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Taxonomic and phylogenetic significance of leaf venation characteristics in *Dioscorea* plants

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Abstract: We undertook a comparative study of the leaf venation characteristics of *Dioscorea* species from all seven sections distributed in China (33 species, 1 subspecies and 3 varieties). We established that leaf venation has a taxonomic significance in *Dioscorea*. The sections Combili, Opsophyton Lasiophyton and Shannicorea all show consistent acrodromous venation (a subtype of palmate venation), with Botryosicyos exhibiting consistent pinnate venation. However, sections Stenophora and Enantiophyllum show obvious intrasectional differences. For example, *D. simulans* and *D. biformifolia* of section Stenophora have acrodromously veined middle leaflets, whereas the side leaflets are pinnately veined; *D. cirrhosa* and *D. cirrhosa* var. *cylindrica* of section Enantiophyllum show dichotomous veinlets. Other species in the same section show either one-branched veinlets or multiple branched veinlets. In addition to the discrepancy in venation patterns, differences in many other important morphological characteristics and chemical components were also observed. Therefore, *D. simulans* and *D. biformifolia* can be reasonably excluded from section Stenophora, and section Illigerestrum can be rebuilt using these two species. In addition, *D. cirrhosa* and *D. cirrhosa* var. *cylindrica* should be removed from section Enantiophyllum and placed into another new section. *D. simulans* and *D. biformifolia* show pinnate venation, and the complexity of their leaf organization (with both simple and compound leaves) also points to variations between the pinnate and acrodromous veins in *Dioscorea*. The repeated presence of palmate and pinnate venation in multiple angiosperm clades also suggests that the formation and evolution of venation are likely the result of evolutionary adaptation to ecological environments.

Key words: *Dioscorea*; leaf venation; micromorphology; section; adaptation

INTRODUCTION

Leaf architecture, such as the shape, size, margin, leaf base, tip, veins and petioles, plays an important role in angiosperm classification, systematics and ecology [1-3]. Compared with other plastic and variable leaf architecture characteristics, venation patterns are significant features for the classification and evolution of angiosperms because their orientation and quantitative characters are relatively stable at the species level [4-5].

The micromorphology of leaf architecture was first explored by paleobotanists in the 1950s [6]. Hickey [7] developed a comprehensive practical classification system and terminology for the microarchitecture of dicot leaves, and proposed the possibility of a stable micromorphological architecture of leaves in plant classification. Since then, leaf venation has been further studied in both dicots and monocots [2,8-12]. For example, Conover et al. [10] found closely related species with different type of veins in Liliidae (reticulate veins and parallel veins) were associated with different numbers of epidermal cells and stomata distributions. By observing the development of corn leaf veins, Chen [11] noted that the morphogenesis of corn leaf veins originated from the center to both sides, with the number and thickness of veins increasing with the expansion of the leaf. Leaf venation characteristics have also been used for systematic classification in orchid plants [12]. Recently, a comprehensive and detailed classification system to describe venation has been further developed by Ellis et al. [1]. In this system, all venation patterns are divided into two fundamental types, pinnate and palmate, with the following additional subtypes included in palmate venation: actinodromous, palinactinodromous, acrodromous, flabellate, parallelodromous and campylodromous.

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Dioscorea plants are a group of monocots widely distributed in tropical and subtropical areas. This group of plants displays many remarkable characteristics that are not often observed in monocots, such as two-furrowed pollens, differentiated twin cotyledons, an apical germ and venation similar to dicots in certain species [13-15]. Therefore, *Dioscorea* is considered a key group in the evolutionary process from dicots to monocots [16,17]. Over 600 species of *Dioscorea* are found worldwide, and they are divided into 22 sections based on morphology, with seven sections distributed in China. The species from different sections of *Dioscorea* show significant variations in leaves. For example, the sections Enantiophyllum, Shannicorea, Opsophyton and Combili only have simple leaves, whereas the sections Botryosicyos and Lasiophyton only have compound leaves. Most Stenophora section species have simple leaves, while others have mixed simple and compound leaves. Fang et al. [4] investigated the leaf characteristics of 13 species and one variety from four sections of *Dioscorea* in Fujian, China, and found compound leaves with pinnate venation in *D. pentaphylla* L. and *D. hispida* Dennst. They found simple leaves with acrodromous venation in *D. persimilis* Prain & Burkill, *D. benthamii* Prain, *D. opposita* Lour., *D. gracillima* Miq., *D. alata* L., *D. tenuipes* Franch, *D. japonica* Thunb., *D. cirrhosa* Thunb., *D. tokoro* Makino, *D. collettii* Hook. f. var. *hypoglauca* Pei et Ting, *D. futschauensis* Uline and *D. bulbifera* L. Given this variation, leaf morphology, in particular venation, was demonstrated to be prominent in the classification of *Dioscorea* plants. However, this study only covered a limited number of *Dioscorea* plants in a narrow distribution that is especially deficient in the compound leaf species.

