DOI: https://doi.org/10.29298/rmcf.v9i47.167

Article

Taxonomic identification key with leaf anatomical characters for *Pinus* L. species in *Hidalgo*

Cristhian Espinoza-Pelcastre[1], Sergio Hernández-León[1*], David S. Gernandt[2], Oscar Arce-Cervantes[1], Rodrigo Rodríguez-Laguna[1], José González-Ávalos[1]

[1]Área Académica de Ciencias Agrícolas y Forestales, Instituto de Ciencias Agropecuarias. Universidad Autónoma de Hidalgo. México.

[2]Departamento de Biología, Instituto de Biología. Universida Nacional Autónoma de México. México.

[*]Autor por correspondencia; correo-e: sergio_hernandez@uaeh.edu.mx

Abstract:

Twelve pine species naturally occur in the state of *Hidalgo*, of which, *Pinus pinceana* and *P. greggii* are listed as threatened. In this context it is necessary to generate information for *Pinus* species identification specially for immature individuals without seed cones. In this study we establish an identification key based on leaf anatomy for the identification of pine species occurring in the state of *Hidalgo*. Specimens from different localities were collected from which, permanent slides were prepared from transverse leaf sections and a character matrix was codified from leaf anatomy features and analyzed with UPGMA. The matrix was used to construct an identification key. The key allow for the identification of 10 out of the 12 species, including *Pinus greggii* a species under risk category. *Pinus cembroides* can only be differentiated from *P. pinceana* when it exhibits a semicircular shape in transverse section. External resin canals were only observed in *Pinus* subgenus *Strobus* and septal resin canals were only recorded for *P. oocarpa*. The number of resin canals may only be used as complementary for identification because of its high variation. UPGMA analysis generated two principal clusters, the first group included two interior clusters corresponding with subgenus *Strobus* species and subgenus *Pinus* species, the second principal cluster included only species from subgenus *Pinus*. This study contributes with a robust identification key for pine species at a local scale exclusively using secondary leaf anatomy characters.

Keywords: Needles, leaf anatomy, resin canals, hypodermis, *Pinus greggii* Engelm. ex Parl., *Pinus pinceana* Gordon.

Fecha de recepción/Reception date: 13 de diciembre de 2017

Fecha de aceptación/Acceptance date: 30 de marzo de 2018

Introduction

Pines are ecologically important in tropical forests close to the sea level, desert areas and forests of the Highlands of Mexico (Farjon *et al.*, 1997), a country regarded as the secondary center of diversification of the genus *Pinus*, with 50 (41.3 %) out of the 121 species listed in the world (Gernandt and Pérez-De La Rosa, 2014; Pérez-De La Rosa and Gernandt, 2017). Twelve taxa are naturally distributed in the state of *Hidalgo*: *Pinus ayacahuite* Ehrenb. ex Schltdl.*, P. cembroides* Zucc.*, P. devoniana* Lindl.*, P. greggii* Engelm. ex Parl.*, P. hartwegii* Lindl.*, P. leiophylla* Schiede. ex Schltdl. & Cham.*, P. montezumae* Lamb.*, P. oocarpa* Schiede.*, P. patula* Schiede. ex Schltdl. & Cham.*, P. pinceana* Gordon.*, P. pseudostrobus* Lindl. and *Pinus teocote* Schiede. ex Schltdl. & Cham. Of these species, *Pinus greggii*, *P. patula*, *P. pinceana* and *P. teocote* are endemic to Mexico. *Pinus pinceana* is included in the list of species of the Mexican Official Standard 059-SEMARNAT-2010, within the category of endangered species (P) (Semarnat, 2010), and *Pinus greggii* is classified as Vulnerable in the IUCN Red List of Threatened Species (Farjon, 2013).

Pines have four types of leaves during their stages of development: cotyledons, primary, cataphylls and secondary leaves. The latter, also known as the needles, begin to appear toward the end of the first early growing season, or in some species, in the second growing season. They are evergreen and metabolically active; they develop in (terminal or axillary) buds, dwarf axillary buds of the cataphylls, and the fascicles are grouped 1-8 needles (Farjon and Styles, 1997). The number of leaves per fascicle varies depending on the species, due to the conditions of the environment in which they develop, three and five are the most common numbers (Martínez, 1945).

The anatomy of needles comprises three tissues that are defined by their function: the dermis, the mesophyll, and the vascular tissue (Hernández-León, 2011). The dermis is constituted by the cuticle, the epidermis and the hypodermis. The tissue of the mesophyll is constituted by long cells with thin walls that fill up the space between the hypodermis and the endodermis; it is there that the resin duct canals develop, appearing in different positions according to the variation of the species. The vascular tissue is formed by the endodermis (a layer of oval cells that surround the vascular tissue) and contains the vascular bundles (the xylem and the phloem) (Farjon and Styles, 1997).

Leaf anatomical characters are important sources of information for species identification. The dichotomous key is a method for identifying species with short descriptions; it consists in the selection of one of two alternative statements. The identification keys are of two types: regional or by morphological group (Farjon *et al.*, 1997).

