

Pollen Morphology of *Pedicularis* sect. *Cyathophora*, a Group Endemic to the Eastern Himalaya-Hengduan Mountains Region

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Abstract

Pedicularis sect. *Cyathophora* is a distinctive group endemic to the eastern Himalaya-Hengduan Mountains region. It was regarded as a 'grex' or section and included all four general corolla types of *Pedicularis*. A 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. Pollen morphology of seven species in sect. *Cyathophora* was investigated by scanning electron microscopy (SEM) and light microscopy (LM). Two different pollen apertures could be distinguished (i.e. trisyncolpate and bisyncolpate) using LM, while examination with SEM made it possible to recognize three types of exine ornamentation (i.e. microscabrate, microfoveolate and microreticulate). The microfoveolate exine ornamentation was found in trisyncolpate pollen grains for the first time. Possible relationships between pollen data and the corolla types were discussed. Comparisons of floral and phyllotaxy characters of the genus *Pedicularis*, together with the pollen characters of sect. *Cyathophora*, could help us to better understand the evolutionary trends in *Pedicularis*.

Key words: eastern Himalaya; Hengduan Mountains; *Pedicularis*; pollen morphology; sect. *Cyathophora*.

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The genus *Pedicularis* L. is traditionally defined as a member of the family Scrophulariaceae (Tsoong 1963; Mill 2001; Wu et al. 2003; Zhang et al. 2006). More than 600 species are recognized, and most of the species are semi-parasitic and primarily occur in the arctic and alpine regions of the Northern Hemisphere (Li 1951; Mill 2001; Wang 2006). There are about 351 species in China (Yang et al. 1998; Yang et al. 2003a), among which about two-thirds of the species are endemic to the east Himalaya-Hengduan Mountains region (Yang et al. 1998; Yang et al. 2003b), where it was treated as a center of

the species diversity and differentiation of this genus (Li 1951; Hong 1983; Ree 2005).

The floral diversifications of *Pedicularis* are likely unequalled by any other genus of flowering plants. The variation of corolla shape is dramatic. The hood-like upper lip, enclosed four introrse anthers and style, was called galea, and can vary greatly, for example, short-, long- and curved-beak, beakless, toothed, toothless, and with a crest or no crest. Additionally, great variation of the length of the corolla tube ranged from 5 mm to 120 mm. Based on the corolla-structure, Li (1951) recognized four general corolla types. According to Li, the two beakless ones, which may be toothed or toothless, were considered as more archaic, and the beaked and long-tubed ones were treated as derived from the beakless ones. In regard to the toothless or toothed type, it was difficult to say which was more primitive (Li 1951; Tsoong 1955; Yang et al. 2002; Wang et al. 2003b). According to traditional viewpoints, the foraging behavior of pollinators may be a selection force determining floral structure and function diversity (Grant and Grant 1965; Faegri and van der Pijl 1979; Nilsson 1988; Yang and Guo 2005). More studies further showed that bumblebees (*Bombus* Latr.) were the primary and the most efficient pollinators of

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Pedicularis (Macior 1988, 1993, 1995; Macior and Tang 1997; Wang and Li 1998, 2005; Macior et al. 2001; Yang et al. 2005). It seems that corolla types and pollination patterns are highly correlated (Macior and Sarvesh 1991; Wang et al. 2003a). Others studies indicated that variation in stigma morphology contributed to pollination efficiency (Yang et al. 2002), and floral diversity may be a result of the extensive parallelism (Yang et al. 2003b; Ree 2005).

Established in 1956 by Tsoong PC, sect. *Cyathophora* was named as a 'grex' (a rank not recognized by the International Code for Botanical Nomenclature). However, it was generally accepted as a section (Li 1948, 1949; Mill 2001). It is endemic to the eastern Himalaya-Hengduan Mountains region, a key geographic region in the distribution of *Pedicularis*. Due to the remoteness and inaccessibility, many aspects of the basic biological data of *Pedicularis* in this region are preliminarily unknown (Ree 2001). Section *Cyathophora* is an amazing group of the genus *Pedicularis*. Despite having few species, this group

is highly diverse in floral variation, including all four general corolla types. Additionally, the most conspicuous morphological characters, whorled leaves and bract bases are fused together to form a cup-like structure around the stem at each node, appearing to have arisen independently from other opposite and whorled-leaved species of *Pedicularis*. Section *Cyathophora* has been recognized in all classification systems for *Pedicularis*, and was divided into four series based on corolla types, namely series *Reges*, *Cyathophylloides*, *Superbae* and *Cyathophyllae*. There are six species in the group (Tsoong 1963). Later, Yang published a new species (i.e. *P. xiangchengensis* H. P. Yang, Yang 1990). In *Flora of China*, seven species were recorded (Yang et al. 1998). The distribution of the group is very interesting (Figure 1). *Pedicularis rex* C. B. Clarke ex Maxim. is a widespread species distributed in the whole eastern Himalaya region, while *P. conanta* Li and *P. xiangchengensis* are described on the basis of solitary collections (type specimens).

