*, showing detail of exine sculpture at aperture (bar=10um).

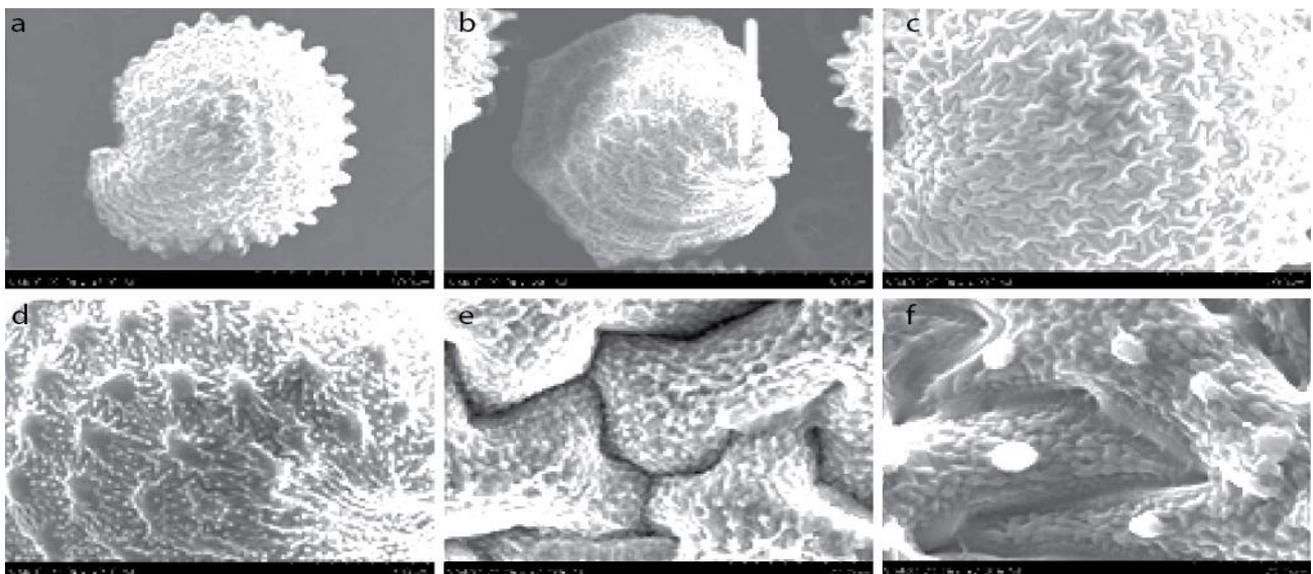


Fig.10. SEM micrographs of seeds of *M. aquaticum* and *St. media*, a mature *M. aquaticum* seed (bar=500um); b mature *St. media* seed (bar=500um); c *M. aquaticum*, showing detail of seed coat sculpture (bar=200um); d *St. media*, showing detail of seed coat sculpture (bar=200um); e *M. aquaticum*, showing detail of papillae in outer surface (bar=20um); f *St. media*, showing detail of papillae in outer surface (bar=20um).

