

# VARIATION IN THE TRACHEIDS OF SEEDS FROM THE SUBTRIBE PITHECOCTENIINAE (BIGNONIEAE: BIGNONIACEAE) AND THEIR CONTRIBUTION TO THE SYSTEMATICS OF THE GROUP

CARLOS MANUEL BURELO-RAMOS<sup>1</sup>, FRANCISCO GERARDO LOREA-HERNÁNDEZ<sup>2</sup>  
AND GUILLERMO ANGELES<sup>3, 4</sup>

<sup>1</sup>Herbario UJAT, División Académica de Ciencias Biológicas. Universidad Juárez Autónoma de Tabasco

<sup>2</sup>Instituto de Ecología, A.C. Red de Biodiversidad y Sistemática

<sup>3</sup>Instituto de Ecología, A.C., Red de Ecología Funcional

<sup>4</sup>Corresponding author: guillermo.angeles@inecol.edu.mx

**Abstract:** The diversity of ornamentations present in the tracheids of seed surface from species of the subtribe Pithecocteniinae (genera *Amphilophium*, *Distictella*, *Distictis*, *Glaziovina*, *Haplophium*, and *Pithecoctenium*) in the Bignoniaceae is described. Three distinct ornamentation types were observed on the tracheid surfaces: (1) Tracheids without ornaments (in the genera *Amphilophium*, *Glaziovina* and *Haplophium*), (2) ornaments in true helices (in the genera *Distictis* and *Distictella*), and (3) ornaments in pseudo-helices (in the genus *Pithecoctenium*). The taxonomic value of these tracheid ornaments to establish possible relationships within the subtribe Pithecocteniinae is discussed.

**Key words:** Bignoniaceae, cell-wall thickening, Pithecocteniinae, tracheids, winged seeds.

**Resumen:** Se describe la diversidad de ornamentaciones presentes en los traqueoides de las semillas de especies de la subtribu Pithecocteniinae (Bignoniaceae), la cual incluye a los géneros *Amphilophium*, *Distictella*, *Distictis*, *Glaziovina*, *Haplophium* y *Pithecoctenium*. Se observaron tres tipos de ornamentación sobre las superficies de los traqueoides: (1) Traqueoides sin ornamentaciones en tres géneros (*Amphilophium*, *Glaziovina* y *Haplophium*), (2) ornamentaciones formando hélices verdaderas (en los géneros *Distictis* y *Distictella*) y (3) ornamentaciones en pseudo-hélices (en el género *Pithecoctenium*). Se discute el posible valor taxonómico de las ornamentaciones de los traqueoides seminales dentro de la subtribu.

**Palabras clave:** Bignoniaceae, engrosamiento de pared celular, Pithecocteniinae, semillas aladas, traqueoides.

Plants have multiple dispersal mechanisms; apparently, they have arisen as ecological adaptations that provide a species with the opportunity to distribute its seeds to wider areas, thus increasing the possibility of occupying such sites under favorable germination conditions (Werker, 1997). Seed dispersal can be classified using several criteria, such as the dispersive mechanism, the type of dispersal unit, and the dispersive agent itself. Although no classification system is ideal, the one based on dispersive agent is the most practical and has been used by several authors (Ridley, 1930; Fahn and Werker, 1972; van der Pijl, 1982; Howe and Smallwood, 1982; Levin *et al.*, 2003). In this system, the wind is considered the best agent due to a phenomenon

known as anemocory. This mechanism is favored by intrinsic characteristics of seed morphology and anatomy such as small size, low weight, and presence of accessory structures such as wings and feathers that keep it afloat in air currents (Werker, 1997). Anatomically, it has been shown that seed wings are an extension of one or all of seed coat layers, although on rare occasions it is formed by interwoven exotestal hairs (Werker, 1997). Winged seeds are common, among other families, in the Bignoniaceae, Scrophulariaceae, and Vochysiaceae (Cronquist, 1981; Werker, 1997).