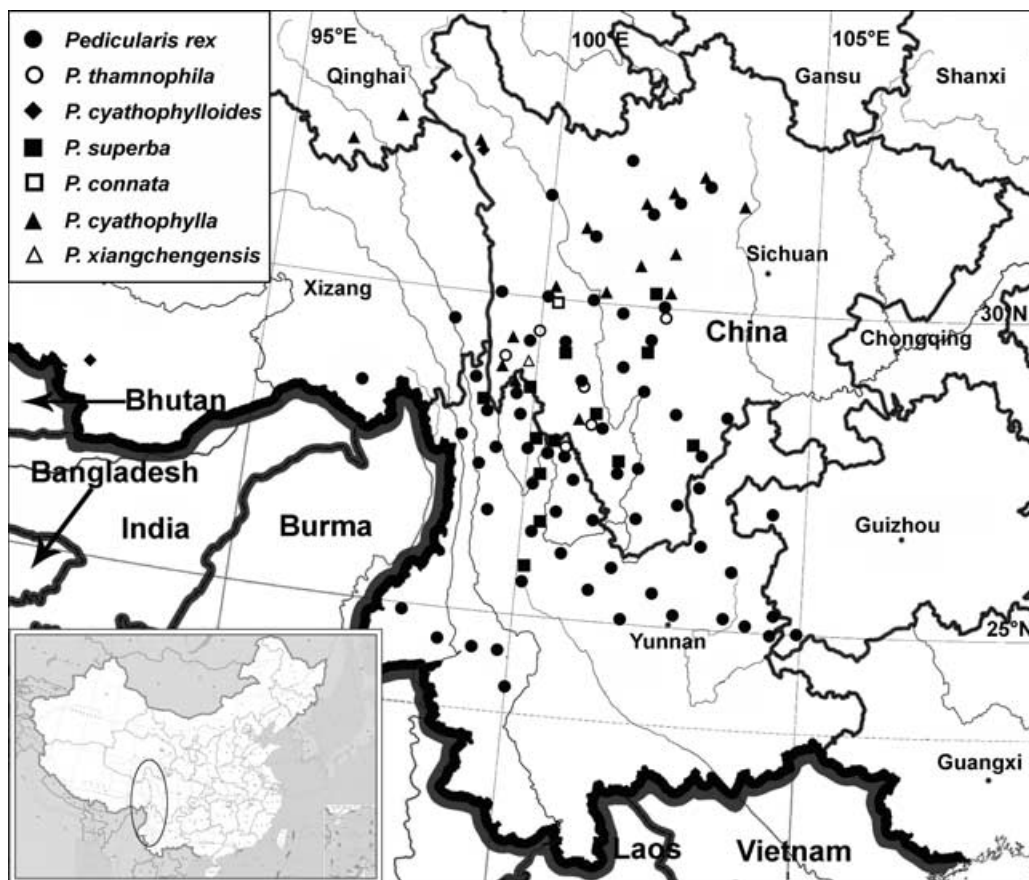


Figure 1. Map showing the distribution of sect. *Cyathophora*, based on the collections from KUN, PE and E herbaria.

Ellipse on the map indicates the distribution of sect. *Cyathophora* in the eastern Himalaya-Hengduan Mountains region.

In recognizing sect. *Cyathophora*, most classification systems have retained the species of *Cyathophora* as a cohesive unit (see reviewed by Tsoong 1955; Ree 2001). However, its systematic position is uncertain in different classifications. For example, Li (1948) put sect. *Cyathophora* into the *Cyclophyllum* group, and Tsoong (1963) named sect. *Cyathophora* as grex *Cyathophora*. According to Macior (1982), in sect. *Cyathophora*, it is questionable that the short-tubed, non-rostrate flowers of *P. rex* were evolved to an extremely long-tubed rostrate form in *P. cyathophylla* Franch.. If saltatory evolution of floral form is possible, the gradual sequence may not represent a historical series of events. Recent molecular phylogenetic analyzes showed that sect. *Cyathophora* is monophyletic, but its systematic position is not yet clear (Yang et al. 2003b; Ree 2005).

