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NOTES ON THE FLORAL MORPHOLOGY AND ANATOMY OF *TESSMANNIANTHUS CARINATUS* (MELASTOMATACEAE)

Notas sobre la morfología y la anatomía floral de *Tessmannianthus carinatus* (Melastomataceae)

Ricardo Kriebel

The New York Botanical Garden, 200th St. & Southern Blvd., Bronx, NY 10458, USA. The Graduate Center, City University of New York, New York, NY, USA. Current address: Department of Botany, University of Wisconsin, Madison, USA. kriebelr@gmail.com

ABSTRACT

This work reports the second collection of *Tessmannianthus carinatus* Almeda, a species previously known only from the type. Two flowering trees were encountered at the type locality of Cerro Jefe, Panama. Species in this genus are very rare and the colors and posture of fresh floral parts have seldom been observed and described. The first known images of flowers at anthesis are here provided. In addition, observations on the posture and color of the stamens were made, including dissections of a flower preserved in spirit, and scanning electron micrographs of the unusual anther apices. Lastly, anatomical sections were conducted of these flowers which revealed the presence of styloids in the hypanthium, anthers and styles. These crystals had only been reported from the wood of one species in the genus and their presence suggests a relationship to the tribes Astronieae and Henrietteeae.

Key words. Floral anatomy, floral morphology, heteranthery, styloids, *Tessmannianthus carinatus*.

RESUMEN

En este trabajo se reporta la segunda colección de *Tessmannianthus carinatus* Almeda, una especie previamente conocida solamente del ejemplar tipo. Se encontraron dos individuos con flores en la localidad tipo de Cerro Jefe, Panamá. Las especies de este género son muy raras y los colores y las posturas de sus partes florales rara vez han sido observadas. Aquí se presentan las primeras fotografías conocidas hasta el momento de flores vivas. Adicionalmente, se reportan observaciones de la postura y el color de los estambres, así como disecciones de una flor preservada en alcohol y micrografías de los ápices inusuales de las anteras. Por último, se realizaron secciones anatómicas de las flores, las cuales revelaron la presencia de cristales alargados de tipo estilodios en el hipanto, anteras y estilo, los cuales se habían reportado solamente de la madera de una especie del género. La presencia de estos cristales sugiere una relación de *Tessmannianthus* con especies de las tribus Astronieae y Henrietteeae.

Palabras clave. Anatomía floral, morfología floral, heterantería, estilodios, *Tessmannianthus carinatus*.

INTRODUCTION

The genus *Tessmannianthus* Markgr. has seven species and is restricted to the Neotropical countries of Panama, Colombia, Ecuador, and Peru (Almeda 1990). It is considered to be a member of the tribe Merianieae (Renner, 1993). Species in the genus are medium to large trees reaching 40 meters tall making them amongst the largest members of Melastomataceae (Almeda 1990). *Tessmannianthus* flowers are also unusual in that they are extremely heterantherous, with the small set of stamens bifurcating at the apex into two distinct pores whereas the large stamens have a single pore. This extreme heteranthery is used to diagnose the genus and gives the name to the type species, *T. heterostemon* Markgr. (Markgraf 1927). Another less uncommon but noteworthy feature of the androecium in *Tessmannianthus* is that the larger series of stamens are opposite the sepals rather than the petals, as is the more frequent condition in heterantherous taxa (Almeda 1989, Mendoza-Cifuentes & Fernández-Alonso 2010).

Species of the genus *Tessmannianthus* are very rare. For example, of the three Panamanian endemics, *T. carinatus* Almeda and *T. cereifolius* Almeda are known only from the type, and *T. gordonii* Almeda is known from the type and a paratype. For this reason, little is known about their floral morphology or biology. The posture of the strongly heterantherous stamens has not previously been described and there are few detailed descriptions of colors of its floral parts (Almeda, 1989). Herein, the first collection after the type of *T. carinatus* Almeda (1989) is reported. This species is endemic to Cerro Jefe, Panama. Two flowering individuals were observed near the summit of Cerro Jefe in September 2011. In order to better characterize the flowers of this enigmatic genus, the first photographs of flowers at anthesis of a species in the genus are provided, as well as floral dissections, scanning electron micrographs of the unusual anther apices, and observations on its floral morphology and anatomy.

