PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(2): 742–749
https://doi.org/10.14719/pst.2751

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Comparative study of the leaf anatomy of *Dendrobium* species (Orchidaceae) from South Kalimantan, Indonesia and its taxonomic significance

Muhammad Irfan Makruf, Maryani & Ratna Susandarini*

Faculty of Biology, Universitas Gadjah Mada, Yogyajarta 55281, Indonesia

*Email: ratna-susandarini@ugm.ac.id

OPEN ACCESS

ARTICLE HISTORY
Received: 05 July 2023
Accepted: 28 December 2023

Available online
Version 1.0 : 22 May 2024
Version 2.0 : 25 May 2024

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE

Makruf MI, Maryani, Susandarini R. Comparative study of the leaf anatomy of *Dendrobium* species (Orchidaceae) from South Kalimantan, Indonesia and its taxonomic significance . Plant Science Today. 2024; 11(2): 742–749. https://doi.org/10.14719/pst.2751

Abstract

Dendrobium Sw. is a genus with high species diversity in the Orchidaceae family. One of the difficulties in identifying *Dendrobium* species is when the specimens bear no flowers or other fertile structures. The use of leaf anatomical characters for species identification is one of the possible solutions in dealing with sterile specimens. In this study, leaf anatomy was examined to assess its taxonomic significance in differentiating species and constructing phenetic relationships between sections within the genus *Dendrobium*. Sixteen specimens of *Dendrobium* from 14 species belonging to 9 sections were collected from South Kalimantan. Observations of leaf cross-sections resulted in 60 anatomical characters used for revealing taxonomic relationships between species using cluster analysis and principal component analysis. The result of the cluster analysis showed that the 14 species of *Dendrobium* were grouped into 3 clusters. The grouping patterns of species were corresponded to sectional classification. The results of the principal component analysis indicated that the characters of the vascular bundles, hypodermis, epidermis and cuticle showed a major role in recognizing species from different sections. This study confirmed the role of leaf anatomy as taxonomic evidence for the identification of sterile specimens and for supporting classification at a sectional level in the genus *Dendrobium*.

Keywords

foliar anatomy; Dendrobium; classification; phenetics; taxonomy

Introduction

Orchidaceae is one of the plant families with high species diversity. Based on their growth habit, the members of this family are divided into 2 groups, namely terrestrial and epiphytic orchids (1). Terrestrial orchids are characterized by the absence of bulbs and relatively thin and delicate leaves, while epiphytic orchids are characterized by the presence of large bulbs and relatively thick leaves (2). *Dendrobium* Sw. is one of the 3 largest genera in the Orchidaceae family with approximately 1500 species in the world, mostly known as an ornamental plant and is popular as a parental for hybridization in the orchid breeding programme (3). Most of the *Dendrobium* species are epiphytic with a wide range of geographical distribution including all tropical regions of Asia, spreading northward to Japan, eastward to Tahiti and southward to New Zealand (4). The genus

Dendrobium is divided into 41 sections based on morphological characters (4, 5).

Inventory studies of orchids using the field exploration method are often hindered by the problem of species identification when dealing with specimens without any flowers at the time of collection. The identification of these sterile specimens is then reliant on the morphological characters of vegetative organs, with leaves being the most available ones. Considering that leaf morphological characters often show similarities between species, identification of sterile specimens using leaf anatomical characters becomes a reasonable and scientifically acceptable alternative. Taxonomic studies on various taxa showed the important role of anatomical characters, as reported in genus *Aechmea* in which leaf anatomy was useful for species characterization and delimitation of species groups (6). Similarly, the important role of leaf anatomy in species delimitation and classification was reported in the genus *Microcos* (7).

Kalimantan is an island recognized as a biodiversity 'hotspot' in Indonesia, as a part of the floristic region known as Borneo. The number of orchids that have been described from the whole region of Borneo is around 2500 - 3000 species, of which 40% are endemic to Kalimantan. This number is equivalent to 10% of the total orchid species in the world (8). Taxonomic research based on anatomical characterization of the genus *Dendrobium* from South Kalimantan has never been reported before. Given the great diversity of *Dendrobium* species in Kalimantan, this anatomical study is very important to reveal role of leaf anatomy in species identification and sectional classification within the genus *Dendrobium.* In this study, the leaf anatomical characters of 16 specimens from 14 *Dendrobium* species were examined and analyzed using numerical taxonomy methods.

Materials and Methods

Collection and Identification of Dendrobium spp.

