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*Full Length Research Paper*

# Macro research on growth and development of *Cremastra appendiculata* (D.Don.) Makino (Orchidaceae)

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**The process of growth and development of *Cremastra appendiculata* (D. Don.) was studied by combined both natural habitat observation and artificial facility cultivation, mainly involved the organogenesis, morphological characteristics, structural composition, dynamic changes in time and space of the vegetative organs and reproductive organs, etc. This study was designed to supplement the basic research data relating the biological characteristics and growth and development of *C. appendiculata*, in order that to provide the essential reference for the resources conservation and sustainable use of this rare species.**

**Key words:** *Cremastra appendiculata* (D. Don.) Makino, vegetative organ, reproductive organ, growth and development, macro research.

## INTRODUCTION

*Cremastra appendiculata* (D. Don.) Makino (Orchidaceae), a medicinal orchid and a perennial herb, is one of the famous wild orchids in Eastern Asia (Kitamura et al., 1986; Mi et al., 2004; Joong et al., 2004), especially in China (Chen et al., 1998; Chen and Chen, 2003). *C. appendiculata* has high medicinal and ornamental value. Its pseudobulbs are important and shortage of medicinal materials (Li et al., 1995; National, 2005), it has been used internally against tumors and cancers of the liver, breast, cervix, uterus and blood vessels (Joong et al., 2004; Li et al., 1996; State, 1999; Jeong et al., 2007; Xia et al., 2005); or externally as Chinese traditional medicine for treating carbuncle, boil or burn skin lesions by a poultice, snake or insect bite (National, 2005). Up today, more than a dozen compounds have been isolated from the pseudobulb, including alkaloids, phenanthrenes, flavanones and aglycones, etc. (Joong et al., 2004; Jeong et al., 2007; Xia et al., 2005; Xue et al., 2005; Yoshitaka et al., 2005; Xue et al., 2006). Yoshitaka et al. (2005) found that the extracts of *C. appendiculata* had strong blocking activity and confirmed one of the active ingredients was pyrrolizidine alkaloids by the active tracking method. A Japanese patent (1982) reported two compounds, named cremastosin 1 and 11, and also obtained an active alkaloid (that is cremastrine) from *C. appendiculata*. Peng et al. (2009) adopted the method of histochemical localization to ascertain basically the accumulation areas of alkaloids in this plant.

The important value of *C. appendiculata*, coupled with the difficulty of natural breeding, led to the mad excavation for this resource from humans, making the wild resources come to the verge of extinction. So the effective protection of *C. appendiculata* has been pressing. However, the studies relate to *C. appendiculata* in worldwide have been lacking, only a few reports focus on the taxonomy (Chen and Chen, 2003; The, 1999; Chen, 2004), reproduction (Naoto, 1996; Mi and Myong, 2003; Zhang et al., 2005; Zhang et al., 2006; Zhang and Yang, 2008; Zhang et al., 2009), chemistry and pharmacology (Joong et al., 2004; Li et al., 1996; State, 1999; Jeong et al., 2007; Xia et al., 2005; Xue et al.,

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2005; Yoshitaka et al., 2005; Xue et al., 2006). In accordance with the requirement of the resources conservation, authors carried out the basic researches to base on the growth and development of *C. appendiculata*, to complement the foundational information in this field.

## MATERIALS AND METHODS

The experimental material which was used in this study was *C. appendiculata* (D. Don.) Kakino distributing in Guizhou of China. It belonged to *Cremastra* in Orchidaceae, a perennial herb with pseudobulbs and a leaf. During the experiment, we adopted the method both natural habitat observation and artificial facility cultivation. The areas of natural habitat observation involved Guiyang, Anshun, South Guizhou and Southeast Guizhou. Because individual numbers of the population of *C. appendiculata* were rare extremely, and their distributions were also not law, therefore, the areas where population's individual numbers were relatively large were chosen as the sample plots of natural habitat observation. The experiments of artificial facility cultivation were arranged in the campus of Guizhou University, potted soil was humus, light transmission rate of the shade was about 25% and with natural temperature and precipitation. The essential trials were to track the indices relating to the plant growth and development of *C. appendiculata*, and the specific means included morphological description, structural anatomy, measurement and count, artificial pollination, etc. The entire experiment lasted for 6 years (2004 - 2009).