MATERIALS AND METHODS

Flowers of *T. carinatus* were collected in Cerro Jefe, which is located inside Chagres National Park in the Province of Panama, Panama, at 915 m of elevation on the 14 of September 2011. Flowers from the spirit collection were dissected and photographed under a Nikon SMZ1500 stereoscope equipped with a Nikon DXM1200F camera connected to a computer and using the the Nikon ACT-1 software.

For flower anatomy, floral buds were fixed in 70% ethanol in the field, subsequently fixed in formalin-acetic acid-ethanol (FAA; 3.7% formaldehyde; 5% glacial acetic acid; 50% ethanol), vacuum-infiltrated overnight, and then stored in 70% ethanol. For light microscopy, fixed material was dehydrated through an alcohol-toluene series in a Leica TP-1020 automatic tissue processor, and embedded in Paraplast X-tra (Fisher Healthcare, Houston, Texas, USA). The samples were sectioned at 10 μm with an AO Spencer 820 rotary microtome (GMI Inc. Minnesota, US). Sections were stained with Johansen's safranin (Johansen 1940) and 0.5% Astra Blue in 2% tartaric acid w/v in distilled water (Maácz & Vágás 1961, Kraus *et al.* 1998) and mounted in Permount (Fisher Scientific, Pittsburgh, Pennsylvania, USA). Sections were viewed and digitally photographed with a Zeiss Axioplan compound microscope equipped with a Nikon DXM1200C digital camera with ACT − 1 software. To detect the presence of crystals, sections were observed under polarized light.

To document the anther apices in detail, a large and a small stamen were transferred to acetone via an ethanol-acetone series,

then dried by critical point, mounted on aluminum stubs with adhesive tabs (Electron Microscopy Sciences), and sputter coated with gold palladium in a Hummer 6.2 sputter coater (Anatech, Springfield, VA). The samples were examined and photographed in a Jeol JSM-5410 LV Scanning Electron Microscope operated at 10 kV. The final plates were prepared with GIMP 2.8 (http://www.gimp.org). Voucher specimens were deposited at NY and PMA (*Kriebel & Burke 5672*).

RESULTS

Two individuals of *T. carinatus* were encountered near the summit of Cerro Jefe (Fig. 1A-D). Observations of live flowering branches of *T. carinatus* confirm what appeared evident on the type specimen, the flowers are positioned at angle of about 90 to 140 degrees with respect to the inflorescence axis (Fig. 1D). The flowering hypanthia are light pink. Anatomical sections of the hypanthium showed the presence of styloid crystals (Fig. 2A). These crystals were also observed in the sepals, petals, placenta, style, anther connective and endothecium. The clawed petals are light pink as is characteristic in the genus (Fig. 1I).

In the second specimen of this species reported here from the same population as the type (and only population known of this species), it is documented that the color of the small stamens is yellow, but the color of the large stamens to be mostly pink to reddish on the distal half (Fig. 1C and D). In addition, Almeda's (1989) description of the staminal filaments as declinate was corroborated but I further describe their strange posture here. The two pairs of lateral large stamens are placed around the flower and have their anther tips pointed to the center of the flower, which is easy to see on the live flowering plants and in material preserved in spirit (Fig 1 C, D and J). Almeda (1989) described in the stamens a geniculation in the filament insertion. A more marked geniculation was observed in the area were the stamens bend about 45 degrees to the side of the flower which is about 1.5 mm below the filament insertion, particularly in the large set of stamens (Fig. 1C and D).

The apex of the small stamens with bifurcating apices is also quite distinctive. Each divergent pore shows a folded area towards the inner side of the pore. This has been noted in three of the illustrations of species in the genus (Almeda 1989, Wurdack 1989) but appear absent in *Tessmannianthus cereifolius* (Almeda 1990). A micrograph of the anther apices for the large and small set of stamens is presented in figure 1 (1L and 1M).

The style in *Tessmannianthus carinatus* is very thick and slightly sigmoid. The stigmatic surface is relatively small and narrower than the width of the style. A transverse section of the style showed three laminar protrusions from the center towards the periphery (Fig. 2B).

