A Survey of Leaf Epicuticular Waxes and Trichomes in the Genus Aristolochia (Aristolochiaceae) using Scanning Electron Microscopy (SEM)

Hafez Mahfoud*[1], Stefan Wanke*[2], Christoph Neinhuis*[3]
*Technische Universität Dresden, Institut für Botanik, D-01062 Dresden, Germany.

Abstract

A scanning electron microscopic survey of leaf surface features in the genus Aristolochia was performed including samples from the New and Old World with a special emphasis on the Mediterranean species. The present SEM-study revealed that the investigated taxa can be divided into three groups according to the type of leaf epicuticular waxes. Furthermore, the Mediterranean Aristolochia species can be divided into two groups: all west Mediterranean species are characterized by wax platelets whereas the Caucasian and Near East species show wax rodlets known as the "Aristolochia type". Other investigated characters are the absence or presence and the type of trichomes on the adaxial leaf surface. In general a glabrous group (~ 19% of the investigated taxa) and the pubescent group (~ 81%) could be separated. The latter can further be subdivided into four basic types of trichomes: a) hook-shaped trichomes (two cells) present in most taxa, b) conical multi-cellular and erect to sub-erect trichomes being characteristic for some East Mediterranean species, c) multicellular-elongated trichomes with cylindrical base and an elongated acute apex (Aristolochia grandiflora complex and Aristolochia subgenus Siphisia), and d) glandular trichomes with rounded tip found only in A. triactina (subgenus Pararistolochia).

Keywords:

Aristolochia, Micromorphology, taxonomy, leaf waxes, trichoms, SEM.

I. INTRODUCTION

Originally systematic studies addressing the systematics of Aristolochiaceae were preliminary based on morphological and anatomical characters, especially seeds ([1], [2], [3]), flowers [4], and leaf characteristics [5]. In general, characteristics of the leaf surface, especially the morphology and chemical composition of epicuticular waxes, has been proofed to be of high taxonomical value in both major plant lineages ([5], [6], [7]) and among species ([8], [9], [10]). In addition to the wide range of usability of epicuticular wax morphology, this research was enhanced by the ease to obtain data by scanning electron microscopy (SEM) from e.g. air-dried samples [11]. More elaborate preparation methods like chemical fixation [12] or glycerol substitution [13] have been established allowing conservation of cellular structures of the leaf samples.

Micromorphology of epicuticular waxes in Magnoliids and other lineages also included 21 species of Aristolochiaceae [6]. The results showed that more than two third of Aristolochiaceae species have a special type of wax rodlets known as "*Aristolochia type*", while six species showed wax platelets some of which were arranged according to the "*Convallaria type*". Barthlott and coauthors [5] screened more than 13000 species representing all major groups of seed plants for epicuticular wax crystals and identified at least 23 wax types [5]. For Aristolochiaceae, initial results could be confirmed characterizing the "*Aristolochia type*" as transversally ridged rodlets being characteristic for "ancestral woody angiosperms", and the "*Convallaria type*" as parallel oriented platelets, typically found in some monocotyledonous families ([5], [6]). The latter, also being present in all major land plant groups, i.e. angiosperms, gymnosperms and mosses.

Another type of characters that can serve for morphological systematics, are the form and density of trichomes. Trichomes can be found on the leaf epidermis as well as other plant organs. Trichome characteristics were used for classification as well as the identification by many systematists on different taxonomic levels ([14], [15], [16], [17]). Plant-hair types have been successfully used in the classification of genera and species and in the recognition of hybrids within certain groups ([18], [19]). In Brassicaceae, for example, the type of hair has been used as a major criterion for the subdivision of the family into tribes

and genera [20]. In Rhododendron (Ericaceae), trichomes afford useful characters for taxonomic separation on the infrageneric level as well as the species level [21]. A generic key for the Indian members of Asteraceae on the basis of trichome was provided by [22]. More recently, the model family Brassicaceae was studied [23] and discovering a great diversity of trichome forms and concluded that this diversity provides by far the most important taxonomic characters of the epidermis and in many cases, are as valuable as any of the other morphological character of the plants. In addition, most researchers agree that leaf hairs could differ among related species and were used as taxonomic characters among species [24] or even among subspecies and varieties [23], others found no difference in hair type within plant groups. They concluded that leaf trichomes could be useful for taxonomic purposes among some related species but might not be useful among others [25].

Aristolochia, a genus of Piperales ([26], [27]), shows a nearly worldwide distribution with the highest diversity in tropical and subtropical regions. The adaptation to different abiotic conditions is likely to be mirrored on leaf characteristics. The systematics of *Aristolchia* has changed considerably, as in many other angiosperm groups, from a pre-molecular era to the molecular phylogenetic era. A recent study [28] summarizing molecular and morphological results from published phylogenetic hypothesis ([29], [27], [30]) as well as new molecular data subdivided *Aristolochia* into three monophyletic subgenera. However, flower and seed attributes were essential for the differentiation between infrageneric groups of the genus; molecular studies shaded light into natural relationships and largely confirmed pre-molecular studies. In recent years the focus has shifted from generic und subgeneric level to species level systematics. The Mediterranean species represent one of these groups. Most important contributions to the knowledge of species from the Mediterranean were published by Nardi and others ([31], [32], [33], [34], [35], [36], [37], [38]). [39] provided the first phylogeny of all West Mediterranean *Aristolochia* species also including few members of Caucasia and the East Mediterranean region.

Here we present a detailed survey of epidermal trichomes and the leaf micromorphology including epicuticular waxes using a broad taxonomic sampling. The present study evaluates the presence of such structures and their phylogenetic importance for the systematics of the genus. Results are discussed in the light of traditional classification as well as hypotheses derived from molecular data.

