

Bumblebee pollination of *Cypripedium macranthos* var. *rebunense* (Orchidaceae); a possible case of floral mimicry of *Pedicularis schistostegia* (Orobanchaceae)

N. Sugiura¹, M. Goubara², K. Kitamura³, and K. Inoue⁴

¹Department of Environmental Science, Faculty of Science, Kumamoto University, Kumamoto, Japan

²United Graduate School of Agricultural Sciences, Tottori University, Tottori, Japan

³Division of Environmental Biology, Faculty of Life and Environmental Science, Shimane University, Matsue, Japan

⁴Biological Institute and Herbarium, Faculty of Science, Shinshu University, Matsumoto, Japan

Received April 23, 2002; accepted August 23, 2002

Published online: November 28, 2002

© Springer-Verlag 2002

Abstract. Nectarless flowers of *Cypripedium macranthos* var. *rebunense* are pollinated by only queen *Bombus pseudobaicalensis*, which also pollinates nectar-producing flowers of *Pedicularis schistostegia*. Our previous study (Sugiura et al. 2001) suggested that they form a floral mimicry system: (1) Flowering phenology of both plants overlapped greatly. (2) *Cypripedium* occurred with lower frequency relative to *Pedicularis*. And (3) in a mixed stand of both plants, foraging bumblebees were sometimes confused between the mimic and model flowers. The present study clarified the system with new evidence. (4) Flower colour of *Cypripedium* and *Pedicularis* would be similar within the range of a bumblebee's visual spectrum. And (5) both species largely overlapped in spatial distribution. Based on these findings, we discuss how *C. macranthos* var. *rebunense* differs in pollination mechanism from other congeneric species, especially *C. calceolus*.

Key words: *Bombus*, *Cypripedium*, floral mimicry, *Pedicularis*, pollination

Approximately 47 species of the genus *Cypripedium* L. are known to occur in Europe,

temperate Asia, North America, and south to Guatemala and Honduras (Cribb 1999). All flowers, well known as “lady’s slippers”, have a pouched labellum but no nectar or collectable pollen, so that they are functionally regarded as “trap flowers”. Reported typical pollinators are either solitary (mainly *Andrena*, *Halictus*, and *Lasioglossum*) or social (*Bombus*) bees (see review by Stoutamire 1967; Nilsson 1979; van der Cingel 1995, 2001; Cribb 1997). Other than bees, a syrphid fly *Syrphus torvus* Osten et Sacken is the main pollinator for *C. reginae* Walter (Vogt 1990) in Vermont, while leaf-cutter bees *Megachile melanophaea* Smith and *M. centuncularis* (L.) were observed pollinating this orchid in another locality (? Canada, Guignard 1886). Flowers of some Asian species (*C. margaritaceum* Franch., *C. lichiangense* P. J. Cribb et S. C. Chen and their allies, and *C. debile* Rchb.) have a mushroom-like scent, suggesting pollination by fungivore flies (Stoutamire 1967, Cribb 1997). Catling (1990) reported auto-pollination in *C. passerinum* Richardson var. *passerinum* and *C. dickinsonianum* Hågsater.

Of the many studies on *Cypripedium* pollination, the most detailed one is by Nilsson (1979) of *Cypripedium calceolus* L. in three Swedish populations over a four-year period. The fruity scented flowers were visited by over 50 insect species belonging to Hymenoptera, Diptera, Coleoptera, Lepidoptera, and Thysanoptera. Effective pollinators were short-tongued bees of the genera *Andrena*, *Lasioglossum*, and *Halictus*, but above all, female *Andrena haemorrhoa* (Fabricius) was most effective. The author concluded that the flowers adopt a deceptive pollination mechanism without a specific flower model as follows. The pollinators were attracted by coloration of the flower, especially the yellow labellum and spot patterns of the staminode and labellum (=false nectar guide), and the acetate-rich floral fragrance. Nilsson explained that flying close to the flower, the bees are strongly attracted to the colour of yellow, due to an innate sensitivity, and also to the fragrance which mimics the bee's volatile secretions which function as landing control pheromone, thereby increasing the probability of the bee entering the saccate labellum.