DISCUSSION

It is here confirmed that individuals of *Tessmannianthus carinatus* still persist in the forests near the summit of Cerro Jefe in Panama, where it is endemic. These forests are considered to have been island refugia from the middle of the Miocene until the land bridge between North America and South America formed about 2.4-3.5 million years ago (Almeda *et al*. 2014 and references therein). The top of Cerro Jefe is dominated by the palm *Colpothrinax cookii* Read and the number of endemic species of Melastomataceae is six including *Tessmannianthus carinatus* (Almeda, 2014). Because the individuals of *T. carinatus* encountered are inside Chagres National Park, it is believed the species is well protected. Based on the very limited known distribution of *T. carinatus*, the species is provisionally assigned a conservation status of Endangered (EN) based IUCN guidelines

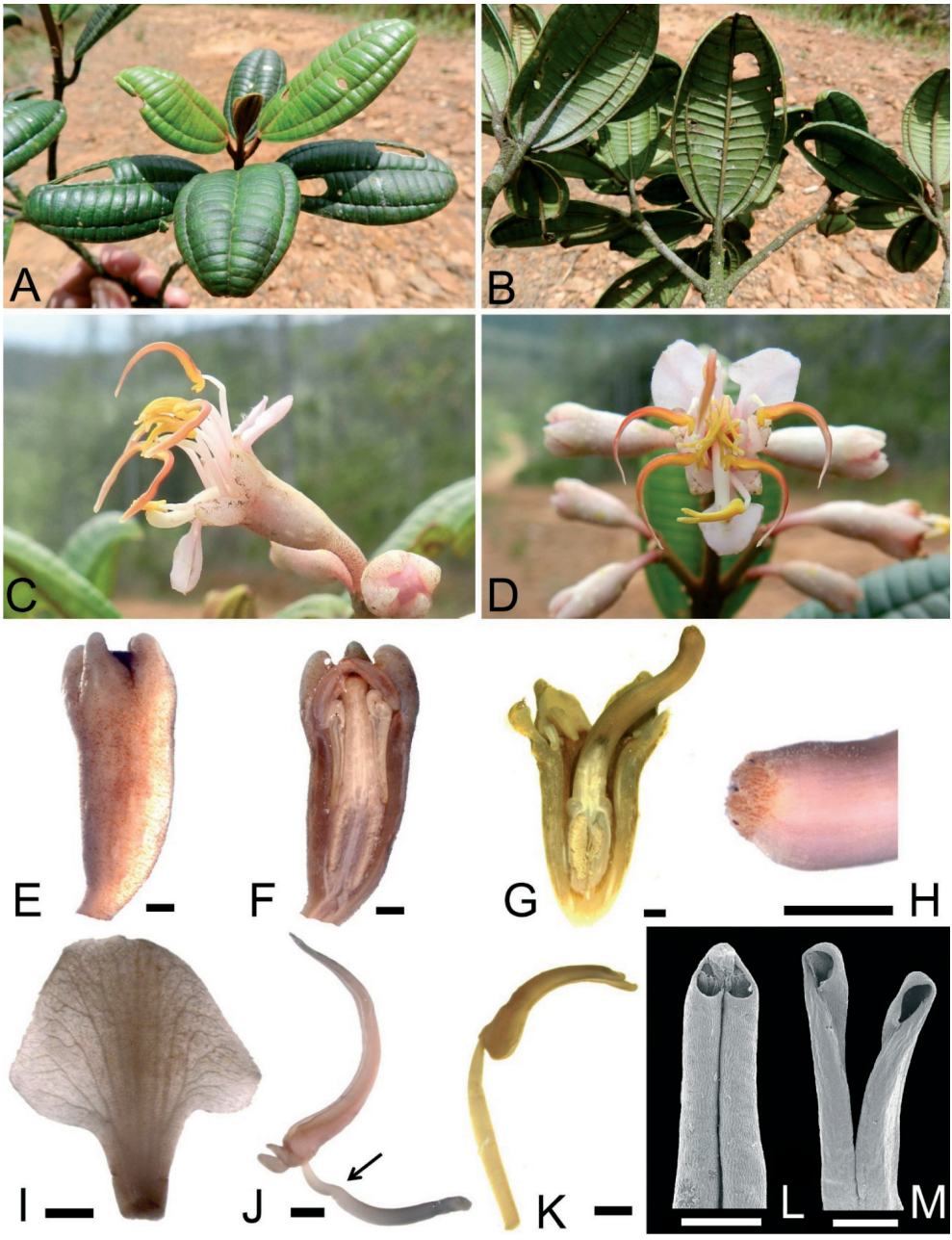

Figure 1. *Tessmannianthus carinatus* Almeda. A. Branch showing leaf adaxial surfaces. B. Branch showing leaf abaxial surfaces. C. Lateral view of a flower at anthesis. D. Ventral view of a flower at anthesis. E. Flower bud. F. Longitudinal section of a flower bud. G. Longitudinal section of a flower at anthesis with petals and stamens removed. H. Close up of the stigma. I. Petal, adaxial view. J. Lateral view of the large stamen (note the arrow pointing to the geniculation). K. Lateral view of the small stamen. L and M. Scanning electron micrographs of ventral view of the large stamen apex (L) and the small stamen apex. Scale bars = 1 mm (E-K), 100 µm (L and M). From *Kriebel & Burke 5672* (NY, PMA).

