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MACROMORPHOLOGICAL, ANATOMICAL AND MOLECULAR STUDIES OF SOME TAXA OF ARALIACEAE IN EGYPT

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Abstract

The present study investigated morphological features, leaf and stem anatomy, leaf architecture, epidermal characteristics, and molecular characters of some taxa of Araliaceae to trace out the diversity and the diagnostic significance of these attributes. The studied taxa based on combination of 260 characters representing 182 morphological and 78 molecular characters which were subjected to a numerical analysis using NTSYS-PC program. The generated dendrogram explained the similarities and the differences between the examined taxa. The specific similarities are discussed and compared with some current classification systems. The generated dendrogram from morphological attributes confirmed the separation of Aralieae and Schefflerieae as two tribes of Araliaceae and supported the separation of simple leaved taxa from compound leaved.

Introduction

Araliaceae comprises 47 genera and over 1.350 species (Wen *et al.*, 2001) Five of the six largest genera with 50 or more species (*Schefflera* J.R.Forst., *Oreopanax* Decne. & Planch, *Dendropanax* Decne. & Planch., *Polyscias* J.R.Forst. and *Osmoxylon* Miq.) are best represented in tropical or subtropical zones although several smaller genera (*Brassaiopsis* Decne. & Planch, *Panax* L., *Macropanax* Miq., *Hedera* L., *Oplopanax* Torr. & A.Gray, and *Gamblea* C.B.Clarke) are found in the North Temperate Zone. Araliaceae are also well-developed in the Old Worldin southeastern Asia, the Pacific, and Indian Ocean basins. New World araliads include only a few genera, most of them are also largely the Old World such as *Aralia*, *Oplopanax*, *Panax*, *Pseudopanax* K.Koch, and *Dendropanax*. After the inclusion of *Sciadodendron* Griseb., in *Aralia* by Wen (2002), *Oreopanax* is now the only genus in the New World. Araliaceae trees or shrubs, sometimes woody vines. Leaves simple, palmately compound or 1-3 pinnately compound, lobed. Fruits drupe or berry.

A significant step was taken in resolving the placement of Araliaceae among the main genealogy of the order Apiales (Plunkett *et al.*, 2004; Plunkett *et al.*, 2001a) and in knowing the relationships within and between related genera of Araliaceae (EIBL *et al.*, 2001; Plunkett *et al.*, 2001b; Wen *et al.*, 1996). Harms (1894–1897) classified the family into three tribes. The tribe Araliaeae with imbricate aestivation and Mackinlayeae and Schefflereae with valvate aestivation are separated from one another by petal insertion based on petal aestivation and base insertion. Bentham (1867) provided nearly similar tribes Mackinlayeae and Aralieae, but the genera that placed in Schefflereae by (Harms, 1894–1897) were treated as tribes Panaceae and Hedereae (with smooth or ruminate endosperm, respectively), in addition to Plerandreae (where stamen number exceeded petal number).

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Based on morphological features by Harms (1898) and Judd *et al.*, (1994) and anatomical evidence by Metcalfe and Chalk-Vol (1950), Araliaceae have been put with Apiaceae which is supported by the recent molecular studies (Plunkett *et al.*, 1996; Plunkett *et al.*, 1997).

Jacobs *et al.* (2010) studied fruit set in *Hedera helix*. Mourad (2013) showed the separation of simple leaved *Meryta denhamii* from lobed (*Hedera helix* and *Tetrapanax papyrifer*) and compound leaved *Polyscias* spp. Amini *et al.* (2020) studied the micromorphology of *Hedera* species in Iran. Lestari and Elya (2019) made macroscopic studies of *Polyscias guilfoylei* leaves. The essential use of leaf architectural character as an aid in the delimitation of genera and species was performed in paleobotany (Dilcher, 1974; Mouton, 1966). Zhernova *et al.* (2021) made comparative wood for the anatomy of *Astropanax* Seem., and *Neocussonia* (Harms) Hutch. Săvulescu and Luchian (2009) studied the diagnostic value of *Hedera* epidermis and epidermis that is made up of one cell layer with polygonal cells and thin lateral wall. studied epidermal cell descriptions of *Hedera* species in Iran. Kotina *et al.* (2010) surveyed the leaf anatomy of Araliaceae and some related taxa. Rout *et al.* (2007) used RAPD and ISSR markers to study the genetic relationship between *Polyscias* and *Schefflereae*. Hoi *et al.* (2021) used Inter Simple Sequence Repeat (ISSR) markers to assess the genetic diversity of *Panax bipinnatifidus*.