Recently, we carried out a baseline study on the sexual reproduction of a Japanese lady's slipper, *Cypripedium macranthos* Sw. var. *rebunense* (Kudô) Miyabe et Kudô (Sugiura et al. 2001), including its functional flower morphology, flowering phenology, pollination, and level of natural pollination. We suggested that it visually mimics *Pedicularis schistostegia* Vved. (Scrophulariaceae but now in Orobanchaceae, see Olmstead et al. 2001) and these species form a floral mimicry system. In the present study of *Cypripedium* and *Pedicularis* flowers, we accumulated data on their colour (as seen by *Bombus* spp.), spatial distribution and height in order to verify our "mimic hypothesis".

Materials and methods

Cypripedium macranthos var. *rebunense* is endemic to Rebun Island, Hokkaido, Japan. It is primarily a plant of the grasslands developing on coastal

hillsides, vegetational succession being largely prevented by strong winds from the sea. The flowers are self-compatible but need a pollen vector for capsule production, and the only pollinators so far recorded are queens of *Bombus pseudobaicalensis* Vogt (Sugiura et al. 2001).

Studies were conducted within the protected area (14.1 ha) for *Cypripedium macranthos* var. *rebunense* in Teppu, Funadomari on the island on June and July, 2001. And the same two study sites, A and B, were used as in Sugiura et al. (2001). Site A was on a hillside facing the sea, where human disturbance was minimal while site B was set along a trail for many visitors (over 4000/day in maximum), who come for *Cypripedium* flowers.

Spatial distribution of flowers. During the period of full bloom of *Cypripedium* (June 8 and 9), a test area (4 m × 15 m) was established on a gentle slope at site A. All inflorescences of *Cypripedium* and *Pedicularis* within the quadrat were individually marked with position, and height recorded. The height of both species was defined as follows; (1) in *Cypripedium*, the distance from the ground to the highest point of the dorsal sepal and (2) in *Pedicularis*, to the uppermost opening flower in each inflorescence.

Analysis of flower colour. Colour vision in bumblebees is known to be an ultraviolet-blue-green trichromacy (Briscoe and Chittka 2001; Chittka et al. 2001). In order to examine the similarity in the floral reflectance of both species with respect to bumblebee vision, we photographed flowers under natural light conditions through a filter which transmits only a particular range of wavelengths, i.e. ultraviolet (360 nm), blue (450 nm), or green (530 nm), produced by Toshiba Glass Co. UV-D36A, Wratten gelatine filter No. 47, and Fuji Photo Film Co. band pass filter BPB53, respectively. A gray-scale (Eastman Kodak Co., Q-13, CAT 152 7654) was used to control for proper exposure as in Kevan et al. (1973). Photographs were made on 400 ASA black and white film (Neopan 400 Presto, Fuji Photo Film Co.) with an Olympus OM-4 camera equipped with an Olympus 50 mm macro lens.

Pollinating success. In order to estimate the level of natural pollination, 400 and 171 flowers were individually marked at sites A and B, respectively, on June 10 and 11. On July 23 to 26, all marked flowers were examined to determine if their ovary had developed into a firm green capsule.

Results

Spatial distribution of flowers. 96 inflorescences of *Cypripedium* and 148 inflorescences of *Pedicularis* were found within the quadrat. Of these, all *Cypripedium* flowers had already bloomed while about 55% (81/148) *Pedicularis* inflorescences had bloomed. Figure 1 shows the spatial distribution of flowering *Cypripedium* and *Pedicularis* within the quadrat. *Pedicularis* tended to thrive in the upper area of the slope relative to *Cypripedium*, but their distribution largely overlapped. This tendency was often found at other places within the protected area (Fig. 2). The height of *Cypripedium* flowers (mean \pm s.d., 18.7 ± 4.8 cm, $n=93$) and *Pedicularis* inflorescences (13.7 ± 3.5 , $n=81$) was significantly different ($P < 0.05$, t -test) but adjacent plants of *Cypripedium* and *Pedicularis* were often similar in their height (Fig. 1).

Because *Pedicularis* preferentially thrives in places where tall herbs are rarely thick (personal observation), its inflorescences were

usually visually conspicuous, along with the large *Cypripedium* flowers. However, *Sorbus sambucifolia* (Cham. et Schldl.) Roem. and some herbs grew densely within a limited portion of the test area (mostly, portion between 0 m and 2 m) and here, flowers of *Cypripedium* and *Pedicularis* were hidden under the foliage.