As a pivotal taxon in the evolution of monocotyledons, Dioscoreaceae occupies a basal position among all extant monocotyledonous plants. The Himalayan-Hengduan Mountains are proposed to be the center of origin and they harbor 52 (21 endemic) *Dioscorea* species with a high diversity of leaf morphology. In this study, a collection of *Dioscorea* species representing all seven sections of distribution in China were used to investigate the characteristics of leaf venation in *Dioscorea* plants. These leaf venation patterns were found to contribute to the taxonomy of *Dioscorea*, and they also provided benchmark data for the adaptation of leaves to different environments. Here we address the following issues: (i) variation of leaf venation in *Dioscorea* by genus, section and species level; (ii) the taxonomic and phylogenetic significance of leaf venation morphology in *Dioscorea*; and (iii) the relationships of leaf venation morphology in *Dioscorea* to the ecological environment.

MATERIALS AND METHODS

Collection of plant materials

Field collection was conducted for 7 sections, 33 species, 1 subspecies and 3 varieties of Chinese *Dioscorea* plants (Table 1). Three to four populations (1-2 individuals per population) per species were investigated,

Table 1. Locations and specimens of the collected Chinese Dioscorea species.

Sect.	Species	Collecting Locations	Voucher Specimen & Location
Stenophora	*D. nipponica*	Linan, Zhejiang Maoxian, Sichuan Tianshui, Gansu Ankang, Shanxi	0648541
	D. nipponica subsp. *rosthornii*	Tianshui, Gansu Badong, Hubei Lishui, Zhejiang Hanzhong, Shanxi	0648571
	D. tokoro	Anhua, Hunan Badong, Hubei Jingdezhen, Jiangxi Xinning, Hunan	0648573
Stenophora	*D. zingiberensis*	Hengshan, Hunan Lijiang, Yunnan Jinfoshan, Chongqing Longnan, Gansu	0646476
	D. sinoparviflora	Lijiang, Yunnan Honghe, Yunnan Guiyang, Guizhou	0648574
	D. deltoidea	Deqin, Yunnan Pingwu, Sichuan Wenshan, Yunnan	0648575
	D. biformifolia	Eshan, Yunnan Guilin, Guangxi Kunming, Yunnan	0648576
	D. gracillima	Huangshan, Anhui Wuyuan, Jiangxi Fuzhou, Jiangxi Yongshun, Hunan	0648542

Table 1. continuited

Stenophora	*D. collettii*	Emei, Sichuan Tianlin, Guangxi Jinghong, Yunnan Badong, Hubei	0648543
	D. collettii var. *hypoglauca*	Linan, Zhejiang Fuzhou, Jiangxi Huangshan, Anhui Lishui, Zhejiang	0648579
	D. futsauensis	Yongtai, Fujian Taojiang, Hunan Lechang, Guangdong	0648580
	D. spongiosa	Hengshan, Hunan Fuzhou, Jiangxi Wenshan, Yunnan Wenzhou, Zhejiang	0648581
	D. tenuipes	Tiantai, Zhejiang Jinfoshan, Chongqing Fuzhou, Jiangxi Xinning, Hunan	0648599
	D. banzuana	Mengzi, Yunnan Jinghong, Yunnan Eshan, Yunnan	0648582
	D. simulans	Guilin, Guangxi Lianzhou, Guangdong Liuzhou, Guangxi	0648583
Combili	*D. esculenta*	Dongle, Hainan Diaoluoshan, Hainan Longzhou, Guangxi Gaozhou, Guangdong	0648585
Shannicorea	*D. subcalva*	Tianlin, Guangxi Mengzi, Yunnan Napo, Guangxi	0648544
	D. subcalva var. *submollis*	Jinfoshan, Chongqin Anning, Yunnan Nandan, Guangxi	0648545
	D. nitens	Mengzi, Yunnan Lijiang, Yunnan Taining, Fujian	0648546
Opsophyton	*D. bulbifera*	Lingshui, Hainan Jinghong, Yunnan Emei, Sichuan Yixing, Jiangsu	0648547
Botryosicyos	*D. melanophyma*	Kunming, Yunnan Mengzi, Yunnan Wangmo, Guizhou	0648548
	D. kamoonensis	Kunming, Yunnan Wenchuan, Sichuan Jinfoshan, Chongqing Ankang, Shanxi	0648549
	D. delavayi	Lingshui, Hainan Mengzi, Yunnan Kunming, Yunnan	0648550
Botryosicyos	*D. pentaphylla*	Nanjing, Fujian Wuyishan, Fujian Lianshan, Guangdong Wenshan, Yunnan	0648551
	D. esquirolii	Longzhou, Guangxi Honghe, Yunnan Xingren, Guizhou	0648552
Lasiophyton	*D. hispida*	Lingshui, Hainan Longzhou, Guangxi Diaoluoshan, Hainan Lincang, Yunnan	0648553
Enantiophyllum	*D. aspersa*	Mengzi, Yunnan Luoping, Yunnan Xingyi, Guizhou	0648588
	D. polystachya	Emei, Sichuan Nanjing, Jiangsu Lishui, Zhejiang Ankang, Shanxi	0648589
	D. japonica	Lin'an, Zhejiang Fenguan, Jiangxi Jinfoshan, Chongqing Wuyuan, Jiangxi	0648590
	D. cirrhosa	Longzhou, Guangxi Wuyuan, Jiangxi Chenzhou, Hubei Lishui, Zhejiang	0648591
	D. cirrhosa var. *cylindrica*	Diaoluoshan, Hainan Wenshan, Yunnan Lincang, Yunnan Lingshui, Hainan	0648592
	D. glabra	Longzhou, Guangxi Badong, Hubei Jinghong, Yunnan Diaoluoshan, Hainan	0648593
	D. fordii	Diaoluoshan, Hainan Dinghushan, Guangdong Wenchuan, Sichuan Kunming, Yunnan	0648594
	D. persimilis	Mingxi, Guangxi Yongshun, Hunan Ruyuan, Guangdong Honghe, Yunnan	0648595
	D. exalata	Tianlin, Guangxi Lishui, Zhejiang Jinghong, Yunnan Diaoluoshan, Hainan	0648596
	D. alata	Nanjing, Fujian Lishui, Zhejiang Lingshui, Hainan Lincang, Yunnan	0648597
	D. decipiens	Jinghong, Yunnan Menglun, Yunnan Tonggu, Jiangxi	0648598