Araliaceae has a taxonomic problem within and between its related genera. Aralieae and Schefflerieae were not accurately delimited and leaf forms were represented within the family of Araliaceae that have a tremendous array. This study aims to try to find the interspecific similarities of the studied taxa by investigating their morphological, anatomical, and molecular characteristics as well as a numerical evaluation of such traits.

Materials and Methods

Sampling

Twelve taxa of Araliaceae representing six genera were collected from the Botanical Garden of Mansoura University and Orman Botanical Garden, Giza, Egypt (Table 1). Identification was confirmed by comparison with herbarium specimens in the herbarium of Ain Shams University, Faculty of Science (CAIA). Voucher specimens of the investigated species were kept in Mansoura herbarium, Botany Department, Faculty of Science, Mansoura University. Nomenclature has been updated according to several websites (https://www.ipni.org/).

Macro-micromorphological investigations

Macromorphological characters of the leaves, inflorescence, flowers, and fruits were described from the fresh specimens. For the anatomical features, the methods were characterized by Johansen (1940) and were adopted by Jensen (1962) and Peacock (1973).

Leaf vein architecture was performed according to the usual method of (JESUDASS *et al.*, 2003). Lamina's architectural terminology follows (Ash, 1999; Hickey, 1973).

Stomatography was performed according to the method of Stace (1965). By using a Reichert Microstar IV microscope, the photomicrographs were taken at the Plant Taxonomy Research Laboratory, Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt. For scanning electron microscope (SEM) small (7 mm²) pieces of the lamina, the material was installed on SEM stubs with double-sided tape, coated with gold in SPI-Module sputter coater, checked, and photographed in Jeol JSM 5200 at various magnifications (500x, 1000x). The description of epidermal characteristics terminology based on (Ash, 1999; Metcalfe and Chalk-Vol, 1950; Murley, 1951; Prabhakar, 2004).

No.	Taxa	Date of Collection	Location
1	<i>Hedera canariensis</i> Willd., Mag. Neuesten Entdeck. Gesammten Naturk. Ges. Naturf. Freunde Berlin 2: 171 (1808). Synonym: <i>H. grandifolia</i> Hibberd, The Ivy 96 (1872).	5/2020 3/2021	Mansoura University Garden
2	<i>H. helix</i> L., Sp. Pl. 1: 202 (1753). Syn: <i>H. donerailensis</i> Hort. ex K.Koch, Dendrologie 1: 680 (1869).	5/2020 3/2021	//
3	Meryta denhamii Seem., Bonplandia 10: 295 (1862). Syn: M. macrocarpa Baill., Adansonia 12: 155 (1878).	5/2020 4/2021	Orman Botanical Garden
4	Oreopanax guatemalensis Decne. & Planch., Rev. Hort. [Paris]. Ser. IV, iii. (1854) 108, nomen. Syn: O. obtusifolius I. O. Williams. Fieldiana. Bot. 31: 20 (1965).	6/2020 2/2021	//
5	Polyscias fruticosa Harms, Nat. Pflanzenfam. [Engler & Prantl] iii. (1894) 45.	5/2020 3/2021	//
	Syn: Aralia tripinnata Blanco, Fl. Filip. [F.M. Blanco] 223 (1837).		
6	<i>P. guilfoylei</i> L.H.Bailey, Rhodora 1916, xviii. 153. Syn: <i>Aralia guilfoylei</i> W.Bull, Cat. New Beautiful Rare Pl. [W. Bull] 83: 4 (1873).	5/2020 3/2021	Mansoura University Garden
7	P. scutellaria (Burm.f.) Fosberg, Occas. Pap. Univ. Hawaii 46: 9 (1948).	5/2020 3/2021	//
	(1783).		
8	<i>Schefflera actinophylla</i> (Endl.) Harms, Nat. Pflanzenfam. [Engler & Prantl] 3(Abt. 8): 36 (1894). Syn: <i>Brassaia singaporensis</i> Ridl., J. Straits Branch Roy. Asiat. Soc.	5/2020 3/2021	//
	75: 38 (1917).		
9	S. arboricola (Hayata) Hayata ex Merr., Lingnan Sci. J. 5(1-2): 139 (1928).	5/2020 3/2021	//
	Syn: <i>Heptapleurum arboricola</i> Hayata, Icon. Pl. Formosan. 6: 23 (1916).	3/2021	
10	S. elegantissima (Veitch ex Masters) Lowry & Frodin, Baileya 23(1): 9 (1989): (1989). Syn: Schefflera fagueti Baill Adansonia 12: 142 (1878)	6/2020 2/2021	Orman Botanical Garden
11	<i>S. pueckleri</i> (K.Koch) Frodin, Baileya 23(1): 10 (1989). Syn: <i>Tupidanthus calyptratus</i> Hook.f. & Thomson, Bot. Mag. 82: t. 4908 (1856).	5/2020 3/2021	Mansoura University Garden
12	<i>Tetrapanax papyrifer</i> (Hook.) K.Koch, Wochenschr. Gärtnerei Pflanzenk. 2: 371 (1859). Syn: <i>Aralia mairei</i> H.Lév., Repert. Spec. Nov. Regni Veg. 13: 342 (1914).	6/2020 2/2021	Orman Botanical Garden