II. MATERIAL & METHODS

This study on micromorphology of *Aristolochia* leaves comprises 54 species representing the three subgenera *Siphisia*, *Pararistolochia* and *Aristolochia* (Table 1). Leaf material was taken from living plants cultivated in the Botanical Garden Dresden. Fresh and uncontaminated leaves were collected preferentially. Squares of leaves (approximately 3 mm x 3 mm) were excised using a razor blade. To obtain a relatively flat surface of the sample the midrib area has been avoided. Leaf samples were prepared for SEM by liquid substitution of glycerol [13]. Samples were fixed to an aluminum sample holder (10 mm in diameter, Plano GmbH, Wetzlar, Germany) using a carbon adhesive tape (Leit Tabs, Plano GmbH) and sputter-coated under argon atmosphere with an approximately 10 nm thin gold layer in EMITECH K550 (Emitech Ltd., Ashford Kent, UK). The adaxial leaf surface was observed using a Leo 420 (Leo Electron Microscopy Ltd., Cambridge, UK) and the software LeoUIF 420 and Diss5. Measurements on wax crystal dimensions were performed using Diss5. The description of wax crystal morphology follows the nomenclature of [5].

III. RESULTS

A. *Epicuticular waxes of the adaxial leaf surface*

Two different types of wax crystals were found on the adaxial leaf surface of *Aristolochia*: transversely ridged rodlets and wax platelets. Different types are characteristic for the specific species and only two cases were observed where both types occur on one single species (Table 2). 71.69% of the investigated species possess transversely ridged rodlets (Figure 1-A, B, C) on the adaxial leaf surface. These waxes of the "*Aristolochia*" type occur likewise in Old World as well as in New World species. Surprisingly, the "*Aristolochia*" type is the only wax type found in East Mediterranean *Aristolochia* species.

According to the variability in length and density of the transversely ridged rodlets, the species showing rodlet type of wax could be divided into two groups: A) short, homogenous and less dense rodlets (Figure 1-A, B) and B) short to long, heterogeneous and denser rodlets (Figure 1-C). All included species from the East Mediterranean region represent the previous form (e.g. *A. guichardii*, *A. krausei*), while the second form is more common within South American species (e.g. *A. brasiliensis*, *A. guentheri*).

Table 1. Investigated *Aristolochia* species: origin of the voucher specimen, its accession number in the Botanical Garden Dresden; as well as the distribution areas of the species are given.

Species	Garden accession number	Origin of the investigated plant	Distribution area
A. acuminata	M. Chiang BG DD (BG Bonn 7417)		S. Asia
A. anguicida			S. America
A. arborea	BG DD1001680-15		C. America
A. austroyunnanesis	J. Murata. Japan		S. Asia
A. baetica	Wanke (034)	Spain, Majorca	W. Mediterranean
A. bottae	Mahfoud (18/2)	Syria, Alzebdane	E. Mediterranean
A. brasiliensis	09867	Brazil	S. America
A. californica	BG DD 013310-08		N. America
A. chlamydophylla	BG DD 01.10.2004		S. Asia
A. clematitis	BG DD 002034-09		Eurasia
A. clusii	Wanke (193)	Italy, Pomarico	W. Mediterranean
A. cucurbitifolia	BG DD 014030-08		S. Asia
A. cymbifera	BG Göttingen 2005		S. America
A. debilis	03.06.2002		S. Asia
A. elegans	BG DD 001306-10		America
A. elongata	Wanke (162)	Greece, Peloponnesus	W. Mediterranean
A. eriantha	BG DD 013305-12		S+C America
A. fimbriata	BG DD 0001870-16		S. America
A. galeata	L.Barabino (BG DD 014075-17) 2003		S. America
A. gigantea	BG DD (BG Bonn 14213)		S. America
A. gorgona	13.03.2006		C. America
A. grandiflora	L. Barabino (BG DD 0038-19-21)		S+C America
A. guentheri		Peru	S. America
A. guichardii	Wanke (186)	Greece, Rhodes	E. Mediterranean
A. hirta	Mahfoud (29/1)	Turkey, Alanya	E. Mediterranean
A. kankauensis	BG DD (BG. Tokio 2262) Japan		S. Asia
A. krausei	Mahfoud (23/1)	Turkey, Erdemli	E. Mediterranean
A. leuconeura	L.Barabino (BG DD 0140332-12) 20.07.2004		C. America
A. lutea	Wanke (W135)	Croatia, Istria	W. Mediterranean
A. maurorum	Mahfoud (20/4)	Syria, Aleppo	E. Mediterranean
A. manschuriensis	BG DD 003860-17 China		S. Asia
A. microstoma	Wanke (171)	Greece, Peloponnesus	W. Mediterranean
A. mollissima	J. Murata, Japan		S. Asia
A. moupinensis	Edinburgh, Japan 11.06.2004		S. Asia
A. nakaoi	BG. Tokio (22617) Japan, Tokio		S. Asia
A. navicularis	Wanke (21)	Italy, Sardinia	E. Mediterranean

Species	Garden accession number	Origin of the investigated plant	Distribution area
A. odoratissima	J. Urban (BG DD)	Bolivia	S+C America
A. paecilantha	Mahfoud (24/1)	Syria, Alzebdane	E. Mediterranean
A. pallida	Wanke (129)	Italy, Alpicelli	W. Mediterranean
A. parvifolia	Wanke (163)	Greece, Rhodes	E. Mediterranean
A. pistolochia	Wanke (199)	France, Cassis	W. Mediterranean
A. pohliana	L. Barabino, Brazil 20.07.04	Brazil	S. America
A. praevenosa	BG DD 014799-30807		Australia
A. prostrata	M. Könen, Bolivia 16.02.2002	Bolivia	S. America
A. rigida	C. Neinhuis	Somalia	Somalia - Yemen
A. ringens	Gonzales (3575) BG. München	Bolivia	S. America
A. rotunda	Wanke (205)	Italy, Sant-Eufemia	W. Mediterranean
A. scabridula	Mahfoud (16/1)	Syria, Msyaf	E. Mediterranean
A. sempervirens	Wanke (106)	Italy, Sicily	Mediterranean
A. shimadai	J. Murata (22624) Japan, Tokio		S. Asia
A. tomentosa	BG DD 006146-17 (BG Bonn 0282) Malaysia		N. America
A. triactina	Neinhuis 119(DR) BG Bonn 12767		Africa
A. westlandi	BG DD 014736-21 (BG Bonn 14211) China		S. Asia

