

## *Holcoglossum nujiangense* (Orchidaceae: Aeridinae) – a new species and its pollination system

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Based on herbarium and field observations, a new species, *Holcoglossum nujiangense*, is described and illustrated. It is morphologically similar to *Holcoglossum sinicum* and *H. weixiense*. *Holcoglossum nujiangense* differs from *H. weixiense* by smaller flowers with broadly rhombic midlobe and from *H. sinicum* by its broadly rhombic midlobe, papillose spur and notable callus on the disc. The chromosome number of this species was found to be  $2n = 38$ . The pollinator for this new species was *Micraphis andreniformis* Smith that carried the pollinia of *Holcoglossum nujiangense* on its head. The visiting behavior of the pollinator is food-search kind, although there is no obvious nectar in the spur.

The orchid genus *Holcoglossum* was established by Schlechter (1919) based on *Saccolabium quasipinifolium* Hayata. Approximately twelve species have been described until recently. Most of them are distributed in southwest China with a few species extending to Malaysia, Myanmar, Thailand and Vietnam (Christenson 1987, 1998, Seidenfaden 1988, Tsi 1999, Jin 2005).

The subtropical mountainous region in northwest Yunnan, southwest Sichuan Province and southeast Xizang (Tibet) is one of the hotspots of biodiversity in the world (Myers et al. 2000). However, its flora may be far from known. During our botanical trip to this region from 2001 to 2005, several undescribed species of *Holcoglossum* along the Mekong, Salween and Dulong River were found. One specimen was collected in May 2002 along Mekong River and published in 2004 (Jin et al. 2004). Another specimen was collected in May of 2005 along Salween River, which will be described in the present paper.

The genus *Holcoglossum* belongs to the subtribe Aeridinae of horticultural significance in Orchidaceae. Until recently, however, few studies have been published that deals with its pollination biology (Dressler 1993, Light 1998, Cingel 2001). Here we attempt to report the pollination system of this new species based on our field observation.

### Material and methods

All examined plants were collected from different localities in Fugong county of Yunnan province, and as well as

specimens of *Holcoglossum* kept in AMES, E, IBSC, K, KUN, P and PE.

Fresh root tips about 0.2 cm long were cut for chromosome observation. They were pretreated in 0.002 M 8-hydroxyquinoline at 20°C for 4 h, then fixed with Carnoy (1:3 glacial acetic acid/absolute alcohol) at 4°C for 2 h, rinsed in distilled water several times and placed in 70% ethanol for 10 min. After this, they were hydrolysed in 1:1 1 N HCl:45% acetic acid at 60°C for 1 min and squashed and stained in 1% aceto-orcein. Permanent slides were made using the standard liquid nitrogen method. Observations were made on nuclei at the somatic mitotic metaphase; chromosome number of three well-spread metaphases of three different root tips were counted.

The pollination of *H. nujiangense* was observed on the east slope of Gaoligongshan mountains at an elevation of 2400–3000 m in May 2005. The total observation time is 34 man-hours. A total of 43 flowers from 13 individuals were observed. The natural fruit set of *Holcoglossum nujiangense* was calculated in 2005 and eight flowers were bagged as self-pollinated in 2005.

The behavior of flower visitors was observed during the day from 06:30 to 19:30, because the previously reported pollinators of the subtribe Aeridinae were all diurnal bees and beetles (Dressler 1993, Cingel 2001, Jin et al. 2005). Visitors were defined as insects that touched any part of the flower, and pollinators were defined as visitors with pollinia attached to their bodies. Visitors were collected from inflorescence by net and killed using ether. Entomologists from the Chinese southwestern Forest College identified the collected insects.