In the family Bignoniaceae, the seed wing is made up of "tracheids", a term used to refer to tracheid-like cells that exhibit diverse ornamentation on their secondary wall, just

as there are variations in the tracheal elements of the xylem according to the Cornerian Terminology (Schmid, 1986). Such degree of diversity as that found in the cell wall ornamentations of tracheoids has not been reported for any other structure in this family (Von Guttenberg, 1971; Lersten *et al.*, 2002).

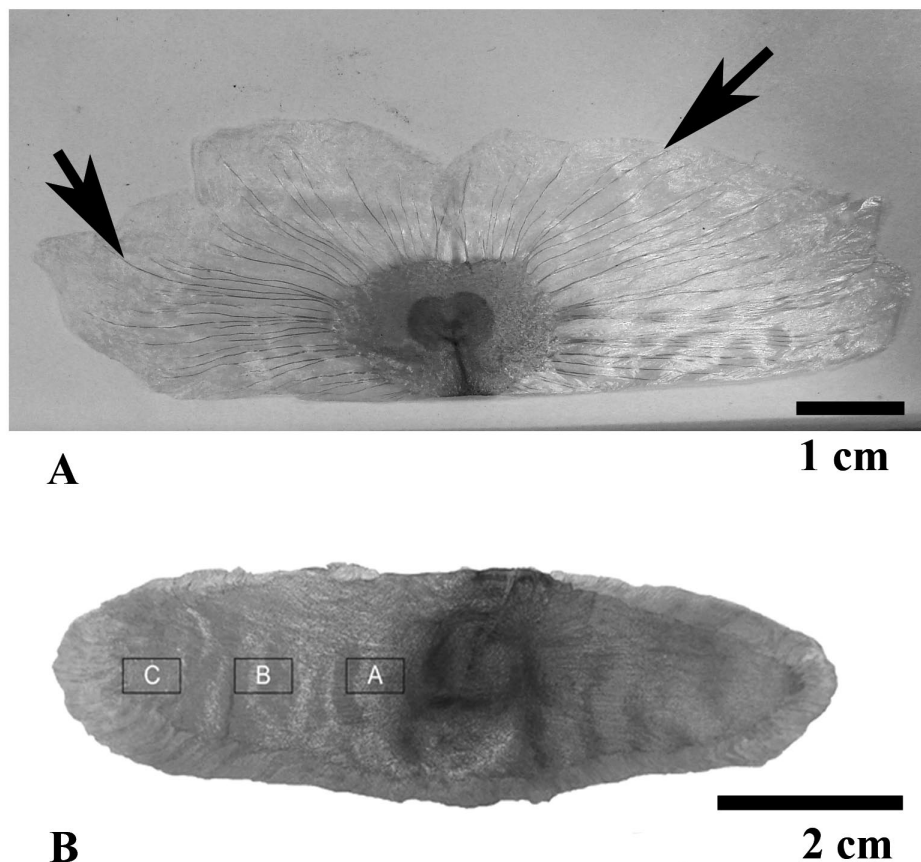
Melchior (1927) established the subtribe Pithecocteniinae, including the genera *Amphilophium*, *Distictis*, and *Pithecoctenium*, all characterized by having hexagonal branches and trifold tendrils. Phylogenetic analyses based on molecular (Lohmann, 2006) and morphological (Burelo and Lorea, in prep.) characters seem to indicate that these three genera, plus *Distictella*, *Glaziovina*, and *Haplophium* form a monophyletic group (the subtribe Pithecocteniinae), comprising 49 species (Gentry, 1973, 1974, 1976, 1993; Pool, 2007 a, b, 2009). The phylogenetic relationships within this group, established by Lohmann (2006), are not resolved definitely, given the low number of species of the group she used in her study (11 species, of a total of 49).