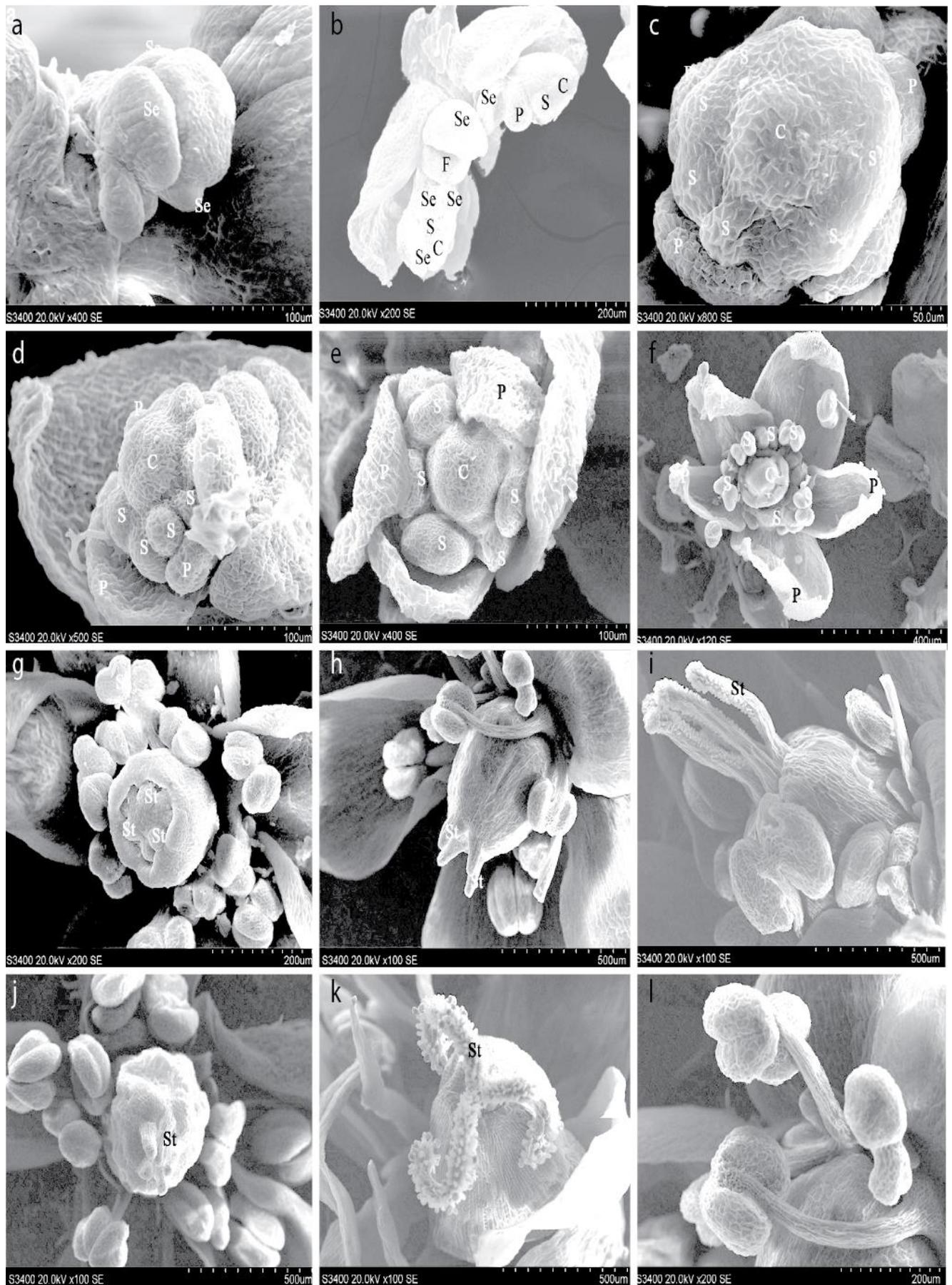


Fig. 11. Floral ontogeny of *M. aquaticum* at various floral stages under SEM. a, b Floral primodium begins to differentiate and five sepal primordia initiate in 2/5 spiral sequence; c,d,e,f Petal primordia initiate and Stamen primordia initiate, sepals removed; g,h,i,j,k Carpel primordia initiate, style initiate and style number; l mature anther.

