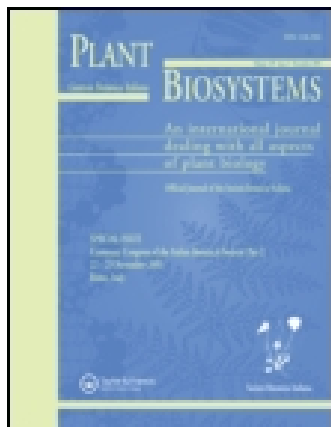


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Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology: Official Journal of the Societa Botanica Italiana

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/tplb20>

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Accepted author version posted online: 26 Nov 2012. Published online: 03 Dec 2012.

To cite this article: C.-L. Xiang, T. Funamoto, E. V. Evangelista, Q. Zhang & H. Peng (2013) Pollen morphology of the East Asiatic genus *Chelonopsis* (Lamioideae: Lamiaceae) and allied genera, with reference to taxonomic implications and potential pollination ecology, *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology: Official Journal of the Societa Botanica Italiana*, 147:3, 620-628, DOI: [10.1080/11263504.2012.748099](https://doi.org/10.1080/11263504.2012.748099)

To link to this article: <http://dx.doi.org/10.1080/11263504.2012.748099>

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Pollen morphology of the East Asiatic genus *Chelonopsis* (Lamioideae: Lamiaceae) and allied genera, with reference to taxonomic implications and potential pollination ecology

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(Received 12 December 2011; final version received 19 June 2012)

Abstract

Pollen grains of 15 taxa of the genus *Chelonopsis* (14 spp. and 1 variety) from China and Japan and 6 species of the closely related genera *Bostrychanthera* (1 species) and *Gomphostemma* (5 species) were examined by light and scanning electron microscopy. Of these, the pollen morphology of 18 taxa was studied for the first time. Pollen grains were found to be tricolpate with polar lengths of 20.8–30.0 μm and equatorial widths of 17.5–27.3 μm . The basic shape of the pollen in most taxa is subprolate or prolate-spheroidal, but spheroidal, subprolate-spheroidal, oblate-spheroidal, and prolate-subprolate grains are also found in some species. The surface is generally reticulate or suprareticulate in *Chelonopsis* and granulate in *Bostrychanthera*. In comparison with those of *Chelonopsis* and *Bostrychanthera*, the pollen grain surfaces of *Gomphostemma* are more diverse. In *Chelonopsis*, pollen is taxonomically useful at the sectional level, and some grains provide enough characters for species delimitation. The potential pollination ecology of cellular hairs on the anthers of *Chelonopsis* and *Bostrychanthera* is also briefly discussed.

Keywords: *Bostrychanthera*, *Gomphostemma*, Lamioideae, taxonomy, tribe Gomphostemmateae

Introduction

The East Asian genus *Chelonopsis* Miq. is one of the three genera currently recognized in tribe Gomphostemmateae (Scheen et al. 2010; Bendiksby et al. 2011). The initially monotypic genus was proposed by Miquel (1865) on the basis of collections from Japan. The genus grew to include 16 species and 2 varieties (Wu & Li 1977; Li & Hedge 1994; Harley et al. 2004), but our prior taxonomic work reduced the number to 13 (Xiang et al. 2008, 2009). Recently, a new hysteranthous species, *Chelonopsis praecox* Weckerle and F. Huber, was described from Sichuan, Southwest China (Weckerle et al. 2009). Thus, the genus *Chelonopsis* currently contains 14 species and 2 varieties, with 3 species endemic to Japan (Murata & Yamazaki 1993), and 13 species endemic to China (Wu & Li 1977; Li & Hedge 1994; Xiang et al. 2010), the presumed center for the genus.

Wu and Li (1965, 1977) originally classified *Chelonopsis* into two subgenera, subg. *Aequidens* C. Y. Wu and H. W. Li and subg. *Chelonopsis*, based on habit and calyx morphology. The former is subdivided into two sections: sect. *Aequidens* C. Y. Wu and H. W. Li and sect. *Microphyllum* C. Y. Wu and H. W. Li. Based on the presence of glandular trichomes, sect. *Microphyllum* is further divided into two series: ser. *Rosea* C. Y. Wu and H. W. Li with glandular trichomes and ser. *Lichiangenses* C. Y. Wu and H. W. Li without glandular trichomes. This classification was supported by the anatomical studies of the trichome morphology (Xiang et al. 2010). For example, the presence of unicellular trichomes and clavate glandular trichomes is highly concordant with the subgeneric division.