The palynology of some *Pedicularis* species was reported (Tsoong and Chang 1965; Belkina 1972; Dutta and Chanda 1978; Inceoğlu 1982; Minkin and Eshbaugh 1989; Wang et al. 2003b). Tsoong and Chang (1965) studied 193 species (excluding 10 species) native to China, while these pollen data were mainly investigated by LM, only Inceoğlu, Minkin and Eshbaugh, and Wang et al. used LM and SEM. The aperture of pollen grains was divided into three types: tricolpate, trisyncolpate and bisyncolpate. However, some studies considered that pollen grains in *Pedicularis* were smooth-surfaced (Tsoong and Chang 1965; Dutta and Chanda 1978; Yang et al. 2002), but others found a distinct variation of exine ornamentation (Inceoğlu 1982; Minkin and Eshbaugh 1989; Wang et al. 2003b). In fact, the exine of pollen grains of *Pedicularis* is extremely thin, and it is difficult to distinguish the exine ornamentation under LM. Wang et al. (2003b) studied 32 species representing 13 groups

of Chinese species and five types of exine ornamentation (i.e. microscabrate, microrugulate, retipilate, microreticulate and microfoveolate) could be distinguished under SEM.

Section *Cyathophora* is an important group in phylogenetic sequences of floral and vegetative evolution in *Pedicularis*. However pollen data were not complete. In the present study, all seven species of sect. *Cyathophora* were observed using LM and SEM. Of these, five species (except *P. rex* and *P. thamnophila* (Hand.- Mazz.) Li) were not studied by SEM previously. The objectives of this study are: (i) to document new information of pollen characters of the sect. *Cyathophora*; (ii) to discuss the evolutionary trends based on pollen data; and (iii) to find some evidence of phylogeny between pollen morphology and corolla types in the genus.

Results

Based on observations under LM and SEM, pollen characters of all seven species of sect. *Cyathophora* are summarized in Table 1.

General descriptions of pollen morphology in sect. *Cyathophora*

Pollen grains were radially symmetrical and isopolar. The size of pollen grains varied from small to medium (P: $20.7 \pm 0.84 - 33.7 \pm 1.80 \mu\text{m}$ × E: $20.4 \pm 0.68 - 33.5 \pm 1.13 \mu\text{m}$), in which the smallest was *P. rex* and the largest was *P. xiangchengensis*. Graphic tests showing a variation of parameters P and E have been given (Figures 2 and 3), and a scatter diagram for the median values of P and E is shown in Figure 4.

Table 1. Summary of pollen data in sect. *Cyathophora*

Taxon	Polar length	Equatorial diameter	P/E	Shape	Aperture	Exine sculpturing	Pollen types (following Wang et al. 2003b)
<i>Pedicularis rex</i>	20.7 ± 0.84 (19.5–22.5)	20.4 ± 0.68 (19.5–21.3)	1.02	OS	3-syncolpate	MS	IIb
<i>P. thamnophila</i>	21.2 ± 0.85 (20.0–23.0)	21.2 ± 1.18 (19.3–23.8)	1.00	S	3-syncolpate	MS	IIb
<i>P. cyathophylloides</i>	31.1 ± 1.94 (27.5–36.3)	31.7 ± 2.16 (28.8–37.5)	0.98	SO	3-syncolpate	MF	IIId ¹
<i>P. superba</i>	33.7 ± 1.80 (31.3–35.5)	33.0 ± 1.03 (31.3–35.0)	1.02	OS	2-syncolpate	MRE	IIIa
<i>P. connata</i>	30.4 ± 1.83 (27.5–33.8)	28.6 ± 1.76 (25.3–31.3)	1.06	OS	2-syncolpate	MRE	IIIa
<i>P. cyathophylla</i>	32.9 ± 1.61 (31.3–36.3)	33.6 ± 2.10 (30.3–38.8)	0.98	SO	2-syncolpate	MRE	IIIa
<i>P. xiangchengensis</i>	33.3 ± 1.24 (30.0–35.0)	33.5 ± 1.13 (31.3–35.5)	0.99	OS	2-syncolpate	MRE	IIIa

MF, Microfoveolate; MRE, Microreticulate. MS, Microscabrate; OS, Oblate spheroidal; P/E, Polar/Equatorial axis ratio; S, Spheroidal; SO, Suboblate; IIb, 3-syncolpate with microscabrate sculpture. IIId¹, 3-syncolpate with microfoveolate sculpture, ¹showing a new subtype; IIIa, 2-syncolpate with microreticulate sculpture. All measurements are in μm .