Table 1. List of the studied Araliaceae taxa and their collection data.

Molecular assessment (ISSR-PCR analysis)

Genomic DNA was extracted from the twelve samples according to the manufacturer protocol of the Gene JET Genomic DNA Purification Kit (K0721/ Thermo fisher). Total genomic DNA was amplified through Gene Amp Polymerase Chain Reaction (PCR) system cycler. PCR for amplified genomic DNA was carried out according to(El-Assal *et al.*, 2011). ISSR-PCR reactions were conducted using 6 primers for the genotype (Table 2). Gel documentation system (Geldoc-it, UVP, and England), was applied for data analysis using Totallab analysis software (Ver.1.0.1), ww.totallab.com.

Table 2.ISSR	primers	names	and	seq	uence
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No	Primers	Sequences
1	iPBS primer 2270	5'-ACCTGGCGTGCCA-3'
2	C1	5'-AGGGCTGGAGGAGGGC-3'
3	G4	5'-ACTGACTGACTGACTG-3'
4	PseCra5	F-5'-CCAGCGTCACCTCCATTATT-3'
		R-5'-TCACAGCCAGCCACTGTATC-3'
5	PseLes1	F-5'-AAGTTGATGGCTTCGCTCAT-3'
		R-5'-ACCACCCCAATACAAAACCA-3'
6	PseCra3B	F-5´-ATGTTTGTGAATTGTGAGTGTGG-3´
		R-5'-CCCCATCTTTTGTCCCTCA-3'

Data analysis

The UPGMA function and SAHN program were used by Sneath and Sokal (1973). All computations were made with the help of NTSYS-PC version 2.02 (Rohlf, 1998).

Results and Discussion

Shape of leaves

Simple in *Hedera canariensis, Meryta denhamii*, and *Oreopanax guatemalensis*, lobed palmate in *Hedera helix*, and *Tetrapanax papyrifer*, compound palmate in 4 species of genus *Schefflera* and compound pinnate in 3species of genus *Polyscias* are as shown in Fig. 1.

Stem and lamina anatomy

Stem investigations

Stem angled in seven taxa viz., Hedera canariensis, Meryta denhamii, Oreopanax guatemalensis, Polyscias fruticosa, Schefflera arboricola, Schefflera elegantissima, Tetrapanax papyrifer and terete in five taxa Hedera helix, Polyscias guilfoylei, Polyscias scutellaria, Schefflera actinophylla and Schefflera pueckleri. All taxa are not glandular except Hedera canariensis. Lenticel present in six taxa viz., Hedera helix, Meryta denhamii, Oreopanax guatemalensis, Schefflera elegantissima, Schefflera pueckleri and Tetrapanax papyrifer, but absence in other six taxa, collenchyma may be angular-lamellar in nine taxa, and angular in Meryta denhamii, Oreopanax guatemalensis and Schefflera arboricola. The aspect of vascular bundles is Siphonostelic in 11 taxa and distinct in Polyscias scutellaria as observed in Fig. 2.