The systematic position of *Chelonopsis* has been controversial. Traditionally, *Chelonopsis* was included in subtribe Melittidinae (Dumort.) Enid., which is delimited based on a set of calyx and corolla

characters (Bentham 1848, 1876; Briquet 1895–1897; Cantino 1985). However, some morphological studies showed that *Chelonopsis* is more closely allied to other East Asian genera, such as *Bostrychanthera* Benth., *Colquhounia* Wall., and *Gomphostemma* Wall. ex Benth. (Cantino 1987; Ryding 1994; Wagstaff et al. 1995). Scheen et al. (2007) abandoned subtribe Melittidinae based on a detailed molecular study that found it to be a non-monophyletic taxon. Consequently, they redefined the tribe Synandreae to accommodate the North American genera which were traditionally included in Melittidinae, but *Chelonopsis* and *Melittis* were unplaced. Recently, Scheen et al. (2010) assigned *Chelonopsis* and two additional Asian genera, *Bostrychanthera* and *Gomphostemma*, into a newly established tribe Gomphostemmateae, as these genera formed a highly supported clade based on three cpDNA markers (*trnL* intron, *trnL-trnF* intergenic spacer, and *rps16* intron).

Investigations of pollen morphology have been an essential aid to classification and phylogenetic reconstruction within the family Lamiaceae (Erdtman 1945; Abu-Asab & Cantino 1989, 1992, 1993a; Moon & Hong 2003; Hong 2007; Salmaki et al. 2008). Some studies also focus on certain subfamilies (Abu-Asab & Cantino 1994), tribes (Abu-Asab & Cantino 1993b), subtribes (Rudall 1980; Moon et al. 2008a,b), or geographic regions (Akgül et al. 2008). However, there is only one prior study (Abu-Asab & Cantino 1994) dealing with the pollen morphology of *Chelonopsis*. To date, the pollen morphology and its taxonomic value within *Chelonopsis* remain undetermined, and the palynology of only two species (*Chelonopsis lichiangensis* and *Chelonopsis odontochila*) has been reported (Abu-Asab & Cantino 1994).

In the course of revising the genus *Chelonopsis* (Xiang et al. 2008, 2009, 2010), our objectives were to provide a detailed account of the pollen morphology using both light microscopy (LM) and scanning electron microscopy (SEM) to evaluate the systematic significance of the resulting data and to correlate these results with traditional classification. Observations of *Chelonopsis* and *Bostrychanthera* species in the field and from herbarium collections revealed the growth of cellular hairs that form a basket over the open pollen sacs. This feature is extremely rare in subfamily Lamioideae, but a similar structure was reported to occur in other lamiids (APG III 2009) such as *Echium* L. (Boraginaceae) and *Esterhazyia* J. C. M. Kian (Scrophulariaceae) (Hesse et al. 2000). These similar structures may function as pollen-connecting vectors in forming pollen dispersal units, or may be involved in pollen presentation (Skvarla et al. 1975, 1978; Hesse et al.

2000); thus, a possible significant correlation to the pollination ecology is discussed.

Materials and methods

Sampling

The sampling materials consist of pollen grains of 21 taxa of tribe Gomphostemmateae, which includes 15 of 16 taxa of *Chelonopsis*, 1 species of *Bostrychanthera*, and 5 species of *Gomphostemma*. The tissue samples of some species were obtained from the herbarium, Kunming Institute of Botany, Chinese Academy of Sciences (KUN), while others were collected from the field and immediately fixed in formalin–acetic acid–70% alcohol in the ratio of 1:1:18. Fifteen grains were measured per species. A set of permanent slides is deposited in the KUN. The list of species and their origins are given in Table I.

Treatment and observation

Fully matured anthers were carefully removed from the specimens and prepared by the prolonged acetolysis procedure of Abu-Asab and Cantino (1989), modified from Erdtman (1960). The collected pollen samples were mounted in Kaiser's glycerol gelatin (Padrón-Mederos & La Serna-Ramos 2011) and sealed with paraffin for LM. Measurements and morphological observations were taken using an Olympus BX-51 microscope (Olympus, Tokyo, Japan). The ratio of polar to equatorial (P/E) axis length was determined for each measured grain, and the mean P/E ratio was calculated from these individual values.