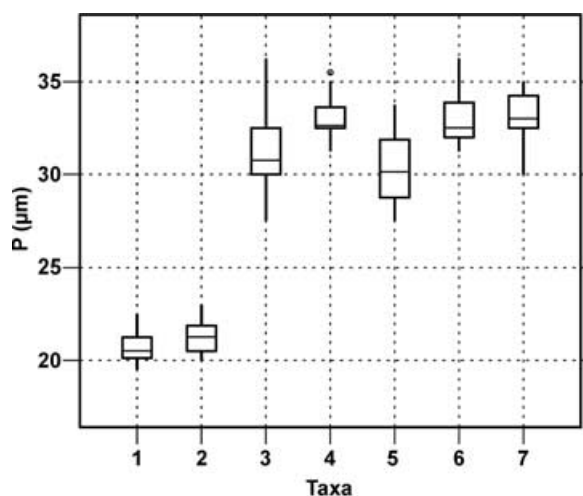


Figure 2. Graphic test (box plot) for median comparisons of polar axis length (P).

Taxa names: 1. *Pedicularis rex*; 2. *P. thamnophila*; 3. *P. cyathophylloides*; 4. *P. superba*; 5. *P. connata*; 6. *P. cyathophylla*; 7. *P. xiangchengensis*

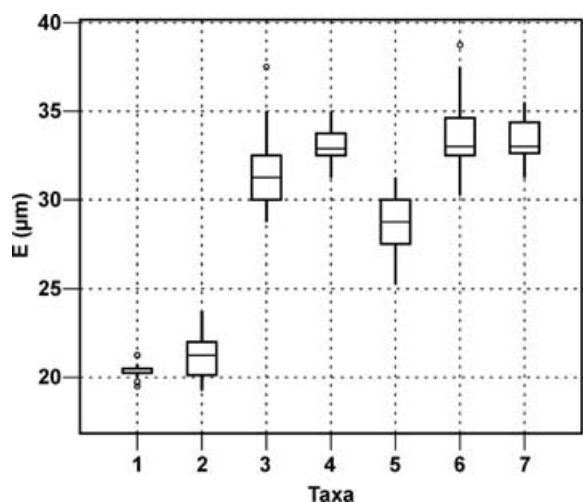


Figure 3. Graphic test (box-plot) for median comparisons of equatorial diameter (E).

Taxa names: 1. *Pedicularis rex*; 2. *P. thamnophila*; 3. *P. cyathophylloides*; 4. *P. superba*; 5. *P. connata*; 6. *P. cyathophylla*; 7. *P. xiangchengensis*

Pollen shapes were spheroidal (P/E 1.00), suboblate (P/E 0.98–0.99) to oblate spheroidal (P/E 1.02–1.06) with broad poles, spheroidal in equatorial view and circular in polar view. According to Wang et al. (2003b), in the genus *Pedicularis*, three types of pollen apertures were recognized under LM, each of which was subdivided (a total of seven sub-types) on the basis of SEM observations. In this study, only two distinct pollen

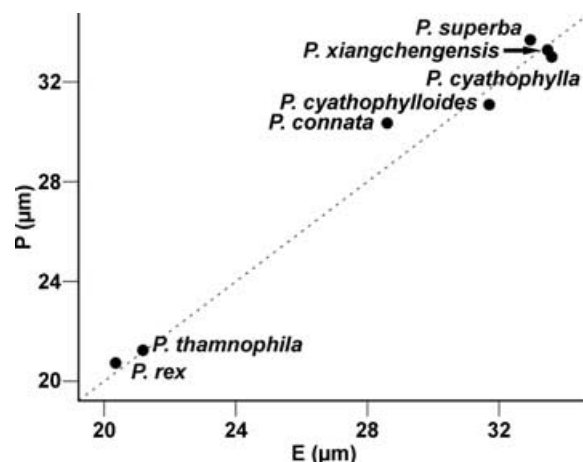


Figure 4. Scatter diagram for P/E ratio.

apertures were recognized: 3-syncolpate and 2-syncolpate. Colpi were usually wide, colpi membranes were often covered with granular elements (Figure 5A), or colpi narrow, smooth and sunken (Figure 5E). SEM observation of exine ornamentation, the microscabrate, microreticulate and microfoveolate sculptures were presented. Also of note, microfoveolate sculpture was found in 3-syncolpate pollen grains for the first time, which was added as a new subtype IId* (Figure 5D–F) (Appendix 1).