and the 5-8 mature leaves close to the lower part of the plant were collected and pressed for drying. After accurate identification, one voucher specimen per species was prepared and preserved in the herbarium of the Institute of Botany, Chinese Academy of Sciences (NAS), Jiangsu Province.

Experimental methods

The Foster [6] method was used to make the leaves transparent. The items observed and the terminologies used refer to the Manual of Leaf Architecture [1] and studies by Hickey [7] and Yu and Chen [18]. According to the manual, there are two fundamental types of leaf venation: palmate and pinnate. Pinnate venation refers to a leaf or leaflet with only one basal vein (mostly midvein) (Fig. 1a1), whereas palmate venation refers to a leaf with three or more basal veins. Acrodromous venation is a subtype of palmate venation where three or more primaries originate from a point and run in convergent arches towards the leaf apex (Fig. 1a2). Percurrent tertiary veins are the joined tertiary veins from the opposite secondary veins (Fig. 1B). Veinlet refers to the highest-order vein (Fig. 1C). Veinlet development is classified into five levels according to the branches: freely ending veinlets (FEVs) absent, unbranched, one-branched, dichotomous branching and dendritic branching. Areoles are the smallest areas of leaf tissue that are completely surrounded by veins; taken together, they form a contiguous field of polygons over most of the area of the lamina (Fig. 1D). Any order of venation can form one or more sides of an areole. The degree of development of areoles is divided into five levels: areolation lacking (venation ramifies into the intercostal area without producing closed meshes), poor development (polygonal areoles and highly irregular size and shapes), moderate development (areoles of irregular shape that are more or less variable in size but with fewer sides than in poorly developed areolation), good development (areoles of relatively consistent size and shape and generally with 3-6 sides) and paxillate (areoles occurring in distinct oriented fields) (Fig. 1D). Marginal ultimate veins refer to veins at the margin of the leaf, and they have four types: absent (ultimate veins join perimarginal veins), incomplete (marginal ultimate veins recurve to form incomplete loops), spiked (marginal ultimate veins form outward-pointed spikes) and looped (marginal ultimate veins recurved to form loops) (Fig. 1E).

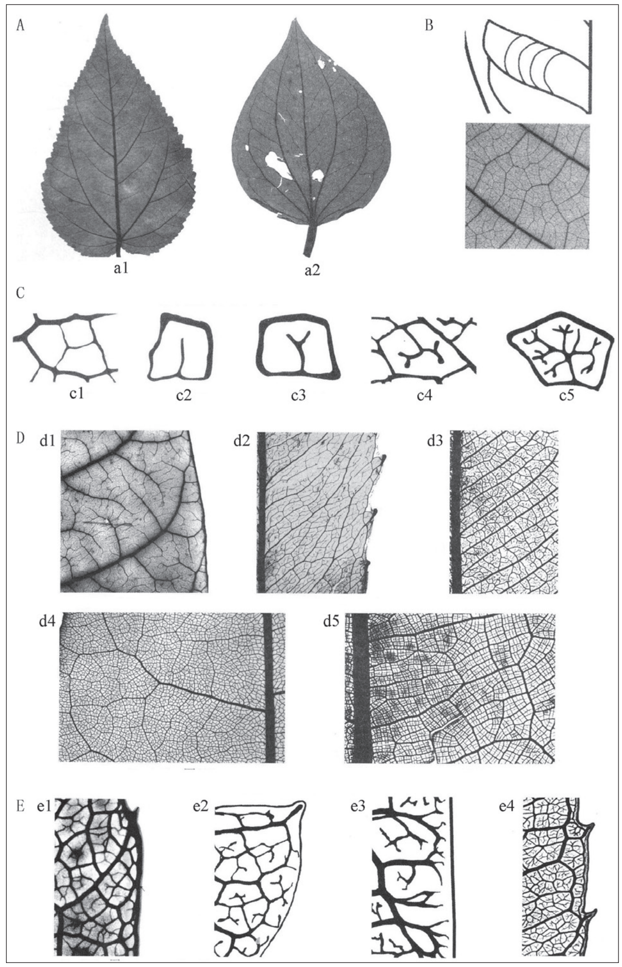

Fig. 1. Graphical presentation of the leaf venation characteristics and terms [11]. **A** – Two types of leaf venation: a1, pinnate venation (*Carrierea calycina*, Salicaceae); and a2, acrodromous venation (*Sarcorhachis naranjoana*, Piperaceae); **B** – Percurrent tertiaries crossing between adjacent secondaries; **C** – Five levels of veinlet development: c1, freely ending veinlets (FEVs) absent; c2, unbranched; c3, one branched; c4, dichotomous branching; and c5, dendritic branching; **D** – Development of areoles: d1, areolation lacking; d2, poor development; d3, moderate development; d4, good development; and d5, paxillate; **E** – Four types of marginal ultimate veins: e1, absent; e2, incomplete; e3, spiked; and e4, looped.

