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Micromorphological Variations of *Camptotheca* Decaisne

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Abstract: The micromorphology of the leaves, pollen, and fruits of different taxa of *Camptotheca* was studied under light microscope and scanning electron microscope. The key diagnostic characters can be used for the identification of taxa within *Camptotheca* include leaf shape, venation type, vein number, stoma size and frequency, shape of outer stomatal rim, subsidiary cell number, and glandular trichome size; cotyledon shape, venation type, and vein number; and fruit surface texture, disc, length, and color. Other characters could not dependably be used for species level discrimination.

Keywords: *Camptotheca*, glandular trichome length, lower leaf surface, pollen, stomatal length, upper leaf surface.

INTRODUCTION

Camptotheca was formerly a monotypic genus, and, prior to this investigation, anatomic and micromorphological data were limited to *C. acuminata* [1-3]. In the present study, we describe the micromorphological variations of leaf, pollen, and fruit surfaces of three different species. Some characters (e.g., size, frequency, outer rim, and subsidiary cell number of stomata, and glandular trichome shape and size) can be used to distinguish the species within *Camptotheca*. For other characters, however, there were no significant variations observed.

MATERIALS AND METHODS

The micromorphology of the lower leaf surface was studied for several populations of each of three different species by stripping fresh leaf epidermis in the morning and observing the samples under a light microscope. Three trees were selected and 20 cells were examined from one leaf of each tree. The sample size (n) of *C. acuminata* was 600 cells, *C. lowreyana* was 120 cells, and *C. yunnanensis* was 60 cells. Stomatal density and size (length and width), subsidiary cell number, and glandular trichome length were recorded for each leaf. Fresh leaf tissue was also prepared for scanning electron microscopy (SEM).

Pollen samples of *C. acuminata* and *C. lowreyana* were collected by Dige Chen in Huaiji, Guangdong, China, in 1996. The pollen sample of *C. yunnanensis* was collected from a dried specimen. At least 30 pollen grains of each species were examined and photographed with SEM.

Three fruits from each of the species were used for SEM examination. Three 2 × 2 mm samples were selected, respectively, from the top, center, and bottom of each fruit.

All fresh samples were prepared for SEM by overnight fixation at 4 °C in a 1:1 mixture of 5% glutaraldehyde and 0.1M potassium phosphate buffer, pH 6.8. The samples were rinsed three times with potassium phosphate buffer 0.05 M at 10 to 15 minute intervals and kept refrigerated. A 1:1 mixture of OsO4 (2%) and Potassium phosphate buffer 0.1 M was added and the samples were refrigerated for two hours. Following osmication, specimens were rinsed twice with distilled water at 10 to 15 minutes each time, then dehydrated in a graded ethanol series at 10 to 15 minutes each step (10%, 40%, 60%, 80%, 95%, 100%, and 100% ETOH). Specimens were then critical point dried using carbon dioxide as the transitional fluid, mounted on SEM stubs, sputter coated with gold palladium, and examined using a Hitachi S405-A scanning electron microscope operating at 15 KeV. Air-dried pollen grains were dusted onto adhesive-coated SEM stubs, then sputter coated as examined as described.

Variation among populations was compared using ANOVA and Tukey's HSD Test for each of the quantitative measures. Means, standard deviations, and ranges of all characters were calculated and are presented for all samples. Statistical significance levels employed are 0.05, 0.01, and 0.001. Frequency distributions of all characters in each sample are also presented to reveal overlap that are often obscured with means-based methods of comparison.

RESULTS AND DISCUSSION

Our phenotypic analysis showed that key diagnostic characters for the identification of taxa within *Camptotheca* include leaf shape, venation type, vein number, stoma size and frequency, shape of outer stomatal rim, subsidiary cell number, and glandular trichome size; cotyledon shape, venation type, and vein number; and fruit surface texture, disc, length, and color. Other characters could not dependably be used for species level discrimination. The informative characters for which microscopic analysis was needed are described below, including stomatal density and size (length and width), subsidiary cell number, and glandular trichome

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length. Like *Nyssa* [2], *Camptotheca* has simple unbranched non-glandular hairs, external unicellular glands, and stomata on the lower surface of the leaf (hypostomatous).

