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Pollen morphology in relation to floral types and pollination syndromes in *Pedicularis* (Orobanchaceae)

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Abstract Floral diversification in the genus *Pedicularis* (Orobanchaceae) is remarkable among flowering plants. In this genus, floral morphology and pollinator behavior are closely co-adaptive. In the current paper, pollen grains of 23 representative species of *Pedicularis* mainly from North America, with two species from Japan and two species from China, whose pollination ecology was previously studied, were examined using light microscopy and scanning electron microscopy. Two pollen aperture types and three kinds of exine ornamentation were recognized among these species. In addition, pollen data from previous and the current studies of *Pedicularis* were integrated and analyzed, together with some pollination characters. There was a significant association between pollen aperture types and corolla types, as well as between pollination syndromes and corolla types. However, there was no association of exine ornamentations with corolla types. The relationships and evolution of this genus were discussed with regards to pollen morphology, corolla types and pollination syndromes.

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Introduction

The genus *Pedicularis* L. is the largest member of the family Scrophulariaceae as traditionally recognized, and comprises over 600 species primarily occurring in the arctic-alpine region of the Northern Hemisphere. More than 50% of the species of this genus are distributed in the Sino-Himalayan region (Ree 2001; Wang and Li 2005; Zhang et al. 2006; Yu and Wang 2008; Yu et al. 2008a), which represents a major center of species diversity and endemism is concentrated (Hong 1983; Yang et al. 1998; Mill 2001).

Pedicularis, along with the other hemiparasitic rhinanthoids have been considered as a bridge between Scrophulariaceae and Orobanchaceae (Hutchinson 1969; Cronquist 1981). However, recently Olmstead et al. (2001) transferred Rhinantheae to the Orobanchaceae on the basis of the molecular phylogenetic analyses and their hemiparasitic habits. The flowers of *Pedicularis* are conspicuously diverse in the shape of the corolla and length of the corolla tube (Li 1951; Tsoong 1963; Macior and Sood 1991). Four basic corolla types were first recognized by Maximovicz (1888) and further demonstrated by Li (1951) as: (1) toothless, (2) toothed, (3) short-tubed with a beak, and (4) long-tubed, with a beak.

Clearly, the ontogeny of the different corollas is similar in the initiation of floral organs, whereas meristem activity on the upper lip plays an important role in corolla architecture and may have significant contributions to floral differentiation (Cai et al. 2003; Yu et al. 2008b). It seems likely that these different corolla types of *Pedicularis*

species might be associated with particular pollination syndromes. Earlier studies of pollination ecology primarily focus on species from North America (reviewed by Macior 1982) and Europe (Kwak 1977, 1979). Most recently, studies have also been carried out in Japan, southwest China and the Himalayas (Macior 1982, 1988, 1990; Macior and Sood 1991; Macior and Tang 1997; Wang 1998; Wang and Li 1998, 2005). All of these studies demonstrate that *Pedicularis* species depend exclusively on bumblebees to accomplish pollen dispersal, with the exceptions that pollination has also been reported by solitary bees and hummingbirds (Macior 1970, 1986). Autogamous reproduction has also been reported in a few erostrate species (Eriksen et al. 1993).

As the male gametophytes, pollen grains play an important role in sexual reproduction in flowering plants. Studies on palynology of *Pedicularis* have been carried out since the last century. Based on light microscopy (LM), some scattered pollen data were reported from European (Risch 1939; Belkina 1972) and Indian (Dutta and Chanda 1979) members of *Pedicularis*. The most comprehensive account of palynology in the genus is that of Tsoong and Chang (1965), who examined 193 species mainly from China. Three types of pollen aperture, i.e., tri-colpate, tri-syncolpate and bi-syncolpate were recognized. The pollen exine is extremely thin in the genus, and it is difficult to distinguish the exine ornamentation under LM (Erdtman 1960; Beug 1961; Tsoong and Chang 1965; Dutta and Chanda 1979), therefore details of ornamentation are lacking in all earlier pollen morphological descriptions. Information on exine ornamentation began to emerge when pollen from two Turkish (Inceoğlu 1981) and one Canadian species (Minkin and Eshbaugh 1989) was described and illustrated using LM and scanning electron microscopy (SEM). The extensive investigation of the exine of some Chinese species using SEM, revealed the presence of five kinds of exine ornamentation: i.e., microfoveolate, microreticulate, microrugulate, microscabrate and retipilate (Wang et al. 2003). SEM observations also revealed three types of aperture configurations, each of which could be subdivided, making a total of eight sub-types (Wang et al. 2003; Yu and Wang 2008). Pollen data have now been obtained for more than 2/3 of the 352 species from China.