Analysis of flower colour. Both *Cypripedium* and *Pedicularis* flowers are blue and green reflective, and very strongly ultraviolet absorptive (Fig. 3). Consequently, the reflectance with respect to bumblebee primary colours is similar for both plants.

Pollinating success. From a total of 571 marked flowers, 497 (330 at site A and 167 at site B) were recovered. The level of natural pollination was 14.5 % (48/330) at site A and 16.8 % (28/167) at site B, and was not significantly different (χ^2 test, $P > 0.05$). Within the test area, no firm green capsule was found if *Cypripedium* flowers had been hidden by foliage.

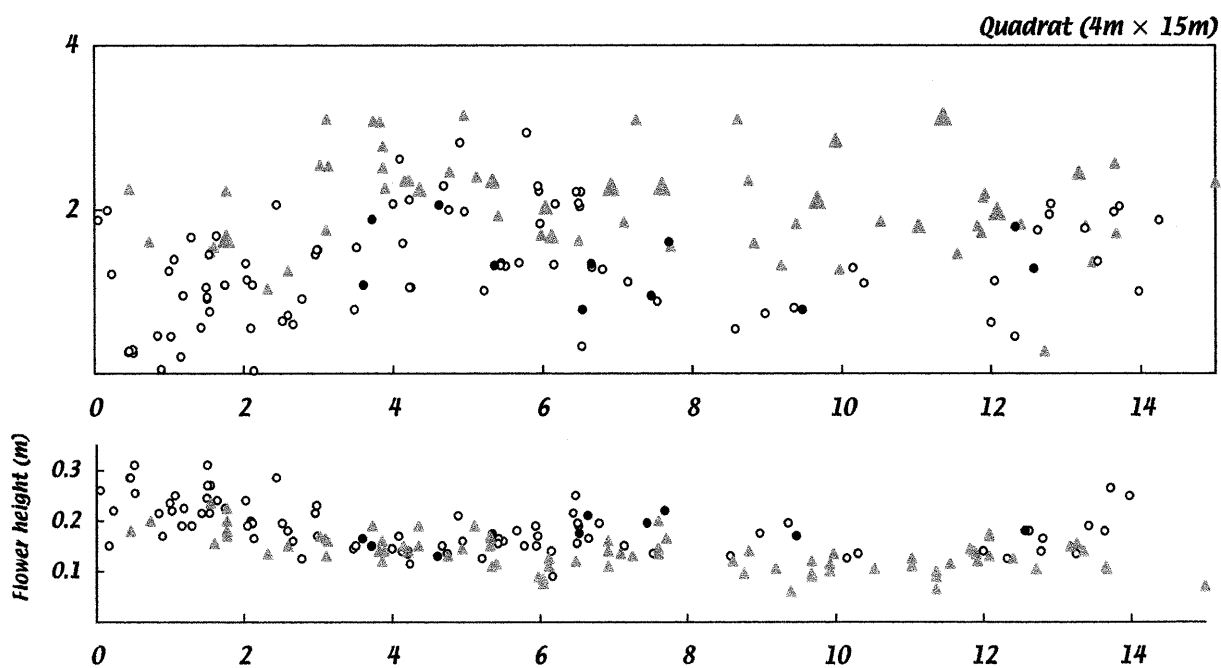


Fig. 1. Spatial distribution and flower height of blooming *Cypripedium macranthos* var. *rebunense* and *Pedicularis schistostegia* within the test area (4 m \times 15 m). \circ *Cypripedium macranthos* var. *rebunense* (unpollinated); \bullet *C. macranthos* var. *rebunense* (naturally pollinated); \triangle *Pedicularis schistostegia* with one inflorescence; \blacktriangle *P. schistostegia* with two inflorescences; \blacktriangle *P. schistostegia* with three inflorescences



Fig. 2. *Cyripedium macranthos* var. *rebunense* growing with *Pedicularis schistostegia*. Note the similarity in flower colour and height of the plants, and difficulty in distinguishing one from the other from a distance