In 24.52% of all investigated species wax platelets with an irregular margin were found. The platelets protrude more or less perpendicular from the underlying surface and are usually connected to it by their narrow side. In some species (e.g. *A. lutea*, *A. parvifolia*, *A. elongata*) the wax platelets are arranged in parallel rows (Figure 1-D), but predominantly arrangement of wax crystals does not show any order (Figure 1-E). With the exception of *A. pistolochia*, which possess transversally ridged rodlets, irregular platelets were found in all investigated species belonging geographically to the West Mediterranean species. Platelets also occurs in few species distributed in Asia and America (e.g. *Aristolochia* subgenus *Aristolochia* and *Aristolochia* subgenus *Siphisia*).

In two species (*A. cucurbitifolia* and *A. mollissima*) belonging to *Aristolochia* subgenus *Siphisia* both irregular wax platelets and transversally ridged rodlets were found. Loosely scattered rodlets are surrounded by platelets forming a bloom like pattern (Figure 1-F).

B. Trichomes on the adaxial leaf surface of Aristolochia

The majority of the investigated taxa are pubescent (Figure 2-A) but 20 % do not show any evidence for trichomes (Table 2). Four different types of trichomes could be observed: hook-shaped trichomes, gland-like trichomes, relatively thick and conical trichomes and long, thin trichomes often with longitudinally cells. Hook-shaped trichomes are at least two-celled, with a long, curved cell forming the hook and one to several cells forming a broad base (Figure 2-B). This type of trichome was found in all three subgenera and 70% of the investigated species. Conical trichomes are inserted with their broad base on the underlying surface. More or less erect trichomes occur only in three species of *Aristolochia* subgenus *Aristolochia* also possessing hook-shaped trichomes (Figure 2-C, D). These more or less long and thin trichomes are cylindrical at their base and possess an elongated acute apex. Cells are always longer than broad and often longitudinally collapsed (multicellular-elongated trichome) (Figure 2-E). Both investigated species of the *Aristolochia grandiflora* complex as well as some species of *Aristolochia* subgenus *Siphisia* are characterized by this type of trichomes. Gland like trichomes (Figure 2-F) with round tips are found in the only investigated African species of *Aristolochia* subgenus *Pararistolochia* (*Aristolochia triactina*). Other species are characterized by a combination of erect and hook-shaped trichomes types.

Figure 1. All magnification 3000x. **A**, **B** and **C** showing both transversally ridged rodlets; **A** *Aristolochia guichardii*, and **B** *Aristolochia triactina*, both with short, homogenous and less dense rodlets; **C** Aristolochia *guentheri* with short to long, heterogeneous and more dense rodlets; **D** and **E** showing wax platelets typical for West Mediterranean species; **D** *Aristolochia parvifolia* has parallel rows of wax platelets, and **E** *Aristolochia clematitis* showing irregular wax platelets; **E** *Aristolochia cucurbitifolia* possessing transversally ridged rodlets and irregular platelets.