Upper Leaf Surfaces

The adaxial surfaces of mature leaves of various *Camptotheca* species are glassy with a thick cuticle and occasional non-glandular trichomes occasionally (Fig. **1**). There is no diagnostic difference among the species.

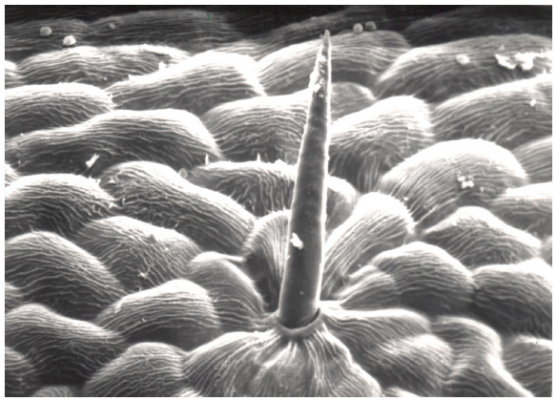

Fig. (1). Upper leaf suface of *Camptotheca acuminata* (× 500).

Lower Leaf Surfaces

Stomata provide an essential connection between the internal air spaces of plants and the external atmosphere [4]. Most authors agree that stomatal size is usually sufficiently stable to be used as a diagnostic character [2]. Related species often have stomata of similar size [2]. Species with smaller stomata usually have a greater number than those species with larger stomata [2, 4]. Smaller stomata (<15 mm long) are often at higher density, while larger stoma (>38 mm) are observed to have lower density [2, 4]. This is not the case in *Camptotheca*, however.

The lower leaf suface has stomata and both glandular and non-glandular trichomes (Fig. **2**). Stomatal size in *Camptotheca* ranges from 20 to 35 μm in length and 10 to 25 μm in width, considered medium-sized in the vascular plants. *C. acuminata* has smaller stomata (average 22.85 ×15.30 mm) than both *C. lowreyana* and *C. yunnanensis* (29.33 × 20.08 mm and 31.05 × 20.23 mm, respectively).

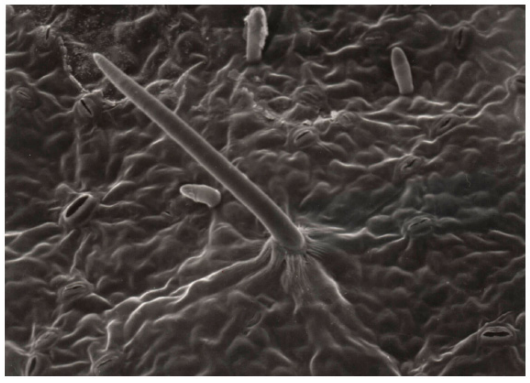

Fig. (2). Lower leaf suface of *Camptotheca lowreyana* 'Katie' (× 300).

The species with larger stomata (*C. lowreyana* and *C. yunnanensis*) have greater stomatal frequency (average 263.20 and 221.22 stomata per mm² of leaf area) than the species with smaller stomata (*C. acuminata*: 180.39) (Fig. **3**). In addition, we found that the thickness and form of the outer stomatal rim are of diagnostic value at the species level. Similar to *Nyssa sylvatica* Marsh [2], *C. lowreyana* has the outer rings of striae of stomata and not the concentric rings of striae, but differs from the other species of *Camptotheca*.

Subsidiary cell number per stoma is higher in *C. yunnanensis* (average 5.83 cells) than the other two species (*C. acuminata*: 4.86, *C. lowreyana*: 5.23) (Fig. **3**).