Fig. 1. (A-D) Leaves photographs of some studied taxa; A) Simple; B) Simple lobed palmate; C) Compound pinnate; D) Compound palmate.

Lamina anatomy

Raised adaxially in 11 taxa and flattened adaxially in *Schefflera actinophylla happen*. All taxa are not glandular except in *Hedera helix* is peltate eglandular, while *Tetrapanax papyrifer* is multicellular branched eglandular. Collenchyma annular in five taxa *Hedera canariensis*, *Hedera helix*, *Polyscias fruticosa*, *Schefflera actinophylla*, *Schefflera elegantissima*. In addition, annular-lamellar is in 5 taxa *Meryta denhamii*, *Oreopanax guatemalensis*, *Schefflera arboricola*, *Schefflera pueckleri* and *Tetrapanax papyrifer*, angular-lamellar in *Polyscias guilfoylei* and angular in *Polyscias scutellaria*. Vascular system partially continuous is in 6 taxa *Hedera canariensis*, *Hedera helix*, *Oreopanax guatemalensis*, *Polyscias guilfoylei* and *Schefflera actinophylla* and distinct in other six taxa. All taxa have druses-raphides except druses in *Polyscias scutellaria* (Fig. 2).

Lamina vein architecture

Primary vein pinnate in six taxa viz., Meryta denhamii, Oreopanax guatemalensis, Polyscias fruticosa, Schefflera actinophylla, Schefflera elegantissima and Schefflera pueckleri, suprabasal in Hedera canariensis, Hedera helix, acrodromous (basal) in Polyscias guilfoylei, suprabasal actinodromous in Polyscias scutellaria, suprabasal actrodromous in Schefflera arboricola, palinactinodromous in Tetrapanax papyrifer. Secondary vein brochidodromousis in four taxa, namely, Hedera canariensis, Hedera helix, Polyscias guilfoylei, and Polyscias scutellaria, reticulodromous in Meryta denhamii, Schefflera arboricola, festooned brochidodromous in Oreopanax guatemalensis, Schefflera actinophylla, Schefflera pueckleri, weak brochidodromous in Polyscias fruticosa, intramarginal vein in Schefflera elegantissima, interior (seven basal veins) in Tetrapanax papyrifer. Third vein category random reticulate in seven taxa, alternate percurrent in four taxa, namely, Oreopanax guatemalensis, Polyscias fruticosa, Polyscias guilfoylei, and Polyscias scutellaria, dichotomizing in Schefflera elegantissima. 4° vein RPR (regular polygonal reticulate) in 9 taxa, alternate percurrent in Meryta denhamii, dichotomizing in Schefflera elegantissima, absence in Oreopanax guatemalensis. 5° category RPR in five taxa viz., Hedera canariensis, Hedera helix, Meryta denhamii, Schefflera arboricola and Tetrapanax papyrifer. Dichotomizing is in five taxa Polyscias guilfoylei, Polyscias scutellaria, Schefflera actinophylla, Schefflera elegantissima and Schefflera pueckleri, absence in Oreopanax guatemalensis, Polyscias scutellaria, Schefflera elegantissima and Schefflera pueckleri, absence in Oreopanax guatemalensis, Polyscias scutellaria, Schefflera elegantissima and Schefflera pueckleri, absence in Oreopanax guatemalensis, Polyscias fruticosa as Fig. 3.



Fig. 2. (A-I) Photographs of some stem anatomy of studied taxa; A) Angled, egland unicellular unbranched trichome, siphonostelic vascular bundle; B) Terete, lenticel; C) Distinct vacular bundle; D) Angular collenchyma. E-I) Photographs of some lamina anatomy of studied taxa; E) Raised adaxially, peltate eglandular trichome, annular collenchyma, druses & raphides crystal, partially continuous vascular bundle; F) Druses crystal, angular collenchyma, distinct vascular bundle. G) Flattened adaxially; H) Multicellular branched eglandular trichome; I) Angular & lamellar. Abbreviations: Tr. trichome; Se. sub epidermal periderm; Vb. vascular bundle; Len. lenticel; AC. angular collenchyma; RC. raphides crystal; DC. druses crystal. Pc. palisade cells; RC. raphides crystal; Tr. trichome; AC. angular collenchyma.