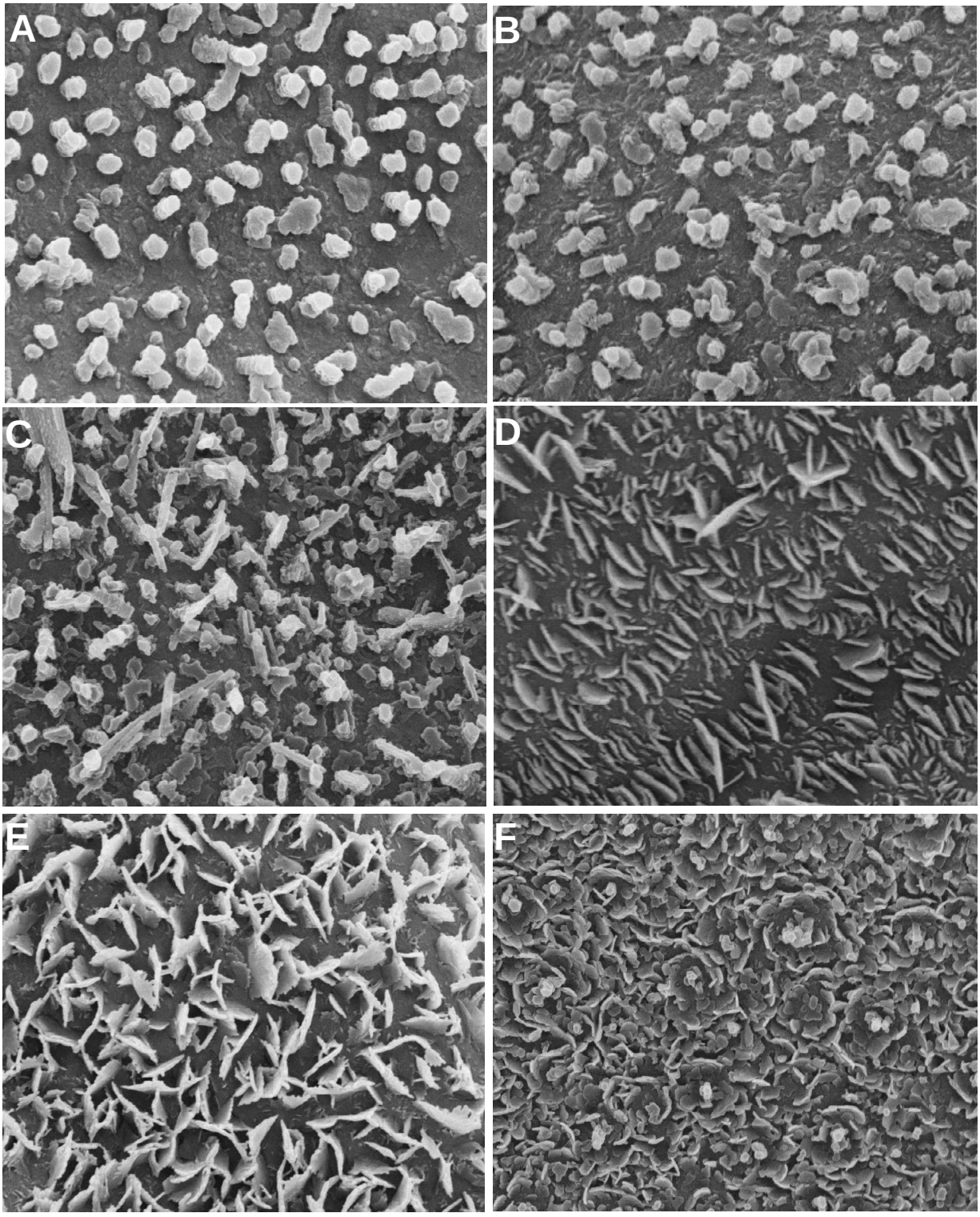


Figure 2. **A** *Aristolochia clematitis* with glabrous leaves; **B** *Aristolochia hirta* with hook-shaped trichomes; **C** conical trichomes and **D** conical together with hook-shaped trichomes both found in *Aristolochia paecilantha*; **E** *Aristolochia tomentosa* shows multicellular-elongated trichome together with hook-shaped trichomes; **F** glandular trichome observed in *Aristolochia triactina*. All pictures are at the same magnification.

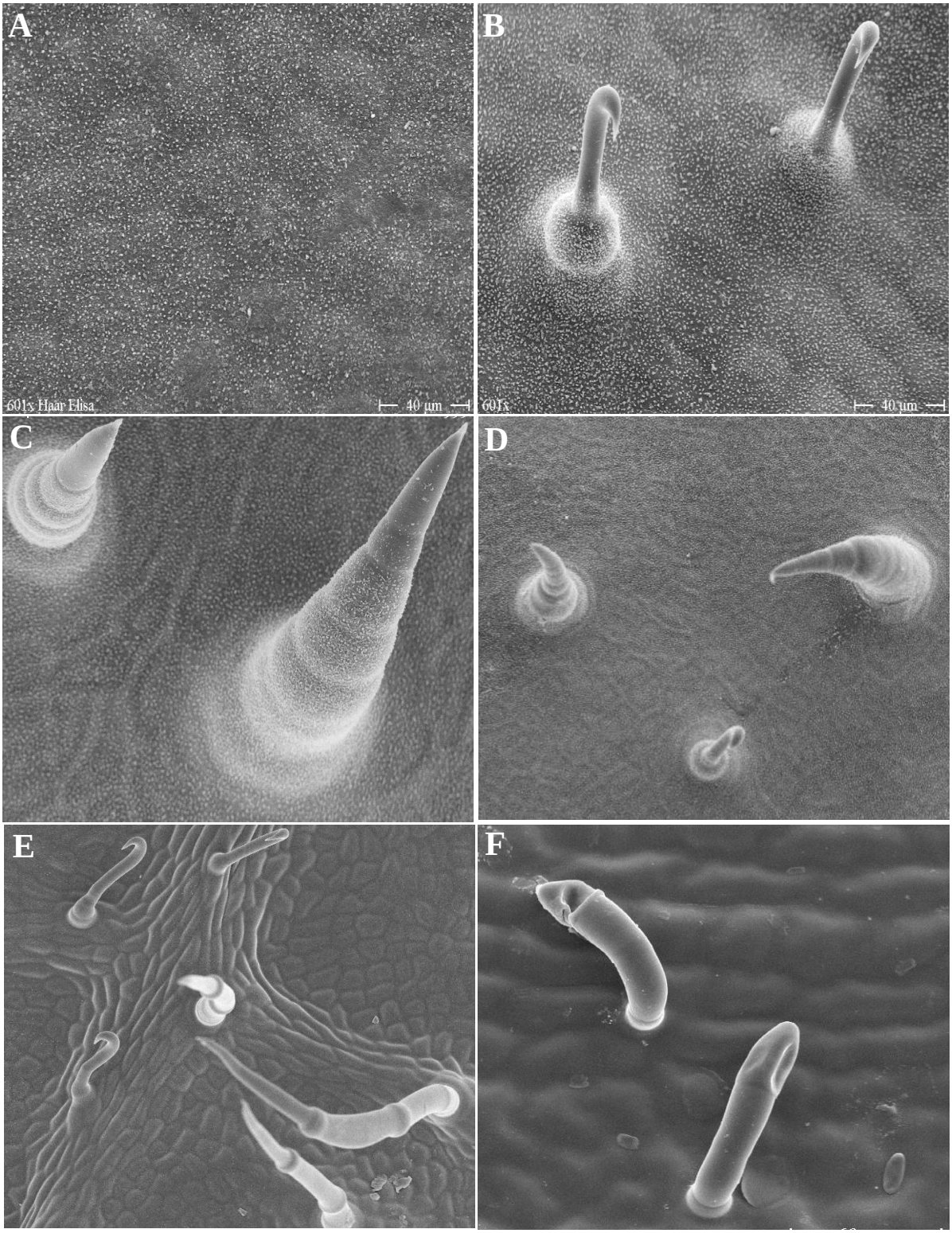


Table 2. Presence and morphology of trichomes and epicuticular wax crystals on the adaxial leaf surface of Aristolochia species.