Lersten *et al.* (2002) studied the seed wing structure of 20 Bignoniaceae species and described in detail the tracheoid characteristics found in them. In their study, they included some species of the subtribe Pithecocteniinae, namely,

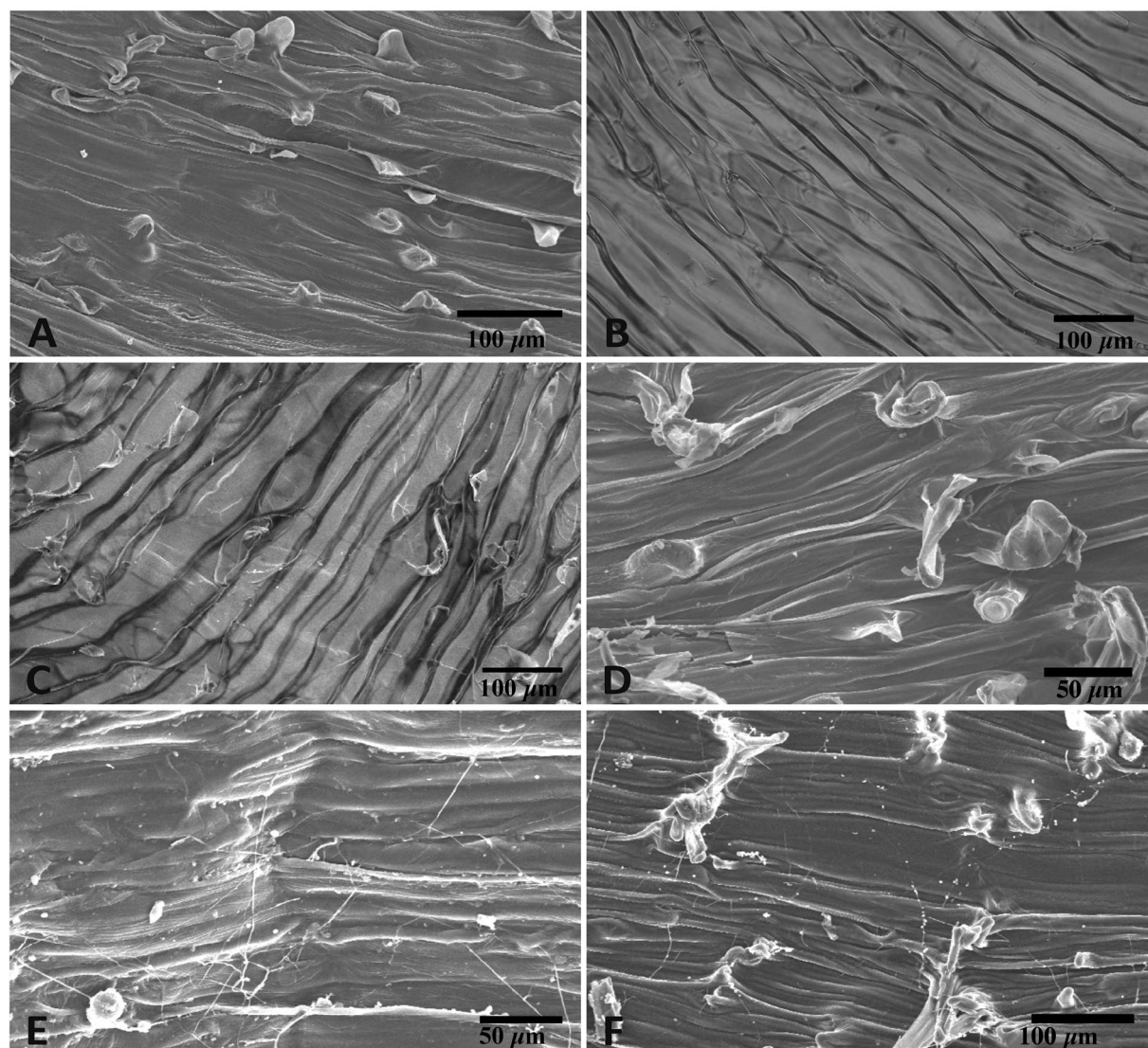
*Amphilophium paniculatum* (L.) Kunth, *Distictella magnoliifolia* (Kunth) Sandw., and *Pithecoctenium crucigerum* (L.) A.H.Gentry. They found several thickening patterns in the secondary wall of tracheoids of seed wings in the Bignoniaceae, a diversity as rich as that found in the tracheary elements of the xylem. From that finding, it is not difficult to imagine the great opportunity offered by such simple system to explore the possibilities of exploiting these traits as a tool for species identification. On the basis of their results, the present research expanded the survey within the six genera of the subtribe Pithecocteniinae and assesses the taxonomic value of tracheoid features present in the seeds of this subtribe.