(IUCN 2001, 2011). The specimen reported here is only the second one collected after the type, 28 years after. The two individuals observed were flowering and allowed the documentation of floral morphology in live plants. The known flowering time of August is extended to include the collections reported here which were made in September.

The colors of anthers in heterantherous Melastomataceae tend to differ between the large set (usually pollen placing) and the small set of stamens (usually pollen rewarding) (Luo *et al*., 2008). Anther color dimorphism was confirmed in *T. carinatus*. Previously, the anthers of different species of *Tessmannianthus* have been described as all being yellow on collector labels and therefore in the protologue of *T. carinatus* (Almeda 1989). The finding here of different colored stamens may apply to other species in the genus. It should be noted that stamen color change is known to occur in several Melastomataceae such as *Leandra subseriata* Gleason and *Miconia mirabilis* (Aubl.) L.O. Williams (obs. pers.). The main reason why stamen color change likely does not occur in *T. carinatus* is that both sets of stamens differed in color at anthesis and do not show evidence of color change. The position of the large set of stamens in fresh material is documented here in detail and is highly unusual and perhaps unique among heterantherous Melastomataceae. The lateral, large set of stamens have a conspicuous filament geniculation that enables them to bend towards the outside of the flower. This positioning of the lateral stamens was not observed in the uppermost stamen which presented an almost unbent filament geniculation. It remains to be determined if the position of the stamens observed in *T. carinatus* is the rule or the exception in the genus and how exactly these awkwardly positioned stamens interact with the bee visitors.

Styloid crystals are elongate calcium oxalate crystals and are not common in the Melastomataceae. They are said to be common in the tribe Henrietteeae (Penneys *et al.* 2010) and have also been recorded in the tribe Astronieae (Baas 1981). The finding of elongated styloid crystals in the flowers of *Tessmannianthus carinatus* as well as anther apices that diverge into two pores in the small set of stamens, suggest a relationship to the tribe Astronieae and challenges previous suggestions of a placement if *Tessmannianthus* in the Merianieae. This is the first report of these crystals in flowers of the genus *Tessmannianthus*. Styloid crystals had only been previously reported in the wood anatomy of *Tessmannianthus calcaratus* (ter Welle & Koek-Norman 1981).

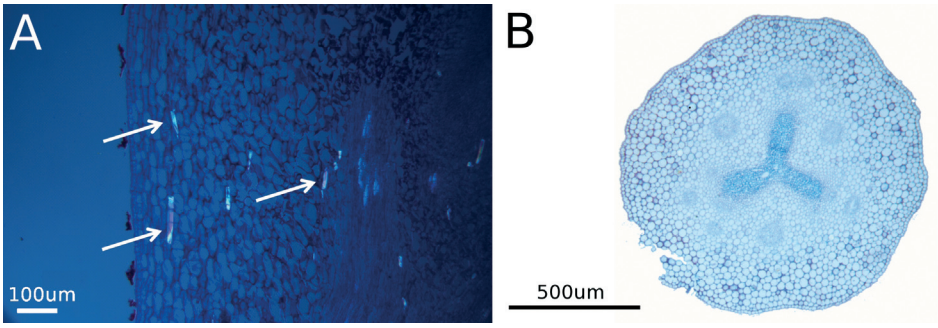

Figure 2. Floral anatomy in *Tessmannianthus carinatus*. A. Longitudinal section of the hypanthium under polarized light showing the presence of styloids (pointed by arrows). B. A transverse section of the style.

When the phylogenetic placement of *Tessmannianthus* is determined, it will reveal if styloid crystals evolved independently in *Tessmannianthus* or, if the latter genus is placed within a clade comprised of the tribes Astronieae and Henrietteeae, then confirming these crystals might have evolved only once in the Melastomataceae.

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