In relation to the taxonomic identification of pine trees, it is important to generate information for species identification based on the anatomy of the needles, particularly in young individuals that lack female cones. For this reason, the objective of this research was to establish a dichotomous key for the identification of pine trees with natural distribution in the state of *Hidalgo*, based on the anatomy of the needles.

Materials and Methods

The state of *Hidalgo* has a surface area of approximately 20 905 km^2, which represent 1.1 % of the national territory; it is located within the Mexican Transition Zone, which includes the biogeographic provinces of the *Sierra Madre Oriental* (Eastern Sierra Madre), the *El Altiplano* (Mexican High Plateau), the *Golfo de México* (the Gulf of Mexico) and the *Eje Neovolcánico Transversal* (Trans-Mexican Volcanic

Belt) (Rzedowski, 1978; Conabio, 1997; Morrone, 2005; Inegi, 2016). According to Gernandt and Pérez-de la Rosa (2014), the *Sierra Madre Oriental* is one of the regions with the greatest diversity of conifers.

Field Work

The collection of specimens of *Pinus* was carried out in various localities of the state of *Hidalgo* (Table 1).

Table 1. Detailed data collection of *Pinus* species of the state of *Hidalgo*.

Species	Collection Num.	Collection locality	Coordinates	
			Latitude	Longitude
Pinus ayacahuite	SHL089	*Acaxochitlán*	20°13.601' N	98°13.351' W
	SHL121	*Zimapán*	20°51.991' N	99°12.916' W
P. cembroides	SHL114	*Jacala*	20°47.377' N	99°17.139' W
	SHL036	*Zimapán*	20°51.092' N	99°15.784' W
P. devoniana	SHL092	*Huasca de Ocampo*	20°12.259' N	98°36.995' W
P. greggii	SHL115	*Jacala*	20°48.462' N	99°15.591' W
	SHL137	*Tlanchinol*	20°59.037' N	98°38.478' W
	SHL095	*San Bartolo Tutotepec*	20°23.865' N	98°14.866' W
	SHL143	*Molango*	20°44.579' N	98°42.520' W
P. hartwegii	SHL149	*Singuilucan*	19°58.245' N	98°28.314' W
	SHL077	*Mineral del Chico*	20°10.264' N	98°43.873' W
P. leiophylla	SHL152	*Singuilucan*	19°58.323' N	98°26.960' W
	SHL022	*Cuautepec de Hinojosa*	19°57.007' N	98°15.6263' W
P. montezumae	SHL150	*Singuilucan*	19°58.675' N	98°26.994' W
	SHL155	*Singuilucan*	19°57.348' N	98°24.768' W
	EUAC002	*Zimapán*	20°50.246' N	99°15.314' W
P. oocarpa	SHL138	*Calnali*	20°52.934' N	98°35.247' W
	SHL145	*Xochicoatlán*	20°44.321' N	98°42.443' W

	SHL146	*Xochicoatlán*	20°44.302' N	98°42.461' W
P. patula	SHL144	*Molango*	20°44.581' N	98°42.513' W
	SHL030	*Huasca de Ocampo*	20°09.064' N	98°33.637' W
	SHL100	*San Bartolo Tutotepec*	20°23.974' N	98°14.955' W
P. pinceana	APO001	*El Cardonal*	20°38.308' N	98°59.436' W
	AAE002	*El Cardonal*	20°38.431' N	98°59.332' W
	No.003	*El Cardonal*	20°38.431' N	98°59.332' W
P. pseudostrobus	SHL123	*Zimapán*	20°51.989' N	99°13.742' W
	SHL130	*Metepec*	20°28.234' N	98°18.835' W
P. teocote	SHL154	*Singuilucan*	19°58.329' N	98°26.312' W
	SHL131	*Metepec*	20°16.906' N	98°18.939' W
	SHL021	*Cuautepec de Hinojosa*	19°57.026' N	98°15.660' W

Leaf sections and staining

The needles were fixed in FAA (formaldehyde: Glacial acetic acid: 50 % ethyl alcohol, in a proportion of 5: 5: 90) for an indefinite period. Transverse sections were obtained manually, with a single-edged razor; of these, we selected the best for histological staining by means of the procedure utilized by Hernández-León (2011); Citrisolv 2 (15 min); EtOH 100 % (5 min); EtOH 96 % (5 min); EtOH 70 % (5 min), EtOH 50 % (5 min); ddH_2O (5 min); Alcian blue (30 min); ddH_2O (5 min); EtOH 50 % (3 min); safranin (2 hr); 50 % EtOH (3 min); 70 % EtOH (3 min); 96 % EtOH (3 min); 100 % EtOH (3 min); Citrisolv (15 min). Once the sections were stained, they were mounted in permanent preparations using Entellan (Merk Co., Germany) synthetic resin, and observed at different magnifications under an optical microscope.