In contrast with the situation for China, palynology in some key areas within the distribution range of *Pedicularis*, especially North America and Japan, has been neglected. It has been important to fill this gap in our knowledge in order to look for close parallels between pollination mechanisms in North America and East Asia and to investigate paths of migration and triggers for speciation in *Pedicularis*. Pollen morphology may be correlated with pollination vectors (Hesse 2000) and certain pollen characteristics, in particular, aperture configuration and exine ornamentation are prone to co-adaptation with specific pollinators (Proctor et al. 1996; Tanaka et al. 2004). Furthermore, the functional significance of floral characters in pollination is important in understanding the evolution of floral diversity in *Pedicularis* (Ree 2001). Thus, further effort is needed to understand the possible evolutionary relationships between pollen morphology, floral diversity and pollination behavior in *Pedicularis*. The current palynological study focuses primarily on species from North America and compares the findings with previously reported data on pollination ecology. The aims of the current study are to: (1) provide detailed pollen data of more American species; (2) make an integrative analysis in relation to pollen data to understand whether pollen morphology is possibly correlated with corolla type and pollinator behavior; (3) try to better understand the co-adaptive significance and evolution of pollen morphology within the genus.

Materials and methods

Pollen samples of 23 species of *Pedicularis* were mainly obtained from the herbarium of Royal Botanic Garden Edinburgh (E), Scotland, UK, with two Chinese species from the herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (KUN). A list of voucher specimens is given in Table 1.

Pollen grains were examined by LM and SEM, the pollen characters were summarized (Table 2). The samples for LM investigation, pollen grains were treated by acetolysis (Erdtman 1960) and mounted in glycerine jelly. Size measurements were based on 20 pollen grains, the value of P (polar axis length) and E (equatorial diameter) were measured and P/E ratio was calculated. However, acetolysis causes the delicate exine of *Pedicularis* pollen grains to rupture and is therefore unsuitable for SEM observation (Wang et al. 2003).

For SEM investigations pollen grains were prepared from herbarium material which was reconstituted by softening in warm water for 5–10 min and then soaked in formalin–acetic acid–alcohol for 24 h. The anthers were then dehydrated in an ethanol series followed by acetone and critical-point-dried (Emitech K850). Anthers were broken to release the pollen onto aluminum stubs and sputter coated (Emscope SC500) with gold palladium. Observations were made using SEM at 30 kV (KYKY-1000B; Science Instrument Company, Beijing). The micrographs were digitized using a Uniscan-O-2000 scanner (Tsinghua Unisplendour Co. Ltd, Beijing). Descriptive terminology follows Punt et al. (2007) and Wang et al. (2003).

To explore the correlations among pollen characters, corolla types and pollination patterns, the integrative

Table 1 A list of voucher specimens

Pedicularis species	Voucher specimen
P. alaschanica Maxim.	Qing-hai, China; Qing-Zang Exped. 72 (KUN)
P. attollens A. Gray.	California, U.S.A; J. Henrikson 2584 (E)
P. bracteosa Benth. ex Hook.	Wyoming, U.S.A; A. Nelson 834 (E)
P. canadensis L.	Quebec, Canada; E. Roy 3124 (E)
P. capitata L.	Alaska, U.S.A; I. W. Hutchison 317 (E)
P. contorta Benth. ex Hook.	Montana, U.S.A; J. W. Blankinship s.n. (E)
P. cranolopha Maxim.	Yunnan, China; T. T. Yu 12822 (KUN)
P. crenulata Benth.	Colorado, U.S.A; L. Goodding s.n. (E)
P. cystopteridifolia Rydb.	Wyoming, U.S.A; A. Nelson & E. Nelson 5810 (E)
P. densiflora Benth. ex Hook.	California, U.S.A; H. E. Brown 615 (E)
P. grayi A. Nelson.	Colorado, U.S.A; L. N. Goodding 1661 (E)
P. groenlandica Retz.	Colorado, U.S.A; R. F. Thorne 18900 (E)
P. lanceolata Michx.	Minnesota, U.S.A; E. D. Sheldon s.n. (E)
P. langsdorfii Fisch.	Canada; J. M. Macoun s.n. (E)
P. oederi Vahl.	Alaska, U.S.A; G. Halliday A319 (E)
P. palustris L.	Alaska, U.S.A; E. P. Walker 844 (E)
P. parryi A. Gray	Colorado, U.S.A; L. N. Goodding1661 (E)
P. pulchella Pennell.	Montana, U.S.A; F. W. Pennell & F. B. Cotaer 23713 (E)
P. racemosa Douglas ex Hook.	Colorado, U.S.A; L. N. Gooddling 1766 (E)
P. resupinata L.	Japan; O. G. Honda 382 (E)
P. sudetica Willd.	Alaska, U.S.A; Y. Mexia 2030 (E)
P. verticillata L.	Japan; H. Takedo s.n. (E)
P. yezoensis Maxim.	Japan; M. Jakeuchi s.n. (E)

List of *Pedicularis* species for which pollen samples were obtained from the herbaria of the Royal Botanic Garden Edinburgh (E) and the Kunming Institute of Botany, Chinese Academy of Science (KUN)

analyses were performed statistically with chi-square test. The level of statistical significance was set at $P < 0.05$. Pollen data of 216 species were used from the current study and previous reports (Tsoong and Chang 1965; Wang et al. 2003; Yu and Wang 2008), in which only of 59 species have descriptions of exine ornamentation. Pollination patterns of 31 species were summarized on the basis of previous pollination studies, the cited literatures were listed within Table 3.