Species	wax crystals	Trichomes
subg. *Aristolochia*		
A. bottae	rodlets	hook-shaped
A. guichardii	rodlets	hook-shaped
A. hirta	rodlets	hook-shaped + conical
A. krausei	rodlets	hook-shaped
A. maurorum	rodlets	hook-shaped
A. paecilantha	rodlets	hook-shaped + conical
A. scabridula	rodlets	hook-shaped
A. baetica	rodlets	hook-shaped
A. clusii	platelets	hook-shaped
A. elongata	platelets	hook-shaped
A. lutea	platelets	absent
A. microstoma	platelets	hook-shaped
A. navicularis	platelets	absent
A. pallida	platelets	hook-shaped
A. parvifolia	platelets	hook-shaped
A. pistolochia	rodlets	hook-shaped
A. rotunda	platelets	absent
A. sempervirens	rodlets	hook-shaped
A. clematitis	platelets	absent
A. debilis	platelets	absent
A. rigida	rodlets	hook-shaped
A. acuminata	rodlets	hook-shaped
A. chlamydophylla	rodlets	hook-shaped
A. kankauensis	rodlets	hook-shaped
A. gorgona	rodlets	hook-shaped + long
A. grandiflora	rodlets	hook-shaped + long
A. anguicida	rodlets	hook-shaped
A. brasiliensis	rodlets	hook-shaped
A. cymbifera	rodlets	hook-shaped
A. elegans	rodlets	hook-shaped
A. eriantha	rodlets	hook-shaped + conical
A. fimbriata	platelets	hook-shaped
A. galeata	rodlets	hook-shaped
A. gigantea	rodlets	absent
A. guentheri	rodlets	absent
A. leuconeura	rodlets	absent
A. odoratissima	platelets	hook-shaped
A. pohliana	rodlets	absent
A. prostrata	platelets	hook-shaped
A. ringens	rodlets	hook-shaped
subg. *Siphisia*		
A. arborea	rodlets	absent

A. californica	rodlets	hook-shaped + long
A. austroyunnanesis	rodlets	long
A. cucurbitifolia	mixed	hook-shaped
A. manschuriensis	rodlets	hook-shaped
A. mollissima	mixed	hook-shaped + long
A. moupinensis	rodlets	long
A. nakaoi	rodlets	long
A. shimadai	rodlets	hook-shaped + long
A. tomentosa	rodlets	hook-shaped + long
A. westlandi	rodlets	long
subg. *Pararistolochia*		
A. triactina	rodlets	glandular
A. praevenosa	rodlets	hook-shaped

IV. DISCUSSION

Based on the classification and nomenclature of plant epicuticular waxes by [5], and after examining nearly all Mediterranean species and a representative selection belonging to other clades, two completely different types of wax crystals occur within the genus. This is in accordance with previous studies [6]. Platelets sometimes arranged according to the "*Convallaria*" type are widely known from angiosperms [40]. This type of wax seems to be characteristic for the West Mediterranean species (*Aristolochia* subgenus *Aristolochia*). However, few species of *Aristolochia* subg. *Siphisia* and other lineages within *Aristolochia* subgenus *Aristolochia* show these features as well (Fig. 3).

Rodlets have been found among all Caucasian and East Mediterranean species as well as for most of the Old and New World taxa, indicating that this is likely a plesiomorphic character in East Mediterranean species. Although, *A. pistolochia* is geographically occurring in the Western Mediterranean area (France, Spain), molecular phylogenetic studies showed this species as sister to all Mediterranean species being more closely allied to *A. clematitis* ([28], [39]), which has a Eurasian distribution and is not part of the Mediterranean clade neither ([28], [39]). Also from a morphological point of view these species are separated from other Mediterranean *Aristolochia* species as they do not possess a rootstock but have either a rhizome or show fleshy roots [39]. Previously published molecular hypotheses as well as macromorphological characters are therewith supported by micromorphological characters studied here. Our results show that rodlets type of wax is most common in *Aristolochia* and could be found among species belonging to all clades. Our results support the findings of [6], although the present study is based on more representative sampling and include many more species.

The presence or absence as well as the type and density of trichomes of the adaxial leaf surface seem not to correlate with the systematic relationships of the species in most cases.

Plant hairs are among the most interesting features of plant surfaces. The woolly, velvety, or bristly appearance of many plants is mostly a result of trichome types and density. The systematic value in angiosperms is documented in the botanical literature as well ([14], [15]). In the present study, pubescence or glabrous appearance of the leaf surfaces were found to be less informative systematically. for the Mediterranean *Aristolochia* species glabrous leaves are present in all Caucasian and East Mediterranean species, while for the West Mediterranean species both pubescent and glabrous leaves are observed. Although the pubescence of leaves might be influenced by abiotic factors and adaptation to e.g. drier habitats co-occurring with higher sun radiation, this study does not support this assumption. As for example, species, which are usually growing in extreme sun exposure, do not show any trichomes (*A. navicularis*) whereas species preferring shady places show trichomes (*A. pallida*). In the *Aristolochia grandiflora* complex, belonging to *Aristolochia* subgenus *Aristolochia* ([28], [39]) a special type of trichomes was observed. This group can be characterized by hook-shaped trichomes, which are elongated compared to other hooked-shaped trichomes present in other clades.