Character Encoding

Through a review of the literature and the observation of the permanent preparations, nine characters and their corresponding character states were established, as shown in Table 2 (Coulter and Rose, 1886; Shaw, 1914; Martínez, 1945; Lanyon, 1966; Mirov, 1967; Farjon and Styles, 1997; Ortiz, 1999). The characters and character states were described with observation of the permanent preparations at different magnifications, with the help of a (Carl Zeiss) Axioscope 40 optical microscope. Leaf sections were photographed with a digital camera system coupled to the microscope, and images were edited in Adobe® Photoshop® CS6 Extended, version 13.0.1 x32 (2017), in order to highlight the relevant leaf anatomical characteristics. A morphoanatomical data matrix was built based on the observations.

Table 2. Characters and character states used for leaf anatomical description of the *Pinus* species of the state of *Hidalgo*.

Character		**Character states**
1.	Shape of the transverse section (STS)	(0) Semi-circular (1) Transversally triangular (2) Widely triangular
2.	Stomatal position in the needles (SPN)	(0) Epistomatal, (1) Amphistomatal
3.	Hypodermal cell wall thickness (HCT)	(0) Evenly thin, (1) Evenly thick, (2) Thin in the first layers and thickened in the rest, (3) Gradual increase in thickness toward the inside
4.	Hypodermal intrusion (HI)	(0) Absent, (1) Present
5.	Number of resin canals (NRC)	(0) Zero (1) One, (2) Two, (3) Three (4) Four (5) Five (6) Six (7) Seven (8) Eight
6.	Position of the resin canals (PRC)	(0) External, (1) Internal, (2) Intermediate, (3) Septal

7.	Endodermal cell walls (ECW)	(0) Evenly thickened, (1) Externally thickened
8.	Vascular bundles (VB)	(0) Separate, (1) Fused only in the region of the xylem, (2) Connate or fused
9.	Vascular sclerenchyma (VS)	(0) Absent, (1) Present

Data analysis

The leaf anatomical data of the 12 species were analyzed with the unweighted pair group method with arithmetic mean (UPGMA) (Sokal and Michener, 1958), using a presence/absence matrix (Table 3). The analysis utilized Jaccard's Similarity Coefficient with the online tool DendroUPGMA (Garcia-Vallvé *et al.*, 1999).

Results and Discussion

Field Work

A total of 30 specimens were collected, processed and deposited in the collection of the Herbarium of the *Universidad Autónoma del Estado de Hidalgo* (Academic Area of Agricultural and Forestry Sciences at the Institute of Agricultural Sciences at the Autonomous University of the State of *Hidalgo*), campus *Tulancingo*, and in the National Herbarium of Mexico (MEXU). The specimens correspond to 1 - 3 individuals of the 12 pine species collected in Hidalgo (Table 1).

Leaf sections and staining

The thirty permanent preparations (30) of needle cross-sections were incorporated into the collection of the Herbarium of the Academic Area of Agricultural and

Forestry Sciences at the Institute of Agricultural Sciences of the Autonomous University of the State of *Hidalgo* and are available for subsequent consultations.

Character encoding

The data matrix of characters and character states are presented in Table 3. The shape of the epidermal cells exhibited inter- and intra-specific variation, consistently with previous records (Farjon *et al*., 1997).

Table 3. Matrix of morphoanatomical data for the needles of the *Pinus* species naturally distributed in the state of *Hidalgo*.

