

Cytotaxonomical Study of *Pedicularis* (Spermatophyta; Scrophulariaceae) in Annapurna Himal, Nepal

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Abstract The chromosome numbers and karyotype of eight species of *Pedicularis* from Annapurna Himal, Nepal were examined: *P. anserantha* var. *elevatogaleata*, *P. confertiflora*, *P. gracilis*, *P. hoffmeisteri*, *P. longiflora* ssp. *tubiformis*, *P. nodosa*, *P. pauciflora* and *P. scullyana*. Four different chromosome numbers, $2n=14$, 16 , 28 and 32 were counted. All except *P. anserantha* var. *elevatogaleata* ($2n=28$, $28+1B?$) and *P. longiflora* ssp. *tubiformis* ($2n=14$) were counted as $2n=16$, which is the most common chromosome number in *Pedicularis*. Two different chromosome numbers, $2n=16$ and 32 , were counted in the same individuals of *P. pauciflora* and *P. hoffmeisteri*. Chromosome numbers of the following six species and one subspecies were counted for the first time, i.e., *P. anserantha*, *P. confertiflora*, *P. hoffmeisteri*, *P. nodosa*, *P. pauciflora* and *P. scullyana* and *P. longiflora* ssp. *tubiformis*. The chromosome number of *P. gracilis* ($2n=16$) was in agreement with that of previous reports. From the present study and previous reports it is considered that Himalayan *Pedicularis* has diversified mainly at diploid level, and the differentiation of karyotype has taken place among the closely related species.

Key words: *Pedicularis*, chromosome, Nepal.

The genus *Pedicularis* is one of the most interesting genera in the alpine flora of the Himalayan region because of highly variable floral morphology and extraordinary species diversity (63 species have been recorded in Nepal; Yamazaki, 1988). The shape of upper lip, lower lip, corolla tube and their spatial arrangement are quite variable. Furthermore, almost all combinations of characters are found. It is very interesting to assess variability of karyomorphological features and correlation with morphological features. However, Himalayan *Pedicularis* have still been insufficiently studied in this field, and chromosome numbers were previously known of only eight Nepalese species (Verma and Dhillon, 1967; Mehra and Gill, 1968; Gill, 1972; Mehra and Vasdevan, 1972; Vasdevan, 1975; Malla *et al.*, 1977a, 1977b, 1979). Recently, I had an opportunity to examine eight species of *Pedicularis* collected from Annapurna Himal, Nepal. In this paper the chromosome numbers and karyomorphological features are described for eight species of *Pedicularis*.

Materials and Methods

For this study material of the following eight species was collected in 1996 from three localities in the Annapurna Himal: *Pedicularis anserantha* T. Yamaz. var. *elevatogaleata* (T. Yamaz.) T. Yamaz., *P. confertiflora* Prain, *P. gracilis* Wall. ex Benth., *P. hoffmeisteri* Klotzsch, *P. longiflora* Rudolph ssp. *tubiformis* (Klotzsch) Pennell, *P. nodosa* Pennell, *P. pauciflora* (Prain) Pennell and *P. scullyana* Prain ex Maxim. (Table 1). The collection localities are: 1. Ommang: Nepal, Dhaulagiri Zone, Mustang District, Ommang, $28^{\circ}44'N$ $83^{\circ}45'E$.—2. Titigaon: Nepal, Dhaulagiri Zone, Mustang District, Titigaon, $28^{\circ}39'N$, $83^{\circ}37'E$.—3. Ghasa: Nepal, Dhaulagiri Zone, Mustang District, Ghasa, $28^{\circ}36'N$ $83^{\circ}39'E$.

Meristems of shoot apices used for cytological observations were treated in the field as follows. Pretreatment in 0.05% colchicine solution for about 4 hours was followed by fixation in Newcomer fixative.

In the laboratory the shoot tips were put into water for 1 hour to rinse out the fixative

Table 1. Collection data and chromosome numbers of the plant used in the present study.

