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Research article

Comparative vascular anatomies of some orchid species

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Bazı orkide türlerinin karşılaştırmalı damar anatomileri

Abstract: In this study, we examined the vascular anatomy of leaves with different morphological features of 11 orchid species. Plant samples were collected from various localities in the Black Sea Region. Fresh leaves were dried and stocked, and their vascular structures were analyzed by clearing and staining. Significant differences were determined in the leaves of taxa in terms of characters such as total leaf perimeter and area, number of veins and nodes, total vein length, total vein area, average vein length, average vein width, average vein surface area, average vein volume, and average areolar area. According to the findings, the topological and morphometric features of the veining can reflect the systematic and phylogenetic relationships of orchids.

Key words: Anatomy, venation network, morphometry, *Orchidaceae*

Özet: Bu çalışmada 11 orkide türüne ait farklı morfolojik özellikleri olan yaprakların damar anatomileri incelenmiştir. Bitki örnekleri Karadeniz Bölgesi'ndeki çeşitli lokalitelerden toplanmıştır. Taze yapraklar kurutularak stoklanmış, saydamlaştırma ve boyama işlemi uygulanarak damar yapıları analiz edilmiştir. Taksonların yapraklarında toplam yaprak çevresi ve alanı, damar ve boğum sayısı, toplam damar uzunluğu, toplam damar alanı, ortalama damar uzunluğu, ortalama damar genişliği, ortalama damar yüzey alanları, ortalama damar hacmi ve ortalama areol alanı gibi karakterler bakımından önemli farklılıklar tespit edilmiştir. Bulgulara göre damarlanmanın topolojik ve morfometrik özellikleri, orkidelerin sistematik ve filogenetik ilişkilerini yansıtabilir.

Anahtar Kelimeler: Anatomi, damar ağı, morfometri, *Orchidaceae*

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1. Introduction

One of the largest families in the angiosperms of *Orchidaceae* display natural distribution with over 200 taxa in Turkey (Güner and Aslan, 2012). Additionally, there are many rare and/or endemic orchid species in our country. In addition to consumption of orchids as drinks (sahlep), use in ice cream production and cultivation as decorative plants, they have economic value as an effective treatment for diseases like tuberculosis, chest pain and asthma (Sezik, 1984; Hossain, 2011). However, due to species displaying distribution in a very large area, excessive intraspecies variation of flowers and leaves especially and high hybridization abilities, there are many systematic problems with orchids (Arditti, 1977; Dressler, 1993). Additionally, mistaken plant identification and different taxonomic opinions further complicate these problems.

The leaves of land plants display diversity in terms of vein architecture (Roth-Nebelsick et al., 2001). Monocot plants have main veins organized parallel to the midrib and anastomosis at the distal and basal tips of the leaf blade. Secondary veins have transverse pattern, and develop between main veins to form short interconnections (Conklin et al., 2019). The architecture of the vascular bundles is affected by the phylogenetic or genetic character of the plant in addition to the ecological conditions it exists in (Sack et al., 2012; Blonder et al., 2020). Additionally, led by carbohydrate and water-carrying capacity, they ensure the occurrence of many basic physiological functions like photosynthesis and perspiration, resistance to injury, mechanical support or lignin-derived costs (Vincent, 1982; Niklas, 1999; Givnish et al., 2005; Brobbrib et al., 2007; Agrawal and Konno, 2009; Katifori et al., 2010; Brodribb et al., 2016; John et al., 2017; Blonder et al., 2011, 2018; Ohtsuka et al., 2018). For this reason, the vein shapes represent the basis for studies in many disciplines. However, there are very limited numbers of studies about the vein anatomy and vein patterns in orchids, especially (Cameron and Dickison, 1998; Mani et al., 2021). This research comparatively investigates the vein shapes and structure of 11 genera among representative species included in the *Orchidaceae* family (*Cephalanthera* Rich., *Coeloglossum* Lindl., *Dactylorhiza* Neck. Ex Nevski, *Gymnadenia* R.Br., *Hymantoglossum* Spreng., *Limodorum* L., *Orchis* L., *Platanthera* Rich., *Serapias* L., *Spiranthes* Rich., *Steveniella* Schltr.) distributed in the Black Sea Region to test the usefulness of vein architecture as a characteristic showing systematic relationships between genera. This study was also applied for the first time on the leaf vein structures of orchids in Turkey.