Results and discussion

In the current study, based on observations under LM and SEM, the detailed information of pollen morphology for 23 species is shown in Table 2.

General description of the pollen grains

Pollen grains radially symmetrical, isopolar. Pollen size ranges from small to medium, P 15.6–32.5 μm × E 14.2–31.3 μm, the largest pollen grain (*P. cranolopha*) up to $P \times E$: 32.5 × 26.0 μm, and the smallest (*P. contorta*) 15.9 × 15.4 μm. Shape in equatorial view variable including suboblate (P/E: 0.83–0.88), oblate spheroidal (P/E: 0.91–0.98), spheroidal (P/E: 1.00), prolate spheroidal (P/E: 1.03–1.13), subprolate (P/E: 1.15–1.29), or prolate (P/E: 1.44).

Amb (polar view) convex or circular to triangular. Apertures tri-syncolpate or bi-syncolpate. Colpi usually wide, long and sunken or not sunken, colpus membrane covered with granules or crustate elements, or colpi narrow, smooth and sunken.

Exine extremely thin, exine ornamentation species specific, three exine kinds being distinguished: microfoveolate, microreticulate and microscabrate.

Description of pollen types

In this study, two out of the three types of pollen aperture configurations known in the genus were observed: 3-syncolpate and 2-syncolpate. Each of these defines a pollen type that can be further divided into two or three subtypes based on examination of exine ornamentation with SEM (pollen types and subtypes following Wang et al. 2003, Yu and Wang 2008).

1. Tri-syncolpate aperture type (Fig. 1a–l): Pollen grains small to medium, mean P and E: 16.7–31.3 × 16.7–31.3 μm, P/E: 0.91–1.44, oblate spheroidal to prolate. Colpi wide or narrow, extending to fuse at the poles, sunken or not sunken. This aperture configuration

Table 2 List of major pollen morphological characters

Pedicularis species	P	E	P/E	Shape	Aperture	Exine sculpturing
P. alaschanica	24.5–(26.0)−28.6	24.7–(26.0)−28.5	1.00	S	Tri-syncolpate	Microfoveolate
P. attollens	20.3–(21.6)−22.8	22.5–(23.8)−24.9	0.91	OS	Tri-syncolpate	Microfoveolate
P. bracteosa	15.8–(16.7)−17.5	15.4–(16.7)−17.9	1.00	S	Tri-syncolpate	Microreticulate
P. canadensis	19.5–(22.1)−23.4	19.0–(22.1)−23.3	1.00	S	Bi-syncolpate	Microreticulate
P. capitata	30.2–(31.3)−32.0	31.3–(30.0)−32.5	1.00	S	Tri-syncolpate	Microscabrate
P. contorta	14.9–(15.9)−17.2	14.6–(15.4)−17.0	1.03	PS	Bi-syncolpate	Microfoveolate
P. cranolopha	31.2–(32.5)−36.0	24.7–(26.0)−28.5	1.25	SP	Bi-syncolpate	Microfoveolate
P. crenulata	16.9–(18.3)−20.1	13.5–(14.2)−15.2	1.29	SP	Bi-syncolpate	Microreticulate
P. cystopteridifolia	20.1–(21.2)−22.5	19.3–(20.4)−21.4	1.03	PS	Tri-syncolpate	Microfoveolate
P. densiflora	28.8–(30.7)−32.1	20.1–(21.3)−22.4	1.44	P	Tri-syncolpate	Microfoveolate
P. grayi	22.5–(23.8)−25.0	22.5–(23.8)−25.0	1.00	S	Tri-syncolpate	Microreticulate
P. groenlandica	21.3–(22.5)−23.7	17.5–(18.8)−20.0	1.20	SP	Bi-syncolpate	Microfoveolate
P. lanceolata	15.1–(16.5)−18.2	20.0–(18.8)−21.1	0.83	SO	Bi-syncolpate	Microfoveolate
P. langsdorfii	19.3–(22.1)−23.6	19.5–(22.1)−23.4	1.00	S	Bi-syncolpate	Microfoveolate
P. oederi	20.8–(19.5−21.8)	20.8–(19.3)−22.1	1.00	S	Tri-syncolpate	Microfoveolate
P. palustris	22.1–(23.4)−24.7	22.0–(23.4)−24.5	1.00	S	Tri-syncolpate	Microreticulate
P. parryi	16.1–(17.3)−19.0	14.5–(15.6)−17.2	1.11	PS	Bi-syncolpate	Microreticulate
P. pulchella	16.5–(18.3)−20.4	14.2–(15.8)−16.6	1.15	SP	Bi-syncolpate	Microfoveolate
P. racemosa	19.5–(20.3)−21.4	19.8–(20.8)−21.1	0.98	OS	Bi-syncolpate	Microreticulate
P. resupinata	19.5–(22.1)−24.5	19.8–(22.1)−24.7	1.00	S	Bi-syncolpate	Microreticulate
P. sudetica	18.2–(19.5)−20.8	15.6–(16.9)−18.2	1.15	SP	Bi-syncolpate	Microreticulate
P. verticillata	15.3–(16.9)−18.5	15.6–(16.9)−18.2	1.00	S	Tri-syncolpate	Microscabrate
P. yezoensis	16.2–(17.8)−19.3	14.6–(15.7)−17.1	1.13	PS	Bi-syncolpate	Microfoveolate