For SEM, acetolysed pollen grains in 70% ethanol were pipetted directly onto aluminum stubs with double-sided adhesive tape, and air dried at room temperature under an inverted flask. Samples were coated with gold–palladium and examined and photographed with KYKY-10000B (Science Instrument Company, Beijing, P.R. China) electronic microscope at an acceleration voltage of 15 kV (Xiang & Liu 2012). General pollen terminology was used according to Abu-Asab and Cantino (1994).

Results

In all examined taxa, the pollen grains are monad and tricolpate traits are shared with other genera of the subfamily Lamoideae sensu Cantino et al. (1992). The main features of the investigated pollen grains are summarized in Table II. The selected micrographs of the pollen grains studied are presented in Figures 1 and 2, while some selected pictures of the anthers are illustrated in Figure 3.

Table I. Collection data of *Chelonopsis* specimens and related genera examined here from morphological point of view.

Taxon/classification	Habit	Voucher	Source
<i>C. chekiangensis</i> C. Y. Wu*	Herb	Xiang CL 0602 (KUN)	Qingliangfeng, Lin'an City, Zhejiang Province, China
<i>C. longipes</i> Makino**	Herb	Funamoto T L060279	Kadoya, Aoi-ku, Shizuoka City, Shizuoka Pref., Japan
<i>C. moschata</i> Miq.**	Herb	Funamoto T L060273	Tatesawa, Tazawako-machi, Senboku City, Akita Pref., Japan
<i>C. yegiharana</i> Hisauchi and Matsuno**	Herb	Funamoto T L060278	Mt. Ashitaka, Nagaizumi-machi, Sunto-gun, Shizuoka Pref., Japan
<i>C. soutiei</i> (Bonati) Merr.*	Shrub	Xiang CL & Peng H 5240 (KUN)	Rendui, Xiangcheng County, Sichuan Province, China
<i>C. forrestii</i> Anthony*	Shrub	Xiang CL & Peng H 5141 (KUN)	Shuiluo, Muli City, Sichuan Province, China
<i>C. giraldii</i> Diels*	Shrub	Xiang CL 025 (KUN)	Baocheng, Hangzhong City, Shaanxi Province, China
<i>C. mollissima</i> C. Y. Wu*	Shrub	Xiang CL 05005 (KUN)	Zhongping, Luquan County, Yunnan Province, China
<i>C. odontochila</i> Diels*	Shrub	Xiang CL 022 (KUN)	Hongyan, Lijiang City, Yunnan Province, China
<i>C. rosea</i> W. W. Sm.*	Shrub	Xiang CL 036 (KUN)	Huiyao, Jingdong County, Yunnan Province, China
<i>C. rosea</i> var. <i>siccana</i> (W. W. Sm.) C. L. Xiang and H. Peng*	Shrub	Forrest G 13414 (IBSC)	Muli County, Sichuan Province, China
<i>C. abbreviata</i> C. Y. Wu and H. W. Li*	Shrub	Forrest G 13587 (IBSC)	Lushui County, Yunnan Province, China
<i>C. bracteata</i> W. W. Sm.*	Shrub	Xiang CL 0607040 (KUN)	Shuiluo, Muli County, Sichuan Province, China
<i>C. lichiangensis</i> W. W. Sm.*	Shrub	Xiang CL 020 (KUN)	Bishuitai, Zhongdian County, Yunnan Province, China
<i>C. praecox</i> Weckerle and F. K. Huber *	Shrub	Xiang CL s. n. (KUN)	Shudi, Lijiang City, Yunnan Province, China
<i>B. deflexa</i> Benth.*	Herb	Xiang CL 048 (KUN)	Tabian, Xingan County, Guangxi Province, China
<i>G. arbusculum</i> C. Y. Wu*	Shrub	Zhou X 550 (KUN)	Ruili City, Yunna Province, China
<i>G. microdon</i> Dunn.	Shrub	Sino-American Expedition 0191 (KUN)	Mengla, Xishuangbanna City, Yunna Province, China
<i>G. parviflorum</i> Wall.	Shrub	Pei SJ 95-0651 (KUN)	Mengla, Xishuangbanna City, Yunna Province, China
<i>G. penduculatum</i> Benth. ex Hook. f.*	Shrub	Liu ED L5611 (KUN)	Mt. Daxueshan, Yongde County, Yunnan Province, China
<i>G. stellatohirsutum</i> C. Y. Wu*	Shrub	Tao DD 879 (KUN)	Mt. Huanglanshan, Luchuan County, Yunna Province, China

Note: Species endemic to China are indicated by asterisk [voucher specimens were deposited in private herbarium of the second author (Tsuneo Funamoto)], while species endemic to Japan are indicated by double asterisks. Location of voucher specimens provided, and herbarium acronyms cited in parentheses.