2. Materials and Method

Plant samples were collected from a variety of localities in the Black Sea region (Table 1). For each species, mean 10 leaf samples were pressed and dried for several days at 60 °C. Leaf samples were taken from at least three individuals. Three different leaves for each individuals were studied, with leaf sizes being small, medium and large. ANOVA test was performed on the obtained data to determine the importance of leaf-anatomical data for taxa. Tests were completed with the aid of SPSS 20 program.

2.1. Clearing

The transparency method was applied by taking Vasco et al. (2014) as reference. Accordingly, dry leaf samples were left for 1-3 days at 40-54 °C in 5% NaOH solution.

Samples were washed with distilled water and treated for durations lasting from 20 s to 10 min with 4.5-5.5% sodium hypochlorite. Leaf samples were passed through a graded ethanol series (50%, 70%, 95%) and stained with safranin by applying the standard protocol.

2.2. Photograph Metadata and Analysis

Leaves with scale added were photographed from the same distance using a NIKON D700 brand camera and analyzed with the aid of the LEAF GUI (Price et al., 2011) program. After a series of cropping and cleaning procedures, RGB photographs were converted to grayscale and vein, node and areole features were calculated. While determining the threshold value, global and local adaptive thresholding approaches were used to obtain binary images representing vein regions with one and nonvein regions with zero, and vein segmentation was achieved. In the vein network, nodes and vein tips were determined with representative single pixel width. Metric calculations for veins and the full leaf were completed using software.

3. Results

When the vein topology of leaves from 11 species belonging to the *Orchidaceae* family are investigated, the taxa displayed clear differentiation based on secondary vein pattern. In *Limodorum abortivum* (L.) Sw., the parallel vein pattern disappears at the leaf tips. For *Cephalanthera kotchyana* Renz & Taubenheim, *Coeloglossum viride* Hartm., *Dactylorhiza urvilleana* (Steud.) H.Baumann & Künkele, and *Gymnadenia conopsea* (L.) R.Br. secondary connections between parallel veins are notable (Fig. 1). These connections intensify toward the leaf tip.

Table 1. List of *Orchidaceae* taxa, localities and collection date

Taxa	Subfamily	Locality	Collection date	Voucher	Habitats	Elevation
Cephalanthera kotschyana	E	Kavak, Samsun	April, 2015	Ss, 29	Deciduous forests	750
Cephalanthera kotschyana	E	Kurupelit, Samsun	April, 2015	Omuhb, 7711	Open forests	225
Coeloglossum viride	O	Köprübaşı, Trabzon	June, 2015	Omub, 8253	Meadows	1820
Dactylorhiza urvilleana	O	Köprübaşı, Trabzon	June, 2015	Omub, 7787	Open forests, meadows	850
Dactylorhiza urvilleana	O	Kavron, Rize	June, 2015	Omub, 7818	Alpine to subalpine meadows	2000
Dactylorhiza urvilleana	O	Santa, Gümüşhane	June, 2015	Omub, 8066	Alpine to subalpine meadows	1600
Gymnadenia conopsea	O	Köprübaşı, Trabzon	July, 2015	Mka, 27	Alpine to subalpine meadows	1670
Himantoglossum caprinum	O	Kurupelit, Samsun	June, 2015	Omuhb, 7739	Coniferous forests, open forests	245
Himantoglossum caprinum	O	Boyabat, Sinop	June, 2015	Mka, 28	Coniferous forests,	300
Limodorum abortivum	E	Kavak, Samsun	June, 2015	Mka, 30	Deciduous forests	450
Limodorum abortivum	E	Kurupelit, Samsun	June, 2015	Omuhb, 3036	*Quercus* forests	150
Orchis mascula subsp. *pinetorum*	O	Abant, Bolu	May, 2015	Ss, 16	Edges of coniferous forests	985
Orchis mascula subsp. *pinetorum*	O	Çambaşı, Ordu	May, 2015	Omuhb, 7829	Edges of coniferous forests	1515
Orchis mascula subsp. *pinetorum*	O	Kurupelit, Samsun	May, 2015	Omuhb, 7712	*Quercus* forests	*163*
Orchis mascula subsp. *pinetorum*	O	Köprübaşı, Trabzon	June, 2015	Omuhb, 7725	Open areas, meadows	2300
Platanthera chlorantha	O	Kavak, Samsun	June, 2015	Mka, 33	Deciduous forests	870
Platanthera chlorantha	O	Kurupelit, Samsun	June, 2015	Omuhb, 4123	Deciduous forests, *Quercus* forests	157
Serapias bergonii	O	Ünye, Ordu	May, 2015	Mka, 36	Meadows	60
Serapias bergonii	O	Kale, Giresun	May, 2015	Mka, 34	Meadows	90
Spiranthes spiralis	O	Köprübaşı, Trabzon	Sep, 2015	Mka, 49	Open forests, meadows	860
Spiranthes spiralis	O	Kurupelit, Samsun	Sep, 2015	Mka, 38	Meadows	205
Steveniella satyrioides	O	Ünye, Ordu	May, 2015	Mka, 50	Edges of deciduous forests	90
Steveniella satyrioides	O	Bafra, Samsun	May, 2015	Mka, 40	Edges of deciduous forests	120
Steveniella satyrioides	O	Kurupelit, Samsun	May, 2015	Omuhb, 3041	Deciduous forests, *Quercus* forests	210