Taxa	Coll. No.	Place	Altitude(m)	Chromosome number
<i>P. anserantha</i> var. <i>elevatogaleata</i>	TI9666134	Ommang	3730	2n=28, 29(28+1B?)
<i>P. confertiflora</i>	TI9666146	Ommang	3900	2n=16
<i>P. gracilis</i>	TI9666182	Titigaon	2610	2n=16
<i>P. hoffmeisteri</i>	TI9666184	Ghasa	2250	2n=16, 32
<i>P. longiflora</i> ssp. <i>tubiformis</i>	TI9666159	Ommang	2700	2n=14
<i>P. nodosa</i>	TI9666133	Ommang	3730	2n=16
<i>P. pauciflora</i>	TI9666156	Ommang	3760	2n=16, 32
<i>P. scullyana</i>	TI9666147	Ommang	3800	2n=16

and then transferred to 1% cellulase (3.2.1.4 WAKO) for 20 minutes at 37°C for maceration. Staining was carried out in 1% lacto-propionic orcein for 4 hours at 20°C, and the extreme apices were then squashed. Voucher specimens are deposited in the Herbarium of Natural History Museum and Institute, Chiba (CBM).

Results

The chromosome numbers of eight Himalayan species are presented in Table 1 with their collection data. Metaphase chromosomes of each species are shown in photographs and interpretative sketches in Fig. 1. Brief descriptions of the karyomorphological features of each species are as follows:

***Pedicularis hoffmeisteri* Klotzsch:** 2n=16 (Fig. 1-1a, b) and 32; first record for this species

Chromosomes were observed from one individual. Two different chromosome numbers (2n=16 and 32) were counted in the same individual. The karyotype of this species is monomodal and symmetric, and length of chromosomes ranges from 3 to 6 μ m.

Voucher specimen. TI9666184 (CBM-BS-145047).

***Pedicularis scullyana* Prain ex Maxim.:** 2n=16 (Fig. 1-2a, b); first record for this species
Chromosomes were observed from two in-

dividuals. The karyotype of this species is monomodal and symmetric, and length of chromosomes ranges from 3 to 7 μ m.

Voucher specimen. TI9666147 (CBM-BS-145046).

***Pedicularis longiflora* Rudolph ssp. *tubiformis* (Klotzsch) Pennell:** 2n=14 (Fig. 1-3a, b); first record for this subspecies

Chromosomes were observed from two individuals. The karyotype of this species is monomodal and symmetric (six pairs of metacentric and one pair of submetacentric chromosomes), and length of chromosomes ranges from 2 to 3 μ m (Fig. 2d).

Voucher specimen. TI9666159 (CBM-BS-145044).

***Pedicularis nodosa* Pennell:** 2n=16 (Fig. 1-4a, b); first record for this species

Chromosomes were observed from three individuals. The karyotype of this species is monomodal and asymmetric (three pairs of submetacentric chromosomes, three pairs of subtelocentric chromosomes and two pairs of acrocentric chromosomes), and length of chromosomes ranges from 3 to 6 μ m (Fig. 2b).

Voucher specimen. TI9666133 (CBM-BS-145042).