All measurements are in μm

OS oblate spheroidal, *P* prolate, *PS* prolate speroidal, *S* spheroidal, *SO* suboblate, *SP* subprolate

includes species with three kinds of exine sculpturing: microfoveolate, microreticulate and microscabrate.

Microfoveolate: five species, *P. alaschanica* (Fig. 1a–c), *P. attollens*, *P. cystopteridifolia* (Fig. 1d–f), *P. densiflora* (Fig. 1g, h) and *P. oederi*.

Microreticulate: three species, *P. bracteosa*, *P. grayi* (Fig. 1j–l) and *P. palustris*.

Microscabrate: two species, *P. capitata* and *P. verticillata* (Fig. 1i).

2. Bi-syncolpate aperture type (Fig. 2a–i): The pollen grains small to medium, mean *P* and *E*: 15.9–32.5 μm × 15.4–26.0 μm, *P/E*: 0.83–1.20, oblate spheroidal to subprolate. Colpi usually wide, long and sunken, extending to fuse at the poles, colpus membrane covered with granules and crustate elements. This aperture configuration includes species with two kinds of exine sculpturing, i.e., microfoveolate and microreticulate.

Microfoveolate: seven species, including *P. contorta*, *P. cranolopha* (Fig. 2f), *P. groenlandica* (Fig. 2g), *P. lanceolata*, *P. langsdorfii*, *P. pulchella* (Fig. 2a–c) and *P. yezoensis* (Fig. 2d, e).

Microreticulate: six species, including *P. canadensis* (Fig. 2h), *P. crenulata*, *P. parryi*, *P. racemosa*, *P. resupinata* (Fig. 2i) and *P. sudetica*.

Pollen morphology in *Pedicularis*

In the current study, no new or previously unreported aperture configuration type or exine ornamentation was observed. Neither tri-colpate pollen grains, nor microrugulate and retipilate pollen grains were found in the species investigated from North America and Japan. To date, only 59 species were studied using SEM, therefore, it is difficult to provide relatively complete data of the exine ornamentation in *Pedicularis*. The ancestral pollen type in this genus was considered to be tri-colpate (Tsoong and Chang 1965; Wang et al. 2003), which is usually present in some 'primitive' or early diverging species. This is supported by data from gross morphology, corresponding either to alternate leaves or to the galea with a densely bearded margin (Li 1951; Tsoong 1963). Recent molecular data revealed that the early diverging clades of this genus all possess tri-colpate pollen (Ree 2005). Moreover, tri-colpate pollen grains do not occur in