### Materials and methods

Herborized seed samples from 22 species of the genera *Amphilophium*, *Distictella*, *Distictis*, *Glaziovina*, *Haplophium*, and *Pithecoctenium* were analyzed (Appendix 1). Five out of the eight *Amphilophium* species, seven of the eighteen *Distictella*, four of the eleven *Distictis*, the only *Glaziovina* species, and all four of *Pithecoctenium* species were included. For *Haplophium* only one of the four spe-



**Figure 1.** Morphology of a winged seed of Pithecocteniinae, showing the sections of the wing analyzed in this research. The embryo is located in the dark spot toward the center of the seed. A. Thin, membranaceous wing. B. Thicker, papyraceous wing. Arrows indicate the tracheoid extending close to the wing's margins. Squares marked A-C correspond to the areas surveyed in the study (see text).



**Figure 2.** Surface view of seed wings of (A) *Amphilophium paniculatum* var. *molle*. Scale bar = 100 µm. Zone A; (B) *A. paniculatum* var. *imatacense*. Scale bar 100 µm. Zone A; (C) *A. pannosum*. Scale bar = 100 µm. Zone C; (D) *A. aschersonii*. Scale bar = 50 µm. Zone C; (E) *Glaziovia bahuiniodes*. Scale bar = 50 µm. Zone B; (F) *Haplolophium glaziovii*. Scale bar = 100 µm. Zone A.

cies was studied, because it was impossible to obtain seeds from the other three species (since for these, neither fruits nor seeds have been collected yet).

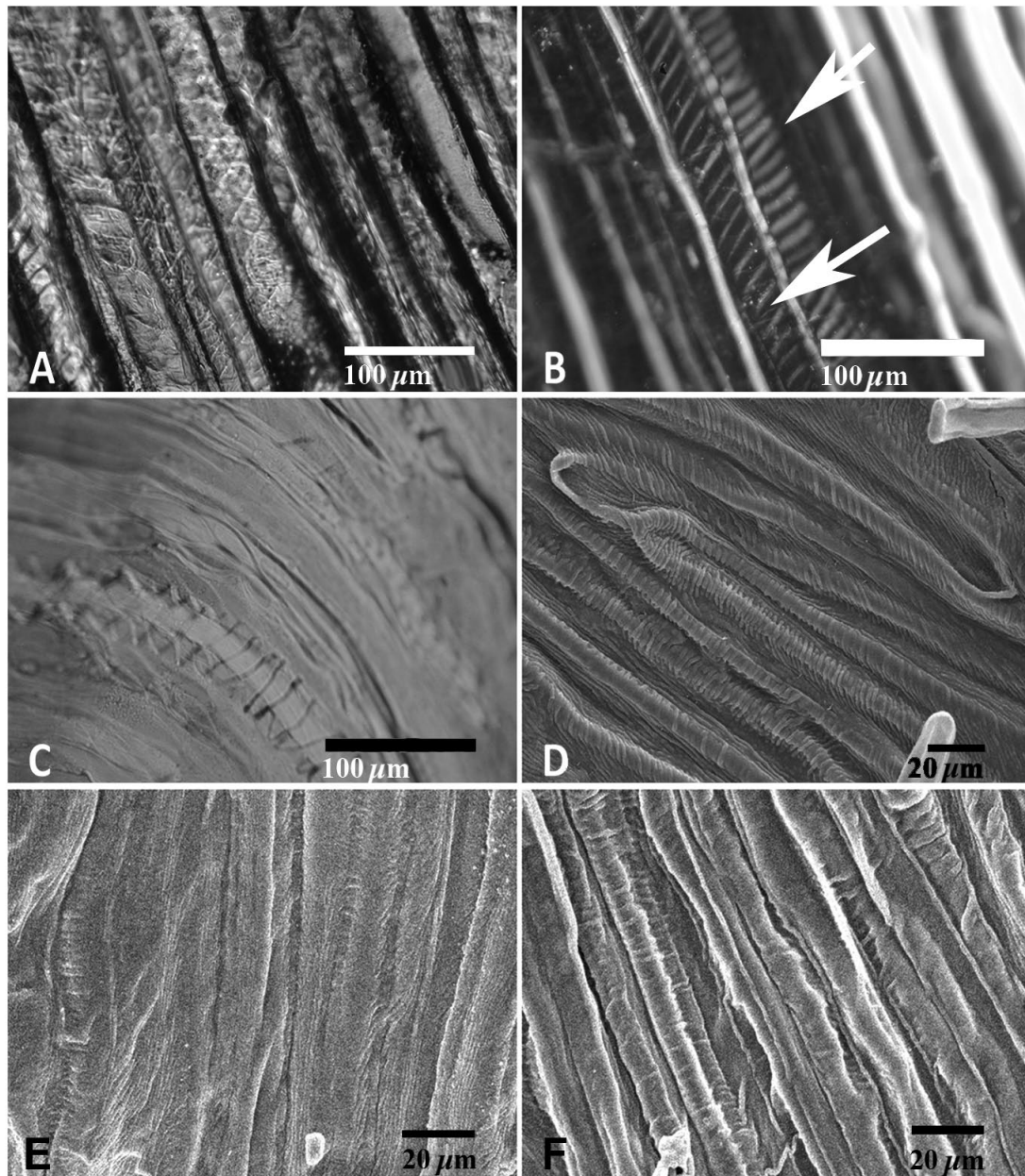
**Light Microscopy (LM).** The clearing technique proposed by Lersten *et al.* (2002) was employed with some modifications. Seeds were rehydrated, placed in NaOH for 15 min, immersed in 5% sodium hypochlorite for 15 min, and washed in ethanol; the wing tissue was separated from the seed body and divided in three parts, as illustrated in Figure 1. Samples were mounted in synthetic resin and observed with the light microscope. Photomicrographs were taken at several magnifications.