Plant materials used in this study were fresh leaf samples from living specimens of *Dendrobium* species collected from South Kalimantan (Table 1). For species that show variations in flower color, namely *D. anosmum* and *D. flos-wanua*, 2 specimens are used that represent these variations. The specimens were obtained from 3 regencies in South Kalimantan Province, namely Hulu Sungai Selatan, Tanah Bumbu and Banjar (Fig. 1). Specimen identification was carried out based on morphological characters referring to a previous account on *Dendrobium* from Borneo (9). The specimens used in this study are maintained as living collections by the first author. The reason for maintaining voucher specimens as living collections is because they can be used in molecular studies using a DNA-based method that requires fresh materials. Samples of *Dendrobium* leaves were covered with water-absorbing tissue, and put in plastic clips filled with silica gel crystals to keep the leaves in good condition during shipment from South Kalimantan to the Faculty of Biology, Universitas Gadjah Mada.

Procedures for preparing leaf cross-sections, observation of microscopic slides and analysis of anatomical data

Fresh leaves were cut into small pieces of 2-3 cm and placed in FAA solution (formaldehyde, glacial acetic acid, 70% ethanol with the ratio of 0.5: 0.5: 9). Microscopic slides of leaf cross-section was made using an embedding method with paraffin and double staining technique with safranin - fast green (10). Observation of the anatomical characters was carried out using a light microscope equipped with an OptiLab microscope camera. Anatomical characters consisting of qualitative and quantitative data were then converted into numerical values for cluster analysis and principal component analysis. Cluster analysis was

Table 1. Species of *Dendrobium* used in this study.

No.	Species	Section	Collection locality
1.	*Dendrobium aloifolium* (Blume) Rchb.f.	Aporum	Hulu Sungai Selatan
2.	*Dendrobium anosmum* Lindl.	Dendrobium	Tanah Bumbu
3.	*Dendrobium anosmum* Lindl. (2)	Dendrobium	Tanah Bumbu
4.	*Dendrobium calcariferum* Carr	Calcarifera	Tanah Bumbu
5.	*Dendrobium compressum* Lindl.	Platycaulon	Hulu Sungai Selatan
6.	*Dendrobium crumenatum* Sw.	Crumenata	Banjar
7.	*Dendrobium cymboglossum* J.J.Wood & A.Lamb	Calcarifera	Hulu Sungai Selatan
8.	*Dendrobium derryi* Ridl.	Calcarifera	Tanah Bumbu
9.	*Dendrobium flos-wanua* Metusala, P.O'Byrne & J.J.Wood	Calcarifera	Hulu Sungai Selatan
10.	*Dendrobium flos-wanua* Metusala, P.O'Byrne & J.J.Wood (2)	Calcarifera	Tanah Bumbu
11.	*Dendrobium macrostachyum* Lindl.	Dendrobium	Hulu Sungai Selatan
12.	*Dendrobium ovipostoriferum* J.J.Sm.	Formosae	Hulu Sungai Selatan
13.	*Dendrobium paathii* J.J.Sm.	Calcarifera	Hulu Sungai Selatan
14.	*Dendrobium plicatile* Lindl.	Plicatiles	Tanah Bumbu
15.	*Dendrobium sanguinolentum* Lindl.	Calcarifera	Tanah Bumbu
16.	*Dendrobium spurium* (Blume) J.J.Sm.	Fugacia	Hulu Sungai Selatan

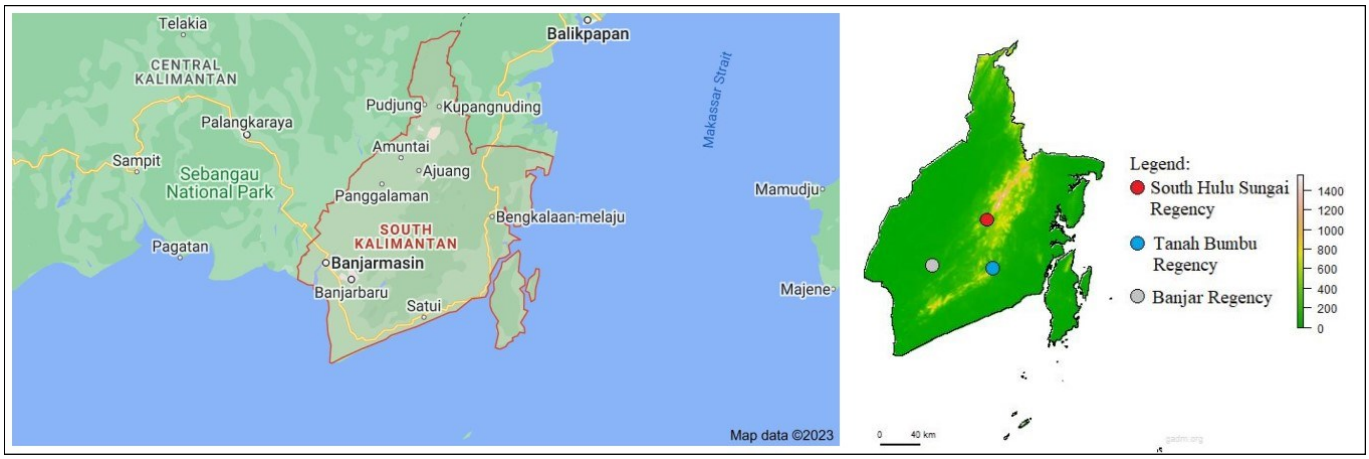

Fig. 1. Map of sampling locations (Google Earth).