Description of pollen morphology for the series

Ser. *Reges* H. L. Li

The series included two species, namely *P. rex* and *P. thamnophila*. The former species occurred in the whole eastern Himalaya, while the latter species was mainly distributed in south-western and western Sichuan, south-eastern Xizang, and north-western Yunnan (Figure 1). Pollen grains of both species were small in size and oblate spheroidal to spheroidal in shape (Table 1; Figure 4). The aperture was trisyncolpate (Figure 5A, C), exine ornamentation was microscabrate (visible only at high magnifications 20 000 \times in SEM; Figure 5B).

Ser. *Cyathophylloides* H. L. Li

P. cyathophylloides Limpr. was the sole species of the series that was distributed in north-western Sichuan and north-eastern and south-eastern Xizang (Figure 1). The pollen grains were medium in size and suboblate in shape. The aperture was trisyncolpate (Figure 5E, F). Exine ornamentation was microfoveolate, with an uneven, undulating surface (visible only at high magnifications 20 000 \times in SEM; Figure 5D).

Ser. *Superbae* Maximowicz

Two species, *P. superba* Franch. ex Maxim. was distributed in south-western Sichuan and north-western Yunnan, and *P. connata* was endemic to western Sichuan (Litang county), which was represented only by Rock JF (No. 16 742) (Figure 1). Pollen grains of both species were medium in size and oblate

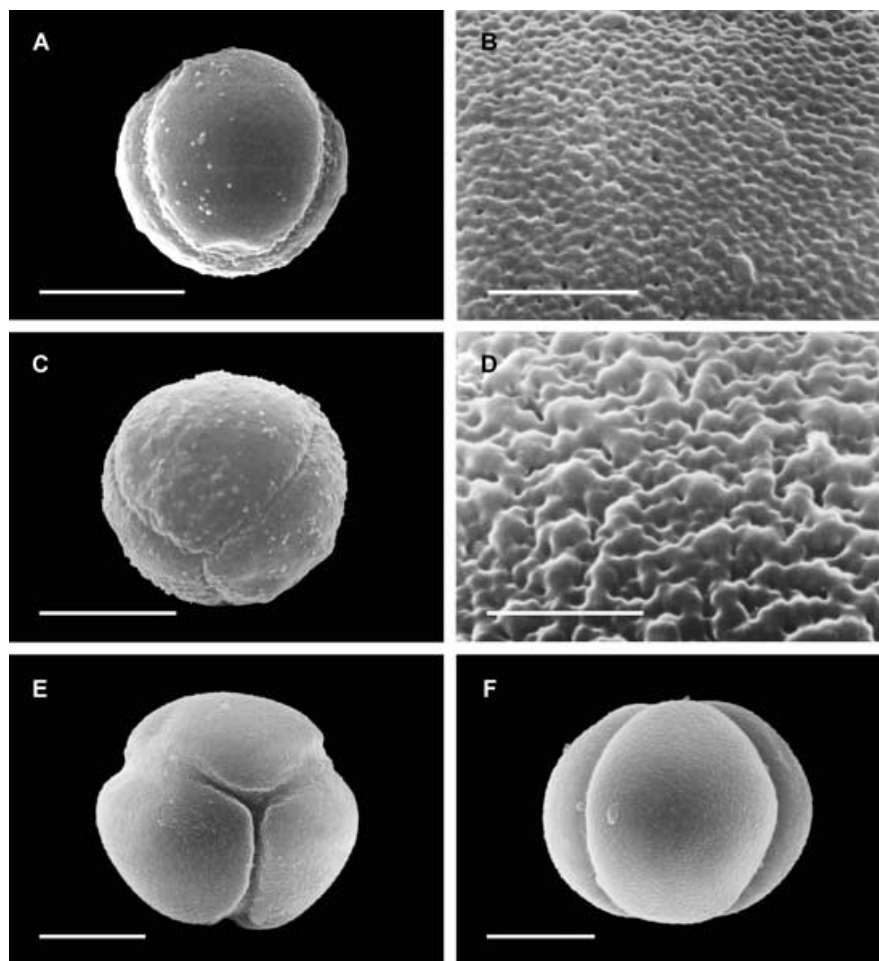


Figure 5. Characteristic features of 3-syncolpate pollen grains (scale bar 10 μm for whole pollen grains, 1 μm for detail of exine ornamentation).

(A) *Pedicularis rex*.

(B)–(C) *P. thamnophila*.

(D)–(F) *P. cyathophylloides*.

spheroidal in shape. The aperture was bisyncolpate (Figure 6A–C), and exine ornamentation of both species was microreticulate (visible only at high magnifications 30 000 \times in SEM; Figure 6D).