E: *Epidendroideae,* O: *Orchidoideae*

In *Orchis mascula* subsp. *pinetorum* (Boiss. & Kotschy) G.Camus, *Serapias bergonii* E.G.Camus and *Steveniella satryoides* (Spreng,) Schltr the secondary vein network is distributed homogeneously throughout the full leaf blade, different to other species. In *Himantoglossum caprinum* (M.Bieb.) Spreng and *Platanthera clorantha* (Cruster) Rchb. the secondary vein network between the primary parallel veins displays net-like pattern. Additionally, the free tipped secondary veins are distributed between areoles in these taxa. In *Spiranthes spiralis* (L.) Chevall, the secondary veins have wave-like pattern and as a result of this pattern, *Spiranthe spiralis* has irregular areoles compared to the rectangular shape of areoles in other species (Fig. 1).

For vein morphometric properties, metric features like total leaf circumference and area; total vein network length and area; vein and node numbers; mean vein length, width, 2D, 3D area and volume; and mean areole equivalent diameter, circumference, area and convex area were investigated. Leaves from photosynthetic or saprophyte species cultivated in different habitats were identified to have significant differences in terms of general leaf properties, vein and areole features. Total leaf circumference and area, vein and node numbers, total vein network length and total area covered by the vein network were higher for *Dactylorhiza urvilleana, Himantoglossum caprinum* and *Platanthera chlorantha* compared to other species. In terms of total leaf features, the lowest values were for *Coeloglossum viride* and *S. spiralis,* while lowest vein and node numbers were observed in *Cephalanthera kotchyana* and *Limodorum abortivum* (Table 2).