RESULTS

Characteristics of leaf venation in *Dioscorea* plants

Leaf venation of *Dioscorea* plants is either acrodromous with 5-11 basal veins, or pinnate in a few species/sections. Pinnate venation is mainly observed in species with compound leaves. A number of secondaries of acrodromous venation have branches that form at an angle of approximately 65° with the pri-

Table 2. Leaf venation of *Dioscorea* species in China.

Taxon	Leaf type	Major Topology	Primary Vein(s)		Highest Vein Order	Branches of Second-Order Veins	FEVs	Areoles		Marginal Vein Branches
			Number	Convergence At Apex				Shape	Development	
Sect. Stenophora										
D. nipponica	Simple	Acrodromous	11	Yes	6, 7	Multiple	Multiple	Irregular	Poor	Looped
D. nipponica subsp. *rosthornii*	Simple	Acrodromous	11	Yes	6, 7	Multiple	Multiple	Irregular	Poor	Looped
D. tokoro	Simple	Acrodromous	9	Yes	6, 7	Multiple	Multiple	-	Lacking	Looped
D. zingiberensis	Simple	Acrodromous	7	Yes	4, 5	Multiple	One branched	-	Lacking	Incomplete
D. sinoparviflora	Simple	Acrodromous	7	Yes	4, 5	Multiple	One branched	-	Lacking	Looped
D. deltoidea	Simple	Acrodromous	7	Yes	4, 5	Multiple	Multiple	Irregular	Poor	Incomplete
D. biformifolia	Simple & compound	Acrodromous & Pinnate	1[a]	No	5, 6	Multiple	One branched	Irregular	Poor	Looped
D. gracillima	Simple	Acrodromous	9	Yes	4, 5	Multiple	Multiple	Irregular	Poor	Incomplete
D. collettii	Simple	Acrodromous	9	Yes	6, 7	Multiple	Multiple	Irregular	Poor	Looped
D. collettii var. *hypoglauca*	Simple	Acrodromous	9	Yes	4, 5	Multiple	One branched	Irregular	Poor	Incomplete
D. futsauensis	Simple	Acrodromous	7	Yes	6, 7	Multiple	Multiple	Irregular	Poor	Looped
D. spongiosa	Simple	Acrodromous	9	Yes	5, 6	Multiple	One branched	Irregular	Poor	Looped
D. tenuipes	Simple	Acrodromous	9	Yes	5, 6	Multiple	Multiple	Irregular	Poor	Looped
D. banzuana	Simple	Acrodromous	7	Yes	5, 6	Multiple	Multiple	Irregular	Poor	Looped
D. simulans	Simple & compound	Acrodromous & Pinnate	1[a]	No	5, 6	Multiple	One branched	Irregular	Poor	Looped
Sect. Combili										
D. esculenta	Simple	Acrodromous	7	Yes	6, 7	Few	Multiple	Irregular	Poor	Looped
Sect. Shannicorea										
D. subcalva	Simple	Acrodromous	9	Yes	5, 6	Multiple	Multiple	-	Lacking	Looped
D. subcalva var.*submolis*	Simple	Acrodromous	9	Yes	5, 6	Multiple	Multiple	Irregular	Poor	Looped
D. nitens	Simple	Acrodromous	9	Yes	5, 6	Multiple	Multiple	Irregular	Poor	Looped
Sect. Opsophyton										
D. bulbifera	Simple	Acrodromous	11	Yes	4, 5	Multiple	Multiple	-	Lacking	Incomplete
Sect. Botryosicyos										
D. melanophyma	Compound	Pinnate	1[a]	No	3, 4	Multiple	One branched	Irregular	Poor	Incomplete
D. kamoonensis	Compound	Pinnate	1[a]	No	3, 4	Multiple	One branched	-	Lacking	Incomplete
D. delavayi	Compound	Pinnate	1[a]	No	5, 6	Multiple	One branched	Irregular	Poor	Looped
D. pentaphylla	Compound	Pinnate	1[a]	No	5, 6	Multiple	One branched	Irregular	Poor	Incomplete
D. esquirolii	Compound	Pinnate	1[a]	No	5, 6	Multiple	One branched	Irregular	Poor	Incomplete
Sect. Lasiophyton										
D. hispida	Compound	Acrodromous	5	Yes	5	Multiple	One branched	Irregular	Poor	Looped
Sect. Enantiophyllum										
D. aspersa	Simple	Acrodromous	9	Yes	4, 5	Multiple	One branched	Quadrangular & Pentagonal	Moderate	Looped
D. polystachya	Simple	Acrodromous	7	Yes	4, 5	Multiple	Multiple	Quadrangular & Pentagonal	Moderate	Looped
D. japonica	Simple	Acrodromous	9	Yes	4, 5	Multiple	One branched	Irregular	Poor	Looped
D. cirrhosa	Simple	Acrodromous	5	Yes	5, 6	Multiple	dichotomous	Quadrangular & Pentagonal	Moderate	Looped
D. cirrhosa var. *cylindrica*	Simple	Acrodromous	5	Yes	5, 6	Multiple	dichotomous	Quadrangular & Pentagonal	Moderate	Looped
D. glabra	Simple	Acrodromous	7	Yes	4, 6	Multiple	Multiple	Quadrangular & Pentagonal	Moderate	Looped
D. fordii	Simple	Acrodromous	7	Yes	4, 6	Multiple	Multiple	Quadrangular & Pentagonal	Moderate	Looped
D. persimilis	Simple	Acrodromous	7	Yes	6	Multiple	Multiple	Irregular	Poor	Looped
D. exalata	Simple	Acrodromous	7	Yes	4, 5	Multiple	One branched	Irregular & Quadrangular	Moderate	Looped
D. alata	Simple	Acrodromous	7	Yes	5, 6	Multiple	Multiple	Quadrangular & Pentagonal	Moderate	Looped
D. decipiens	Simple	Acrodromous	5	Yes	5, 6	Multiple	Multiple	Irregular	Poor	Looped