Ser. *Cyathophyllae* H. L. Li

There were two species in the series, *Pedicularis cyathophylla* that occurred in western Sichuan, north-western Yunnan and south-eastern Qinghai, and *P. xiangchengensis* endemic to south-western Sichuan (Xiangcheng county) (Figure 1). The latter was only found in its type locality. Pollen grains of both species were medium in size, suboblate and oblate spheroidal in shape. The aperture was bisyncolpate (Figure 6E, G, H), and exine ornamentation of both species was microreticulate (visible only at high magnifications 20 000 \times in SEM; Figure 6F).

Discussion

A new subtype of pollens in *Pedicularis*

In sect. *Cyathophora*, pollen grains with microfoveolate ornamentation and rough surfaces were found in trisyncolpate pollen grains. This is recognized in all examined species of the genus *Pedicularis* for the first time. Previously this exine ornamentation was found in species with bisyncolpate pollen grains, and with short corolla tubes and S-shaped beaks, and long corolla tubes and curved or coiled beaks (Wang et al. 2003b). There are three species with trisyncolpate pollen (*P. rex*, *P. thamnophila* and *P. cyathophylloides*) in sect. *Cyathophora*. The new subtype was found in *P. cyathophylloides*, which is a very distinct species having relatively longer corolla tubes, but belonging to the 'short-tubed' type with a toothless or obscure beak. Li

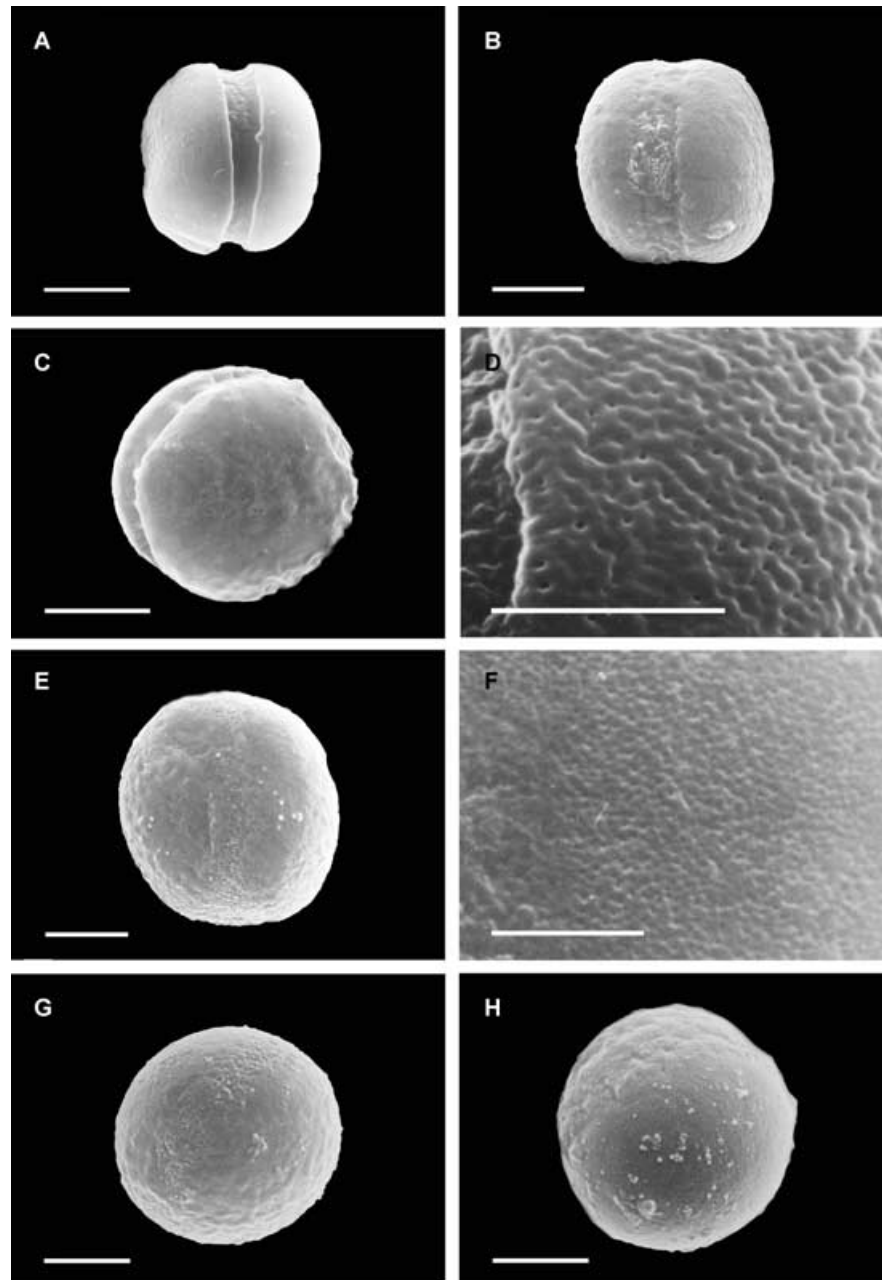


Figure 6. Characteristic features of 2-syncolpate pollen grains (scale bar 10 μm for whole pollen grains, 1 μm for detail of exine ornamentation).