Fig. 3. (A-H) The main categories of lamina vein architecture with LM. A) Suprabasal 1°V, brochidodromous 2°V, random reticulate 3°V, regular polygonal reticulate 4°V 5°V; B) Pinnate 1°V, weak brochidodromous 2°V, alternate percurrent 3°V; C) Acrodromous 1°V, dichotomizing 5°V; D) Suprabasal actinodromous 1°V; E) Suprabasal acrodromous 1°V, reticulodromous 2°V; F) Dichotomizing 3°V, 4°V, 5°V. G) Festooned brochidodromous 2°V; H) Palinactinodromous 1°V.

Epidermal cell description

Cell shape was irregular in 4 taxa, namely, *Hedera canariensis*, *Hedera helix*, *Meryta denhamii*, and *Tetrapanax papyrifer* and polygonal in the rest taxa. Anticlinal wall sinuous in four taxa *Hedera canariensis*, *Hedera helix*, *Meryta denhamii*, and *Tetrapanax papyrifer* and slightly curved in 8 taxa. Stomatal shape elliptical in all taxa. Stomatal type anomocytic and anisocytic is in *Hedera canariensis* and *Hedera helix*. Anisocytic in seven taxa, anisocytic and diacytic in *Polyscias fruticosa*, *Polyscias guilfoylei* and *Schefflera elegantissima*. Sculpture ruminate in four taxa *Hedera canariensis*, *Oreopanax guatemalensis*, *Schefflera actinophylla*. *Schefflera pueckleri* arepusticulate in *Hedera helix* and *Meryta denhamii*, reticulate-aerolate in *Polyscias fruticosa*, *Polyscias scutellaria* reticulated in *Schefflera arboricola*, favulariate in *Schefflera elegantissima* and striate in *Tetrapanax papyrife* (Fig. 4).



Fig. 4. (A-C) Major categories of stomatography as revealed with LM; A) Anomocytic & anisocytic stomata, irregular cell shape, sinuous anticlinal wall; B) Anisocytic stomata, polygonal cell shape, slightly curved anticlinal wall; C) Anisocytic & diacytic. D-F) Major types of lamina surface sculpture with SEM; D) Ruminate; E) Pusticulate; F) Reticulate-aerolate. G-I) Major types of lamina surface sculpture with SEM; G) Reticulate; H) Favulariate; I) Striate.

Molecular assessment

All primers produced 78 monomorphic and polymorphic bands (Table 3). Primer iPBS primer 2270 produced one monomorphic band and 9 polymorphic bands (7 common and 2 unique), C1 produced one monomorphic band and 14 polymorphic bands (13 common and 1 unique), G4 produced one monomorphic band and 13 polymorphic bands (12 common and 1 unique), PseCra5

produced no monomorphic bands, and 13 polymorphic bands (13 commons). While no unique bands were produced, PseLes1 produced no monomorphic bands, and 13 polymorphic bands (13 commons). While no unique bands were produced, PseCra3Bproduced no monomorphic bands, and 13 polymorphic bands (13 commons), while no unique bands were recorded (Fig. 5).

Table 3. Type of	bands and j	percentage of	polymorphism	of ISSR	primers	applied	on the	studied	taxa
of family Ara	liaceae.								

Primer	Monomorphic	Polymor	phic bands	Total bands	Polymorphism	
	bands	Common	Unique	_	%	
iPBS primer 2270	1	7	2	10	90	
C1	1	13	1	15	93.33	
G4	1	12	1	14	92.86	
PseCra5	0	13	0	13	100	
PseLes1	0	13	0	13	100	
PseCra3B	0	13	0	13	100	



Fig. 5 (A-F) ISSR profile of the studied taxa of Araliaceae generated by A) iPBS primer 2270; B) primer C1; C) primer G4; D) primer PseCra5; E) primer PseLes1; F) primer PseCra3B.