[a]Only Midvein exists.

mary vein. The secondaries of the pinnate venation are curved and form an angle less than 45° with the primary vein. The tertiaries are all percurrent. The highest order vein in *Dioscorea* is the 7th. The veinlet has branches and the number of branches vary among different species. Areoles are present in most of the surveyed species, but poorly or moderately developed, and the shapes of areoles in most species are irregular. Marginal ultimate venations of some species recurve to form loops while in the other species they are incomplete (Table 2). The micromorphological characteristics of leaf venation were conserved within species.

In section Stenophora, most species show simple leaves and acrodromous venation (Fig. 2A) except for *D. biformifolia* C. Pei & C. T. Ting and *D. simulans* Prain & Burkill, which show mixed simple and compound leaves. The primary leaves of *D. biformifolia* and *D. simulans* have 2-4 simple leaves with acrodromous venation, whereas the metaphylls have 3 or 5 small leaflets. The two small leaflets at both sides show pinnate venation and semicraspedodromous secondaries (secondaries that branch near the margin) that extend towards the apex, and 1 or 3 middle leaflets show acrodromous venation (Fig. 2B). Seven to 11 primary veins originate from the base of the acrodromous venation, and multiple secondary veins have branches that form an angle of approximately 65° with the primary veins. The highest vein order is the 7th. The veinlet of this section forms multiple branches, although *D. zingiberensis* C. H. Wright, *D. sinoparviflora* C. T. Ting et al., *D. biformifolia*, *D. collettii* var. *hypoglauca* C. T. Ting et al., *D. spongiosa* J. Q. Xi et al. and *D. simulans* have one branch. Most areoles are poorly developed with irregular shapes (Table 2, Fig. 3A), although *D. tokoro* Makino, *D. zingiberensis* and *D. sinoparviflora* do not show clear areoles. The marginal ultimate veins form either complete or incomplete loops (Fig. 3B & C).

Section Combili shows simple leaves with acrodromous venation (Fig. 2C). Seven primaries originate from the base, the secondaries have few branches, and the angle formed with the primaries is larger than 65°. The highest vein order is the 7th. The veinlets have many branches and the areoles are poorly developed and show irregular shapes (Fig. 1d2). The marginal ultimate veins are looped (Fig. 1e4).