## RESULTS

### Composition of vegetative organs

The vegetative organs of *C. appendiculata* were consisted of the roots, pseudobulbs, rhizomes and leaf (Figure 1). The pseudobulb was an approximate spherical shape with nodes and adventitious buds, its diameter was 1.5 - 3 cm, and there were many filament fibers from dry-rot scale leaf around the node. The pseudobulbs connected together by the rhizomes (Figures 1B, E, F and G), a number of fleshy fibrous roots born from the pseudobulb's base. The leaf was large and oblong; out from the pseudobulb's top, only 1 leaf usually, 2 leaves rarely in a plant. The length of leaf blade was 18 - 45 cm, width of 4 - 8 cm, there were 3 main vein and many arcuate-parallel veins in leaf blade (Figure 1A and G). The apex of leaf blade was acuminate gradually, and its bottom shrank nearly wedge. The leaf stalk's length was 7 - 17 cm and its lower part was often enwrapped by the remnants of sheath.

### Morphological changes of the vegetative organs

From September to next February, it was the main period of vegetative organs growth of *C. appendiculata*. During this period, starting the pregnant procedure of adventitious bud which originated from basal or middle adventitious bud eye on the pseudobulb (Figure 1-B2), but initial adventitious bud did not continue to the differentiation and development, they remained dormant state until May or June. Coming to May, the leaf dried with the plant flowering, following the adventitious bud began on the differentiation and development. A few weeks later, the adventitious bud appeared up-down two parts by a node separated them (Figure 1-C4). The down part was intumescent and subsphaeroidal, and it would develop into a new pseudobulb. The upper was cone-shaped and elongated rapidly to form a young leaf by membranous sheath wrapped (Figure 1-D5), up to July mid and late, the tip of cone shot out of the soil. Afterwards, rapid growing young leaf broke through membranous sheath (Figure 1-E6). A number of fleshy fibrous roots born from the bottom of new pseudobulb at the same time of the new leaf growth (Figure 1-E7), so that to provide water and mineral nutrients what the plant needed in growth and development.

Usually, a plant of *C. appendiculata* yielded only a few pseudobulb in a year. The pseudobulbs each year were gathered through the rhizomes, but the adventitious bud on the latest pseudobulb could develop into a new plant (Figure 1E and G). In addition, we found a special phenomenon in this experiment, namely, once the leaves were injured during the growing period, they would not be healed.

### Floral organogenesis and morphological changes

The gestation of flower bud proceeded from a side of the top of more than three-year-pseudobulb beginning in September, afterward, a series of morphological changes occurred in the floral organs (Figure 2). The flower bud wrapping in bracts (3 - 4 layers) germinated in early spring of following year (Figure 2A), and it differentiated quickly and formed numerous small flower buds (Figure 2B). In late March, the flower bud shot out of the soil (Figure 2C) and the flower stalk elongated rapidly, the anthotaxy came out of the bracts (Figure 2D) and soon grew to 20 - 40 cm height of scape. The scape was erect, stout, higher than leaf and with two sparse tubular sheaths. The raceme consisted of dozens of flowers to decentralize on the rachis. The date to begin flowering in April late and the flowering sequence from the bottom up (Figure 2G). For young anthotaxy, the tops of small flowers were all upward to arrange on the rachis (Figure 2E). The upcoming-open flowers and the opened flowers were all the tops drooping on the rachis (Figure 2F, G and H). After flowering, all flowers turned to a side of the rachis (Figure 2H). It is noteworthy that *C. appendiculata* had a very strict requirement to light intensity during the growth and development, under the direct light, the growth of plant was extremely slow, and almost no bolting and flowering.