Table 3 Summary of *Pedicularis* species and their pollination syndromes

Pedicularis species	Corolla types	Pollinator types	Pollination patterns	Foraging	Country	References
P. alaschanica	Short-tubed and beaked	W	NT	N, P	China	Macior and Tang (1997)
P. attollens	Short-tubed and beaked	Q, W	ST	P	America	Sprague (1962), Macior (1977)
P. bracteosa	Toothless	Q, W	NT, ST	N, P	America	Macior (1973, 1996), Aluri and Robart (1991)
P. canadensis	Toothed	Q	NT	N, P	America	Macior (1968a)
P. capitata	Toothless	Q, W	NT, ST	N, P	Canada	Macior (1975)
P. contorta	Short-tubed and beaked	Q, W	ST	P	America	Macior (1973)
P. cranolopha	Long-tubed and beaked	W	ST	P	China	Macior et al. (2001)
P. crenulata	Toothed	W	NT	N, P	America	Sprague (1962)
P. cyathophylla	Long-tubed and beaked	W	ST	P	China	Tang and Xie (2006)
P. cystopteridifolia	Toothless	Q, W	NT, ST	N, P	America	Macior (1983)
P. davidii	Short-tubed and beaked	W	ST	P	China	Macior and Tang (1997)
P. densiflora	Toothless	H, W	NT	N, P	America	Sprague (1962), Macior (1986)
P. densispica	Toothless	W	NT, ST	N, P	China	Wang and Li (1998, 2005)
P. grayi	Toothed	H, Q, W	NT, ST	N, P	America	Macior (1970)
P. groenlandica	Short-tubed and beaked	Q, W	ST	P	America	Sprague (1962), Macior (1968b, 1973, 1983)
P. lanceolata	Short-tubed and beaked	W	ST	P	America	Macior (1969)
P. langsdorfii	Toothed	Q, W	NT, ST	N, P	Canada	Macior (1975)
P. longiflora	Long-tubed and beaked	W	ST	P	America	Wang and Li (1998), Macior et al. (2001)
P. lutescens	Toothed	W	NT, ST	N, P	China	Wang and Li (2005)
P. oederi	Toothless	Q, W	NT, ST	N, P	America, Japan	Macior (1983, 1988)
P. palustris	Toothed	Q, W	NT, ST	N, P	Canada	Macior (1993)
P. parryi	Short-tubed and beaked	W	NT	N, P	America	Macior (1970)
P. plicata	Toothless	Q	NT, ST	N, P	China	Macior and Tang (1997)
P. pulchella	Toothed	Q, W	NT, ST	N, P	America	Macior (1986)
P. racemosa	Short-tubed and beaked	W	ST	P	America	Sprague (1962), Macior (1970, 1973)
P. resupinata	Short-tubed and beaked	W	ST	P	Japan	Macior (1988)
P. rex	Toothed	Q, W	NT, ST	N, P	China	Wang and Li (1998)
P. sudetica	Toothed	M, W	NT, ST	N, P	America, Canada	Macior (1970, 1975)
P. torta	Short-tubed and beaked	W	ST	P	China	Tang and Xie (2006)
P. verticillata	Toothless	W, Q	NT	N, P	Canada, China, Japan	Macior (1975, 1988), Macior and Tang (1997)
P. yezoensis	Short-tubed and beaked	W	ST	P	Japan	Macior (1988)

M bumblebee male, *Q* bumblebee queen, *W* bumblebee worker, *H* hummingbird, *NT* nototribic, *ST* sternotribic, *P* pollen, *N* nectar

Fig. 1 Scanning electron microscopy micrographs of pollen grains of *Pedicularis* species (tri-syncolpate pollen), scale bar 10 μm for whole pollen grains, 1 μm for details of ornamentation. **a–c** *P. alaschanica*. **a** Equatorial view; **b** polar view; **c** microfoveolate ornamentation, with a rough surface. **d–f** *P. cystopteridifolia*. **d** Equatorial view; **e** polar view; **f** exine with a microfoveolate ornamentation. **g, h** *P. densiflora*. **g** Equatorial view; **h** microfoveolate ornamentation at the mesocolpium. **i** *P. verticillata*, ornamentation microscabrate with few tectal perforations. **j–l** *P. grayi*. **j** Equatorial view; **k** polar view; **l** ornamentation microreticulate with few tectal perforations
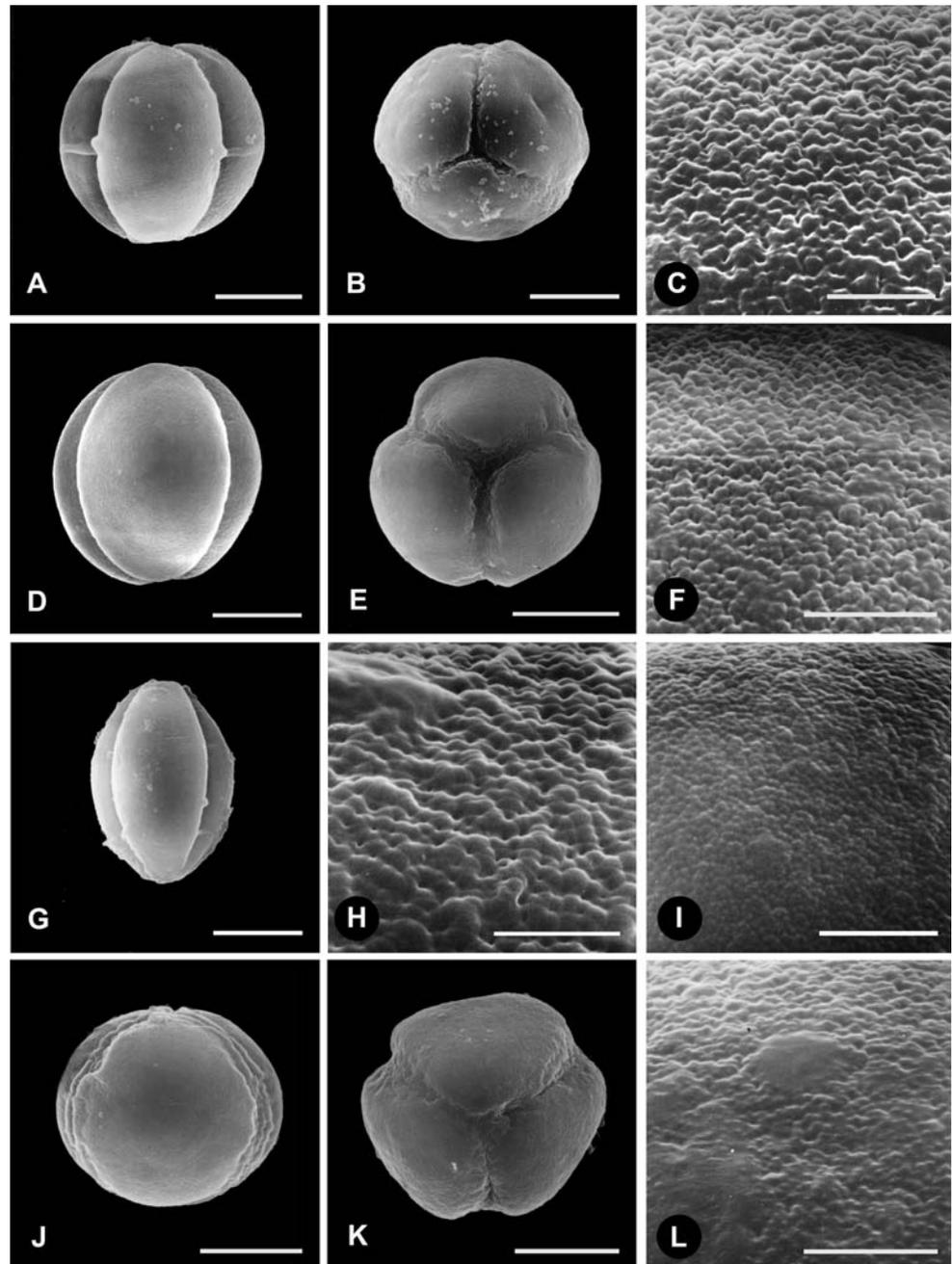
a monophyletic group *Cyathophora*, which is endemic to the eastern Himalaya-Hengduan Mountains region. The unique feature of it is that the leaf and bract bases are fused together to form a cup-like structure around the stem at each node. The group includes all four corolla types, and is regarded as derived in the genus (Yu and Wang 2008).