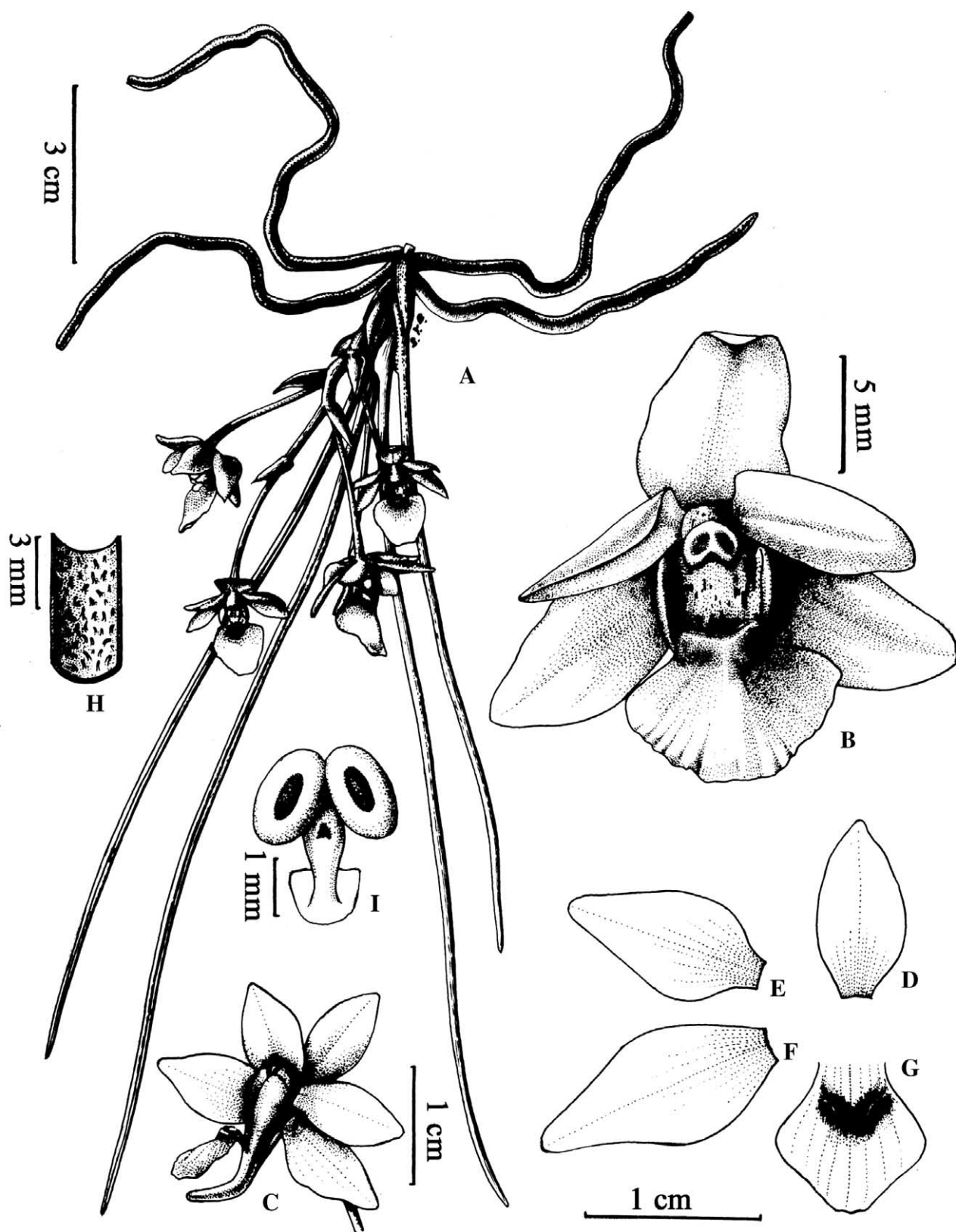


Fig. 1. *Holcoglossum nujiangense*. (A) habit, (B) front view of flower, (C) side view of flower, (D) dorsal sepal, (E) petal, (F) lateral sepal, (H) inner wall of spur, (I) pollinarium.

Vouchers of the plant specimens and insects are kept in the herbarium, Inst. of Botany, Chinese Acad. of Sciences (PE).

***Holcoglossum nujiangense* X. H. Jin & S. C. Chen sp. nov. (Fig. 1)**

*Habitu H. sinico subsimile, sed labello medio late rhombico et callo notabili, calcari intus papillato differt.*

Holotype: China, Yunnan, Fugong, Jiakeding, 2400 m, 16 May 2005, X. H. Jin 6930 (PE!)