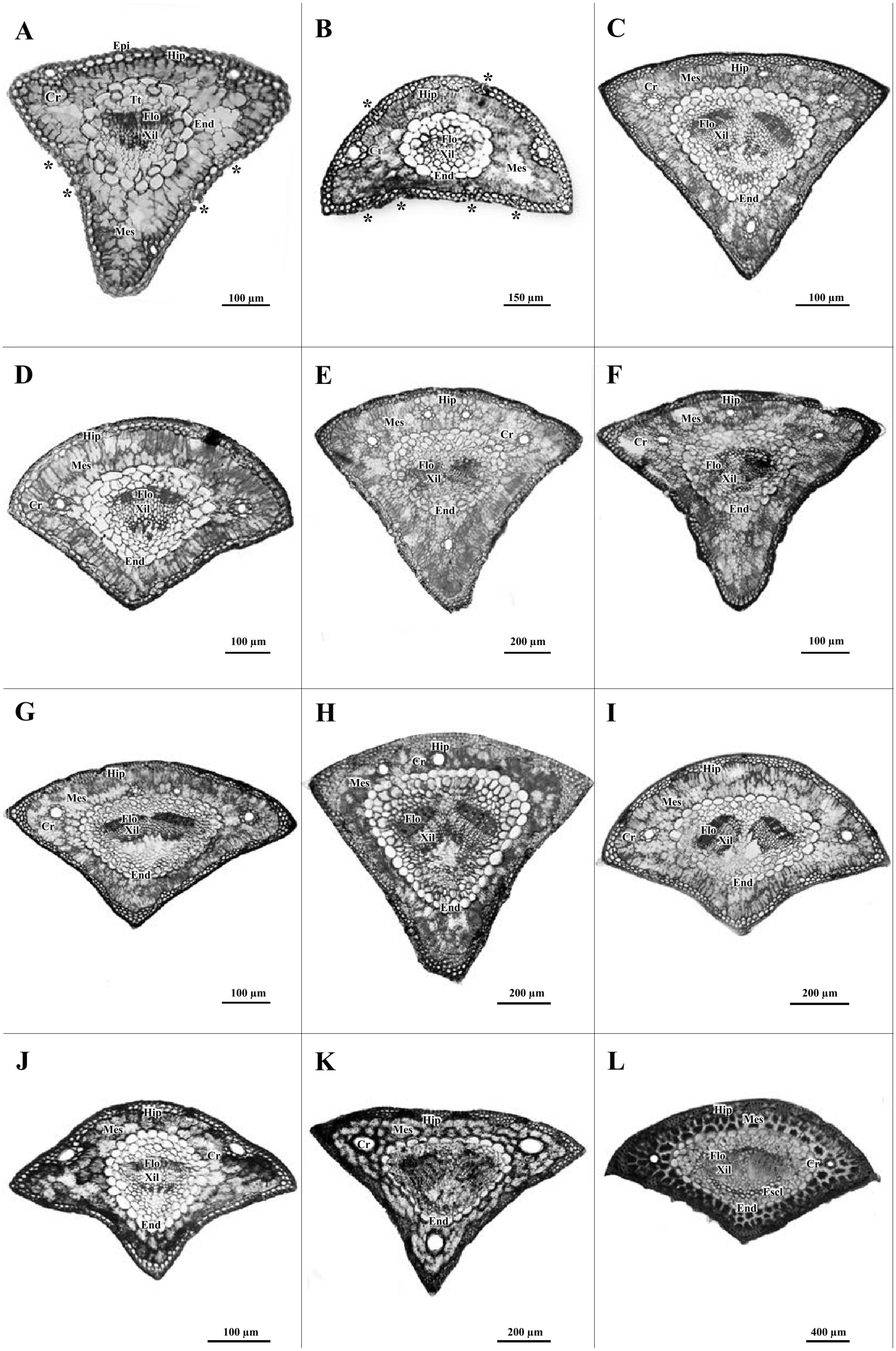
Species	**Collection Num.**	**Dendrogram key**	**Character**								
			STS (1)	SPN (2)	HCT (3)	HI (4)	NRC (5)	PRC (6)	ECW (7)	VB (8)	VS (9)
Subgenus *Strobus*											
Pinus ayacahuite	SHL089	Pa1	1	0	0	0	2	0	0	2	0
	SHL121	Pa2	1	0	0	0	6	0	0	2	0
P. cembroides	SHL114	Pc1	0	1	1	0	2	0	0	2	0
	SHL036	Pc2	1	1	1	0	2	0	0	2	0
P. pinceana	APO001	Ppi1	1	1	2	0	2	0	0	2	0
	AAE002	Ppi2	1	1	2	0	2	0	0	2	0
	No.003	Ppi3	1	1	1	0	2	0	0	2	0
Subgenus *Pinus*											
P. devoniana	SHL092	Pd1	2	1	3	1	4	2,0	1	0	0
P. greggii	SHL115	Pg1	1	1	0	0	2	2	0	0	0
	SHL137	Pg2	1	1	0	0	2	2	0	0	0
	SHL095	Pg3	1	1	0	0	2	2	0	0	0
	SHL143	Pg4	1	1	0	0	2	2	0	1	0
P. hartwegii	SHL149	Ph1	2	1	2	0	5	2	1	0	1
	SHL077	Ph2	2	1	2	0	4	2	0	0	1

P. leiophylla	SHL152	Pl1	2	1	0	0	2	2	1	1	1
	SHL022	Pl2	2	1	0	0	3	2	1	1	1
P. montezumae	SHL150	Pm1	2	1	0	0	4	2,1	1	0	1
	SHL155	Pm2	2	1	0	0	4	2,1	1	0	1
	EUAC002	Pm3	2	1	0	0	4	2,1	1	0	1
P. oocarpa	SHL138	Po1	2	1	2	0	6	3	0	0	1
	SHL145	Po2	2	1	2	0	1	3	0	0	1
	SHL146	Po3	2	1	2	0	3	3	1	0	1
P. patula	SHL144	Ppa1	1	1	0	0	5	2,1	1	0	1
	SHL030	Ppa2	1	1	0	0	2	2,1	1	0	1
	SHL100	Ppa3	1	1	0	0	2	2,1	0	0	1
P. pseudostrobus	SHL123	Pps1	2	1	3	0	3	2	1	0	1
	SHL130	Pps2	2	1	3	1	3	2	1	0	1
P. teocote	SHL154	Pt1	1	1	2	0	2	2	1	0	0
	SHL131	Pt2	1	1	2	0	4	2	1	0	0
	SHL021	Pt3	1	1	2	0	4	2	0	0	0

STS = Shape of the transverse section; SPN = Stomatal position in the needles; HCT = Hypodermal cell wall thickness; HI = Hypodermal intrusion; NRC = Number of resin canals; PRC = Position of the resin canals; ECW = Endodermal cell walls; VB = Vascular bundles; VS = Vascular sclerenchyma