### Composition and characteristics of the flower

The flower's structure of *C. appendiculata* was very

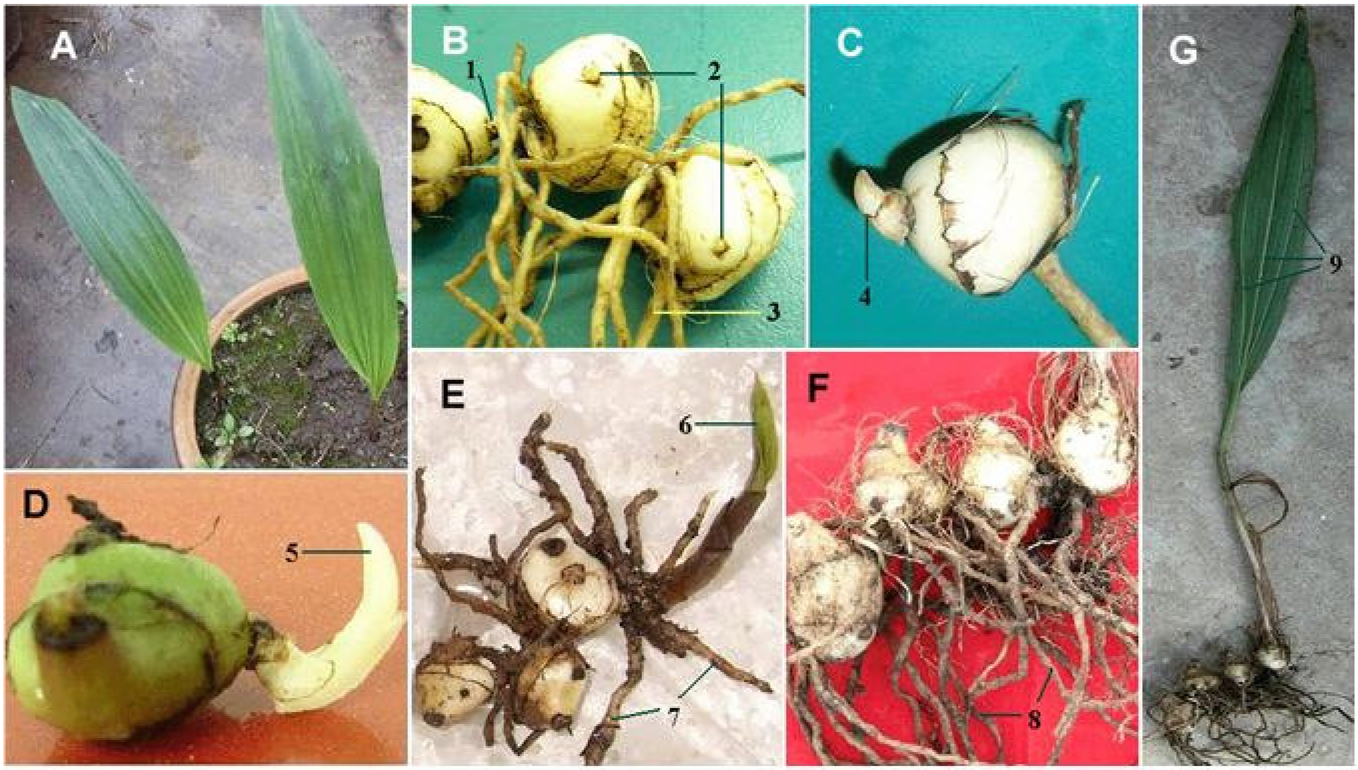


**Figure 1.** The vegetative organs of *C. appendiculata*: A: Aboveground parts of the plant; B: Pseudobulbs (1. rhizome; 2. adventitious buds; 3, 7, 8. fleshy fibrous roots); C: Differentiated node from adventitious bud (4. node); D: Elongating upside of the node of adventitious bud (5. elongated adventitious bud was wrapped by membranous sheath); E: New plant from adventitious bud (6. young leaf just crossed the membranous sheath); F: Gathering pseudobulbs; G: A whole plant (9. three main veins on the leaf).