(A), (D) *Pedicularis superba*.

(B)–(C) *P. connata*.

(E)–(F) *P. cyathophylla*.

(G)–(H) *P. xiangchengensis*.

(1948, also see Tsoong 1955) put ser. *Cyathophylloides* in the basal position of sect. *Cyathophora*. However, Tsoong (1955) considered that the corolla of *P. cyathophylloides* possessed a swelled, obscure-beaked galea and an observably spreading

lower lip, which was most probably more advanced than the other two species (*P. rex* and *P. thanmophila*) with the toothed and beakless galea. Actually, our pollen data backed Tsoong's viewpoint.

Pollen morphology in relation to floral evolutionary trends

In general, more pollen apertures usually offer a selective advantage because of the increased germination sites and fertilization rates (Furness and Rudall 2004). Some experimental studies showed that pollen grains with more aperture numbers germinated faster, but at the same time, they had other disadvantages in bad environmental conditions (Dajoz et al. 1991). In addition, longer corolla tubes, larger corolla and pollen sizes in flowering plants may be more adaptive (Cruden and Miller 1981; Harder and Barrett 1995; Harder 1998; Torres 2000; Sarkissian and Harder 2001; Yang and Guo 2004). Tsoong and Chang (1965) recognized tricolpate, trisyncolpate and bisyncolpate pollen grains, and suggested that tricolpate pollen grains were the primitive type, however bisyncolpate pollen was only found in *Pedicularis* from reported pollen data of the family Scrophulariaceae. Wang et al. (2003b) stated that tricolpate pollen also exhibited putatively primitive exomorphological characters, such as alternate leaves, toothed and beakless, and the galea with a densely bearded margin etc., while species with long-tubed corolla and beaks were mostly bisyncolpate and regarded as very advanced.

In the present study, major differences in pollen morphology of sect. *Cyathophora* include pollen size and shape, aperture type and exine ornamentation, though these pollen characteristics were very homogeneous among species of the same series. These may provide additional information for the understanding of the phylogenetic relationships in this genus. The tricolpate pollen grains did not occur in all seven species of sect. *Cyathophora*. This group is restricted to the eastern Himalaya-Hengduan Mountains region, in which the most highly developed long-tubed species of *Pedicularis* were distributed. More comprehensive studies on pollen grains demonstrated that sect. *Cyathophora* should be young and active. Our results showed that the bisyncolpate pollen grains, which are larger in size, present in the species of the short-tubed and beaked type (ser. *Superbaea*) and the long-tubed, and beaked type (ser. *Cyathophyllae*), while the trisyncolpate pollen grains were relatively smaller in size, and were found in the toothed and beakless or obscure-beaked types (series *Reges* and *Cyathophylloides*) (Figure 4). This was consistent with previous reports (Tsoong and Chang 1965; Wang et al. 2003b).

Section *Cyathophora* as a model of evolution of *Pedicularis*

Several classification systems of *Pedicularis* based on floral and vegetative characters have been proposed (see reviewed by Tsoong 1955; Tsoong 1956, 1963). Most of the systems prior to Li mainly emphasized floral characters. However, the systems of Li (1948, 1949) and Tsoong (1955, 1956, 1963) thought that floral characters were shaped by pollinators or environmental conditions, involving parallel evolution in floral

morphology of different species. They also paid special attention to the functional importance of beaked galea in pollination. In contrast, phyllotaxy is steadier under selection pressure and more reliable for diagnosing primary subgeneric groups, and exhibits much less evolutionary lability than floral characters. Despite the floral differences among the series, these species of *Cyathophora* are a nature unit.