performed using Gower General Similarity Coefficient and Unweighted Pair Group Method with Arithmetic Average (UPGMA) clustering technique. Character contribution for the grouping of species was determined based on character loading values obtained from principal component analysis. Data analysis was performed using MVSP software version 3.1 (11).

Results

Observations on leaf cross-sections resulted in 60 anatomical characters for the analysis of taxonomic relationships (Table 2). High variations were found on the cuticle, epidermis, hypodermis, mesophyll and vascular bundles. Variation in cuticle was the thickness of the cuticular layer on the upper and lower surfaces of the leaves. Variation in epidermal tissue was found in the shape and size of the cells.The presence or absence of hypodermal tissue varies between species. The mesophyll showed a variation in the shape and arrangement of the parenchyma cells, the presence of fiber bundles, and the presence of calcium oxalate crystals. Examination of the leaf cross-section revealed that vascular bundles showed the highest variation. Three types of vascular bundles were recognized in *Dendrobium* leaves, namely major vascular bundles, peripheral vascular bundles and accessory vascular bundles. The major vascular bundle was commonly found in the midrib, except for *D. aloifolium* (Blume) Rchb.f. since the leaves of this species are very thick with sharp edges and the major vascular bundles are located at the edges of the leaves. Each type of vascular bundle showed variations in shape, size, existence of xylem fiber cap layer, size of xylem fiber cap, size of xylem cell, existence of phloem fiber cap layer, size of phloem fiber cap, size of phloem cells and the number of stegmata, a typical sclerenchymatous tissue commonly found in the leaves of Orchidaceae. The cross sections of *Dendrobium* species leaves are shown in Fig. 2.

The result of the cluster analysis was shown in the dendrogram showing taxonomic relationships of *Dendrobium* species (Fig. 3). The dendrogram constructed based on 60 leaf anatomical characters showed the formation of 3 clusters which represented the grouping of species from 9 sections. Cluster I composed of 4 species from 3 sections, namely *D. plicatile* Lindl. from the section of Plicatiles, *D. aloifolium* from the section of Aporum and *D. crumenatum* from the section of Crumenata. Cluster II consisted of 2 specimens of *D. anosmum* Lindl., from section *Dendrobium* and *D. spurium* (Blume) J.J.Sm. from the section of Fugacia. Meanwhile, 6 species belonging to the section of Calcarifera were grouped in cluster III with 2 species belonging to the sections of Formosae and Platycaulon.

The anatomical feature that clearly characterized *Dendrobium* species in cluster I was the presence of hypodermis tissue. The hypodermis tissue was found in all 3 species forming cluster I, namely *D. crumenatum, D. aloifolium* and *D. plicatile.* The hypodermis tissue was found in both adaxial and abaxial surfaces. These 3 species also had similarities in the existence of fiber bundles in mesophyll tissue, which became a specific character that was not shared by the other 11 species.

Taxonomic relationships of species in cluster II which consisted of *D. anosmum-1, D. anosmum-2* (section *Dendrobium*) and *D. spurium* (section Fugacia) was marked by similarities in 3 anatomical characters. The 2 specimens of D. *anosmum* showed the highest similarity coefficient of 0.792. The characters shared by these 3 specimens were mesophyll with rounded parenchyma cells, irregular arrangement of parenchyma cells and size of accessory vascular bundles. These anatomical features were identified as the distinguishing characters of species in cluster II from those belonging to cluster III.

Results of cluster analysis showed that there are nine species representing 3 sections grouped into cluster III. These 3 sections are Calcarifera, Formosae and Platycaulon. In this cluster, there are 2 specimens of *D. flos-wanua* Metusala, P.O'Byrne & J.J.Wood which have a similarity coefficient of 0.757. The placement of *D. ovipostoriferum* J.J.Sm. (section Formosae) in the same cluster with 6 species belonging to the section of Calcarifera was mainly due to the similarity in the number of stegmata. The 6 species from the section of Calcarifera shared the characters of the size of the fiber cap in the xylem and the number of fiber caps in the phloem of peripheral vascular bundles. The fiber cap which is composed of thick-walled cells functions as supporting tissue for the leaves. The number of fiber caps in the phloem was thought to be related to the relatively larger size of phloem tissues in spe-

Table 2. List of anatomical characters observed in this study.