complex, it included bracts, tepals, sepals, petals, labellum, gynostemium and other parts (Figure 3). The length of narrow lanceolate bract equaled or fell short to the peduncle, the apex of tubular tepals unfolded slightly. A flower was composed of three sepals, two petals and one labellum, purple red or pink-white color. Two petals sited on both sides of confrontation, and with the same size and shape (Figure 3-B7). The three sepals had same size and shape (oblanceolate) with acute apexes, to arrange like delta shape, about 3.5 cm of length and the mid-upper width of about 4 mm. The dorsal sepal (Figure 3-B9) was opposite with the labellum, the lateral sepals (Figure 3-B8) separated on both sides of dorsal sepal. The length of lateral sepals and dorsal sepal were equal to petals. The labellum looked like a spoon and the length was near to the sepals, its base was a shallow cystic and two edges curled upward slightly. The front-end of labellum expanded and split to three, the central lobe was similar to an obround (Figure 3-B3) and both sides were the narrow lateral lobes (Figure 3-B4). There was a protuberant callosum in the center of central lobe of the labellum (Figure 3-B5), and with an appendage on its bottom.

The slender gynostemium stood in the center of the flower (Figure 3-B6), length was about 2 cm, and a number of stamens attached on its top. The yellow calotte in the end of gynostemium was called anther cap (Figure 3-C10), it protected 4 pieces of pollinium (Figure 3-D13) produced from stamens, the sticky substance connecting 4 pieces of pollinium was the viscidium (Figure 3-D12), and the hollow beneath androecium with sticky secretions was the stigma (Figure 3-C11). Both gynostemium and lateral sepals composed of the mentum with a protuberance (Figure 3-A1). It was difficult to distinguish the inferior ovary and the peduncle before the ovary intumesced (Figure 3-A2).

## Characteristics of pollination and fructification

Under natural conditions, *C. appendiculata* was little bearing fruit, but it had higher fruit sets by artificial pollination. The blossoming period was from early April to early May, and the fruiting period was from mid-May to mid-August. Figure 4 showed that the morphological changes both flowers and fruits of *C. appendiculata* after the artificial pollination. The unpollinated flowers kept still fresh and bright after blossoming a week (Figure 4-Af), but the pollinated flowers in time began to wilting soon (the rest flowers except f-marker in Figure 4-A). The ovary enlarged gradually by pollination, finally, the ovary formed the fruit through a period of growth and