Discussion

Pollination mechanism of *Cyripedium macranthos* var. *rebunense*. An idea of floral mimicry of *Pedicularis schistostegia* by *Cyripedium macranthos* var. *rebunense* was derived from the following findings of our previous report (Sugiura et al. 2001). (1) Flowering phenology of *Cyripedium* (the mimic) and *Pedicularis* (its putative model) greatly overlapped. (2) The mimic occurred with lower frequency relative to the model. And (3) some bumblebees visiting *Pedicularis* patches in succession were observed being momentarily attracted to *Cyripedium* flowers, and an individual bumblebee visiting *Pedicularis* inflorescences was actually found with a *Cyripedium* pollen mass. From the present study, several lines of evidence in

support of our “mimic hypothesis” were further obtained. (4) Because of the similarity in flower reflectance of *Cyripedium* and *Pedicularis*, their flower colour would be perceived similar within the range of the bumblebee’s visual spectrum. In field experiments with species of different flower shapes and colours, Wilson and Stine (1996) found that bumblebees visited flowers of similar colour in different shapes, but not vice versa. (5) No capsule was set in *Cyripedium* flowers hidden under foliage, suggesting no significant role of olfactory attraction in this pollination mechanism. Finally (6) spatial distribution of both *Cyripedium* and *Pedicularis* was well overlapping, and adjacent plants of both species were similar in flower height.

We believe that our evidence makes floral mimicry the most likely pollination mechanism for *C. macranthos* var. *rebunense*, as reported in other orchid taxa such as European *Cephalanthera rubra* (L.) Rich. (Nilsson 1983), Australian *Diuris maculata* Sm. (Beardsell et al. 1986), or South African *Disa ferruginea* (Thunb.) Sw. (Johnson 1994). However, we have yet to examine whether fruiting success of *Cyripedium* is significantly decreased by the absence of *Pedicularis*.

Why floral mimicry has evolved only in *C. macranthos* var. *rebunense* in this genus is unknown. Flowering phenology and light condition in *Cyripedium* habitats may be key factors. In early June, when queen bumblebees have just commenced visiting flowers, there are few good native flowers, except for *Pedicularis*, which completely conceals nectar into a long corolla tube and is pollinated solely by bumblebees (unpublished observation). Consequently, its flowers might be considered as a good model to mimic for *Cyripedium*. Besides, growing in open grassland seems to contribute to mimicry because flowers are exposed to sunlight.

Cyripedium macranthos (sensu lato) usually have pinkish purple or deep red purple flowers (Cribb 1997). They are also found at our study sites although the frequency is extremely low. This may indicate that *C. macranthos* at Rebut