Plant perennial, epiphytic. Roots white, terete, fleshy, 2 mm in diam. Stems pendent, 1–2 cm long, enclosed by persistent leaf sheaths, 1.5 mm in diam. Leaves semi-terete, fleshy, adaxially channelled, acuminate, 20–30 cm long, 1.5 mm in diam., jointed basally, base enlarged and sheathed. Inflorescence lateral, 1 to 3-flowered; peduncle about 1 cm long. Floral bracts ovate, 7 mm long, concave. Pedicel and ovary 2 cm long. Flowers fully opening, white. Dorsal sepal erect, elliptic, 1.4 cm long, 6 mm wide, obtuse. Lateral sepals oblique at base, 1.3 cm long, 5 mm wide, narrowly elliptic. Petals longly ovate, 1.2 cm long, 6 mm wide. Lip 3-lobed. Lateral lobes erect, triangular, adaxially with red stripes; middle lobe broadly rhombic, entire, obtuse, 6 mm long, 8 mm wide, with a yellow fleshy callus at its base. Spur funnel-shaped, curved forwards, 6 mm long, papillose inside. Column 3 mm long, white, with a column foot 1 mm long. Pollinia two, porate; stipe 2 mm longly, tapering. Viscidium longly semi-orbiculate.

Paratype. China. prov. Yunnan, Fugong county. Shangpa district, Qimigunu, 2400 m, 8 Jun 1982, Qinghai-Tibet plant expedition team 7122 (KUN); Shangpa district, Zhuminglin, 14 May 2005, X. H. Jin 6981(PE).

The new species is only found in southwest China, on the east slope of Gaoligongshan mountains in a forest dominated by oaks, alt. 2500–3000 m.

**Chromosome number of *Holcoglossum nujiangense***

The chromosome number of *Holcoglossum nujiangense* is  $2n = 38$  (Fig. 2A), which is consistent with previously reported chromosome numbers of most species of *Holcoglossum* (Jin et al. 2007).

**Pollination system of *Holcoglossum nujiangense***

During our 34 h of observation, a total of 12 visitors of *H. nujiangense* were observed. They were all bees and flies of which eleven were pollinators. Seven of these pollinators were seen carrying a pair of pollinia on their head (Fig. 2C, 2E).

The pollinators were identified as bees of Apidae, *Micrapis andreniformis* Smith (Fig. 2C–2E). They were caught when visiting the flower and carrying the pollinia of *H. nujiangense*. They exhibited typical food-seeking behavior, continuously visiting one to three clusters of flowers, each usually containing one to four flowers. They landed on the midlobe and then entered the spur and visited the flower no more than 5 s. When they retreated along the passage from the spur and later the midlobe, the viscidium was attached to their head, after which the pollinia were removed. The stipe was observed to bend in the middle for about ten seconds when it was removed from the rostellum. The pollinator with pollinia on its head then entered the spur of another flower, and the pollination of the flower took place. *Micrapis andreniformis* usually entered the spur