**Scanning Electron Microscopy (SEM).** Fragments from the three regions of the seed wing indicated in Figure 1 were

removed. Afterwards they were placed on a brass microscope sample holder, sputter-coated with gold-palladium in a JEOL Fine Coat JFC-1100 device, and then observed and photographed at various magnifications under a JEOL JSM 5600 LV electron microscope.

## Results

**Anatomy.** Analysis of seed wings revealed that tracheoids are distributed throughout the wing body, but the largest numbers of them are concentrated at the wing base, because the way tracheoids extend outwards, in a fan-like pattern (Figure 1). Patterns of tracheoid wall ornamentation do not appear to be influenced by size, texture, or wing shape.



**Figure 3.** Surface view of the seeds of (A) *Distictella obovata*. Scale bar = 0.1 mm. Zone A; (B) *Distictella obovata*. Scale bar = 100  $\mu$ m. Zone C; (C) *Distictis granulosa*. Scale bar = 100  $\mu$ m. Zone B; (D) *D. granulosa*. Scale bar = 20  $\mu$ m. Zone A; (E) *D. gnaphalantha*. Scale bar = 20  $\mu$ m. Zone A; (F) *D. lactiflora*. Scale bar = 20  $\mu$ m. Zone B. Arrows point to two tracheoids with helical thickenings.