Pollen characters correlation with corolla types

As above mentioned, four corolla types are recognized in *Pedicularis*. The majority of species have beaked corollas

exhibiting considerable diversity and with most of them being long-beaked (Yang et al. 1998). On the basis of molecular evidence, long corolla tubes seem to be derived from short ones, galeas with teeth to be derived from toothless galeas, and the diverse forms of galea beak may be both gained and lost over the course of floral evolution (Ree 2001).

To understand the correlation between the pollen aperture configuration and exine ornamentation and the four major corolla types, pollen aperture data of 216 species were analyzed based on the current and previous studies (Fig. 3). One hundred and thirty-two species are

Fig. 2 Scanning electron microscopy micrographs of pollen grains of *Pedicularis* species (bi-syncolpate pollen), scale bar 10 μm for whole pollen grains, 1 μm for details of ornamentation. **a–c** *P. pulchella*. **a** Equatorial view; **b** mesocolpium view; **c** perforated tectum with microfoveolate ornamentation. **d, e** *P. yezoensis*. **d** Mesocolpium view; **e** microfoveolate ornamentation, with a rough, undulating surface. **f** *P. cranolopha*, microfoveolate ornamentation, with a rough, undulating surface. **g** *P. groenlandica*, microfoveolate ornamentation at the mesocolpium. **h** *P. canadensis*, perforated tectum with microreticulate ornamentation. **i** *P. resupinata*, microreticulate ornamentation at the mesocolpium
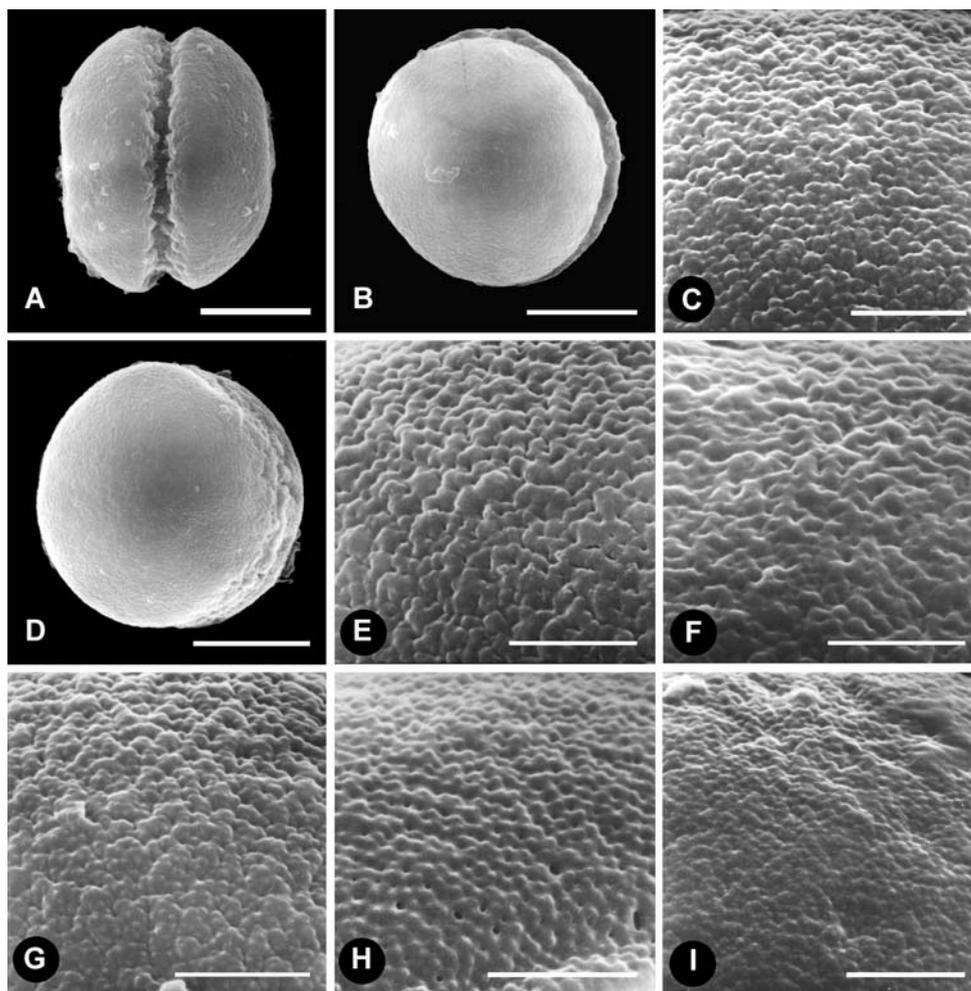