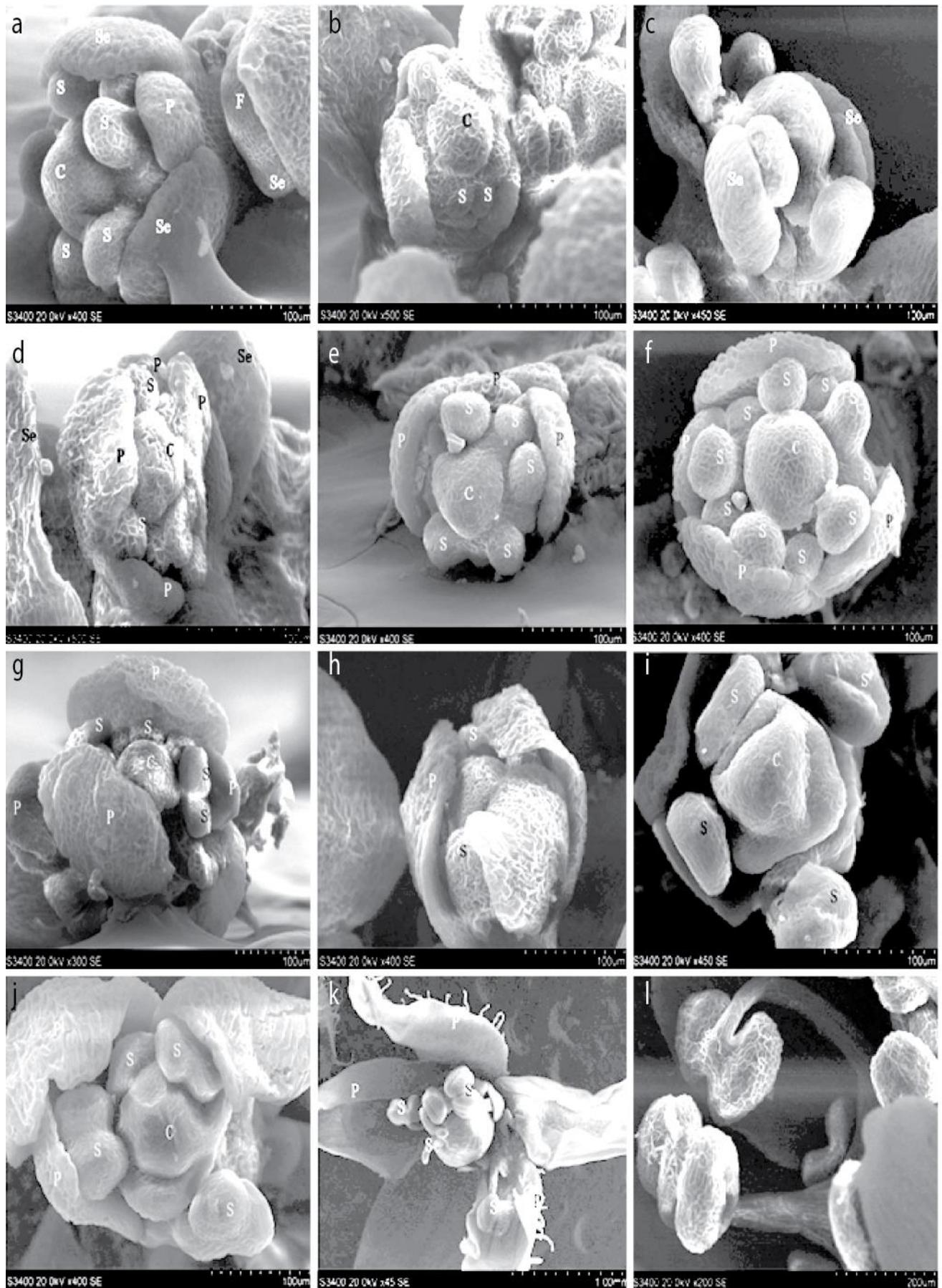


Fig.12. Floral ontogeny of *St. media* at various floral stages under SEM. a,b,c Floral primordium begins to differentiate and five sepal primordia initiate in 2/5 spiral sequence; d,e,f,g,h Petal primordia initiate and Stamen primordia initiate, sepals removed; i,j,k Carpel primordial initiate, floral apex sunken and style initiate; l mature anther.

Discussion

The anatomical result of *M. aquaticum* and *St. media* show that, they have same anatomy characters: (1) Root is composed of uniseriate epidermis, parenchyma cortex, endodermis, pericycle and central cylinder, and primary xylem of root is tetrarch. (2) Stem epidermis is arranged closeness and parallel, and have distinct shuttle-shape tuber. (3) Leaf is dorsiventral mesophyll with palisade layer and spongy parenchyma. (4) Anomocytic type of stomatas are found in the epidermis of the leaf, stem and sepal. (5) Pollen is single and spheroidal. A number of germ pore is observed and no pollen aperture, that is completely sunken, papillate and polyaperturate. (6) Crassuloid type of seeds have simple papillae and no strophiole. Seed coat is rounded shape minute secondary sculpturing with an outer surface formed by foveolate epidermal walls. (7) From the globular floral primordium develop to complete bisexual flower, the process of floral initiation are nearly the same. From here we see that the higher similarity in comparative anatomy of *M. aquaticum* and *St. media*, justifies it is appropriate that *M. aquaticum* belongs to *Stellaria*.

Based on morphological characters of Caryophyllaceae, taxonomic treatment is not so reliable (Smitsen *et al.*, 2002). Wu, (1991) regarded as the evolution tend of morphological Caryophyllaceae become to similarity, such as decrease stamen and style number, petal absent, which difficult identify Caryophyllaceae species. At present, style number is a key trait of identification *Myosoton* and *Stellaria*. However, the number of *M. aquaticum* is variable by outdoor investigation, from 3 to 5. So the view of Moench is not reliable, just based on the morphological characters.

In the last decennium, some molecular systematic studies of Caryophyllaceae have been published. For instance, Fior (2006) collected 40 species, Harbaugh (2010) included 126 species, and Greenberg (2011) sampled 630 species. Their sampling species included *M. aquaticum* and *St. media*. From their phylogenetic tree for Caryophyllaceae, we can say *M. aquaticum* is very close to *St. media*, *M. aquaticum* should be placed to *Stellaria*. Specially, Zhao *et al.*, (2009) agreed with the systematic treatment in the context of the resulting N-J systematic tree using data from the ITS region and *trnL-F* gene of *M. aquaticum*, *St. media* and relative species. In the molecular systematic viewpoint, it is appropriate that *M. aquaticum* belongs to *Stellaria*.

Conclusions

As supplementary viewpoint for molecular systematic, comparative anatomy of *M. aquaticum* and *St. media* show that they are the higher similarity, no matter what vegetative organ, reproductive organ, and floral ontogeny. Our result support the taxonomic treatment that *M. aquaticum* belongs to *Stellaria*.

Acknowledgements

The authors declare that they have no conflict of interest and have read publication ethics. The authors are grateful to Dr. LIU Zhong-wei for valuable comments on

SEM. The study was supported by the earmarked fund for China Agriculture Research System (nycytx-19-E09), Collaborative Innovation Center for Multiple cropping system and modern production in Southern paddy crop, China (MCSMP20120802), Hunan Provincial Key Laboratory for Biology and Control of Weeds (2015TP1016), and Scientific-Innovative team of Hunan Academy of Agricultural Sciences (2014TD01).

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(Received for publication 4 August 2015)