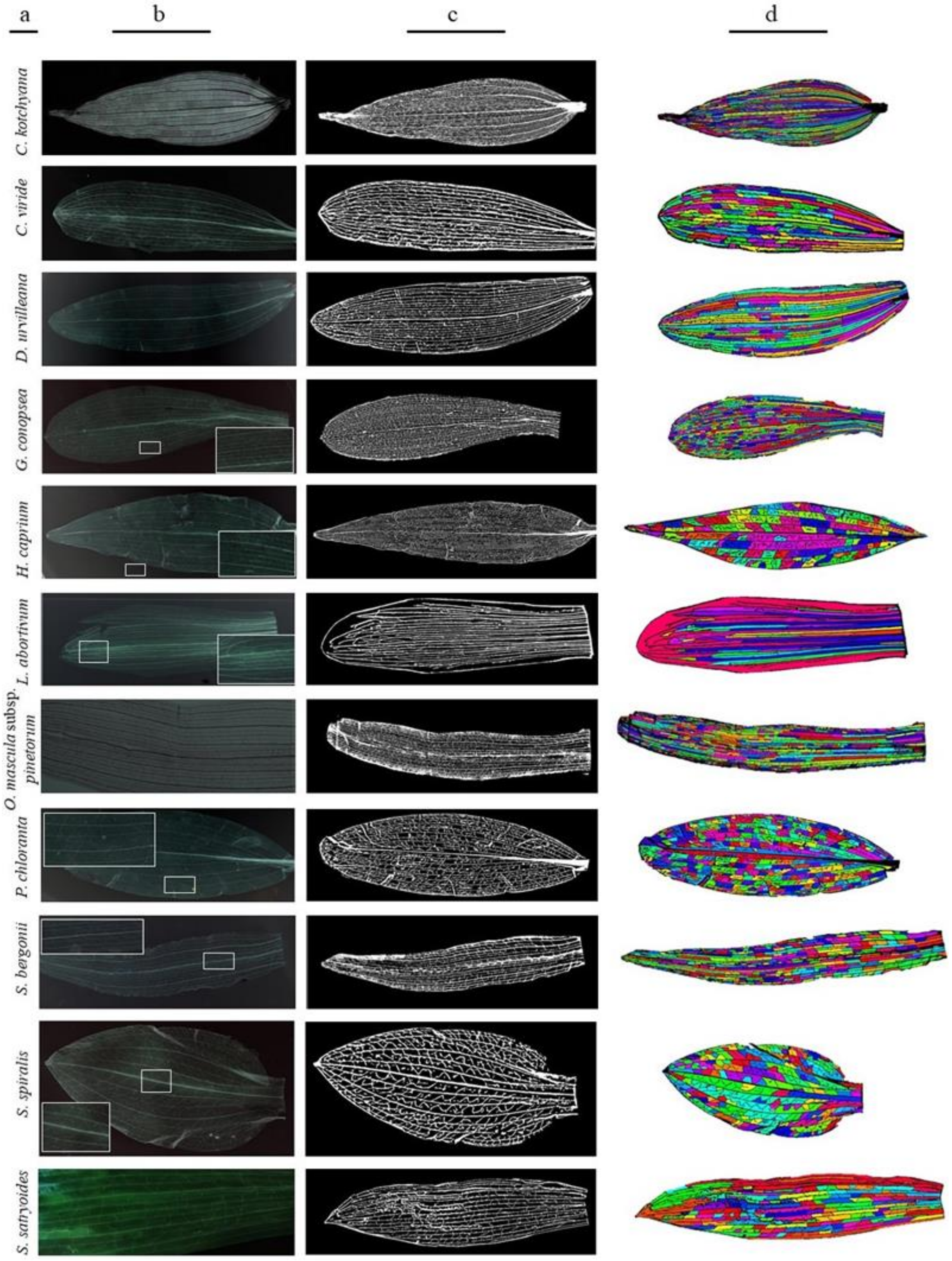

Figure 1. Vein segmentation of taxa. a: taxon b: RGB photographic samples, c: binary images showing veins white and non-vein regions in black, d: image of color labeled areoles (each color represents different areoles)

Table 2. Average morphometric data of the leaf vein characters of taxa

	Leaf						Vein					Areole				
Taxa/ Character	Area (mm^2)	Perimeter (mm)	Total Length of Network (mm)	Total Network Area (mm^2)	Number of Veins	Number of Nodes	Length (mm)	Width (mm)	2D Area (mm^2)	3D Area (mm^2)	Volume (mm^3)	Area (mm^2)	Convex Area (mm^2)	Perimeter (mm)	Equivalent Diameter (mm)	Average Distance (mm)
Cep kot	560.0	146.1	2159.1	210.0	1241	1285	1.69	0.06	0.09	0.29	0.01	1.8	7.3	17.0	0.85	0.04
Coe vir	284.8	88.7	1009.0	110.9	3317	2888	0.23	0.05	0.01	0.05	0.00	0.3	0.5	2.9	0.37	0.04
Dac urv	2867.4	309.1	5230.9	869.0	14033	13106	0.29	0.08	0.03	0.09	0.01	1.5	3.0	5.9	0.49	0.05
Gim con	795.7	187.2	2779.5	280.4	7792	7078	0.30	0.05	0.02	0.05	0.00	0.4	0.6	3.7	0.53	0.05
Him cap	2390.1	256.5	5682.6	717.3	18005	17926	0.23	0.06	0.02	0.05	0.00	1.0	2.2	3.2	0.20	0.03
Lim abo	599.7	110.7	1166.1	178.7	2133	1981	0.48	0.07	0.04	0.13	0.01	1.8	4.1	8.4	0.56	0.04
Orc mas	782.8	234.9	2699.3	307.6	6857	5842	0.33	0.07	0.02	0.07	0.00	0.4	0.5	3.0	0.40	0.04
Pla chl	2562.8	232.5	5200.6	783.9	16740	15361	0.23	0.08	0.02	0.07	0.01	1.1	1.4	4.6	0.63	0.04
Ser ber	351.8	130.9	1106.7	115.3	2417	2140	0.39	0.06	0.02	0.07	0.00	0.4	0.6	3.3	0.47	0.07
Spi spi	327.4	87.3	1201.0	100.9	5370	4735	0.15	0.05	0.01	0.02	0.00	0.2	0.3	1.8	0.28	0.05
Ste sat	842.2	166.7	1818.8	231.4	4454	4077	0.34	0.06	0.02	0.08	0.00	1.1	1.7	5.4	0.63	0.04