Table II. Summary of pollen morphological data for *Chelonopsis* and related genera.

Taxon/Classification	Polar axis	Equatorial axis	P/E ratio	Colpi	Shape	Sculpturing	Figures
<i>Chelonopsis</i>							
Subg. <i>Chelonopsis</i>							
<i>C. chekiangensis</i>	24.3 (20–26)	22.5 (21–23)	1.08	3	Prolate-spheroidal	Suprareticulate	Figure 1A
<i>C. longipes</i>	23.5 (20–26)	19.3 (18–20)	1.21	3	Subprolate	Perforate	Figure 1B and C
<i>C. moschata</i>	20.8 (17–22)	19.1 (18–21)	1.08	3	Prolate-spheroidal	Suprareticulate	Figure 1D
<i>C. yagiharana</i>	22.8 (19–25)	20.4 (19–23)	1.11	3	Prolate-spheroidal	Suprareticulate	Figure 1E and F
Subg. <i>Aequidens</i>							
Sect. <i>Aequidens</i>							
<i>C. forrestii</i>	23.0 (21–25)	23.4 (22–25)	0.98	3	Oblate-spheroidal	Suprareticulate	Figure 1G
<i>C. souliei</i>	24.6 (20–26)	23.3 (20–24)	1.06	3	Prolate-spheroidal	Suprareticulate	Figure 1H and I
Sect. <i>Microphyllum</i>							
Ser. <i>Rosea</i>							
<i>C. girdalii</i>	30.0 (28–32)	25.1 (23–28)	1.19	3	Subprolate	Suprareticulate	Figure 1J and K
<i>C. mollissima</i>	23.0 (21–24)	22.3 (18–25)	1.03	3	Prolate-spheroidal	Reticulate	Figure 1L
<i>C. odontochila</i>	27.7 (25–29)	25.7 (23–27)	1.05	3	Prolate-spheroidal	Reticulate	Figure 1M
<i>C. rosea</i>	24.5 (23–25)	24.0 (22–26)	1.02	3	Prolate-spheroidal	Reticulate	Figure 1N
<i>C. rosea</i> var. <i>siccanea</i>	28.1 (25–32)	24.5 (23–27)	1.14	3	Subprolate	Reticulate	Figure 1O
Ser. <i>Lichiangenses</i>							
<i>C. abbreviata</i>	26.6 (24–27)	20.8 (18–22)	1.27	3	Subprolate	Suprareticulate	Figure 2A and B
<i>C. bracteata</i>	26.3 (24–28)	22.7 (20–23)	1.16	3	Subprolate	Suprareticulate	Figure 2C and D
<i>C. lichiangensis</i>	27.9 (24–30)	26.8 (23–28)	1.04	3	Spheroidal	Suprareticulate	Figure 2E and F
<i>C. praecox</i>	22.4 (19–25)	20.8 (24–28)	1.07	3	Spheroidal	Suprareticulate	Figure 2G and H
<i>B. deflexa</i>	29.2 (27–32)	24.3 (23–25)	1.20	3	Subprolate	Granulate	Figure 2I and J
<i>G. arbusculum</i>	29.0 (27–32)	25.4 (22–27)	1.14	3	Subprolate	Reticulate	Figure 2K
<i>G. microdon</i>	22.0 (18–24)	17.5 (15–19)	1.25	3	Subprolate	Scrobiculate	Figure 2L
<i>G. parviflorum</i>	27.1 (26–29)	26.2 (24–28)	1.04	3	Subprolate-spheroidal	Granulate	Figure 2M
<i>G. penduculatum</i>	28.8 (27–30)	27.3 (25–29)	1.05	3	Subprolate	Foveolate	Figure 2N
<i>G. stellatohirsutum</i>	26.5 (23–29)	20.3 (17–23)	1.30	3	Prolate-subprolate	Scrobiculate	Figure 2O