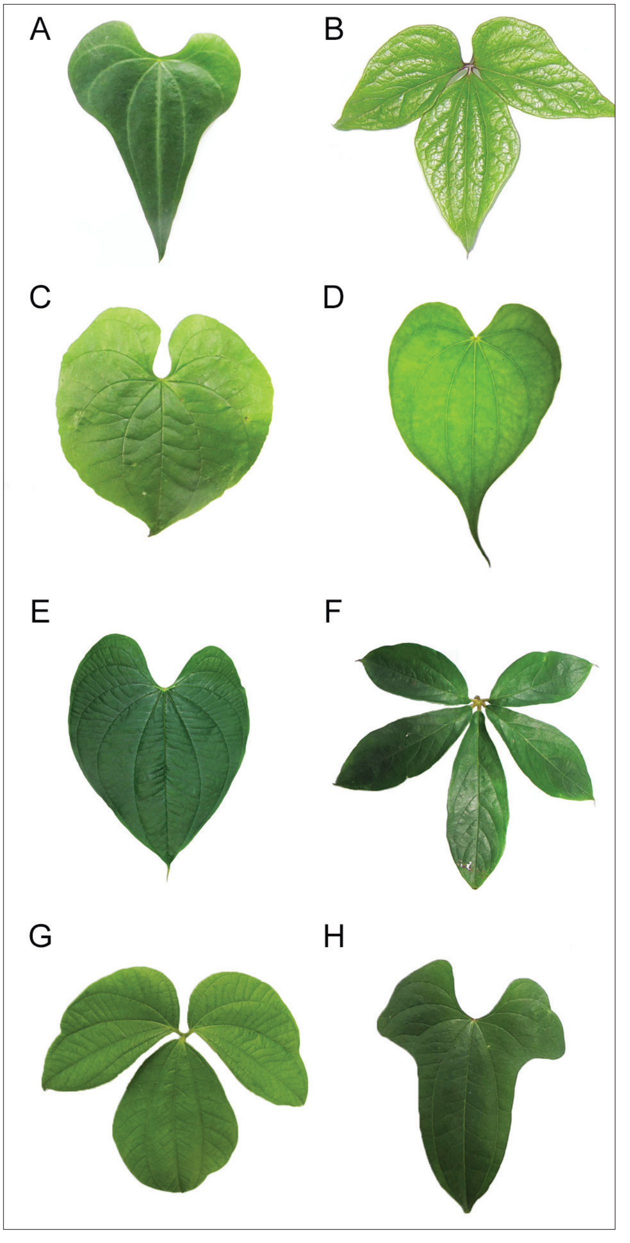

Fig. 2. Venation of *Dioscorea* species from seven sections in China exhibiting acrodromous, pinnate or mixed venation types in different sections. **A** – Sect. Stenophora: acrodromous in species with simple leaves (*D. zingiberensis*). **B** – Sect. Stenophora: mixed in species with compound leaves, middle leaflet acrodromous and side leaflets pinnate (*D. simulans*). **C** – Sect. Combili: acrodromous (*D. esculenta*). **D** – Sect. Shannicorea: acrodromous (*D. subcalva*). **E** – Sect. Opsophyton: acrodromous (*D. bulbifera*). **F** – Sect. Botryosicyos: pinnate (*D. pentaphylla*). **G** – Sect. Lasiophyton: acrodromous (*D. hispida*). **H** – Sect. Enantiophyllum: acrodromous (*D. polystachya*).

Section Shannicorea shows simple leaves with acrodromous venation (Fig. 2D). Nine primaries originate from the base, and there are multiple secondaries that have branches, and the angle formed with the primary vein is larger than 65°. The highest vein order is the 6th. The veinlets have many branches, and the areoles are poorly developed with irregular shapes (Fig. 1d2). However, *D. subcalva* Prain & Burkill does not have areoles and the marginal ultimate veins are looped (Fig. 1e4).

Section Opsophyton shows simple leaves with acrodromous venation (Fig. 2E). Eleven primaries originate from the base: there are multiple secondaries with branches and the angle formed with the primary vein is larger than 65°. The highest vein order is the 5th. The veinlets have many slender branches, but the areoles are lacking in this section (Fig. 1d1). The marginal ultimate veins form incomplete loops (Fig. 1e2).

Section Botryosicyos shows compound leaves with 3-7 leaflets with pinnate venation (Fig. 2F). There are multiple secondaries with branches and the angle formed by the secondaries with the primaries is smaller than 45°. The highest vein order is the 6th. The veinlets have one branch. The areoles are poorly developed with irregular shapes (Fig. 3D), except for *D. kamoonensis* Kunth, which does not have areoles (Fig. 1d1). The marginal ultimate veins all form incomplete loops (Fig. 1e2), except for *D. delavayi* Franch., which displays complete loops (Fig. 3E).

Section Lasiophyton shows compound leaves with 3 leaflets with acrodromous venation (Fig. 2G). Five veins originate from the base; there are many secondaries with branches and the angle formed with the primaries is larger than 65°. The highest vein order is the 5th. The veinlets have one branch, and the areoles are poorly developed with irregular shapes (Fig. 1d2). The marginal ultimate veins form loops (Fig. 1e4).

Section Enantiophyllum shows simple leaves with acrodromous venation (Fig. 2H). Five to nine veins originate from the base; there are many secondaries with branches, and most angles formed with the primaries are larger than 65°. The highest vein order is the 6th. The veinlets have complex branches: *D. aspersa* Prain & Burkill, *D. japonica* Thunberg and *D. exalata* C. T. Ting & M. C. Chang have one branch (Fig. 3F), *D. cirrhosa* Loureiro and its variety *D. cirrhosa* var. *cylindrica* C. T. Ting & M. C. Chang have dichotomous branches (Fig. 3G) and other species have multiple branches. The areoles of most species in this section are moderately developed and show quadrangular and pentagonal shapes (Fig. 3H), although those of *D. japonica*, *D. persimilis* Prain & Burkill and *D. decipiens* J. D. Hooker are poorly developed with irregular shapes (Fig. 1d2). The marginal ultimate veins form loops (Fig. 1e4).