***Pedicularis confertiflora* Prain:** 2n=16 (Fig. 1-5a, b); first record for this species

Chromosomes were observed from one individual. The karyotype of this species is

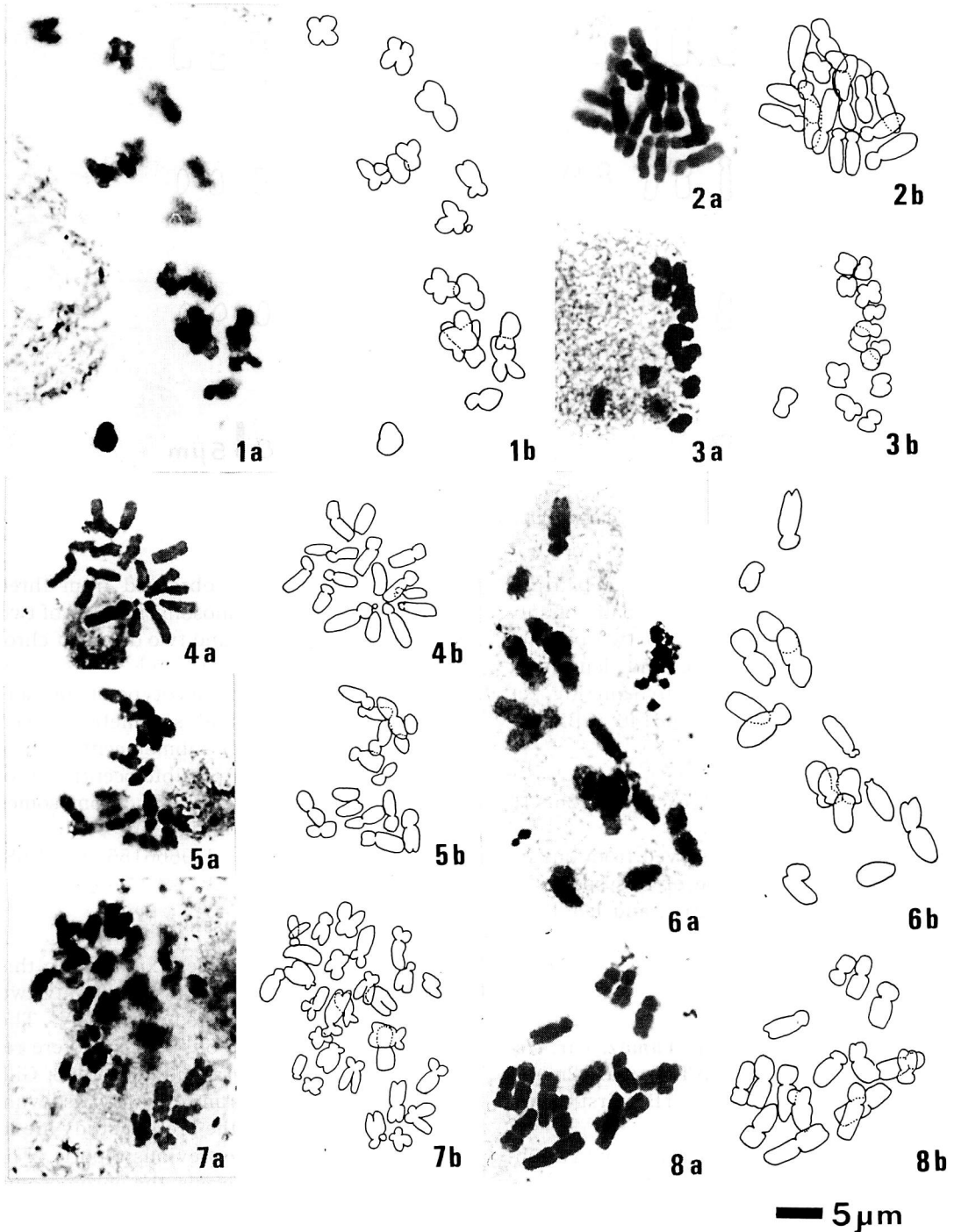


Fig. 1. Chromosomes of *Pedicularis*. 1a, b, *P. hoffmeisteri* ($2n=16$); 2a, b, *P. scullyana* ($2n=16$); 3a, b, *P. longiflora* ssp. *tubiformis* ($2n=14$); 4a, b, *P. nodosa* ($2n=16$); 5a, b, *P. confertiflora* ($2n=16$); 6a, b, *P. gracilis* ($2n=16$); 7a, b, *P. anserantha* var. *elevatogaleata* ($2n=28$); 8a, b, *P. pauciflora* ($2n=16$).