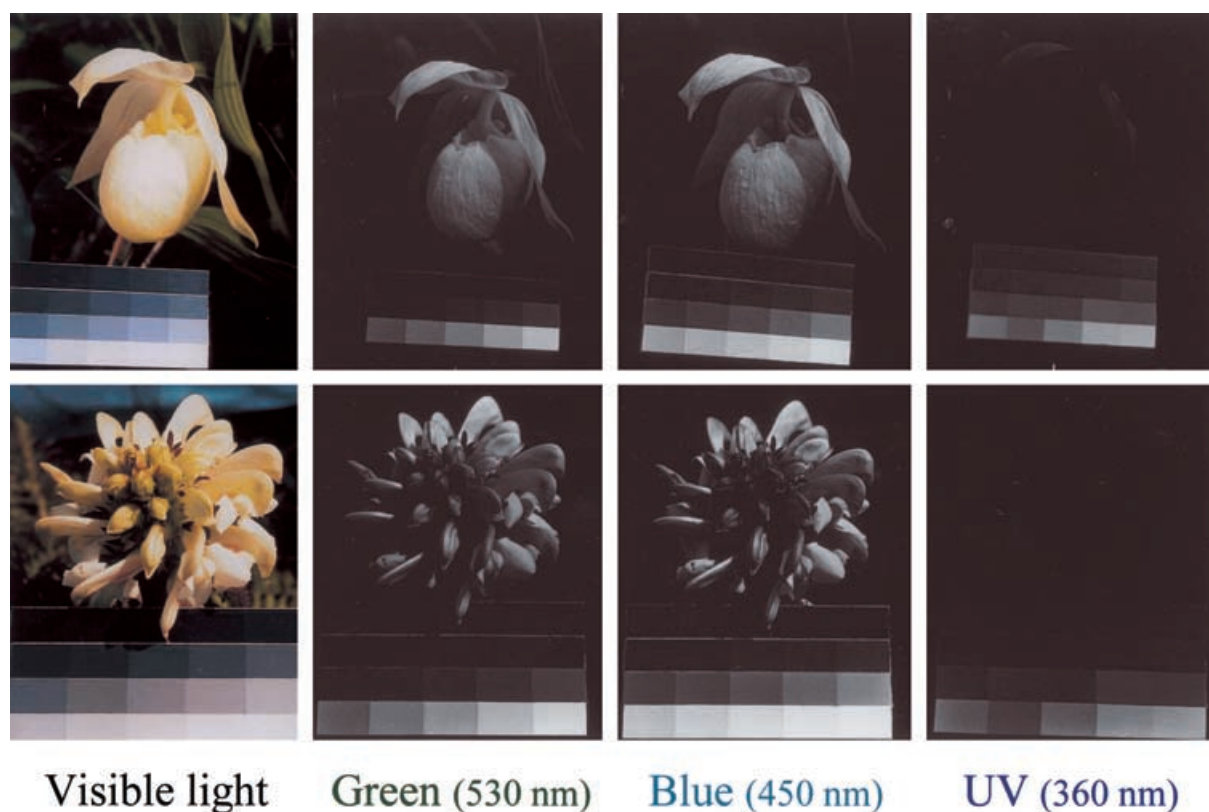


Fig. 3. Flower colours of *Cypripedium macranthos* var. *rebunense* and *Pedicularis schistostegia* as bumblebees might see them

Island had previously changed its flower colour similar to *Pedicularis*.

Therefore, the pollination mechanism of *C. macranthos* var. *rebunense* is essentially different from that of other *Cypripedium* taxa, including *C. calceolus* (Nilsson 1979, Cribb 1997). Inflorescences of *C. macranthos* var. *rebunense* are usually only one-flowered. The flowers are yellowish white or white, without any spots or false nectar guide, and scent is faint or lacking (Sugiura et al. 2001). In contrast, in *C. calceolus* inflorescences are sometimes two-flowered, and the flowers are smaller but more showy; with dark red brown perianth parts except for the yellow labellum, reddish spot markings on the whitish staminode and labellum, and lateral petals long and spirally twisted. They emit a distinct scent.

Cypripedium flowers have a saccate labellum without exception but its size, shape, and

colour vary greatly among species or varieties of the same species (Cribb 1997). Variation in fragrance is also expected, as Bergström et al. (1992) showed in three geographically separated intraspecific taxa. This indicates that a variety of pollination mechanisms exist in the genus. More well-documented studies, such as Nilsson (1979) on *C. calceolus*, are required to broaden our knowledge on the pollination of the genus.

Pollinating success and implications for conservation in native habitats. Sugiura et al. (2001) reported that in 2000 the level of natural pollination of *Cypripedium* at sites A and B was 8.3 % (30/361) and 1.2 % (2/173), respectively. They assumed that such a significant difference partially resulted from human disturbance (i.e. presence of many visitors at site B). However, the level of natural pollination was significantly higher in 2001 than in 2000 (χ^2 -test, Site A,

$P < 0.05$; Site B, $P < 0.01$), and did not differ between sites in 2001 ($P > 0.05$). Why do the results differ so markedly between the years? It is possible that negative effects of human disturbance were also present in 2001 but pollination conditions, including abundance of *B. pseudobaicalensis* queens and weather conditions during the flowering period, were much better in 2001, and greatly outweighed any negative effect of human disturbance. Two-year studies on the level of natural pollination strongly suggest that fruiting success in *C. macranthos* var. *rebutense* varies considerably annually as in other *Cypripedium* species (Curtis 1954, Nilsson 1979). Long-term monitoring of reproductive success of *C. macranthos* var. *rebutense* in natural habitats should be implemented to save this threatened plant.

We wish to express our sincere thanks to T. Kuwahara (Environment Ministry of Japan) for his help in a variety of ways including study permits, T. Miyake (Kyushu University) and Y. Hosoda (Shimane Agricultural Experimental Station) for their assistance in the field, and Adam L. Cronin (Hokkaido University) and Y. Hosoda for valuable comments on the manuscript. This research was financially supported in part by the protection and propagation project for *Cypripedium macranthos* var. *rebutense* (Environment Ministry of Japan).