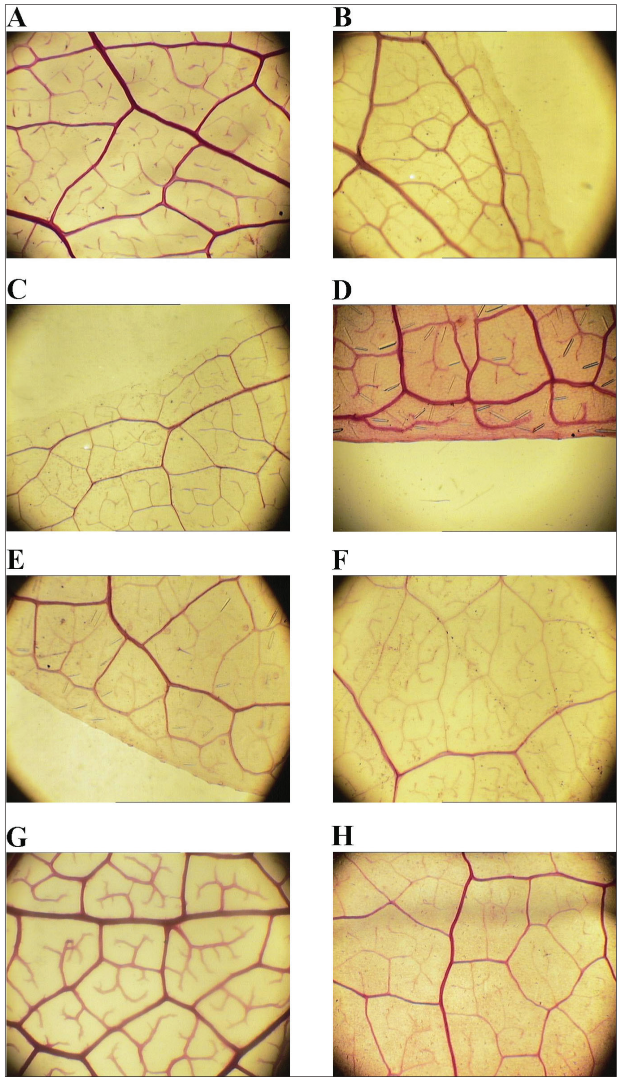

Fig. 3. Micromorphology of the leaf architecture of *Dioscorea* species from different sections displaying veinlets, areoles and marginal ultimate veins. **A** – Irregularly shaped and poorly developed areoles (*D. gracillima*). **B** – Looped marginal ultimate veins (*D. nipponica*). **C** – Incompletely looped marginal ultimate veins (*D. deltoidea*). **D** – Irregularly shaped and poorly developed areoles (*D. esquirolii*). **E** – Looped marginal ultimate veins (*D. delavayi*). **F** – Irregularly square and polygonal areoles (*D. exalata*). **G** – Dichotomous freely ending veinlets (FEVs) (*D. cirrhosa*). **H** – Quadrangular, pentagonal and moderately developed areoles (*D. glabra*).

Table 3. Morphological differences between *D. simulans*, *D. biformifolia* and other species of sect. Stenophora [3,19,21].

Morphological characters	***D. simulans* and *D. biformifolia***	**Other species of sect. Stenophora**
Sex of individuals	Androdioecious	Dioecious
Arrangement of stamens (observed from top)	2 laps	1 lap
Seeds wing	Winged all round, but wing much wider toward capsule base.	Seeds inserted near the middle of axile placentation, and winged all round in most species; seeds inserted near the base of axile placentation, and wing pointing towards the capsule apex in other species.
Staminodes in female flowers	Well developed with intact filaments, anthers and pollens	Undeveloped with filament-like shapes
Leaf organization	Compound and simple mixed	Simple
Trichome hair on leaf epidermis	Absent	Present
Leaf venation	Simple leaves acrodromous; compound leaves with middle leaflet acrodromous, side leaflets pinnate	Acrodromous
Ratios of the thickness of internal to external ground tissue in rhizome	1:0.4	<1:0.2
The average length of rhizomatous myxocytes	323μm	<190μm
Diosgenin content	Very little, almost none	High

Table 4. Morphological differences between *D. cirrhosa, D. cirrhosa* var. *cylindrica* and other species of sect. Enantiophyllum [22-23].

Morphological characters	***D. cirrhosa* and *D. cirrhosa* var. *cylindrica***	**Other species of sect. Enantiophyllum**
Leaf texture	Leathery or leathery-like	Herbaceous
Stem thorn	Present at the lower part of stems	Absent except *D. fordii*
Tuber shape	Ovoid, globose, oblong, or gourd-shaped	Cylindirc
Production of tannin	Yes	No

Key to the sections of *Dioscorea*

The sections of *Dioscorea* can be distinguished using leaf venation characteristics (see also the key below).

1. Leaves simple
2. Marginal ultimate vein fused, looped and leaf blade margin always entire — Sect. Combili
2. Marginal ultimate vein incompletely looped — Sect. Opsophyton
2. Marginal ultimate vein completely looped
3. Areoles irregular shape; blind vein multiple branches — Sect. Shannicorea
3. Areoles mostly quadrate or polygon; blind vein branches complicated — Sect. Enantiophyllum

1. Leaves compound and simple mixed
2. Simple leaves all acrodromously veined; if compound leaves present 3 or 5 leaflets, the middle leaflet acrodromously veined and the side leaflets pinnately veined — Sect. Stenophora
2. Leaflets pinnately veined with 1 midvein — Sect. Botryosicyos
2. Leaflets acrodromously veined — Sect. Lasiophyton

DISCUSSION

Taxonomic significance of the micromorphological architecture of Chinese *Dioscorea* leaves

The morphological architecture of *Dioscorea* leaf venation shows taxonomic significance. Sections Shannicorea and Botryosicyos generally show consistent venation characteristics among species. However, the intrasection variation of venation morphology within sections Stenophora and Enantiophyllum has been illustrated, particularly for controversial species in the traditional taxonomy. For example, *D. simulans* and *D. biformifolia* of section Stenophora have acrodromously veined middle leaflets, whereas the side leaflets are pinnately veined; *D. cirrhosa* and *D. cirrhosa* var. *cylindrica* of section Enantiophyllum show dichotomous veinlets; and other species in the same section show either one-branched veinlets or multiple-branched veinlets.