The situation is different for features like mean vein dimensions, vein surface area and volume. *Cephalanthera kotchyana* and *Limodorum abortivum* had much higher vein length values compared to other species. Additionally, these species had higher values for 2D and 3D vein area compared to other species. *Spiranthes spiralis* had the lowest values for mean vein dimensions (Table 2,3).

For areole features, clear differences were not observed for criteria like the mean distance (mean Euclidean distance between each areole pixel and vein pixel) and equivalent diameter (diameter of the region with the same normalized secondary axis moment). However, there were variability for criteria like areole area, areole convex area (area of the convex shell covering the areole) and areole circumference in many species. These values were high for species like *Cephalanthera kotchyana* and *Limodorum abortivum* and lowest for *Coeloglossum viride* and *Spiranthes spiralis* (Fig. 2, Table 2).

Table 3. Descriptive statistic for vein and areole of taxa

		Vein					Areole					
Taxa		Length (mm)	Width (mm)	2D Area (mm^2)	3D Area (mm^2)	Volume (mm^3)	Area (mm^2)	Convex Area (mm^2)	Perimeter (mm)	Equivalent Diameter (mm)	Average Distance (mm)
Cep kot	Mean	1.686	0.060	0.092	0.288	0.011	1.848	7.294	17.050	0.846	0.039
	Std. Deviation	4.180	0.063	0.199	0.625	0.029	5.001	21.300	39.912	1.283	0.014
Coe vir	Mean	0.234	0.054	0.015	0.047	0.002	0.310	0.451	2.942	0.372	0.041
	Std. Deviation	0.368	0.029	0.031	0.098	0.006	0.698	1.165	5.828	0.506	0.024
Dac urv	Mean	0.286	0.082	0.029	0.091	0.006	1.488	2.985	5.934	0.490	0.048
	Std. Deviation	0.550	0.055	0.080	0.253	0.036	7.974	22.780	26.640	1.287	0.043
Gim con	Mean	0.298	0.051	0.017	0.053	0.002	0.412	0.583	3.667	0.529	0.055
	Std. Deviation	0.430	0.022	0.031	0.098	0.005	1.071	2.067	7.697	0.495	0.025
Him cap	Mean	0.232	0.060	0.017	0.055	0.003	1.010	2.249	3.196	0.204	0.034
	Std. Deviation	0.407	0.033	0.057	0.180	0.046	12.545	30.203	35.144	1.116	0.015
Lim abo	Mean	0.478	0.066	0.041	0.128	0.006	1.763	4.125	8.369	0.558	0.042
	Std. Deviation	1.066	0.035	0.103	0.323	0.018	11.368	37.811	41.429	1.393	0.037
Orc mas	Mean	0.327	0.066	0.022	0.068	0.003	9.579	10.085	6.310	0.482	0.041
	Std. Deviation	0.499	0.042	0.033	0.103	0.006	77.204	81.280	49.989	3.486	0.114
Pla chl	Mean	0.227	0.081	0.022	0.069	0.006	1.077	1.438	4.595	0.628	0.070
	Std. Deviation	0.278	0.059	0.058	0.182	0.070	3.547	5.437	12.131	0.989	0.064
Ser ber	Mean	0.388	0.060	0.023	0.072	0.003	0.393	0.594	3.283	0.471	0.052
	Std. Deviation	0.617	0.034	0.033	0.104	0.004	1.317	3.302	9.572	0.528	0.028
Spi spi	Mean	0.154	0.047	0.008	0.025	0.001	0.218	0.297	1.787	0.277	0.040
	Std. Deviation	0.170	0.024	0.012	0.037	0.002	0.766	1.068	4.585	0.449	0.030
Ste sat	Mean	1.686	0.060	0.092	0.288	0.011	1.114	1.666	5.406	0.634	0.063
	Std. Deviation	4.180	0.063	0.199	0.625	0.029	3.287	6.403	16.438	1.010	0.058