Chelonopsis

All grains examined were single, isopolar, and tricolpate. These features are common to most pollens in the species of subfamily Lamioideae (Abu-Asab & Cantino 1992, 1994). The mean length of the polar axis of *Chelonopsis* varies from 20.8 μm , in *Chelonopsis moschata* (17–22 μm ; Figure 1D), to 30.0 μm , in *Chelonopsis girdalii* (28–32 μm ; Figure 1J and K), but the equatorial axis ranges from 19.1 μm , in *C. moschata* (18–21 μm ; Figure 1D), to 26.8 μm , in *C. lichiangensis* (23–28 μm ; Figure 2E and F). The shape of the pollen grains is mostly subprolate or prolate spheroidal, but occasionally spheroidal, subprolate-spheroidal, or oblate-spheroidal grains occur (Figures 1 and 2, Table II). Three types of exine sculpturing can be distinguished: suprareticulate (Figures 1A, D–K and 2A–H), reticulate (Figure 1L–O), and perforate (Figure 1B and C). Lumina are distinctly patterned; the muri of reticulation are smooth. Lumina are more or less polygonal in all species.

Bostrychanthera

The representative species of this genus examined in this study was *Bostrychanthera deflexa*. The pollen size is 29.2 (27–32) \times 24.3 (23–25) μm (polar axis

\times equatorial axis) while the ratio of length of the polar axis to that of the equatorial axis is 1.20. The sculpture is reticulate (Figure 2I and J).

Gomphostemma

Five species of *Gomphostemma* were examined (Figure 2K–O). The mean polar axis of *Gomphostemma* varies from 22.0 μm in *Gomphostemma microdon* (18–24 μm ; Figure 2L) to 29.0 μm in *Gomphostemma arbusculum* (27–32 μm ; Figure 2K), and the equatorial axis ranges from 17.5 μm in *G. microdon* (15–19 μm ; Figure 2L), to 27.3 μm in *Gomphostemma penduculatum* (25–29 μm ; Figure 2N). The shape of the pollen grains is from subprolate, prolate-subprolate to subprolate-spheroidal (Figure 2L–O). Four types of exine sculpturing were distinguished: reticulate (Figure 2K), scrobiculate (Figure 2L and O), granulate (Figure 2M), and foveolate (Figure 2N).

Discussion

Among the *Chelonopsis* species, *C. girdalii* has the largest grains with an average of 30.0 \times 25.1 μm ; Figure 1J and K), while *C. moschata* has the smallest pollen grain (20.8 \times 19.1 μm ; Figure 1D). Thus far, chromosome numbers have been reported from only