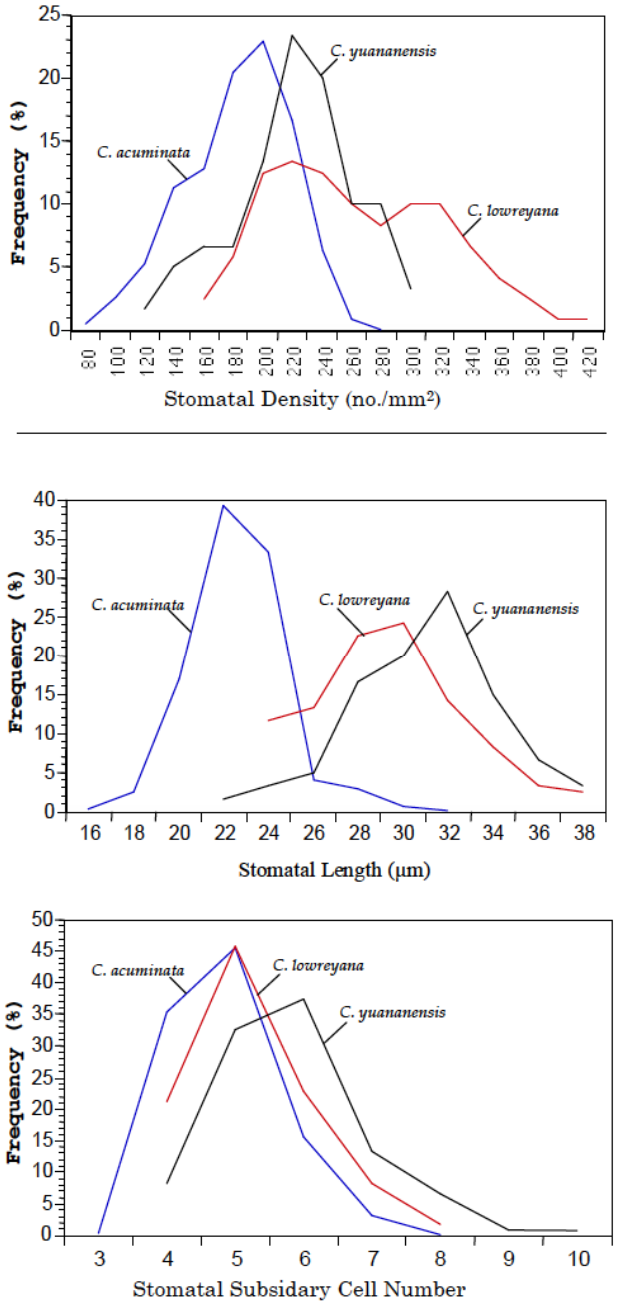

Fig. (3). Leaf stomatal density, stomatal length, and subsidiary cell number of *C. acuminata* (n = 600), *C. lowreyana* (n = 120), and *C. yunnanensis* (n = 60).

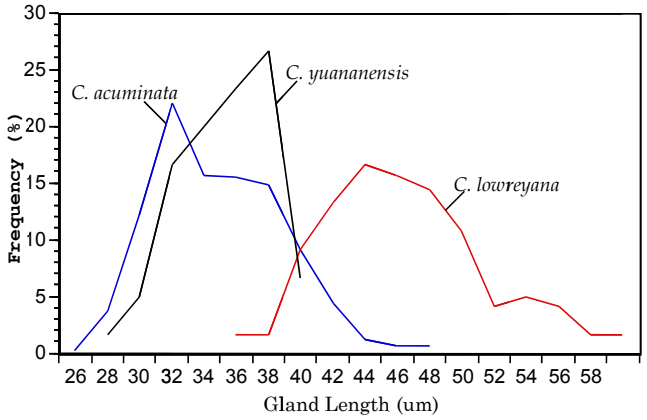

Fig. (4). Leaf glandular trichome length of *C. acuminata* (n = 600), *C. lowreyana* (n = 120), and *C. yunnanensis* (n = 60).