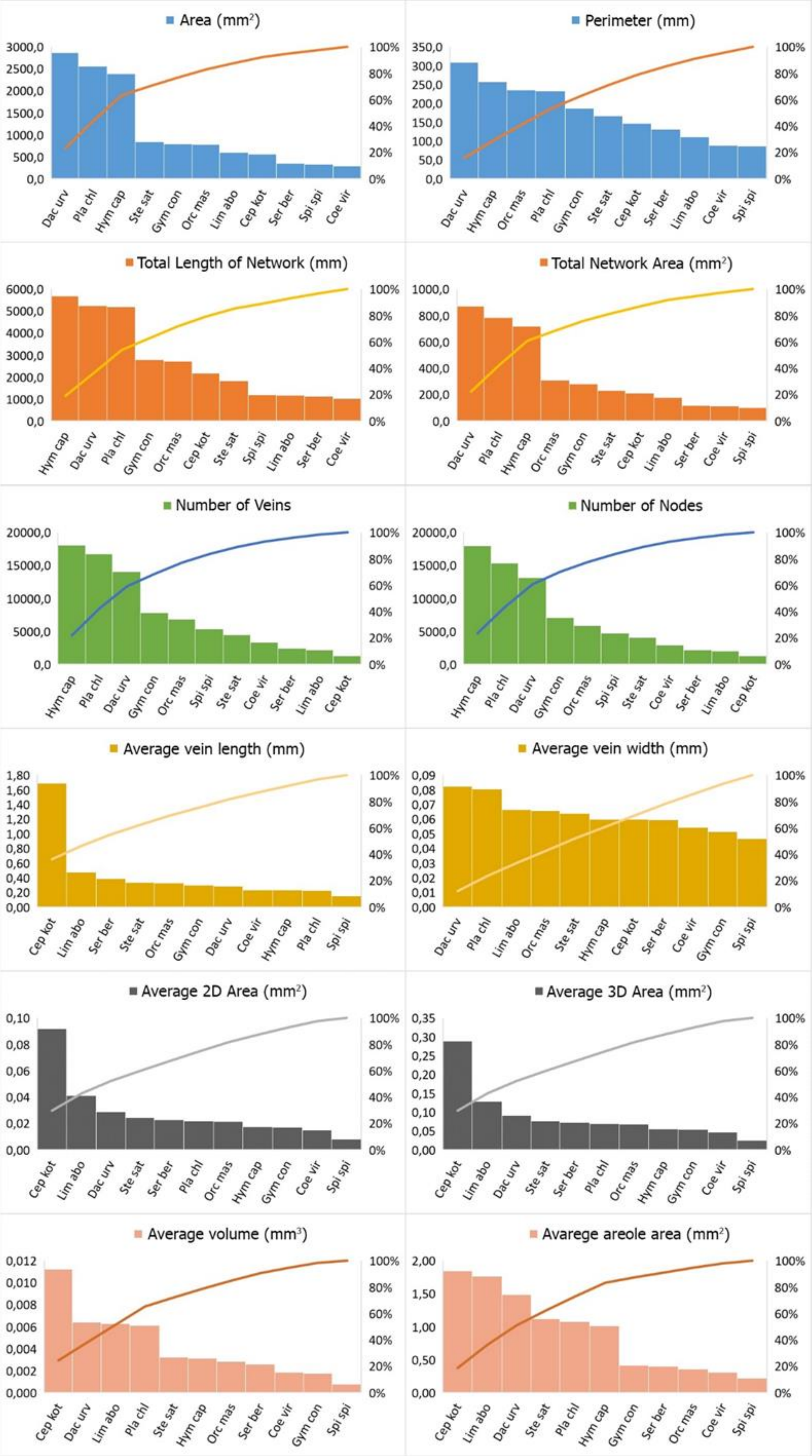

Figure 2. Pareto graphs of vein morphometric data

It was revealed that the characters using ANOVA test were important for at least one taxa (Table 4).