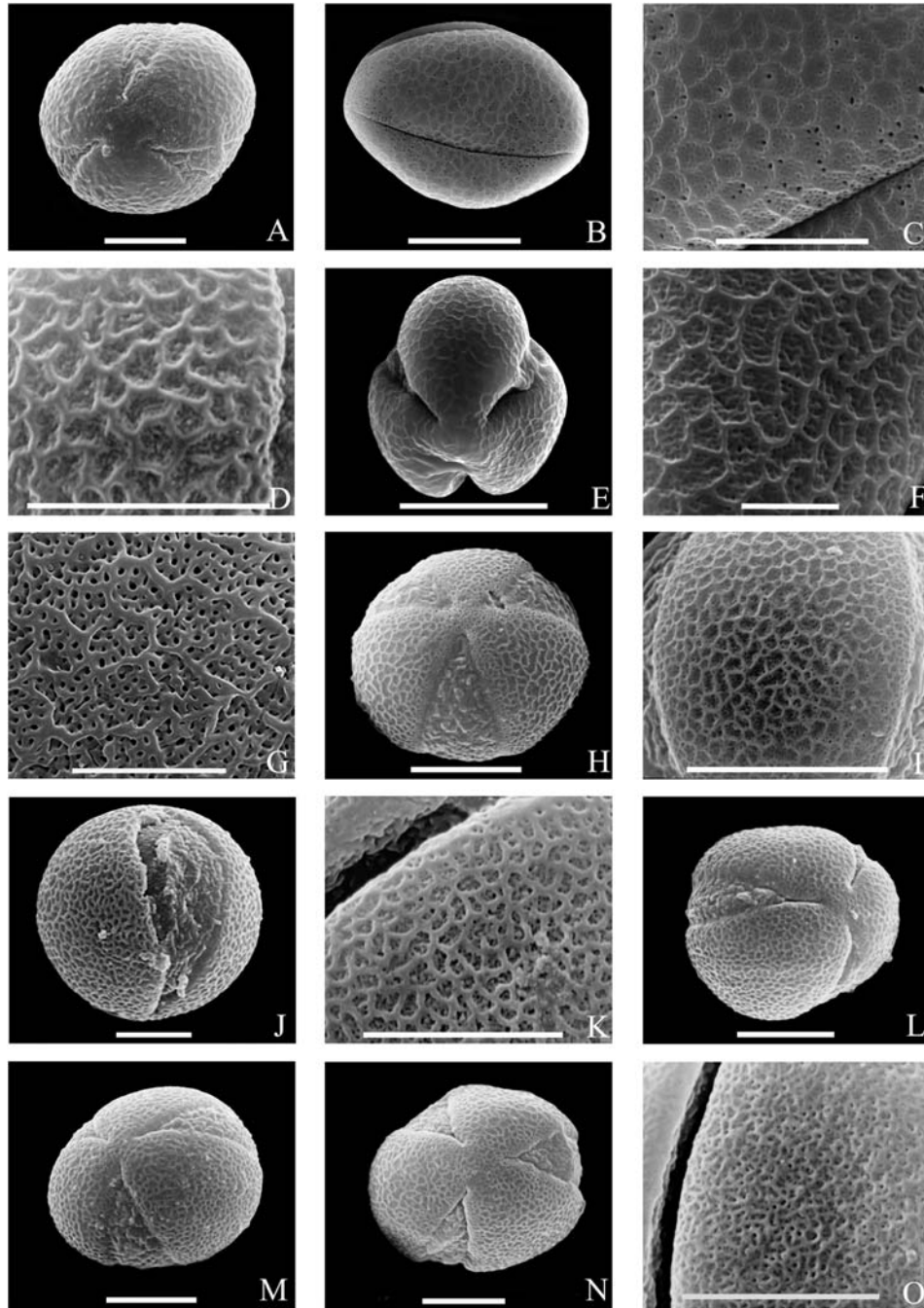


Figure 1. SEM micrographs of pollen grains in *Chelonopsis* spp. (A) *C. chekiangensis*. (B and C) *C. longipes*. (D) *C. moschata*. (E and F) *C. yagiharana*. (G) *C. forrestii*. (H and I) *C. souliei*. (J and K) *C. giraldii*. (L) *C. mollissima*. (M) *C. odontochila*. (N) *C. rosea*. (O) *C. rosea* var. *siccanea*. Scale bars: A–F and H–O = 10 μm ; G = 3 μm .

three taxa endemic to Japan (*Chelonopsis longipes*, *C. moschata*, *Chelonopsis yagiharana*, $2n = 32$; Funamoto 2007), which makes it difficult to ascertain whether that size difference is related to either geographical variations or chromosome number. In comparison with *Chelonopsis*, the pollen grains of *Bostrychanthera* are slightly larger, but the size difference is not significant (Table II, Figure 2I and J). Five species of *Gomphostemma* are examined here, and the largest grains are observed in *G. arbusculum* with

an average of $29.0 \times 25.4 \mu\text{m}$ (Figure 2K). The pollen grains of seven *Gomphostemma* species which were examined by Abu-Asab and Cantino (1994) are larger in size and could be attributed to the difference in procedure in the preparation of treatments (Reitsma 1969; Harley 1992; Schols et al. 2004; Lens et al. 2005; Moon et al. 2008b).