Three types of tracheoid ornamentation were found:

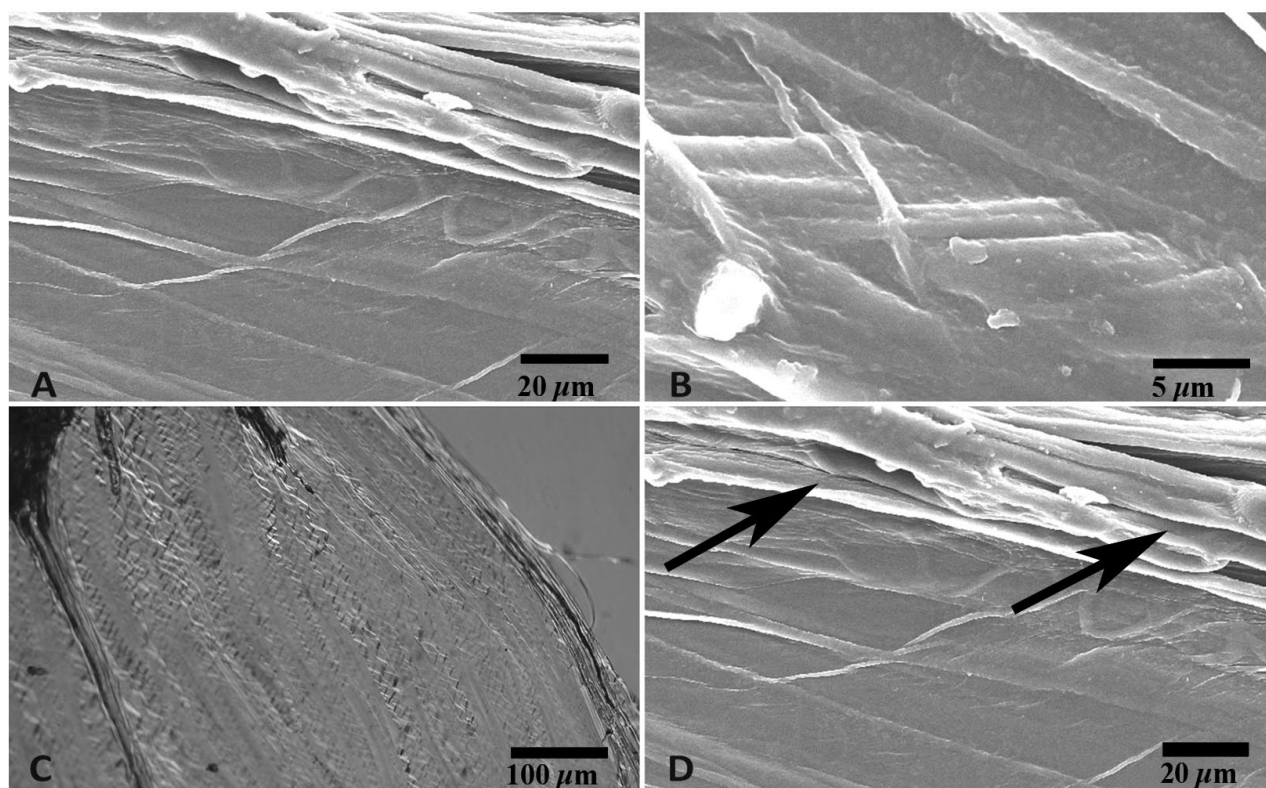
1.- Tracheoids with smooth cell wall, *i.e.* lacking ornamentations; found in *Amphilophium*, *Glaziovia*, and *Haplophium* (Figure 2).

2.- Tracheoids with ornamentations, may be of two types, according to Esau (1977):

2a.- Helical thickening. Cell wall thickening is arranged in true helices that make full turns around the tracheoid; found in the species of *Distictis* and *Distictella* (Figure 3). Helical thickenings are transversally connected by narrow lines;

these may appear as: (i) scattered or (ii) tightly interwoven. In *Distictis pulverulenta*, *D. lactiflora*, and *D. granulosa* helices are observed tightly interwoven or dense, with up to nine helices for each 20  $\mu$ m. We call this helix condition "tight" (Figure 3). It is noteworthy that in *D. lactiflora*, helices are not evenly spaced. The other species of this group exhibit 3-4 helices every 20  $\mu$ m; we call this helix condition "lax."

2b.- Pseudo-helical thickenings. These tracheoids have helices that are found only on the external face of the tracheoid.