Eight of the studied species exhibited the basal sheath of the permanent fascicle. The taxa with the deciduous fascicle basal sheath were *Pinus ayacahuite, P. cembroides*, *P. leiophylla* and *P. pinceana*, with the exception of *P. leiophylla;* these taxa correspond to the soft pines or subgenus *Strobus. Pinus leiophylla* is one of the few species of hard pines or subgenus *Pinus* with a deciduous fascicle basal sheath (Gernandt *et al*., 2005). The character state of the fascicle basal sheath is helpful for allotting specimens to groups; however, in combination with other characteristics, it can be effective for the identification of certain taxa (Farjon *et al*., 1997).

The studied material had two, three and up to five needles per fascicle; this character has been widely used for identifying pine species, generally in combination with other characteristics (Farjon and Styles, 1997; Farjon *et al*., 1997; Eckenwalder, 2009). The needles of a fascicle form a cylinder; the number of needles per fascicle determines its transversal shape, which corresponds to a section of the cylinder; for example, when there is one needle, the transversal shape will be circular; in fascicles with two needles, it will be semi-circular (Figure 1B); in fascicles with three needles, it will be transversally triangular (Figure 1D, G, I, J, L), and in fascicles with four or more needles, it will have a widely triangular shape (Figure 1A, C, E, F, H, K) (Martínez, 1953; Farjon and Styles, 1997).