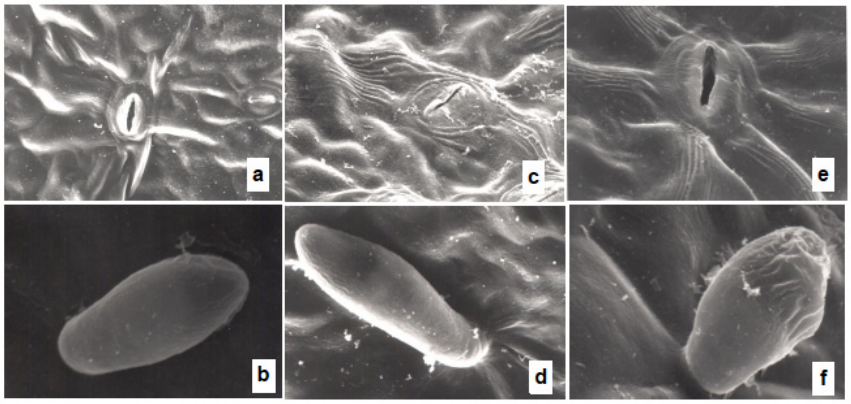

Fig. (5). Scanning electron micrographs of stomata and glandular trichomes on lower leaf surface (stomata × 1,000; glandular trichomes × 1,400): *C. acuminata* (**a** and **b**), *C. lowreyana* (**c** and **d**), and *C. yunnanensis* (**e** and **f**).

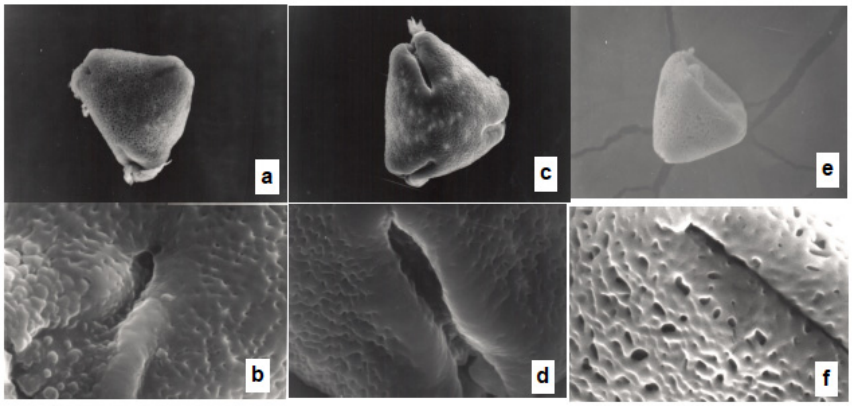

Fig (6). Scanning electron micrographs of pollens of *Camptotheca* species (upper: × 750; Lower: × 3,750) *C. acuminata* (**a** and **b**), *C. lowreyana* (**c** and **d**), and *C. yunnanensis* (**e** and **f**).

The size and shape of glandular trichomes on the lower leaf surface vary in *Camptotheca* and are diagnostic characters for species identification. *C. lowreyana* has pandurate glandular trichomes that are larger (average 46.49 µm in length) than those in the other two species (*C. acuminata*: 34.87 µm and *C. yunnanensis*: 35.20 µm in length and both are obelliptic in shape) (Figs. **4** and **5**).

Leaf micromorphology provides important quantitative and qualitative characters for species identification in *Camptotheca*. Stomatal size and frequency, shape of outer stomatal rim, subsidiary cell number, and glandular trichome shape and size are of diagnostic value.

Pollen and Fruit Surfaces

Pollen grains of *Camptotheca* are 3-colporate, suboblate, obtuse-triangular in polar view, 29-38 µm (polar axis) × 33-54 µm (equatorial axis), and sexine punctitegillate (Fig. **6**). Colpi are not very distinct; colpi margins have nexinous

thickenings [5]. SEM revealed no significant differences in pollen surface texture among the three species. In terms of pollen size, grains of *C. acuminata* are 34.4 (28.6-40.2) µm × 39.8 (34.5-42.5) µm. Pollen of *C. lowreyana* are very similar in size to those of *C. acuminata*, measuring 34.4 (28.6-40.2) µm × 39.8 (34.5-42.5) µm in size. The only difference is that the pollen of *C. lowreyana* is more oblate. Pollen grains of *C. yunnanensis* are very similar to the other two species, but of smaller size, measuring 31.2 X 35.5um.

The fruit surface of *C. acuminata* is rugose, while both *C. lowreyana* and *C. yunnanensis* have smooth and lustrous fruit surfaces.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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Declared none.

PATIENT'S CONSENT

Declared none.

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