The wax type (rodlets) and the presence of the hook-shaped trichomes in the *Aristolochia sempervirens* complex (*A. sempervirens* and *A. baetica*) indicates a closer relationship of this group to the East Mediterranean species, which might be a plesiomorphic trait and would therewith only partly contradict molecular findings as this group is recovered as sister to all remaining West

Mediterranean species and shows many floral features of East Mediterranean species (limb is not elongated, form more similar to tropical representatives of the genus and also the biogeographic distribution (circum Mediterranean) highlight the exceptional combination of traits found in this species complex.

The wax type and trichome form observed in *Aristolochia rigida*, which is occurring in Somalia and Yemen are similar to the West Mediterranean species and therewith are in contrast to other findings ([39], [41]) describing *Aristolochia rigida* as a species displaying morphological affinities to the East Mediterranean and Caucasian species (bilabiate, curved perianth, sessile utricle). The intermediate traits between the West and the East Mediterranean *Aristolochia* species seem to be symptomatic for this species, which could also not be placed in molecular based phylogenetic trees [39]. From a biogeographic point of view a close relationship to other East African species could be assumed but molecular results were unable to assign this species to any monophyletic group.

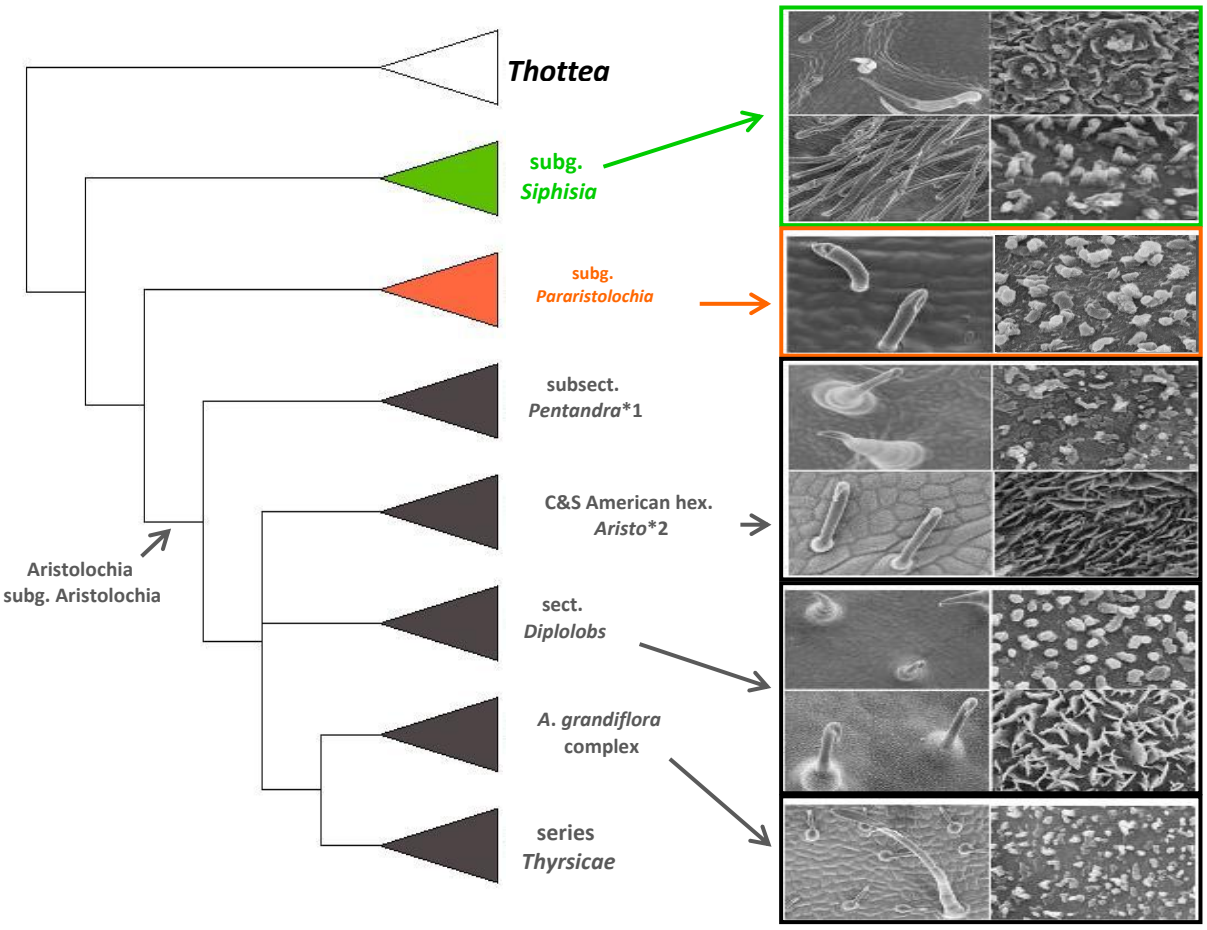
tube opens gradually and it is slightly curved, fruits forming a "basket"). This group also shows a growth

The glandular form of the trichomes observed only in *A. triactina* could be a synapomophy for the African *Aristolochia* subgenus *Pararistolochia* species, although more species from this subgenus need to be investigated to confirm this hypothesis.

V. CONCLUSION

Although more work on leaf epicuticular waxes and trichomes in *Aristolochia* is clearly needed, it is possible to draw some general conclusions. First, the study of the epicuticular wax of *Aristolochia* revealed a number of important micromorphological characters, and these characters exhibit interesting variations that are of significance for identification of some clades. Second, the variation in trichomes is an equally useful character for taxonomic purposes within some clades as well.

Figure 3. Current phylogenetic hypothesis compiled from multiple analyses ([27], [28], [30], [39], [41], [42], [43], [44]) aligned with some epicuticular wax types found in this study. Taxonomic unites, if used, correspond to clades identified in aforementioned studies (subg. = subgenus; *1 includes *A. lindneri*; *2 clade corresponds to Central and South American *Aristolochia* species with hexandrous gynostemium organization but excludes species belonging to the *A. grandiflora* complex and series *Thyrsicae*; sect. = section).