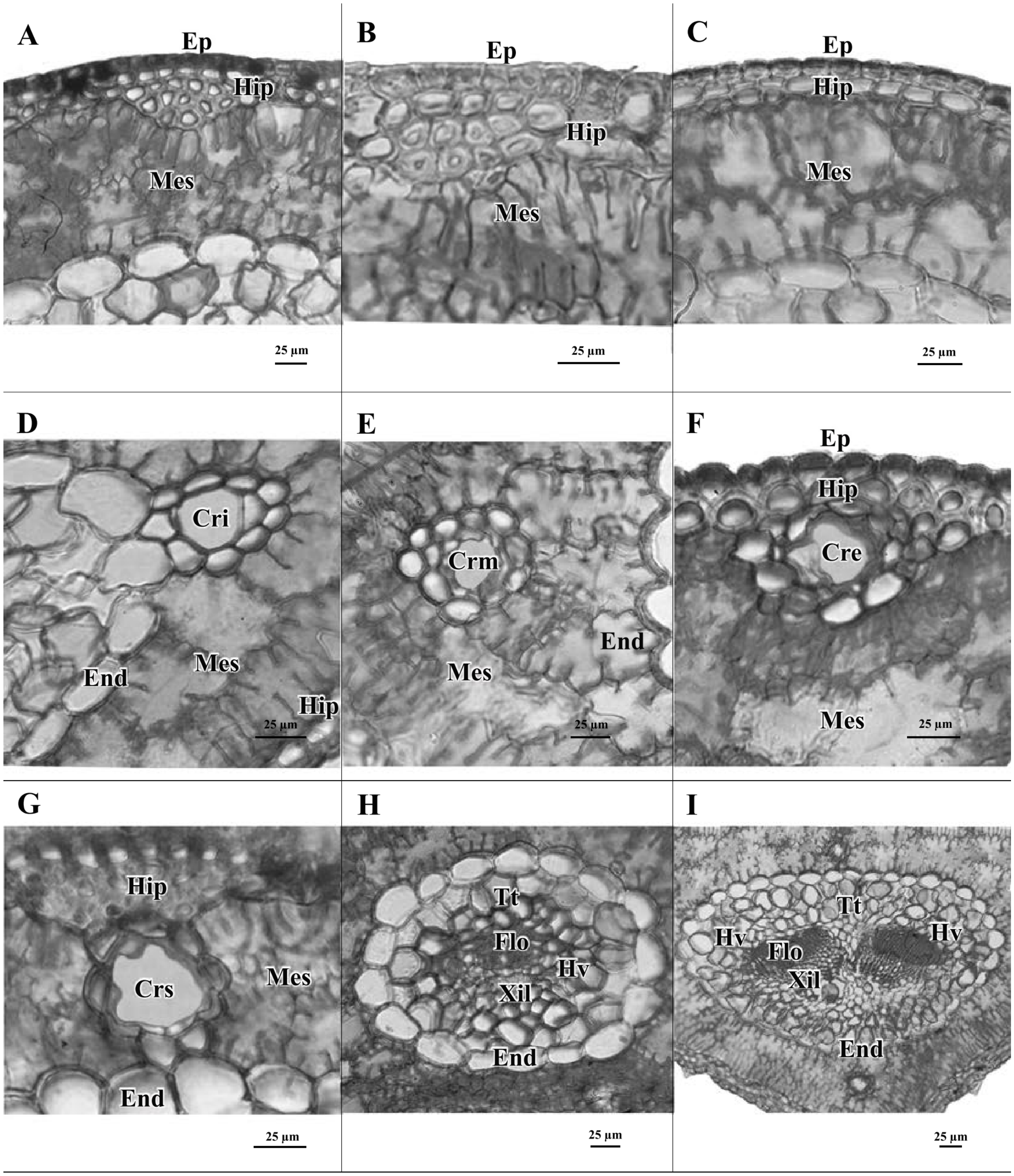

A
Epi
Hip
Cr
Tt
Flo
Xil
End
Mes
100 µm
B
Hip
Flo
Xil
Cr
Mes
End
150 µm
C
Mes
Hip
Cr
Flo
Xil
End
100 µm
D
Hip
Mes
Flo
Xil
Cr
End
100 µm
E
Hip
Mes
Cr
Flo
Xil
End
200 µm
F
Hip
Mes
Cr
Flo
Xil
End
100 µm
G
Hip
Mes
Flo
Xil
Cr
End
100 µm
H
Hip
Cr
Mes
Flo
Xil
End
200 µm
I
Hip
Mes
Flo
Xil
Cr
End
200 µm
J
Hip
Mes
Cr
Flo
Xil
End
100 µm
K
Hip
Mes
Cr
Xil
End
200 µm
L
Hip
Mes
Flo
Xil
Cr
Escl
End
400 µm

A = *Pinus ayacahuite*; B = *P. cembroides*; C = *P. devoniana;* D = *P. greggii*; E = *P. hartwegii;* F = *P. leiophylla;* G = *P. montezumae*; H = *P. oocarpa;* I = *P. patula;* J = *P. pinceana;* K = *P. pseudostrobus;* L = *P. teocote*; * = Indicates the stomatal position; Cr = Resin duct canal; End = Endodermis; Epi = Epidermis; Escl = Sclerenchyma; Flo = Phloem; Hip = Hypodermis; Mes = Mesophyll; Tt = Transfusion tissue; Xil = Xyleme.

Figure 1. Cross-sections of needles of *Pinus* species of the state of *Hidalgo*.

Pinus cembroides was the only species with two leaf shapes in the cross-section. Due to its semi-circular shape, it can be differentiated from the rest of the taxa (Figure 1B); however, when transversally triangular, it is impossible to separate it from *P. pinceana* (Figure 1J). *Pinus ayacahuite* was the only one that exhibited the epistomatal distribution (Figure 1A), while the rest of the species exhibited an amphistomatal distribution.

For the hypodermal cell wall thickness, the most frequently registered character state was "evenly thin hypodermal cell thickness" (*e.g., P. ayacahuite*, *P. greggii* and *P. leiophylla*; Figure 2C), while "hypodermal cell wall thickness gradually increasing inward" was less frequently observed (*e.g., P. devoniana* and *P. pseudostrobus*; Figure 2A). *Pinus patula* and *P. pinceana* exhibited intra-specific variation for this character (Table 3). According to Farjon and Styles (1997), the long leaves, with layers of hypodermal cells with thin walls are restricted mainly to temperate or mesic subtropical climates (e.g., *P. ayacahuite*, *P. greggii*, *P. patula*).



A = *Pinus devoniana*; B, E, I = *P. teocote*; C-D = *P. greggii*; F, H = *P. cembroides*; G = *P. oocarpa*; Cre = External resin duct canal; Cri = Internal resin duct canal; Crm = Intermediate resin duct canal; Crs = Septal resin duct canal; End = Endodermis; Epi = Epidermis; Flo = Phloem; Hip = Hypodermis; Hv = Vascular bundle; Mes = Mesophyll; Tt = Transfusion tissue; Xil = Xyleme.

Figure 2. Cross-cutting sections of needles of *Pinus* species of the state of *Hidalgo*.

The existence of different degrees of hypodermal intrusions in the mesophyll has been observed (Martínez, 1945). In this research, *Pinus devoniana* and *P. pseudostrobus* exhibited hypodermal intrusions (Table 3, figures 1C, K and 2A); no intrusions are present in other species (e.g., *P. greggii*; Figure 2C). Hypodermal tissue concentrations have been observed to occur in marginal areas, whereas in the rest of the leaf the hypodermis persists in two cell layers (Farjon and Styles, 1997).

In certain taxa, such as *Pinus oocarpa,* the number of resin duct canals was highly variable, ranging between 1 and 6, while *P. pinceana,* for example, consistently exhibited two resin duct canals (Figure 1J). In the latter case, the character can be of use for identifying the species at state level (Table 3) (Perry, 1991; Farjon and Styles 1997; Cole *et al*., 2008). Previous studies indicate an interval of 1-12 resin duct canals in the leaves of pine species of Latin America, and in the case of the state of *Hidalgo*, 1-6 resin duct canals were found in each needle (Farjon and Styles, 1997).