D. simulans of section Stenophora was officially named by Prain and Burkill in 1931 and was once classified into the section Illigerestrum based on characteristics including number of flowers in the base cymule of male inflorescence, stamens, wing of seeds and leaves. However, careful observation by Pei et al. [19] suggests that the classification characteristics used by Prain and Burkill were biased, and based on the rhizome and stamens characteristics, *D. simulans* was placed into section Stenophora, which was further confirmed in "Flora of China". *D. biformifolia*, which is from the same section, is a novel species determined by Pei et al. [20] based on characteristics of leaf and petiole; apart from flower color, leaflet texture and male stalk, all other characteristics are essentially the same as in *D. simulans*. Both *D. simulans* and *D. biformifolia* have mixed simple and compound leaves. The micromorphological architecture of their leaves indicated that the two small compound leaflets on both sides have pinnate venation and the middle leaflets have acrodromous venation; thus, these species present a completely different morphological architecture compared with that of other species in section Stenophora. Considering the apparent morphological differences in the fruits, seed wings, stamens of mature flowers, proportion of androdioecy, characteristics of leaf trichomes, mucous cells of underground stems and amount of diosgenin [3,19,21], we suggest that section Illigerestrum should not be abolished but rather rebuilt by including *D. simulans* and *D. biformifolia* instead of section Stenophora.

D. cirrhosa and *D. cirrhosa* var. *cylindrica* belong to the advanced evolved section Enantiophyllum. However, the morphology of their leathery leaves, stem thorns and spherical tubers, as well as production of tannins differ from other species in the same section [22,23]. Therefore, certain taxonomists have suggested the exclusion of these two species from Enantiophyllum and the construction of a new section [22]. We observed that the veinlet branches of *D. cirrhosa* and *D. cirrhosa* var. *cylindrica* are dichotomous and show a distinguishable morphological difference compared with those of other species not only in section Enantiophyllum but also in *Dioscorea*. Therefore, our results suggest that *D. cirrhosa* and its variety *D. cirrhosa* var. *cylindrica* should be excluded from section Enantiophyllum and included in a new independent section.

Phylogenetic significance of heterophyllous venation in *Dioscorea*

Dioscorea plants have simple leaves, compound leaves and mixed simple and compound leaves. According to the internationally accepted classification system by Burkill [13], the compound leaves of *Dioscorea* mainly belong to five sections, with the following three distributed in China: Botryosicyos, Lasiophyton (with compound leaves only) and Stenophora (*D. simulans* as well as *D. biformifolia*, with mixed simple and compound leaves).

Whether primary veins converge at the apex determines the type of venation. In *Dioscorea*, the decisive factor is whether leaves are simple or compound. The primary veins of species with simple leaves converge at the apex and all exhibit acrodromous venation, while the species with compound leaves, including *D. simulans*, *D. biformifolia* and section Botryosicyos, show pinnate venation.

Parallelodromous venation is considered a typical morphological characteristic of monocots. Dahlgren et al. [16] posited that monocots without parallelodromous venation, including Dioscoreaceae, Smilacaceae, Alismataceae and Taccaceae, should be the primitive lineages, and this hypothesis was further supported by molecular phylogenetic analyses [24]. Therefore, we propose that the species with pinnate venation was the primitive in *Dioscorea*, and the evolutionary trend from pinnate to acrodromous venation in *Dioscorea* plants could be inferred in *D. simulans* and *D. biformifolia*. The venation of side leaflets is pinnate, whereas that of the middle leaflets is acrodromous. Although the degree of development of monocotyledonous pinnate venation is even below that of poorly developed dicotyledonous venation, obvious characteristic dicotyledonous venation properties are exhibited compared with that of the typical parallelodromous venation of monocots, such as mesh-like areoles, percurrent intercostal tertiaries between secondaries, independent tracheids, a vascular bundle sheath, loops and free ending veinlets [15].

Variations in leaflet number and heterophyllous properties are common among species with compound leaves in *Dioscorea* and typical for *D. simulans*. Other species, such as *D. quartiniana* A. Rich, which is distributed in Africa, even exhibit three types of heterophylls [13].

Relationship of leaf architecture and ecological environment

The leaf venation morphology of all angiosperm orders was investigated [25] and the results showed that pinnate and palmate venation occurs in Dioscoreales, Liliales, Asparagales and Alismatales of monocots, Myrtales, Gentianales and Lamiales of eudicots, and Piperales and Laurales of magnoliids. Therefore, at the systematic scale, the generation and evolution of venation appears unrelated to the evolution of angiosperms. In addition, morphological and molecular analyses all showed that in monocots the characteristics of compound leaves did not originate monophyletically but likely resulted from convergent evolution [10,12,26].

Compound leaves were shown to be associated with shady conditions [27]. Compound leaves with a high leaf surface area are more active with respect to photosynthesis and transpiration than simple leaves. In fact, *Dioscorea* species with compound leaves were more likely to be distributed in tropical region; for example, section. Lasiophyton was observed in a forest in the Philippines, and it had a leaf surface area of up to 996 cm^2; and *D. cochleari-apiculata* De Wild of the section Botryosicyos was observed in the Tanganyika rainforest, and it had a leaf area of up to 1900 cm^2 [13]. In our study, pinnate venation was observed in many species with compound leaves, including *D. simulans* and *D. biformifolia* and section Botryosicyos, which could be explained by the observation that broad leaves which favor shady conditions cannot support themselves mechanically and thus require longitudinal and lateral reinforcement from pronounced primary and secondary veins [26]. Therefore, the generation of venation morphology has a closer relationship with the ecological environment. The compound leaves and different venation morphologies in *Dioscorea* may be the evolutionary result of the plant's adaptation to environmental conditions.

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Supplementary Data

Fig S1. The venation of angiosperms (phylogeny based on Angiosperm Phylogeny Group III) [25].