REFERENCES

[1] W. Barthlott, "Epidermal and seed surface characters of Plants: Systematic applicability and some evolution aspects," Nordic. J. Bot., vol. 1(3), pp. 345-355. 1981.

[2] H. HUBER. "Samenmerkmale und Gliederung der Aristolochiaceen". Bot. Jahrb. Syst. 107: 277-320. 1985.

[3] A. S. Adams, M. J. Baskin, and C. C. Baskin, "Comparative morphology of seeds of four closely related species of Aristolochiaceae subgenus Siphisia (Aristolochiaceae, Piperales) ," Bot. J. Linn. Soc., vol. 148(4), pp. 433-436. 2004.

[4] F. González, and D. W. Stevenson, "Perianth development and systematics of Aristolochia," Flora., vol. 195(4), pp. 370-391. 2000.

[5] W. Barthlott, C. Neinhuis, D. Cutler, F. Ditsch, I. Meusel, I. Theisen, and H. Wilhelmi, "Classification and terminology of plant epicuticular waxes," Bot. J. Linn. Soc., vol. 126(3), pp. 237-260. 1998.

[6] S. Hennig, W. Barthlott, I. Meusel, and I. Theisen, "Mikromorphologie der Epicuticularwachse und die Systematik der Magnoliidae, Ranunculidae und Hamamelididae," Tropische und subtropische Pflanzenwelt., vol. 90, pp. 5-60. 1994.

[7] W. Barthlott, I. Theisen, T. Borsch, and C. Neinhuis, "Epicuticular waxes and vascular plant systematics: integrating micromorphological and chemical data. In: Stuessy, Mayer, Hörandl (Eds.): Deep morphology. Toward a renaissance of morphology in plant systematics," Gantner Verlag Rugell, Liechtenstein. 2003.

[8] Z. Yousuf, K. Z. Shinwar, R. Asghar, and A. Parveen, "Leaf epidermal anatomy of selected Allium species, family Alliaceae from Pakistan," J. Bot., vol. 40(1), pp. 77-90. 2008.

[9] R. Veeramohan, and N. Watharon, "Macromorphological and micromorphological studies of four selected Passiflora species in peninsular Malaysia," Pak. J. Bot. vol. 47(2), pp. 485-492. 2015.

[10] D. T. Leandro, S. E. Dias, and O. C. R. Arruda, "Micromorphology and anatomy of the leaf blade: a contribution to the taxonomy of Luziola (Poaceae, Oryzoideae) from the Pantanal, Brazil," Plant Systematic and Evolution., vol. 302 (3), pp. 265-273. 2016.

[11] B. Mankovska, B. Godzik, O. Badea, Y. Shparyk, and P. Moravcik, "Chemical and morphological characteristics of key tree species of the Carpathian Mountains," Environ. Pollut., vol. 130(1), pp. 41-54. 2004.

[12] R. Riccio, M. Trevisan, and E. Capri, "Effect of surface waxes on the persistence of chlorpyrifos-methyl in apples, strawberries and grapefruits," Food Addit. Contam., vol. 23(7), pp. 683-692. 2007.

[13] H. J. Ensikat, and W. Barthlott, "Liquid substitution: A versatile procedure for SEM specimen preparation of biological materials without drying or coating," J. Microsc., vol. 172(3), pp. 195-203. 1993.

[14] W. L. Theobald, J. L. Krahulik, and R. C. Rollins, "Trichome description and classification," In C.R. Metcalfe and L. Chalk (eds.), Anatomy of the Dicotlydons, 2nd edition, Clarendon Press, Oxford., vol. 1, pp. 40-53. 1979.

[15] M. R. W. Batterman, and T. G. Lammers, "Branched foliar trichomes of Lobeliodeae (Campanulaceae) and the infrageneric classification of Centropogon," Syst. Bot., vol. 29(2), pp. 448-458. 2004.

[16] L. A. Khokhar, T. M. Rajput, and S. S. Tahir, "Taxonomic study of the trichomes in the some members of the genus Convolvulus (Convolvulaceae) ," Pak. J. Bot., vol. 44(4), pp. 1219-1224. 2010.

[17] S. M. Hajiabad, M. Hardt, and B. Honermeier, "Comparative investigation about the trichome morphology of Common oregano (Origanum vulgare L. subsp. vulgare) and Greek oregano (Origanum vulgare L. subsp. hirtum) ," Journal of Applied Research on Medicinal and Aromatic Plants., vol. 1(2), pp. 50-58. 2014.

[18] R. C. Rollins, "Evidence for natural hybridity between Guayule (Parthenium aryentatum) and Mariola (Parthenium incanum) ," Am. J. Bot., vol. 31(2), pp. 93-99. 1944.

[19] R. C. Rollins, "Some known and probable levels of reciprocal introgression between Guayule (Parthenium argentatum) and Mariola (Parthenium incanum)," [Abs.] Genetics., vol. 30(4), pp. 18 -19. 1945.

[20] O. E. Schultz, "Cruciferae," Pp. 227-658 in: Engler, A. & Harms, B. (ed.), Die natürlichen Pflanzenfamilien, ed. 2. 17b. Leipzig. 1936.

[21] J. M. Cowan, "The Rhododendron Leaf: A study of the epidermal appendages," Oliver and Boyd, Edinburgh. pp. 120. 1950.

[22] N. Ramayya, "Studies on the trichomes of some Compositae I. General structure," Bull. Bot. Surv. India., vol. 4, pp. 177-188. 1962.