Pinus oocarpa and *P. ayacahuite* exhibited the largest number of resin duct canals (6); a lower number (1) was observed in sections of *P. oocarpa* needles. According to Helmers (1943), there is a close relationship between the number of resin duct canals and the environment. Eventually, some needles may have no resin duct canals, but this condition is not associated with any particular species. *P. donnell-smithii* is the exception to specific recognition, since many of its needles have no resin duct canals (Perry, 1991). According to certain authors, the number of resin duct canals is highly variable, and therefore they do not regard it as a systematic element (Martínez, 1945).

The position of the resin duct canals is a characteristic that is commonly used for the classification and identification of species since Engelmann (1880) and Coulter and Rose (1886), because most species are characterized by exhibiting a single position, although there are combinations like the ones cited in this study for *Pinus devoniana* and *P. montezumae*; (Figure 1C, G). Nevertheless, combinations with more than two positions have never been registered (Farjon and Styles, 1997). The joint use of the position and number of the resin duct canals is very helpful for identifying many species (Perry, 1991). In taxa with a variable number of canals, those that are largest and whose position is fixed are regarded as primary, and the smaller ones are considered to be subsidiary (Farjon and Styles, 1997). Since their position is less variable than their number, it is more helpful for identification purposes (Martínez, 1945). The intermediate position of the resin duct canals was predominant among the pines of the state of *Hidalgo* (*e.g*., *Pinus leiophylla*, *P. montezumae*, *P. pseudostrobus*) (Martínez, 1945; Farjon and Styles, 1997) (Figure 1F, G, K), while the septal position was observed only in *P. oocarpa*; therefore, this character state is diagnostic for this species, at least in the state of *Hidalgo* (figures 1H, 2G).

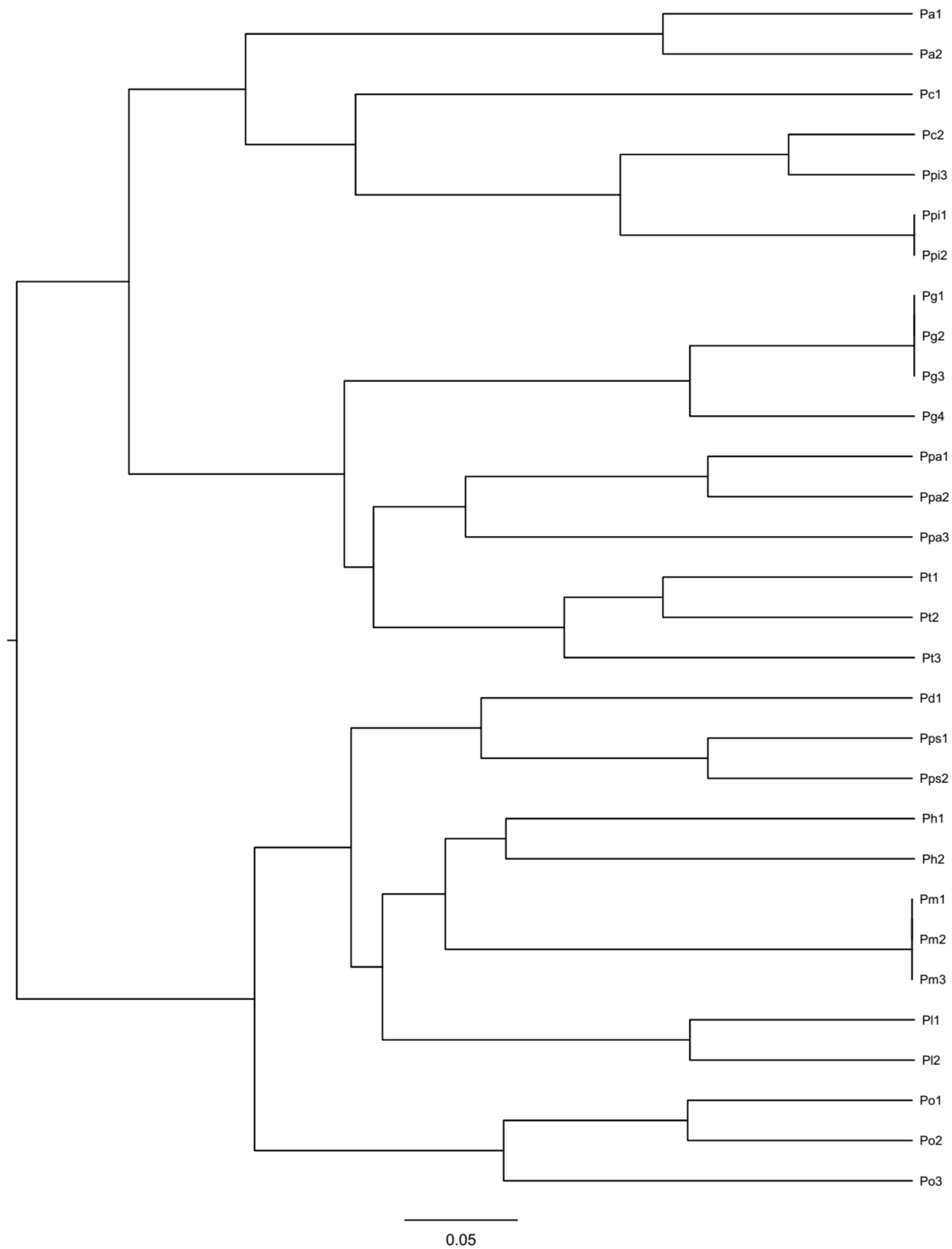
The external thickening of the endodermal cell walls (Figure 2I) has been used as a diagnostic character (Farjon and Styles, 1997). However, species with multiple individuals, such as those included in the present research, exhibit inter- and intra-specific polymorphism, which is not adequate for taxonomic identification.