4. Discussions

Orchidaceae is taxonomically divided into five subfamilies (*Epidendroideae, Orchidoideae, Vanilloideae, Cyprepedioideae* and *Apostasioideae*). The species common in Turkey are from the *Epidendroideae* and *Orchidoideae* subfamilies. The members of this family are common in the whole country (Sezik, 1984). The leaf vein topographic and morphometric features of some representative orchid species growing in Turkey were comparatively assessed between genera. These features clearly separated the epidendroid and orchidoid taxa. Software defined the junction point of two or more vein elements as a node and defined the vein segments between the two nodes as veins (Larese et al., 2014). The vein and node numbers in epidendroid species were fewer than for orchidoid species. In other words, sparse location of secondary connecting veins between parallel veins causes higher mean vein and areole dimensions. For this reason, these characteristics reflect the phylogenetic differentiation between two subfamilies (epidendroid and orchidoid) of the investigated species (Cameron et al., 1999).

Among *Orchideae* tribes members which were the topic of the study, *Spiranthes spiralis* was classified within the *Cranichideae* tribes, different to other orchidoid species. This is different to other orchidoid taxa due to leaves characterized by wavy secondary veins and irregular areole shapes. In this group, *Coeloglossum viride*, *Dactylorhiza urvilleana,* and *Gymnadenia conopsea* taxa are closely related on dendrograms obtained with molecular markers (Pridgeon, 1997; Batemann et al., 2003). In these three taxa, secondary vein connections intensify toward leaf tips, and the topology of these veins may indicate characteristics shared at the genera level.

Table 4. ANOVA analysis for vein and areole characters

	Charachters		Sum of Squares	df	Mean Square	F	Sig.
Vein	Length	Between Groups	2967.46	10.00	269.77	534.40	0.00
	Width		12.24	10.00	1.11	580.45	0.00
	2D Area		10.82	10.00	0.98	244.20	0.00
	3D Area		106.76	10.00	9.71	244.20	0.00
	Volume		0.40	10.00	0.04	20.94	0.00
Areole	Area		7098.31	10.00	645.30	8.10	0.00
	Convex Area		19606.59	10.00	1782.42	5.84	0.00
	Perimeter		44539.87	10.00	4049.08	9.39	0.00
	Equivalent Diameter		229.52	10.00	20.87	23.38	0.00
	Average Distance		1.36	10.00	0.12	79.67	0.00

Many species may be characterized in terms of criteria evaluating vein and areole structure. The results show that the topology of secondary veins between parallel veins in orchids are preserved during evolution. For this reason, it is considered that when preliminary preparation procedures are completed correctly and carefully, this method will provide easy, rapid and reliable data which will be beneficial to assess relationships between genera in problematic groups like orchids or to identify possible intergeneric hybrid taxa.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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References

Agrawal AA, Konno K (2009). Latex: A model for understanding mechanisms, ecology, and evolution of plant defense against herbivory. Annual Review of Ecology Evolution and Systematics 40:311-331.

Arditti J (1977). Orchid biology, reviews and perspectives. Ithaca: Cornel Univ. Press.

Bateman RM, Hollingsworth PM, Preston J, Yi-Bo L, Pridgeon AM, Chase MW (2003). Molecular phylogenetics and evolution of *Orchidinae* and selected *Habenariinae* (*Orchidaceae*). Botanical journal of the Linnean Society 142(1): 1-40.

Blonder B, Both S, Jodra M, Xu H, Fricker M, Matos IS, Majalap N, Burslem DFRP, Teh YA, Malhi Y (2020). Linking functional traits to multiscale statistics of leaf venation networks. New Phytologist 228: 1796-1810.

Blonder B, Salinas N, Bentley LP, Shenkin A, Chambi Porroa PO, Valdez Tejeira Y, Asner GP (2018). Structural and defensive roles of angiosperm leaf venation network reticulation across an Andes–Amazon Elevation Gradient. Journal of Ecology 106(4): 1683-1699.

Blonder B, Violle C, Bentley LP, Enquist BJ (2011). Venation networks and the origin of the leaf economics spectrum. Ecology letters 14(2): 91-100.