[23] A. K. Khalik, "Morphological studies on trichomes of Brassicaceae in Egypt and taxonomic significance," Acta. Bot. Croat., vol. 64(1), pp. 57-73. 2005.

[24] P. H. Davis, and V. H. Heywood, "Principles of angiosperm taxonomy," Krieger. pp.559. 1973.

[25] C. Clark, W. C. Thompson, and D. W. Kyhos, "Comparative morphology of the leaf trichomes of Encelia (Compositae: Heliantheae) ," Botanical Society of America, Misc. Publ., pp.158. 1980.

[26] T. Borsch, C. Löhne, K. Müller, S. Wanke, A. Worberg, W. Barthlott, C. Neinhuis, K. W. Hilu, and D. Quandt, "Towards understanding basal angiosperm diversification: recent insights using fast evolving genomic regions," Nova. Acta. Leopold., vol. 342, pp. 85-110. 2005.

[27] C. Neinhuis, S. Wanke, K. W. Hilu, K. Muller, and T. Borsch, "Phylogeny of Aristolochiaceae based on parsimony, likelihood, and Bayesian analyses of trnL-trnF sequences," Plant . Syst. Evol., vol. 250(1-2), pp. 7-26. 2005.

[28] S. Wanke, F. Gonzales, and C. Neinhuis, "Systematics of pipevines: combining morphological and fast-evolving molecular characters to investigate the relationships within Aristolochioideae (Aristolochiaceae)," Int. J. Plant. Sci., vol. 167(6), pp. 1215-1227. 2006.

[29] J. Murata, T. Ohi-Thoma, S. Wu, D. Darnaedi, T. Sugawara, T. Nakanishi, and H. Murata, "Molecular phylogeny of Aristolochia (Aristolochiaceae) inferred from matK sequences," Acta Phytotaxon Geobot., vol. 52(1), pp. 75-83. 2001.

[30] T. Ohi-Toma, T. Sugawara, H. Murata, S. Wanke, C. Neinhuis, and J. Murata, "Molecular Phylogeny of Aristolochia sensu lato (Aristolochiaceae) based on Sequences of rbcL, matK, and phyA Genes, with special reference to differentiation of Chromosome numbers," Syst. Bot. vol. 31(3), pp. 481-492. 2006.

[31] E. Nardi, "The genus Aristolochia L. (Aristolochiaceae) in Italy," Webbia. Vol. 38(1), pp. 221-300. 1984.

[32] E. Nardi, "Renewed proposal to reject Aristolochia longa L. (Aristolochiaceae) ," Taxon., vol. 37(4), pp. 978-980. 1988.

[33] E. Nardi, "The genus Aristolochia L. (Aristolochiaceae) in Greece," Webbia., vol. 45(1), pp. 31-69. 1991.

[34] E. Nardi, and C. N. Nardi, "Taxonomic and chlorogical notes on the genus Aristolochia L. (Aristolochiaceae) from the Central and Eastern Mediterranean area," Bot. Helvetica., vol. 97, pp. 155-165. 1987.

[35] E. Nardi, and C. Recceri, "II genere Aristolochia I. In Corsica," Webbia., vol. 41, pp. 225-239. 1987.

[36] P. H. Davis, and M. S. Khan, "Aristolochia in the Near East," Notes Roy. Bot. Gard. Edinburgh., vol. 23, pp. 515-546. 1961.

[37] P. H. Davis, and M. S. Khan, "Two new Aristolochias from Turkey," Notes Roy. Bot. Gard. Edinburgh., vol. 25, pp. 67-69. 1964.

[38] P. H. Davis, and M. S. Khan, "Aristolochia L. in: Flora of Turkey and the East Aegean Islands," Edinburgh., vol. 7, pp. 552-565. 1982.

[39] S. Wanke, "Evolution of the genus Aristolochia: Systematics, Molecular Evolution and Ecology," Dissertation TU Dresden, Germany (online available). pp. 159. 2007.

[40] I. Theisen, and W. Barthhlott, "Mikromorphologie der Epicuticularwachse und die Systematik der Gentianales, Rubiales, Dipsacales und Calycerales," Tropische und subtropische Pflanzenwelt., vol. 89, pp. 7-62. 1994.

[41] H. De Groot, S. Wanke, and C. Neinhuis, "Revision of the genus Aristolochia (Aristolochiaceae) in Africa, Madagascar, and adjacent islands," Bot. J. Linn. Soc., vol. 151(2), pp. 219-238. 2006.

[42] F. González, S. T. Wagner, K. Salomo, L. Symmank, M. S. Samain, S. Isnard, N. Rowe, C. Neinhuis, and S. Wanke, "Present trans-Pacific disjunct distribution of Aristolochia subgenus Isotrema (Aristolochiaceae) was shaped by dispersal, vicariance and extinction," Journal of Biogeography. Vol. 41, pp. 380–391. 2014.

[43] B J. Bliss, S. Wanke, A. Barakat, S. Ayyampalayam, N. Wickett, P. K. Wall, Y. Jiao, L. Landherr, P. E. Ralph, Y. Hu, C. Neinhuis, J. Leebens-Mack, K. Arumuganathan, S. W. Clifton, S. N. Maximova, H. Ma, and C. W. dePamphilis, "Characterization of the basal angiosperm Aristolochia fimbriata: A potential experimental system for genetic studies," BMC Plant Biology., vol. 13: (13). 2013.

[44] S. Wanke, C. Granados Mendoza, S. Müller, A. Paizanni Guillén, C. Neinhuis, A. R. Lemmon, E. Moriaty Lemmon, and M. S. Samain, "Recalcitrant deep and shallow nodes in Aristolochia (Aristolochiaceae) illuminated using anchored hybrid enrichment," Molecular Phylogenetics and Evolution., vol. 117, pp. 111-123. 2017.