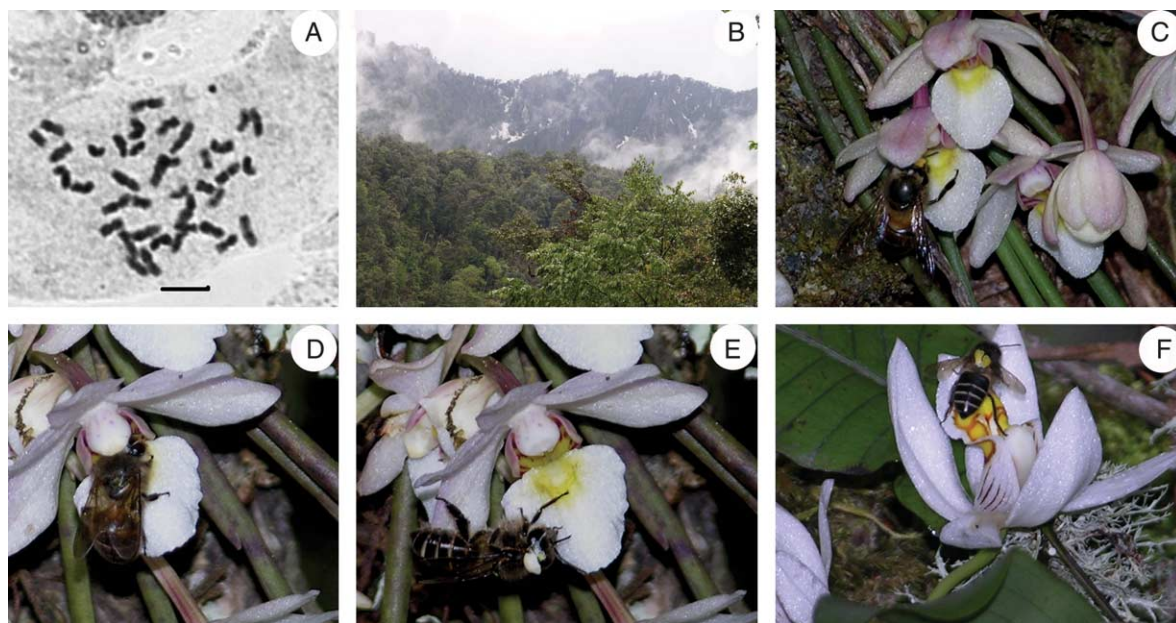


Fig. 2. (A) Metaphase chromosomes of *Holcoglossum nujiangense* (scale bar = 5  $\mu$ m). (B) Habitat of *Holcoglossum nujiangense*. (C) *Micrapis andreniformis* carrying pollinia on its head to visit a flower. (D) *M. andreniformis* retreated from spur along the passage and midlobe. (E) *M. andreniformis* taking away pollinia on its head when it moved on. (F) *M. andreniformis* carrying pollinia of *Coelogyne corymbosa* on its back.

Table 1. Natural fruit set and fruit set after self-pollination of *Holcoglossum nujiangense*.

	Number of flowers	Number of fruits	Fruit set (%)
Natural fruit set	53	6	11.3
Self pollination	8	4	50

along the passage formed by two lateral lobes; a middle lobe and a column. The red stripes on the inner surface of the lateral lobes were very distinct on the white flowers, which may act as an indicator of nectar, although there was no obvious nectar in the spur.

Pollinators of *H. nujiangense* were rather common in its habitat and they were the main visitors to nearly all flowers observed during May 2005, such as *Lyonia* spp., *Rhododendron* spp., *Coelogyne corymbosa* (Fig. 2F).

*Holcoglossum nujiangense* is not fully self-compatible. Natural fruit set and fruit set of hand self-pollinated flowers were recorded in Table 1.

## Discussion

There are about 6–7 species of *Holcoglossum* distributed in the Hengduanshan mountains, northwestern Yunnan. Most of them are endemic to this area and replace each other along the rivers or mountains there. These species share many floral characters with each other and form the section *Sorotylos* (Jin 2005). They differ mainly in growth form, size of flowers and shapes of the midlobe and lateral lobes. *Holcoglossum nujiangense* has a similar habit to *H. sinicum* and *H. weixiense*, but their floral morphology is quite different from the latter species, and therefore clearly represents a separate species. *Holcoglossum nujiangense* differs from *H. sinicum* by its broader rhombic midlobe, papillose spur and notable callus on the disc, and differs from *H. weixiense* by its much small flowers and a broadly rhombic midlobe.

In the section *Sorotylos* there are five species altogether, including the new species described here, whose pollination system also has been observed. *Holcoglossum rupestre* is pollinated by a beetle (Jin et al. 2005), whereas *H. weixiense*, *H. flavescens* and *H. nujiangense* are pollinated by bees and *H. sinicum* is autogamous. In the *Sorotylos*, the only species whose pollination system is unknown is *H. tsii* Yukawa. The flowers of *H. tsii* are similar in shape and smell to those of *H. rupestre* but about twice the size. It has been suggested that *H. tsii* is also pollinated by a beetle or some kind of long-mouth flies (Y. B. Luo pers. comm.). The autogamy of *H. sinicum* may be caused by its stipe bending in bud or during anthesis. Autogamy is rather rare in the genus. It seems like the beetle pollination system is rather advanced

in sect. *Sorotylos*, because the flowers have a special smell, and this kind of pollination is very rare in the genus and even in the family.

## Etymology

The epithet is derived from the Chinese name of Salween River, Nujing River. The new species is distributed in the valley of Nujing River.

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