References

- Beardsell D. V., Clements M. A., Hutchinson J. F., Williams E. G. (1986) Pollination of *Diuris maculata* R. Br. (Orchidaceae) by floral mimicry of the native legumes *Daviesia* spp. and *Pultenaea scabra* R. Br. Austral. J. Bot. 34: 165–173.
- Bergström G., Birgersson G., Groth I., Nilsson L. A. (1992) Floral fragrance disparity between three taxa of lady's slipper *Cypripedium calceolus* (Orchidaceae). Phytochemistry 31: 2315–2319.
- Briscoe A. D., Chittka L. (2001) The evolution of color vision in insects. Annu. Rev. Entomol. 46: 471–510.
- Catling P. M. (1990) Auto-pollination in the Orchidaceae. In: Arditti J. (ed.) Orchid Biology: Reviews and Perspectives, vol. 5. Cornell University Press, Ithaca, pp. 121–158.
- Chittka L., Spaethe J., Schmidt A., Hickersberger A. (2001) Adaptation, constraint, and chance in the evolution of flower color and pollinator color vision. In: Chittka L., Thomson J. D. (eds.) Cognitive Ecology of Pollination: Animal Behavior and Floral Evolution. Cambridge University Press, Cambridge, pp. 106–126.
- Cribb P. (1997) The genus *Cypripedium*. Timber Press, Portland.
- Cribb P. (1999) 3. *Cypripedium*. Distribution. In: Pridgeon A. M., Cribb P. J., Chase M. W., Rasmussen F. N. (eds.) Genera Orchidacearum vol. 1: General Introduction, Apostasioideae, Cypripedioideae. Oxford University Press, Oxford, p. 115.
- Curtis J. T. (1954) Annual fluctuation in rate of flower production by native cypripediums during two decades. Bull. Torrey Bot. Club 81: 340–352.
- Guignard J. A. (1886) Insects and orchids. Ann. Rep. Ent. Soc. Ontario 16: 39–48.
- Johnson S. D. (1994) Evidence for Batesian mimicry in a butterfly-pollinated orchid. Biol. J. Linn. Soc. 53: 91–104.
- Kevan P. G., Grainger N. D., Mulligan G. A., Robertson A. R. (1973) A gray-scale for measuring reflection and color in the insect and human visual spectra. Ecology 54: 924–926.
- Nilsson L. A. (1979) Anthecological studies on the lady's slipper, *Cypripedium calceolus* (Orchidaceae). Bot. Notiser 132: 329–347.
- Nilsson L. A. (1983) Mimesis of bellflower (*Campanula*) by the red helleborine orchid *Cephalanthera rubra*. Nature 305: 799–800.
- Olmstead R. G., dePamphilis C. W., Wolfe A. D., Young N. D., Elisons W. J., Reeves P. A. (2001) Disintegration of the Scrophulariaceae. Amer. J. Bot. 88: 348–361.
- Stoutamire W. P. (1967) Flower biology of the lady's slippers (Orchidaceae: *Cypripedium*). Michigan Bot. 6: 159–175.
- Sugiura N., Fujie T., Inoue K., Kitamura K. (2001) Flowering phenology, pollination, and fruit set of *Cypripedium macranthos* var. *rebutense*, a threatened lady's slipper (Orchidaceae). J. Plant Res. 114: 171–178.
- van der Cingel N. A. (1995) An Atlas of Orchid Pollination: European Orchids. A. A. Balkema Publishers, Rotterdam.
- van der Cingel N. A. (2001) An Atlas of Orchid Pollination: America, Africa, Asia and Australia. A. A. Balkema Publishers, Rotterdam.

Vogt C. A. (1990) Pollination in *Cypripedium reginae* (Orchidaceae). *Lindleyana* 5: 145–150.

Wilson P., Stine M. (1996) Floral constancy in bumble bees: handling efficiency or perceptual conditioning? *Oecologia* 106: 493–499.

Addresses of the authors: Naoto Sugiura (e-mail: sugiura@aster.sci.kumamoto-u.ac.jp), Department of Environmental Science, Faculty of Science,

Kumamoto University, Kumamoto 860-8555, Japan. Masashi Goubara, United Graduate School of Agricultural Sciences, Tottori University, Tottori 680-8553, Japan. Kenji Kitamura, Division of Environmental Biology, Faculty of Life and Environmental Science, Shimane University, Matsue 690-8504, Japan. Ken Inoue, Biological Institute and Herbarium, Faculty of Science, Shinshu University, Matsumoto 390-8621, Japan.