**Fig. 4.** Surface view of the seeds of (A) *Pithecoctenium crucigerum*. Scale bar = 20 µm. Zone A; (B) *P. crucigerum*. Scale bar = 5 µm. Zone B; (C) *P. cynanchoides*. Scale bar = 100 µm. Zone A; (D) *P. cynanchoides*. Scale bar = 20 µm. Zone B.

The ornamentation is not observed in the area where the two tracheoids make contact. Species of the genus *Pithecoctenium* have this type of ornamentation (Figure 4).

## Discussion

The three types of tracheoid wall ornamentation found in the subtribe Pithecocteniinae furnish additional information that strengthens former taxonomic hypotheses. The presence of smooth tracheoids in the genera *Amphilophium*, *Glaziovina*, and *Haplolophium* provides new data to support the suggestion that they are closely linked taxonomically (Gentry, 1976). Previous findings indicate that they share a 7-9 aperturate pollen type (Gentry and Tomb, 1979; Burelo *et al.*, 2009) and a strongly lobulate calyx. All this morphological coherence is supported by the results of the phylogenetic study by Lohmann (2006), who identified a strongly supported clade within the Pithecocteniinae formed by these three genera.

*Distictis* and *Distictella*, genera that have ornamented tracheoids with complete helices, were originally described as a sole taxon; according to Bureau (1864) and Schumann (1894), the nature of their fruits and seeds is what distinguishes them from other genera in the subtribe. Gentry (1976) suggested that *Distictella* and *Distictis* should not be

considered separate genera. In this case, Lohmann (2006) found that *Distictella* is (based on the low number of species surveyed) apparently monophyletic and distinct from *Distictis*. Anyhow, the presence of helical-thickened walls of tracheoids in the seeds of the species of these genera should be taken into account in future phylogenetic analyses of the subtribe in order to take the best taxonomic decisions.

The genus *Pithecoctenium* has always been easily identified, with its characteristic 3 to 5-trifid tendrils. Here, the feature of incomplete helical thickenings on the secondary wall of tracheoids in the species of this genus, clearly distinguish it from the remaining genera in the Pithecocteniinae. Additional characters that help to distinguish the genus *Pithecoctenium* are the presence of pseudo-helical thickenings such as those described herein, along with some pollen features [inaperturate, with radial symmetry, apolar, spheroidal, with granules within the lumina of the reticulum, and curved, simple-baculate muri (Burelo *et al.*, 2009)] and fruit characters (rounded at the base, with a replum that extends beyond the valves, forming a pointed structure). Only one species of *Pithecoctenium* was considered by Lohmann (2006) in her phylogenetic study of the Bignoniaceae, and its relationships to other genera of the Pithecocteniinae are not clear; could it be sister of *Distictis* or to the *Glaziovina-Haplolophium-Amphilophium* clade.

There is no data about the morphology of the tracheoids from species in the sister groups of the Pithecocteniinae. Lersten *et al.* (2002) included in their study only one species of *Anemopaegma*, which showed mostly non ornamented cells in the seed wing. Information from *Bignonia*, *Clytostoma*, *Dolichandra*, *Macfadyena*, *Mansoa*, *Melloa*, and *Pyrostegia*, among other genera, is needed in order to elaborate on the possible evolution of the ornamentation of the tracheoids in the subtribe. Certainly, as concluded by Lersten *et al.* (2002), in the general picture the ornamentation of tracheoids in seed wings of the Bignoniaceae do not indicate any evolutionary trend of this character in the family. But, there can be such trends within subordinate groups, as can be suspected within the Pithecocteniinae; if tracheoid ornamentation is optimized on the phylogenetic hypothesis of the subtribe (Lohmann, 2006), then one possibility is the trend tracheoid wall with full helical thickenings→tracheoid wall with incomplete helical thickenings→tracheoid with smooth wall.

Based on current data, tracheoid characteristics appear to be taxonomically important, since they clearly help us to distinguish between genera. Therefore it is suggested to include the tracheoid characteristics in future phylogenetic research, not only of the subtribe Pithecocteniinae, but within the tribe Bignonieae in general, and other tribes as well, like Tecomeae. We suggest these characters to be further analyzed from a morphological and taxonomic perspective, and that in phylogenetic research to be conducted to evaluate their behavior in conjunction with other characters.