bi-syncolpate, 60 tri-syncolpate and 24 species tri-colpate. Whereas of the bi-syncolpate species, 76 have corollas that are short-tubed and beaked, while 10 species have toothless corollas. Within the species with tri-syncolpate apertures, only one species has a long-tubed corolla. For the tri-colpate species, only one has a toothed corolla and none has a long-tubed corolla. Thus, there is a remarkably significant association between pollen aperture types and corolla types ($\chi^2 = 46.323$, $df = 6$, $P < 0.001$).

When assessing the association between pollen aperture configuration and the corolla types, our analyses indicate that 76 of 112 species with short-tubed and beaked corollas have bi-syncolpate pollen, while 20 of 21 species with long-tubed corollas are found to be bi-syncolpate. Species with long-tubed corollas never have tricolpate pollen. It is of interest that the most long-tubed species, which are limited to the Sino-Himalayan region, are characterized by bi-syncolpate pollen. Furthermore, most species with long-tubed corollas are distributed on alpine meadows or tundra, and specialize as rosette-type plants (except *P. cyathophylla*). It is evident that the
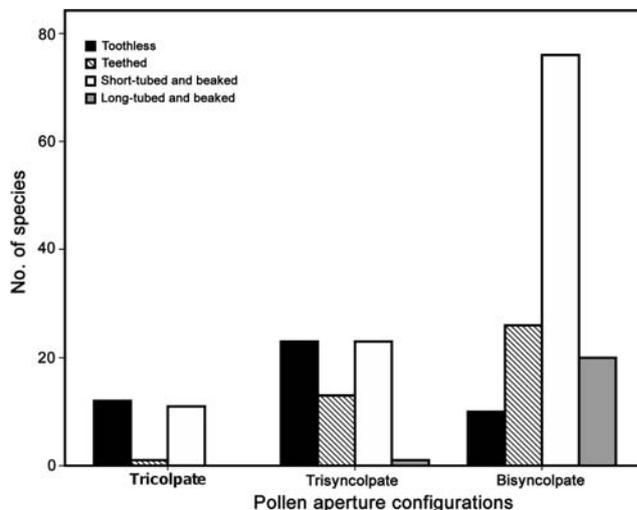
Fig. 3 The correlation between the aperture configurations types and the corolla types