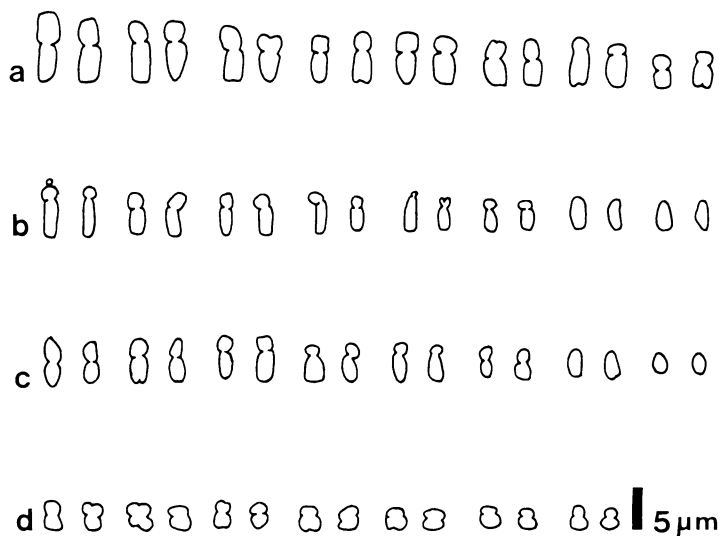


Fig. 2. Karyotypes of four species of Himalayan *Pedicularis*. a, *P. pauciflora* ($2n=16$); b, *P. nodosa* ($2n=16$); c, *P. confertiflora* ($2n=16$); d, *P. longiflora* ssp. *tubiformis* ($2n=14$).

monomodal and symmetric (four pairs of metacentric chromosomes, two pairs of submetacentric chromosomes and two pairs of acrocentric chromosomes), and length of chromosomes ranges from 2 to $5\ \mu\text{m}$ (Fig. 2c).

Voucher specimen. TI9666146 (CBM-BS-126738).

***Pedicularis gracilis* Wall. ex Benth.:** $2n=16$ (Fig. 1-6a, b)

Chromosomes were observed from one individual. The karyotype of this species is monomodal and asymmetric, and length of chromosomes ranges from 3 to $7\ \mu\text{m}$.

Voucher specimen. TI9666182 (CBM-BS-145045).

***Pedicularis anserantha* T. Yamaz. var. *elevatogaleata* (T. Yamaz.) T. Yamaz.:** $2n=28$ (Fig. 1-7a, b) and 29 ($28+1B?$); first record for this species

Chromosomes were observed from two individuals. The karyotype of this species is monomodal and symmetric, and length of chromosomes ranges from 2 to $5\ \mu\text{m}$.

Voucher specimen. TI9666134 (CBM-BS-129230).

***Pedicularis pauciflora* (Prain) Pennell:** $2n=16$ (Fig. 1-8a, b) and 32; first record for this species

Chromosomes were observed from three individuals. The chromosome number of two individuals is $2n=16$, and two different chromosome numbers, $2n=16$ and 32, are counted in the other. The karyotype of this species is monomodal and symmetric (seven pairs of metacentric or submetacentric chromosomes and one pair of subtelocentric chromosomes), and length of chromosomes ranges from 3 to $7\ \mu\text{m}$ (Fig. 2a).

Voucher specimen. TI9666156 (CBM-BS-145043).

Discussion

Among the eight species examined in this study, the chromosome numbers of only two species have been reported previously. The chromosome numbers of *P. gracilis* were recorded as $n=8$ (Mehra and Gill, 1968; Gill, 1972; Vasdevan, 1975) and $2n=16$ (Vasdevan, 1975). The results of the present study are in agreement with these previous reports. For *P. longiflora* ssp. *tubiformis*, the chromosome number ($2n=14$) is different from that of the previous report ($2n=16$) for ssp. *longiflora* from Siberia (Krasnikova *et al.*, 1983).

In this study four different chromosome numbers are found, i.e., $2n=14$, 16, 28 and 32. This suggests that the basic chromosome numbers are $x=7$ and 8. If $x=7$ is one of the basic numbers of *Pedicularis*, *P. anserantha*

var. *elevatogaleata* ($2n=28$ and $28+1B?$) should be a tetraploid species. The nuclear plates of $2n=32$ are always found together with those of $2n=16$ in the individuals of *P. hoffmeisteri* and *P. pauciflora*, so higher ploidy number is thought to result from endopolyploidy. Other five species are considered as diploid species with a basic chromosome number of $x=8$.