Numerical analysis

The data obtained from the whole plant, stem, and leaf anatomy for the examined taxa were amalgamated with the data that was obtained from lamina architecture and stomatographic analyses. Then, they were subjected to numerical analysis to explain and discuss the similarity among the studied taxa based on (182) macro-micromorphological traits that were used for computation and produced dendrogram as shown in Fig. 6. The data extracted from ISSR analysis were subjected to numerical analysis to explain and discuss the similarity among the examined taxa based on (78) molecular traits. These traits were used for computation and produced dendrogram as shown in Fig. 7. Finally, the data extracted from macro-micromorphological attributes were amalgamated with the data from ISSR analysis. They were subjected to numerical analysis to explain and discuss the similarity among the studied taxa based on (260) macro-micromorphological and molecular traits that were used for computation and produced dendrogram as shown in Fig. 8.



Fig. 6. Dendrogram of studied taxa of Araliaceae based on morphological characters (182).

The resulting dendrogram from morphological attributes is compared with the current system treatments. The dendrogram shows that the taxa under investigation were splitted into two main series (I and II), three clusters (A, B, and C), and five groups (Fig. 6). Series I included only one cluster (A) and one group; Cluster A included one group of three studied species. Series II involved two clusters (B & C) and four groups and Cluster B involved two groups; the first group involved two studied species while the second one involved four studied species. Cluster C involved two groups; the first group involved two studied species. The similarities among these taxa are summarized as follows.



Fig. 7. Dendrogram of studied taxa of Araliaceae based on molecular characters (78).



Fig. 8. Dendrogram of studied taxa of Araliaceae based on morphological and molecular characters (260).

Series I, Group 1 includes *Hedera canariensis*, *Hedera helix*, *Tetrapanax papyrifer*. These results are in agreement with Harms (1894-1897 classification systems that put them in the same tribe (Hutchinson, 1967; Bentham, 1867; Chang-Jiang *et al.*, 1982) placed them in different tribes. Calestani (1905) and Viguier (1906) placed *Tetrapanax papyrifer* in the same tribe, but *Hedera canariensis*, *Hedera helix* indifferent tribes. Seemann (1868) placed *Tetrapanax papyrifer* in the same family but different tribe and placed *Hedera canariensis*, *Hedera helix* in a different family.

Series II, Group 2 includes *Meryta denhamii*, *Oreopanax guatemalensis*. These results are in agreement with Harms (1894-1897) classification systems that put them in the same tribe. Hutchinson (1967), Bentham (1867), Chang-Jiang *et al.* (1982) and Seemann (1868) placed *Meryta denhamii* in the same tribe, but *Oreopanax guatemalensis* in a different tribe. Calestani (1905) and Viguier (1906) placed *Oreopanax guatemalensis* in the same tribe, but *Meryta denhamii* a different tribe.

Group 3 includes *Schefflera actinophylla*, *S. pueckleri*, *S elegantissima*, *S. arboricola*. These results are in agreement with Harms (1894-1897), Calestani (1905) and Viguier (1906) classification systems that put them in the same tribe. Hutchinson (1967), Bentham (1867), Seemann (1868), Chang-Jiang *et al.* (1982) placed them in the same family but in different tribes.

Group 4 includes *Polyscias fruticosa*, *P. guilfoylei*. These results are in agreement with Bentham (1867), Seemann (1868), Harms (1894-1897), Calestani (1905), Hutchinson (1967) and Chang-Jiang *et al.* (1982) classification systems that put them in the same tribe. Viguier (1906) placed it in the same family but different tribe.

Group 5 includes *Polyscias scutellaria*. This result is in agreement with Bentham (1867); Seemann (1868); Harms (1894-1897); Calestani (1905); Hutchinson (1967) and Chang-Jiang *et al.* (1982) who put them in the same tribe. Viguier (1906) placed it in the same family but in a different tribe.

Conclusion

Araliaceae has a taxonomic problem within and between its related genera. Araliaea and Schefflerieae were not accurately delimited. The numerical analysis interprets the similarities between the studied taxa based on 260 macro-micromorphological and molecular traits. The data of this study resulted from macro-micromorphological traits suggest the separation of Araliaea and Schefflerieae as two tribes of Araliaceae and simple leaved taxa from compound leaved ones.

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