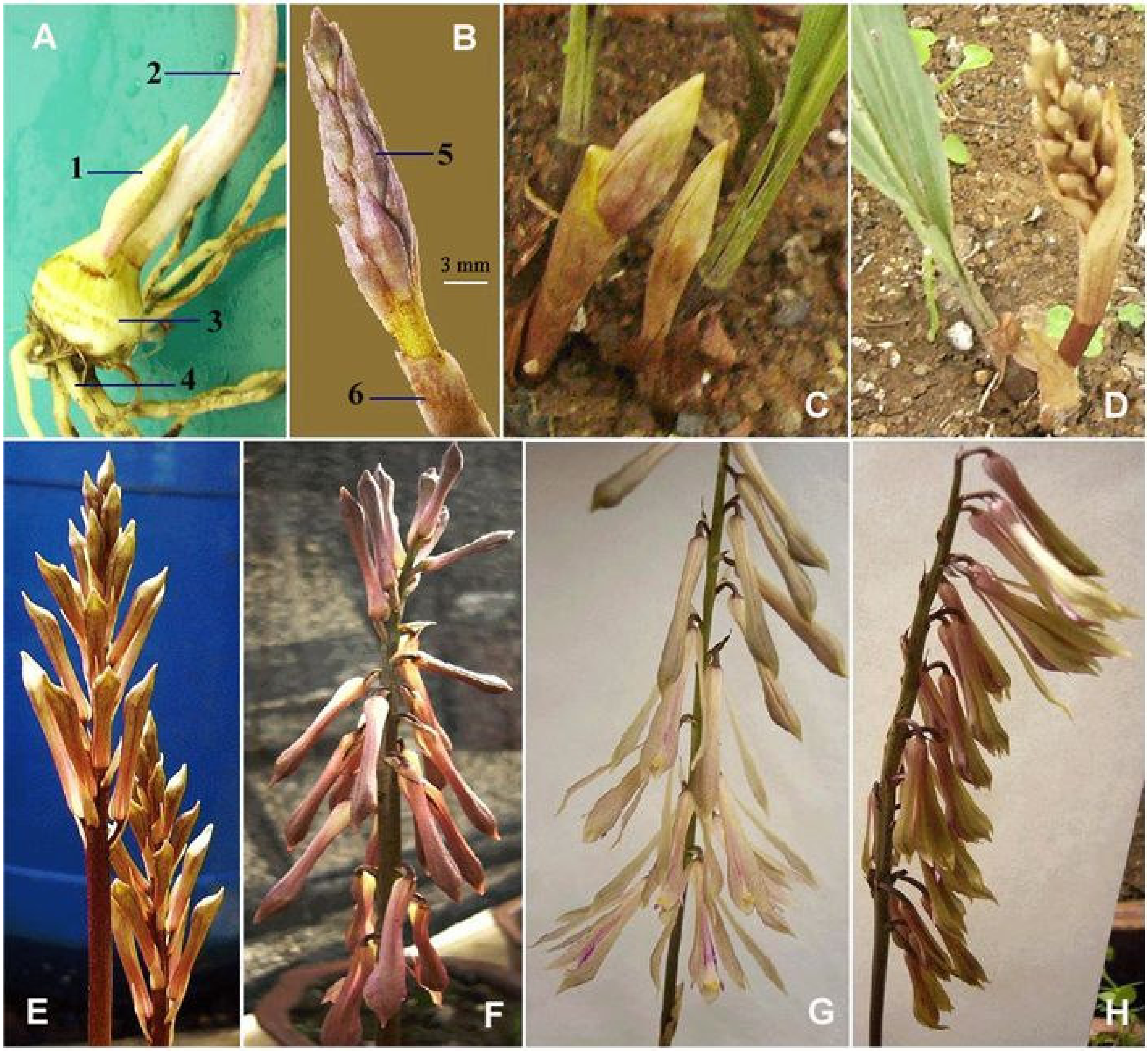


**Figure 2.** Floral organogenesis and morphological changes of *C. appendiculata*: A: Occurrence of flower bud (1. flower bud; 2. leafstalk; 3. pseudobulb; 4. fleshy fibrous root); B: Flower bud of removed the bracts (5. rudiment of the anthotaxy; 6. peduncle); C: Flower bud of break through the soil; D: anthotaxy of break through the bracts; E: Young anthotaxy; F: Anthotaxy of approaching maturity; G: Bottom-up order of flowering; H: Anthotaxy after flowering (all flowers turned to a side of the rachis).

development. Figure 4-B, C and D showed that the state of fruit growth and development after pollinated 20, 40 and 80 days, respectively. The capsule of *C. appendiculata* was similar to the ellipsoid, the length about 3 cm, it had 3 carpels and belongs to the lateral membrane placenta. The seeds were numerous and like flocculence, it had a hypogenetic proembryo and almost could not germinate.