The presence of one (Figure 2H) or two (Figure 2I) vascular bundles supports Koehne's proposal (1893) of dividing the genus *Pinus* into two sections —Haploxylon and Diploxylon—, which correspond with the subgenera *Strobus* and *Pinus* (Gernandt *et al*., 2005), consistently with the results documented herein.

Certain taxa —*Pinus oocarpa* and *P. teocote* (Figure 1L)— exhibit a sclerenchyma in the vascular region that surrounds and sometimes divides the vascular bundles (Farjon and Styles, 1997). However, due to its variation, this is not a diagnostic character.

Cluster analysis

The analysis of similarity of the morphoanatomical characters of the needles resulted in two main groups (Figure 3). Group one comprises *Pinus ayacahuite*, *P. cembroides* and *P. pinceana*, of the subgenus *Strobus*, which are more related with one another than with other species; on the other hand, it includes *P. greggii, P. patula* and *P. teocote*, of the subgenus *Pinus*. The second main group consists of taxa of the subgenus *Pinus*: *P. devoniana, P. hartwegii, P. leiophylla, P. montezumae, P. oocarpa* and *P. pseudostrobus*; *P. oocarpa* was separated from the rest of the species within the group. At the terminals, groups are formed by taxon, except for a sample of *Pinus cembroides* that is grouped together with *P. pinceana*. According to Eckenwalder (2009), the convergence in the characteristics of the leaves between unrelated species is caused by a strong association between the leaf characteristics and the environmental conditions, so that the former can reflect the mechanisms whereby the plants adapt to their environment (Tian *et al*., 2015).



Cophenetic correlation coefficient = 0.847018494508054. The number in each terminal indicates the number of specimens per species.

Figure 3. Dendrogram obtained through an UPGMA group analysis using Jaccard's similarity coefficient in 12 *Pinus* species distributed in the state of *Hidalgo*.

Identification key

Based on the dichotomous key resulting from the research documented herein, it is possible to differentiate 10 of the 12 pine species of the state of Hidalgo. *P. cembroides* and *P. pinceana* are separated only when the shape of the transverse section is semi-circular in the first species.

1.- External resin canals 2

2.- Epistomatal position of the stomata .... *Pinus ayacahuite*

2'.- Amphistomatic position of the stomata 3

3.- Transversally triangular leaf shape.. *P. cembroides* and *P. pinceana*

3'.-Semi-circular leaf shape... *P. cembroides*

1'.- Intermediate, internal or septal resin duct canals..4

4.- Septal resin duct canals .... *P. oocarpa*

4'.- Middle or intermediate resin duct canals 5

5.- Present hypodermal intrusions 6

6.- Intermediate resin duct canals.. *P. pseudostrobus*

6'.- Intermediate and internal resin duct canals .... *P. devoniana*

5'.- Absent hypodermal intrusions 7

7.- Thin hypodermis in the first layer and thickened hypodermis in the rest of the layers....8

8.- Widely triangular leaf shape .... *P. hartwegii*

8'.- Transversally triangular leaf shape .... *P. teocote*

7'.- Evenly thin hypodermis 9

9.- Widely triangular leaf shape 10

10.- Intermediate resin duct canals .... *P. leiophylla*

10'.-Intermediate or internal resin duct canals .... *P. montezumae*

9'.- Transversally triangular leaf shape 11

11.- Intermediate resin duct canals .... *P. greggii*

11'.- Intermediate and internal resin duct canals .... *P. patula*

Conclusion

This study contributes to the obtainment of anatomical leaf data for species of the genus *Pinus*. The generation of taxonomic identification tools based on leaf anatomical characters is of major importance for the taxonomic separation of species, if no other identification source is available. Such is the case of nursery-grown plants, young individuals, commercial plantations, reforestations for restoration purposes at seedling stage, and trees without the presence of female cones. The results made it possible to differentiate the taxa and to develop a robust identification key. For this reason, the methodological approximation utilized in this study is an alternative for the identification of pine species distributed in other states or regions of the country.

Acknowledgments

Ramón Escárcega Vargas, Perla Barrón Hernández, Shamed Popoca Paredes and Edith Jiménez Muñoz, for their support during the field work. *Red Temática Código de Barras de la Vida* (*Continuidad de Redes Temáticas*) [Life Bar-Code Thematic Network (Thematic Networks Continuity)] No. CONACyT: 271108 for financing the field work.

Conflict of interest

The authors declare no conflict of interest.

Contribution by autor

All the authors were involved in field work and preparing the manuscript in all its sections