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Epicuticular Leaf Architecture Confirms a New *Smilax* Species (Smilacaceae) from Northeast Thailand

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ABSTRACT: A new species, *Smilax petiolatumidus* Moore, Narkkong, Moore et Lutat sp. nov. is proposed based upon a specimen from Loei province, Thailand, having leaves with a distinctively swollen petiole and one to many sub-tending thorns. Our recognition of this sterile individual as a new species is confirmed by its unique epicuticular leaf architecture. This is the first instance in which leaf epidermal architecture has been applied as a taxonomic tool in the absence of reproductive structures to diagnose a new Thai species of Smilacaceae.

KEYWORDS: *Smilax*, cuticle, stomata, Smilacaceae, Thailand.

INTRODUCTION

Smilacaceae Vent is a small cosmopolitan family. In Thailand species are climbers or shrubs, often having stems with prickles and leaves with 3 or more campylodromous or acrodromous primary veins. Two genera are represented in Thailand; *Heterosmilax* Kunth and *Smilax* Linnaeus'. The later is comprised of twenty-six species and subspecies. However, little work has been devoted to Smilacaceae since Tetsuo Koyama's taxonomic key was published in the Flora of Thailand, Volume II [1]. When assessing *Smilax*, identification regularly requires the combined examination of foliage and flowers from strictly dioecious plants and often fruits. Because it is unlikely that specimens will be found having all plant structures at the time of collection, it is particularly difficult to make systematic determinations. Further, when using the taxonomic key, leaves often are found to be variable in size, shape and venation within a single species, such as *Smilax verticalis* Gagnepain. Consequently, taxonomic characters break-down in the field and the inability to collect plants in optimal condition may result in questionable systematic assignments. Therefore, a supporting taxonomic method would be valuable for *Smilax* systematics.

The analysis and use of epidermal characters of leaves is recognized as a frequently successful taxonomic method to distinguish between individual taxon where floral material is absent or minute differences in flowers and foliage exist such as in *Smilax*. For instance, paleobotanists have successfully separated and identified fossil leaves based on their epidermal architecture in the absence of reproductive material[2,3,4,5]. In modern studies on extant leaves, numerous workers have also made use of leaf cuticle ornamentation, stomata and epidermal cell geometry to reliably identify a particular species [6,7,8,9]. Thus, considering that *Smilax petiolatumidus* is nominated using a single sterile specimen, we confirm its recognition based on its unique epidermal cell architecture and foliar morphology.

MATERIALS AND METHODS

Sample Source

While curating *Smilax* specimens derived from a recent field-trip to Phu Kradung National Park, Loei province, the first author recognized an individual specimen with foliar characters uniquely different from other reported species in Thailand. The specimen was collected in a steeply rugged area near the main trail leading up to Phu Kradung above 1,150 meters under the closed canopy rainforest. The type specimen, BKF147149, is in the collections at the Forest Herbarium (BKF), Bangkok, Thailand.

Microscopic Examination

For the light microscopic examination, a 0.5 cm x 0.5 cm ablated tissue sample was removed from the mid-leaf margin, followed by oxidation in a 6% sodium hypochlorite solution for 8 hours. The oxidant was decanted and H_2O added for 2 hours to remove any remaining oxidant. The tissue was then removed and placed in a partially water-filled Petri dish. Using a dissecting microscope, the upper and lower epidermis were separated with a fine spatulated-tip wire probe taking care to preserve the joining leaf margin, which acts as a hinge that preserves cuticle orientation [10]. The remaining mesophyll cells were gently brushed away using a fine sable brush. The cuticle was then stained to a desired contrast in 2% methylene blue-H_2O solution, placed on a microscope slide in glycerol, followed by placement of a cover slip. The slip was sealed with clear fingernail polish. Micro-photography was performed using an Olympus BX50 microscopic and Olympus C-7070 camera. Digital images were stored on CD.

A second tissue sample was removed from the same leaf and region as the first tissue. It was halved for examination on both surfaces by scanning electron microscopy. The halved cuticle was sputter coated with gold using an SPI-Sputter Coater. Imaging was then performed using a JEOL,JSM-6460LV microscope at 10 KVA and magnifications of x40 and x200 for storage on CD. All images for figures were managed with Microsoft Office Picture Manager, 2001.

Results

Descriptive Vegetative Morphology

The plant was a climber, 2 meters long, with stem terete, smooth, 5 mm thick, curved and irregularly right twining, internodes 7-13 cm long, with scattered prickles 2-4 mm long (Fig 1a) straight or recurved, usually one or more thorns subtending the petiole (Figs 1b and 1d); stem un-branched. Leaves (n = 16) alternate, elliptic to narrowly elliptic-lanceolate, 16.8 - 21.2 cm long and 4.6 - 8.8 cm wide; base obtuse to rounded, slowly tapering distally from below the middle to form an acute (Fig 1a) or abruptly and suddenly mucrunate apex, leaves are slightly shiny, dark green above and lighter below, dull, sub-coriaceous, not glaucous; costae 5-7 plus an additional fine fimbril pair, all separate from the base (Fig 1c), prominent on both surfaces, the median 3 abaxially convex; lateral reticulate veins irregular, arching upward, further divided into finer veinlets forming polygonal areolae; petioles are 3-3.5 cm long, recurved from the stem to a then distally straightened and swollen region 1.2-2.8 cm long, again narrowed to join the leaf base (Figs 1b and 1c), dehiscence may be at either the distal or proximal ends of the swollen length, tendrils absent.