Yamazaki (1988) divided Nepalese species of *Pedicularis* into two groups; i.e., Group *Cyclophyllum* and *Allophyllum*. The chromosome numbers of three species belonging to *Cyclophyllum* (*P. nodosa*, *P. gracilis* and *P. confertiflora*) are the same ($n=8$ or $2n=16$) (Gill, 1972; Vasdevan, 1975; the present study). However, the karyotypes of *P. nodosa* and *P. confertiflora* differ. In the Series *Claspedotrichae* (Group *Allophyllum*, Sect. *Lasioglossa*) the chromosome numbers of *Pedicularis anserantha* var. *elevatogaleata* ($2n=28$ and $28+1B?$, the present study) and *P. clarkei* ($n=8$, Malla *et al.*, 1977a) are different. In the case of *Pedicularis longiflora*, the chromosome numbers are different between the two subspecies: ssp. *turbiformis* ($2n=14$; the present study) and ssp. *longiflora* ($2n=16$; Krasnikova *et al.*, 1983). *Pedicularis siphonantha* also belongs to Section *Phanerantha* and the chromosome number ($n=8$, Verma and Dhillon, 1967; Mehra and Gill, 1968; Gill, 1972; Mehra and Vasdevan, 1972; $2n=16$, Vasdevan, 1975) is the same as in *P. longiflora* ssp. *longiflora*. In the Section *Sarcochilus* (*P. hoffmeisteri*, *P. pauciflora* and *P. megalantha*) the common chromosome number of $n=8$ or $2n=16$ is found (Mehra and Vasdevan, 1972; Vasdevan, 1975; Malla *et al.*, 1979; the present study). Considering the data of this study together with previous reports it is concluded that Himalayan *Pedicularis* has diversified mainly at diploid level, and differentiation of karyotype has taken place among the closely related species.

Morphological characters of the flower: the shape of upper lip, lower lip, length of corolla tube, spatial arrangement of floral organs etc. are quite variable in *Pedicularis*. However, no clear correlation is found between morphological characters and chromosome numbers.

Two different chromosome numbers were

counted in the same individuals of *Pedicularis hoffmeisteri* and *P. pauciflora*. Disruption of chromosomes cannot cause this phenomenon, because the chromosomes of the nuclear plates with $2n=32$ have two chromatids in each chromosome. Such endopolyploidy is commonly found, especially in herbaceous perennials (Jong, 1987).

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ネパール、アンナプルナ・ヒマール産
シオガマギク属 (ゴマノハグサ科)
の細胞分類学的研究

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ネパール、アンナプルナ・ヒマール産の 8 種のシオガマギク属植物 (*Pedicularis*) の染色体数と核型について観察した: *Pedicularis anserantha* var. *elevatoga-*

leata, *P. confertiflora*, *P. gracilis*, *P. hoffmeisteri*, *P. longiflora* ssp. *tubiformis*, *P. nodosa*, *P. pauciflora*, *P. scullyana*. このうち, *P. gracilis* 以外の 6 種 1 亜種については, 初めての報告である (*P. longiflora* ssp. *tubiformis* については 亜種で初めて). *Pedicularis gracilis* ($2n=16$) については, 従来の報告 ($n=8$, $2n=16$) と一致した. *Pedicularis confertiflora*, *P. hoffmeisteri*, *P. nodosa*, *P. pauciflora*, *P. scullyana* では $2n=16$ が観察された. *P. pauciflora* と *P. hoffmeisteri* に関しては $2n=16$ と 32 の染色体数が同時に観察された個体があった. *Pedicularis anserantha* var. *elevatogaleata* の染色体数は $2n=28$, または $28+1B?$ であり, *P. longiflora* ssp. *tubiformis* の染色体数は $2n=14$ であった. 今回及び従来の研究から, ヒマラヤ産シオガマギク属植物は近縁種間でも核型が異なることと主に二倍体レベルで種分化していることが明らかになった.