The shapes of the pollen in equatorial view vary among taxa, with those of *Chelonopsis* being mostly subprolate and prolate-spheroidal, except for

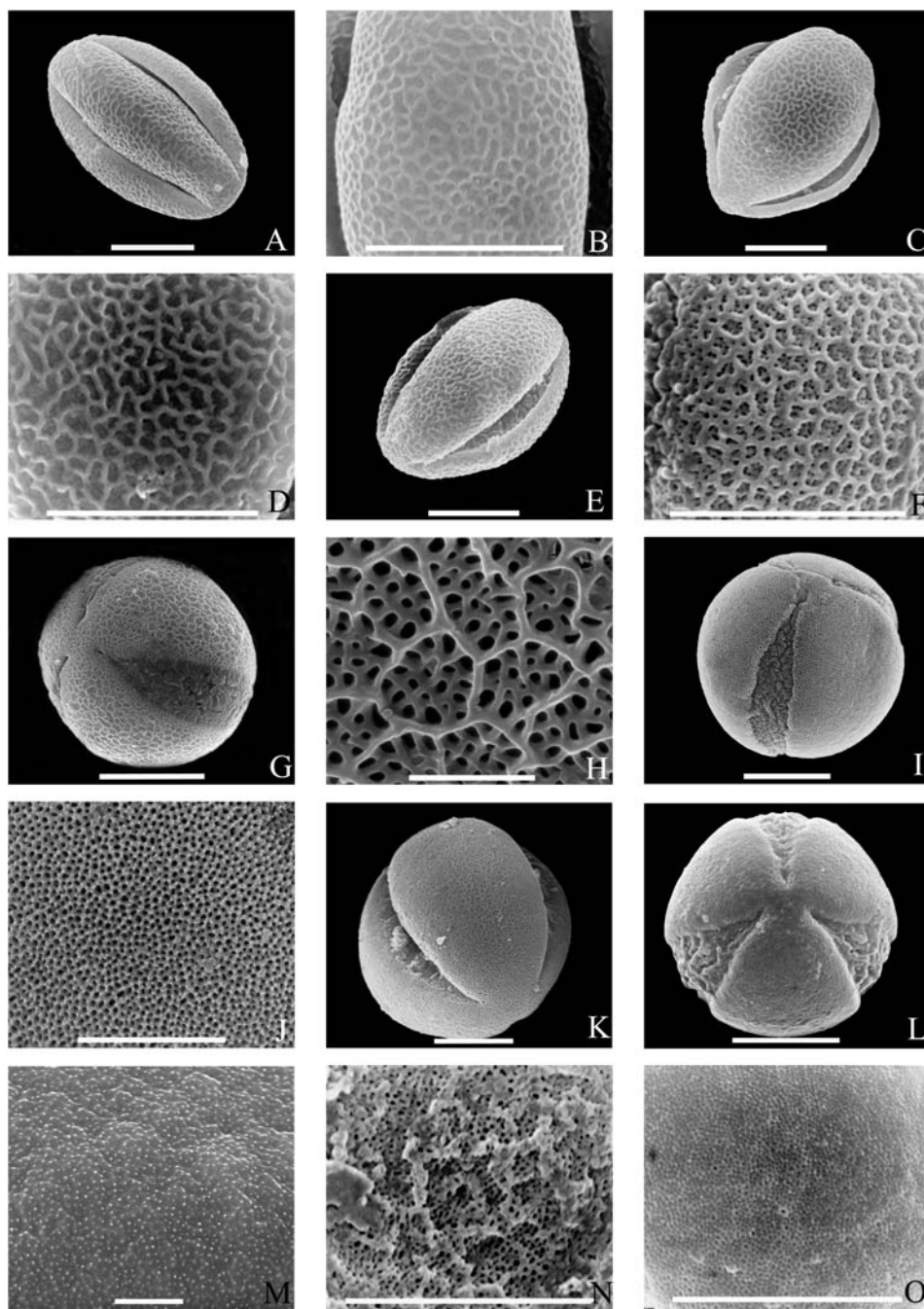


Figure 2. SEM micrographs of pollen grains in *Chelonopsis* spp. and allied genera. (A and B) *Chelonopsis abbreviata*. (C and D) *C. bracteata*. (E and F) *C. lichiangensis*. (G and H) *C. praecox*. (I and J) *Bostrychanthera deflexa*. (K) *Gomphostemma albusculum*. (L) *G. microdon*. (M) *G. parviflorum*. (N) *G. pendunculatum*. (O) *G. stellatohirsutum*. Scale bars: A–G, I, K, L, N, and O = 10 μm ; H = 1 μm ; J = 4 μm ; M = 2 μm .