## DISCUSSION

Through the observation and study for the growth and development of *C. appendiculata*, we found several interesting problems. First, the growth of *C. appendiculata* under the direct light was extremely slow, and hardly stalking and flowering. Secondly, the leaf of this plant died after flowering in early summer, new young

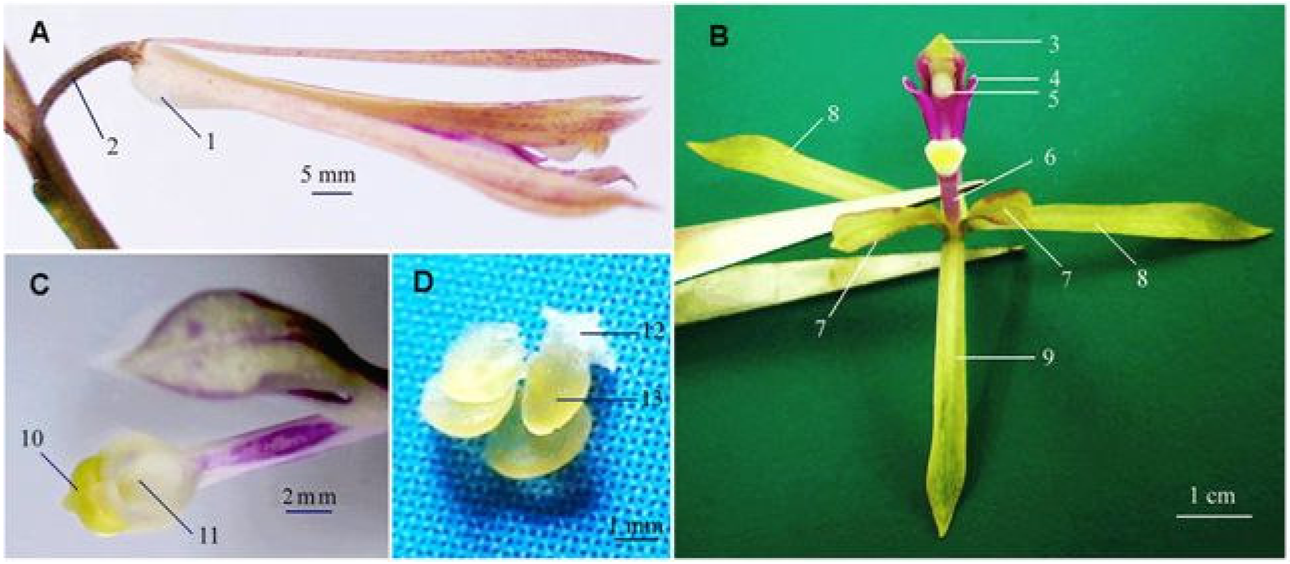


**Figure 3.** Flower structure of *C. appendiculata*: A: External feature of flower (1. mentum; 2. ovary); B: Anatomic structure of flower (3. central lobe; 4. lateral lobe; 5. callosum; 6. gynostemium; 7. petal; 8. lateral sepal; 9. dorsal sepal); C: Gynostemium (10. anther cap; 11. stigma); D: Pollinium (12. viscidium; 13. pollinium).


**Figure 4.** Fruit growth and development of *C. appendiculata*: A: Flowers performance after pollinated (f. non-pollinated fresh flowers); B: 20 days fruits after pollinated; C: 40 days fruits after pollinated; D: 80 days fruits after pollinated.

plant produced from pseudobulb in early autumn, and the plant grew vigorously in autumn and winter, the growth characteristics of dormancy in summer and growth in winter was extremely rare in orchid plants. Thirdly, during the growing period, the injured leaf could not be healed. Fourthly, the pseudobulbs were strung together by short rhizomes, and no matter how many pseudobulbs were strung, only the latest could produce a plant each year and born again a new pseudobulb by this plant, and other pseudobulbs could not bring new plants and new pseudobulbs. However, each of pseudobulbs all could become a new plant and bear a new pseudobulb if the strung pseudobulbs were separated each other. Above enigmas are all pending further to research deeply.

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