Before proposing that the presence of ornamented tracheoids in the seeds of *Distictis*, *Distictella*, and *Pithecoctenium* constitutes an adaptive advantage over the unornamented tracheoids of *Amphilophium*, *Glaziovia*, and *Haplophium* it is necessary to study and compare their dispersal efficiency and evaluate germination. At first sight it seems that there is no clear relationship between tracheoid ornamentation and ecological adaptation, as there are several species with different tracheoid ornamentation occupying extensive areas and sharing the same habitats; like *Amphilophium paniculatum* and *Pithecoctenium crucigerum* that are found from central Mexico down to Brazil.

It has been established that tracheoids have four functions: (1) to channel water flow, (2) to provide resistance against negative pressure, (3) to give structural support, and (4) to provide cells with flexibility (Lersten *et al.*, 2002). Wing tracheoids should therefore be the subject of research that might link their ornamentation type to germination rates, seed dispersal distance, resistance to dehydration, and other factors among species.

## Acknowledgments

We are grateful to the Instituto de Ecología, A.C. and the following organizations for funding the doctoral studies of

the first author: Consejo Nacional de Ciencia y Tecnología (CONACyT; scholarship number 157797) and the Universidad Juárez Autónoma de Tabasco for support through the 2002 Institutional Academic Advancement Program. We thank the herbaria JBR, MEXU, NY, UEC and XAL for the loan of specimens and their facilities. Thanks to Mr. Tiburcio Láez Aponte for its technical assistance in SEM and for editing the figures. Three anonymous reviewers provided key suggestions that helped to improve the quality of the manuscript.

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*Recibido: 6 de mayo de 2011*

*Aceptado: 24 de octubre de 2011*

**Appendix 1.**


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*Amphilophium aschersonii* Ule, Berlin 3541 (NY); *A. ecuadorensis* A.H.Gentry, Steyermark 54023 (NY); *A. paniculatum* (L.) Kunth, Avendaño 683 (XAL); *A. paniculatum* var. *molle* (Cham. & Schltdl.) Standl., Oliva 615 (XAL); Cedillo 1091 (XAL); *Amphilophium paniculatum* var. *imatacense* A.H.Gentry, Thomas et al. 4029 (NY); *A. pannosum* (DC.) Bureau & K.Schum., Pittier 959 (NY); *A. sandwithii* Fabris, Jardim et al. 1998 (NY); *Distictella cuneifolia* (DC.) Sandwith, Silva 228 (NY); *D. elongata* (Vahl) Urb. Broadway s/n (NY), Kirizawa 111 (XAL); *D. laevis* (Sandwith) A.H.Gentry, Ferreira 5808 (NY); *D. magnoliifolia* (Kunth) Sandwith, Galeano et al. 1049 (NY); *D. mansoana* (DC.) Urb., Oliveira & Montovani 109 (XAL); *D. monophylla* Sandwith, Maguire 30662 (NY); *D. obovata* Sandwith, Liesner & Holst 20668 (NY); *Distictis buccinatoria* (DC.) A.H.Gentry, Boutin 3317 (MEXU); *D. gnaphalanthae* (A.Rich.) Urb., González s/n (XAL); *D. granulosa* Bureau & K.Schum., Davidse & González 21917 (MEXU); *D. lactiflora* (Vahl) DC., Britten & Cowell 1268 (NY); *Glaziovía bauhinioides* Bureau ex Baill., Herringer 1583 (SP); *Haplophium glaziovii* (Bureau ex K.Schum.) A. H.Gentry, Rizzini 419 (R); *Pithecoctenium crucigerum* (L.) A.H.Gentry, Nee & Taylor 28772 (XAL); Vázquez 108 (XAL); *P. cynanchoides* DC., Galleto 1025 (XAL); *P. dolichoides* (Cham.) Bureau ex K. Schum.; *Handro* s/n (JBR); *P. hatschbachii* A.H.Gentry, Bernacci 25905 (UEC).

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