Chelonopsis forrestii with an oblate-spheroidal shape (Figure 1G), and *C. lichiangensis* (Figure 2E) and *C. praecox* (Figure 2G) with a spheroidal shape. In contrast, three kinds of shapes can be found in the five species of *Gomphostemma* examined. It is noteworthy that the pollen shape in the Lamiaceae is often affected by the state of hydration and/or fixation (Demissew & Harley 1992; León-Arencibia & La-Serna Ramos 1992; Moon et al. 2008a). Thus,

differences in shape among the pollen grains may not be significant or even applicable in their taxonomy. As suggested by Moon et al. (2008a), in order to preserve a more natural form, careful processing is required, including critical point drying of the fresh material.

Pollen characteristics have shown great taxonomic significance, and have been used in the classification of Lamiaceae (Erdtman 1945). Based



Figure 3. (A–D) Flowers showing the voluminous hair puff formed by the four anthers: (A) *Bostrychanthera deflexa*; (B) *Chelonopsis souliei*; (C) *C. rosea*; (D) *C. mollissima*. (E) *B. deflexa*. (F–J) SEM micrographs showing anther hairs: (F) anther of *C. odontochila*, lateral view; (G) *C. forrestii*, pollen grains sticking to some anther hairs; (H and I) *C. praecox*, trichomes along the side of the thecae; (J) *C. rosea*, beginning of anther dehiscence. Scale bars: E and F = 1 mm; G and J = 300 μm ; H and I = 400 μm .

on the data summarized in Table II and Figures 1 and 2, some characters are diagnostic with respect to the taxa belonging to *Chelonopsis*. For example, subg. *Chelonopsis* is represented in this study by all four species: *C. moschata*, *C. yagiharana*, *C. longipes*, and *Chelonopsis chekiangensis*. Morphologically, *C. longipes* is distinct in comparison with the remaining taxa because of its perforations, a character that is unique to this species. This difference in palynology is also consistent with their macro-morphological characters, e.g. *C. longipes* has much longer peduncles (1.5–3 cm) and pedicels (1–3 mm), whereas only 2–6 mm long for peduncles and 0.5–5 mm long for pedicels in other three species (Murata & Yamazaki 1993).

Chelonopsis subgenus *Aequidens* is subdivided into two sections, sect. *Aequidens* and sect. *Microphyllum* (Wu & Li 1965, 1977). In addition to differences in morphology, the pollen grain size is also different in these two sections. The pollen size of the sect. *Aequidens* is much smaller than that of sect. *Microphyllum* (Table II). This difference is positively correlated with geographical distribution and

morphology. For example, species of sect. *Aequidens* have lanceolate leaves, while species of sect. *Microphyllum* have ovate leaves. However, the pollen morphology does not support any significant difference between ser. *Rosea* and ser. *Lichiangense* within sect. *Microphyllum*. Thus, we can say that the pollen morphology neither supports nor conflicts with Wu and Li's (1965, 1977) series classification.

It is of particular interest that there is a voluminous hair puff formed by the four anthers in all investigated *Chelonopsis* species (Figure 3B–D) and also in *B. deflexa* (Figure 3A and E). These hairs may play a significant role in pollination ecology, such as to hinder dispersal of pollen grains from the open pollen sac. In all *Chelonopsis* species, cellular hairs were found forming a basket over the open pollen sacs (Figure 3B–D and F). Scanning electron micrographs illustrate that some grains stick to the trichomes (Figure 3G) while others are covered with a trichome layer (Figure 3H and I). As reported from the genus *Echium* (Boraginaceae) and *Esterhazyia* (Scrophulariaceae), the primary function of the

anther hairs is not only to hinder the pollen from falling out prematurely, but also to partition and present the pollen to flower visitors (Hesse et al. 2000). Functionally, these hairs are comparable to the *Impatiens* L. (Balsaminaceae) basket equipment (Vogel & Cocucci 1988). However, occurrence of trichomes on anthers is very rare in the family Lamiaceae. Thus, the function and mechanism of anther hairs are in need of further investigation.

Acknowledgments

The authors are grateful to Mr. Xi-Kan Fan and Dr. Zong-Xin Ren for their technical assistance in SEM. The authors specially thank Dr Jacqueline Van De Veire for her valuable suggestions and her great help in editing the text, and also thank Prof. Carlo Blasi and two anonymous reviewers for their constructive suggestions that greatly improved the paper. This study was supported by the National Natural Science Foundation of China (Nos. 31100164 and 31110103911) and the Chinese Academy of Sciences Grants (KSCX2-EW-J24, KSCX2-EW-Z-1).

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