species with beaked or long-tubed corollas have a high capacity for retention of pollen grains (Yang et al. 2002). This may imply that selection constraint favors pollen
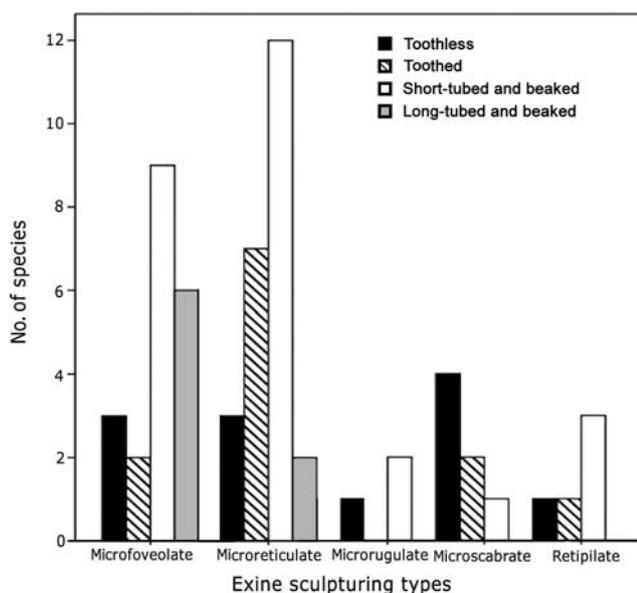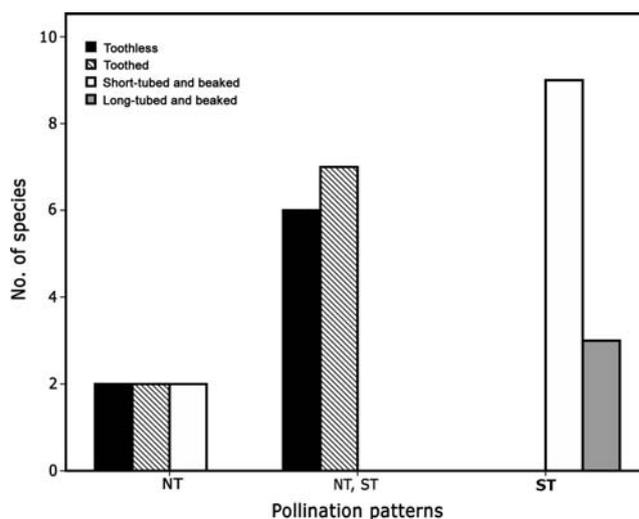
Fig. 4 The correlation between the exine types and the corolla types

Fig. 5 The correlation between pollination syndromes and the corolla types

aperture evolution, and the floral differentiation has possibly been accompanied by corresponding with reproductive and ecological adaptation.

Similarly, we made a statistical analysis for the 59 species studied with data of exine ornamentation (Fig. 4). Twenty species are microfoveolate, 24 species being microreticulate, while 7 species are microscabrate, 5 species retipilate, and 3 are microrugulate. We detect no association of exine ornamentations with corolla types ($\chi^2 = 17.355$, $df = 12$, $P > 0.05$). All five kinds of exine sculpturing are found in species with the different corolla types, but the species with long-tubed corollas exhibit only two kinds of exine ornamentation, i.e., microfoveolate and microreticulate. In the species studied with long-tubed corollas, pollen grains of six of the eight species are microfoveolate. Meanwhile, the species with toothed corollas never have microrugulate sculpturing. The microrugulate pollen is only present in three species that have putatively 'primitive' corolla types: two species with short beaks and densely bearded galea margins, and one species with toothless corolla.

Pollination patterns correlation with corolla types

Thirty-one species that have previously been studied in relation to pollination syndromes were summarized in Table 3. In general, the relationships between flowers and pollinators are established by the means of attractants,

with nectar and/or pollen as the primary rewards to pollinators. We analyzed the species for which pollination data are available (Fig. 5) and the results show that *Bombus* queens tend to forage for both nectar and pollen, while workers favor collecting pollen. Some similar forms of corolla may be pollinated by the same or different species of pollinators with same foraging pattern (Fig. 6). None of the six species that are nototribically pollinated by bumblebees has a long-tubed corolla. Meanwhile, 13 species that are pollinated both nototribically and sternotribically are teethed or teethless. In contrast, there are 12 species with sternotribic pollination pattern, but none of them are teethed or teethless. In short, it is indicated that the correlation between corolla types and pollination patterns is significant ($\chi^2 = 26.245$, $df = 6$, $P < 0.001$).

However, recent molecular phylogenetic analyses indicate that floral characters of *Pedicularis* show a high degree of homoplasy, and morphologically similar corollas were apparently derived independently multiple times, perhaps as a result of adaptive radiation (Ree 2005). It seems that species with similar corollas are visited by bumblebees with similar foraging patterns, while pollen is deposited in different positions on the pollinators, and the complex corollas require more precise behavior of pollinators. The combination of corolla types, pollen and pollination mechanisms may promote reproductive isolation among sympatric species, and the differentiation of floral characteristics may accelerate speciation in *Pedicularis*.

Fig. 6 Flowers of *Pedicularis* and bumblebees. **a–d** Four general corolla types: **a** toothless, *P. oederi* Vahl; **b** toothed, *P. lutescens* Franch. ex Maxim.; **c** short-tubed with a beak, *P. integrifolia* Hk. f.; **d** long-tubed with a beak, *P. cranolopha* Maxim. **e–i** Two pollination patterns (i.e., nototribic: NT; sternotribic: ST). **e, g** Beakless flowers are nototribically pollinated by bumblebee, *P. densispica* Franch. ex Maxim. and *P. rex* C. B. Clarck ex Maxim. respectively; **f** beakless flowers are sternotribically pollinated by bumblebee, *P. densispica*; **h, i** beaked flowers are sternotribically pollinated by bumblebees, *P. rhinanthoides* Schrenk and *P. longiflora* Rudolph respectively

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