It is interesting to note that the pollen characteristics in sect. *Cyathophora*, reflect some differences in corolla morphology, as in the whole genus of *Pedicularis*. The palynology of Tsoong and Chang (1965) considered that these species with beakless and toothless corollas or small boated-galea were primitive in *Pedicularis*, which mostly had tricolpate pollen grains and alternate phyllotaxy (especially in the 'grex' *Scepttrum*). In this genus, most species with advanced corollas bear relatively larger pollen grains with bisyncolpate aperture (Tsoong and Chang 1965; Wang et al. 2003b). These pollen data results (Tsoong and Chang 1965; Wang et al. 2003b, and the present study) may provide a preliminary framework for relationships between pollen morphology and corolla types, and throw a light on the evolution of the genus. As Ree (2001) suggested on the molecular data that long corolla tubes, beaked galeas, and toothed galeas have each evolved independently multiple times within disparate lineages. The most diverse part in the corolla is the amusing beak, meanwhile pollen grains of these species with short corolla tube and various beaks were more diverse and complex. Nevertheless the evolution pattern on pollen morphology is basically correlated to the corolla types in *Pedicularis*.

Materials and Methods

The pollen samples of seven species were obtained from the herbaria of the Kunming Institute of Botany (KUN), the Institute of Botany (PE) and the Royal Botanic Garden Edinburgh (E), or collected in the fields by the authors (Table 2), and investigated by LM and SEM. For LM investigation (Tsoong and Chang 1965), pollen grains were treated with alcohol and mounted in glycerine jelly. Measurements were based on 20 pollen grains; the values of P (polar axis length) and E (equatorial diameter) were measured and the P/E ratio was calculated (Wang et al. 2003b). The interval range for each parameter was established, as well as the mean and standard deviation values, using SPSS version 13.0. Graphic tests (Figures 2 and 3; box plots with indication of median values) showing variation of parameters P and E were elaborated by means of SPSS version 13.0 (Martinez-Ortega et al. 2000).

Generally, acetolysis is regarded as the standard palynological technique (Erdtman 1952). However, it is unsuitable for *Pedicularis* pollen observation, because it causes the exine of pollen grains to rupture (Wang et al. 2003b). Due to the disadvantage of acetolysis in *Pedicularis*, we used a modified

Table 2. List of species, voucher specimens and corolla types investigated

Taxon		Collection	Corolla type
Species	Series		
<i>Pedicularis rex</i>	Ser. <i>Reges</i>	Yunnan: Qiaojia County, Wang H et al. 1427	Short-tubed, toothed and beakless
<i>P. thamnophila</i>		Sichuan: Daocheng County, Yu WB et al. 97	Short-tubed, toothed and beakless
<i>P. cyathophylloides</i>	Ser. <i>Cyathophylloides</i>	Tibet: Jiangda County, Xia GC 1325	Short-tubed, toothless and obscure-beaked
<i>P. superba</i>	Ser. <i>Superbae</i>	Yunnan: Lijiang County, Yu WB et al. 10	Short-tubed, and beaked
<i>P. connata</i>		Sichuan: Litang County, Rock JF 16742	Short-tubed and beaked
<i>P. cyathophylla</i>	Ser. <i>Cyathophylla</i>	Sichuan: Xiangcheng, Boufford DB et al. 30686	Long-tubed, and beaked
<i>P. xiangchengensis</i>		Sichuan: Xiangcheng, Qing-Zang Expedition 3829	Long-tubed and beaked

method for SEM investigation. Anthers were prepared from herbarium materials, which were put into centrifugal pipes by softening in warm water for 5–10 min and then soaked in FAA (Formalin-Acetic Acid-Alcohol) for 24 h. Softened anthers were triturated by glass sticks and then percolated by cuprum net. Pollen materials were centrifuged and dehydrated in an ethanol series from 75% to 95%. Prepared pollen samples were mounted on aluminum stubs and sputter-coated with gold-palladium. Observation and photography were by SEM (KYKY-10 000B; Science Instrument Company, Beijing, China) at 15 kv. Descriptive terminology follows Punt et al. (1994) and Wang et al. (2003b).

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Appendix 1. Key to pollen types, vegetative and corolla characters

1. Trisyncolpate pollen with microscabrate or microfoveolate tectum.....2
 - Bisyncolpate pollen with a microreticulate tectum.....3
 2. Tectum with microscabrate ornamentation.....4
 - Tectum with microfoveolate ornamentation.....*P. cyathophylloides*
 3. Short corolla tube.....5
 - Long corolla tube.....6
 4. Lower lip acute angle spread.....*P. rex*
 - Lower lip right angle spread.....*P. thanmophila*
 5. Corolla yellow, calyx 2-lobed.....*P. connata*
 - Corolla purple, calyx 5-lobed.....*P. superba*
 6. Middle lobe of lower corolla lip entire.....*P. cyathophylla*
 - Middle lobe of lower corolla lip emarginate apically.....*P. xiangchengensis*

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