Brodribb TJ, Bienaimé D, Marmottant P. (2016). Revealing catastrophic failure of leaf networks under stress. Proceedings of the National Academy of Sciences 113(17): 4865-4869.

Brodribb TJ, Feild TS, Jordan GJ (2007). Leaf maximum photosynthetic rate and venation are linked by hydraulics. Plant Physiology 144(4): 1890-1898.

Cameron KM, Dickison WC (1998). Foliar architecture of vanilloid orchids: insights into the evolution of reticulate leaf venation in monocotyledons. Botanical Journal of the Linnean Society 128(1): 45-70.

Cameron KM, Chase MW, Whitten WM, Kores PJ, Jarrell DC, Albert VA, Goldman DH (1999). A phylogenetic analysis of the Orchidaceae: evidence from rbcL nucleotide sequences. American Journal of Botany 86(2): 208-224.

Conklin PA, Strable J, Li S, Scanlon MJ (2019). On the mechanisms of development in monocot and eudicot leaves. New Phytologist 221(2): 706-724.

Dressler RL (1993). Phylogeny and classification of the orchid family. Oregon: Dioscorides Press.

Givnish TJ, Pires JC, Graham SW, McPherson MA, Prince LM, Patterson TB, Millam KC (2005). Repeated evolution of net venation and fleshy fruits among monocots in shaded habitats confirms a priori predictions: Evidence from an ndhF phylogeny. Proceedings of the Royal Society B: Biological Sciences 272(1571): 1481-1490.

Güner A, Aslan S (Eds.) (2012). Türkiye bitkileri listesi:(damarlı bitkiler). İstanbul: Nezahat Gökyiğit Botanik Bahçesi Yayınları.

Hossain MM (2011). Therapeutic orchids: traditional uses and recent advances an overview. Fitoterapia 82(2): 102-140.

John GP, Scoffoni C, Buckley TN, Villar R, Poorter H, Sack L (2017). The anatomical and compositional basis of leaf mass per area. Ecology Letters 20(4): 412-425.

Katifori E, Szöllősi GJ, Magnasco MO (2010). Damage and fluctuations induce loops in optimal transport networks. Physical Review Letters 104(4): 048704.

Larese MG, Namías R, Craviotto RM, Arango MR, Gallo C, Granitto PM (2014). Automatic classification of legumes using leaf vein image features. Pattern Recognition 47(1): 158-168.

Mani M, Rasangam L, Selvam P, Shekhawat MS (2021). Micro-morpho-anatomical mechanisms involve in epiphytic adaptation of micropropagated plants of *Vanda tessellata* (Roxb.) Hook. ex G. Don. Microscopy Research and Technique 84: 712-722.

Niklas KJ (1999). A mechanical perspective on foliage leaf form and function. The New Phytologist 143(1): 19-31.

Ohtsuka A, Sack L, Taneda H (2018). Bundle sheath lignification mediates the linkage of leaf hydraulics and venation. Plant, Cell and Environment 41(2): 342-353.

Price CA, Symonova O, Mileyko Y, Hilley T, Weitz JS (2011). Leaf extraction and analysis framework graphical user interface: Segmenting and analyzing the structure of leaf veins and areoles. Plant Physiology 155(1): 236-245.

Pridgeon AM (1997). Phylogenetics of subtribe *Orchidinae* (*Orchidoideae*, *Orchidaceae*) based on nuclear ITS sequences. 1. Intergeneric relationships and polyphyly of *Orchis* sensu lato. Lindleyana 12: 89-109.

Roth-Nebelsick A, Uhl D, Mosbrugger V, Kerp H (2001). Evolution and function of leaf venation architecture: a review. Annals of Botany 87(5): 553-566.

Sack L, Scoffoni C, McKown AD, Frole K, Rawls M, Havran JC, Tran T (2012). Developmentally based scaling of leaf venation architecture explains global ecological patterns. Nature Communications 3(1): 1-10.

Sezik EE (1984). Orkidelerimiz: Türkiye'nin orkideleri. İstanbul: Sandoz Kültür Yayınları.

Vasco A, Thadeo M, Conover M, Daly DC (2014). Preparation of Samples for Leaf Architecture Studies, a Method for Mounting Cleared Leaves. Applications in Plant Sciences 2(9): 1400038.

Vincent JF (1982). The mechanical design of grass. Journal of Materials Science 17(3): 856-860.