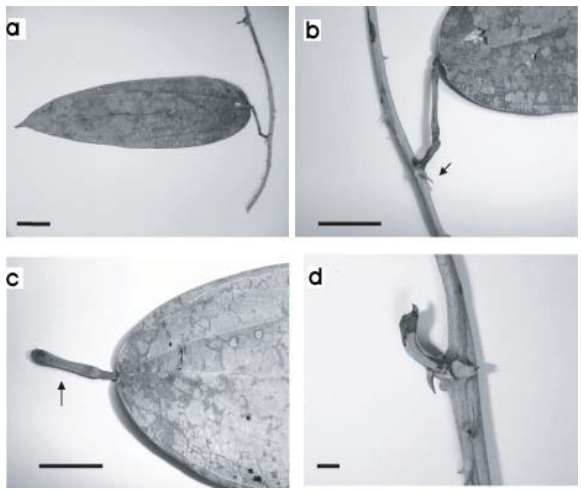

Fig 1. a. Outline of a *Smilax petiolatumidus* leaf attached to stem (Scale bar = 2 cm). b. Attached leaf, petiole morphology, and one sub-tending thorn (Scale bar = 2 cm). c. Swollen leaf petiole (Scale bar = 2 cm). d. Showing multiple thorns sub-tending a petiole (Scale bar = 5 mm).

Flowers and fruits are unknown.

Descriptive Epidermal Morphology

Stomatal Complexes

Hypostomatic stomata are distributed randomly over the leaf surface, their long axis generally oriented toward the midvein (Figs 2a and 2b). Mature stomata complexes are surround by 4 to 7 subsidiary cells (Figs 2a and 2b). Leaf cuticle overlaps the guard cells forming a dome with a slit-like aperture. Stomata are encircled with 1 or 2 raised striae with additional striae radiating outward in spoke-like fashion. (Fig 2c).

Abaxial Epidermis

Marginal cells elongate and buttressed, forming ~16 rows, their anticlinal cell walls are uniformly thickened, periclinal wall perimeters are fluted and punctate with circular thickenings; admedially cells gradually become tetragonal, buttressed and forming ~ 6 rows with mixed convex or concave walls, then pentagonal to polygonal until losing their thickened and fluted anticlinal walls and punctate ornamentation (Figs 2d and 2f) to form elongated epidermal cells that are pentagonal to polygonal in outline with anticlinal walls that are mixed concave, convex or slightly sinuous (Fig 2b).

Adaxial Epidermis

Marginal cells are typical in arrangement and description to those of the abaxial surface (Fig 2d and 2f). Anticlinal cell walls (Fig 2e) are tetragonal to

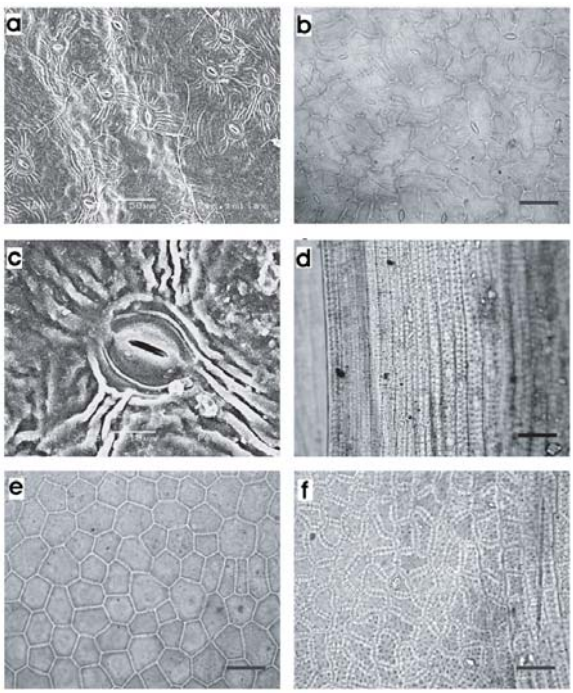

Fig 2. a. SEM (x40) showing distribution of hypostomatic stomata and striate ornamentation (Scale bar = 50 mm). Note the fungal hyphae. b. Slide mounted tissue (x40) showing distribution of stomata and subsidiary cell arrangement (Scale bar = 50 mm). c. SEM (x200) showing a single stomate and striate epidermal ornamentation (Scale bar = 10 mm). d. Slide mounted tissue (x40) showing elongated marginal cells and punctate surface ornamentations. Note the thickened anticlinal walls (Scale bar = 50 mm). e.-f. Slide mounted upper epidermis (x40) showing cells with 4-9 anticlinal walls-mid lamina (Scale bar = 50 mm). f. Cuticle showing circular surface ornaments on epidermal cells near the leaf margin. Note the thickened cell walls (Scale bar = 50 mm).

polygonal (up to 9 straight anticlinal walls). Adaxial surfaces have scattered and minute striae. Bundles of toothpick-like phytoliths are present in the mesophyllous region.

Discussion

While the use of floral characters remains the most robust method to determine taxonomic identification of angiosperms, often leaf epidermal characters are reliable determinative proxy in plant systematics[11]. Taxonomically diagnostic features, such as the stomata complex, epidermal cell architecture and surface ornamentation have been used in some cases as an alternative method to classify at the genus and species level [12, 13]. A number of these epidermal structures have been successfully used to differentiate certain *Smilax* species in Thailand [14]. In this study, uniquely domed stomata and narrowed pore opening , furrowed and punctuate periclinal perimeters on marginal cells and striate epidermal surface were used to diagnose *Smilax petiolatumindus* as a new species. This new taxon also exhibits diagnostically distinct vegetative morphologies in its swollen leaf petiole, from which the specific name is derived, as well as its subtending petiolar thorns. As a taxonomic method, the use of microepidermal leaf characters described in our study overcomes the need for reproductive structures to identify this taxon and provides a robust method for systematic studies of *Smilax* in southeast Asia.

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