No.	Character	Code	No.	Character	Code
1	Thickness of cuticular layer on upper epidermal surface	UEC	31	Length of phloem area on major vascular bundle	VPL
2	Shape of upper epidermal cells	UES	32	Number of stegmata on major vascular bundle	VSN
3	Width of upper epidermal cells	UEW	33	Shape of peripheral vascular bundles	PVS
4	Length of upper epidermal cells	UEL	34	Width of peripheral vascular bundles	PVW
5	Thickness of cuticular layer on lower epidermal surface	LEC	35	Length of peripheral vascular bundles	PVL
6	Shape of lower epidermal cells	LES	36	Number of fibre cap on xylem of peripheral vascular bundles	PFN
7	Width of lower epidermal cells	LEW	37	Width of fibre cap on xylem of peripheral vascular bun-	PFW
8	Length of lower epidermal cells	LEL	38	Length of fibre cap on xylem of peripheral vascular bundles	PFL
9	Hypodermis tissue	HYD	39	Width of xylem area on peripheral vascular bundles	PXW
10	Arrangement of parenchyma cells in mesophyll	MCA	40	Length of xylem area on peripheral vascular bundles	PXL
11	Shape of parenchyma cells in mesophyll	MCS	41	Number of fibre cap on phloem of peripheral vascular bundles	PPN
12	Type of calcium oxalate crystal in mesophyll	MCO	42	Width of fibre cap on phloem of peripheral vascular bundles	PPW
13	The nature of midrib	MMR	43	Length of fibre cap on phloem of peripheral vascular bundles	PPL
14	Number of vascular bundles row across leaf width	MVN	44	Width of phloem area on peripheral vascular bundles	PFW
15	Inter-vascular bundle distances	MVD	45	Length of phloem area on peripheral vascular bundles	PFL
16	Fibre bundles in mesophyll	MFB	46	Number of stegmata on peripheral vascular bundles	PSN
17	Major vascular bundles on leaf margin	VBM	47	Shape of accessory vascular bundles	AVS
18	Position of major vascular bundle	VBP	48	Width of accessory vascular bundles	AVW
19	Shape of major vascular bundle	VBS	49	Length of accessory vascular bundles	AVL
20	Width of of major vascular bundle	VBW	50	Number of fibre cap layer on xylem of accessory vascular bundles	AFN
21	Length of major vascular bundle	VBL	51	Width of fibre cap on xylem of accessory vascular bundles	AFW
22	Number of fiber cap layers on xylem of major vascular bundle	VFN	52	Length of fibre cap on xylem of accessory vascular bundles	AFL
23	Width of fiber cap layers on xylem of major vascular bundle	VFW	53	Width of xylem area on accessory vascular bundles	AXW
24	Length of fiber cap layers on xylem of major vascular bundle	VFL	54	Length of xylem area on accessory vascular bundles	AXL
25	Width of xylem area on major vascular bundle	VXW	55	Number of fibre cap layer on phloem of accessory vascular bundles	APN
26	Length of xylem area on major vascular bundle	VXL	56	Width of fibre cap on phloem of accessory vascular bundles	APW
27	Number of fiber cap layers on phloem of major vascular bundle	VFP	57	Length of fibre cap on phloem of accessory vascular bundles	APL
28	Width of fiber cap layers on phloem of major vascular bundle	VFV	58	Width of phloem area on accessory vascular bundles	APV
29	Length of fiber cap layers on phloem of major vascular bundle	VFH	59	Length of phloem area on accessory vascular bundles	APH
30	Width of phloem area on major vascular bundle	VPW	60	Number of stegmata on accessory vascular bundles	ASN

Note: all measurements were made in µm, based on observations of 10 replicates.

cies from the section of Calcarifera.

In this study, 2 numerical taxonomic methods were used in data analysis, cluster analysis and principal component analysis (PCA). Cluster analysis was performed to determine taxonomic relationships recognized from the grouping pattern of species, while principal component analysis was carried out to identify the contribution of characters in the grouping of species. The results of PCA were presented as a 2-dimensional scatter plot of species (Fig. 4) and character loadings extracted from the first and second principal components (Table 3). Referring to the scatter plot and the character loadings, 6 characters were identified as differentiating features of cluster I from clusters II and III. These characters were indicated by the score of character loadings > 0.2 on the second principal component. These 6 characters were: (a) the thickness of cuticular layer on upper epidermal surface, (b) the thick-

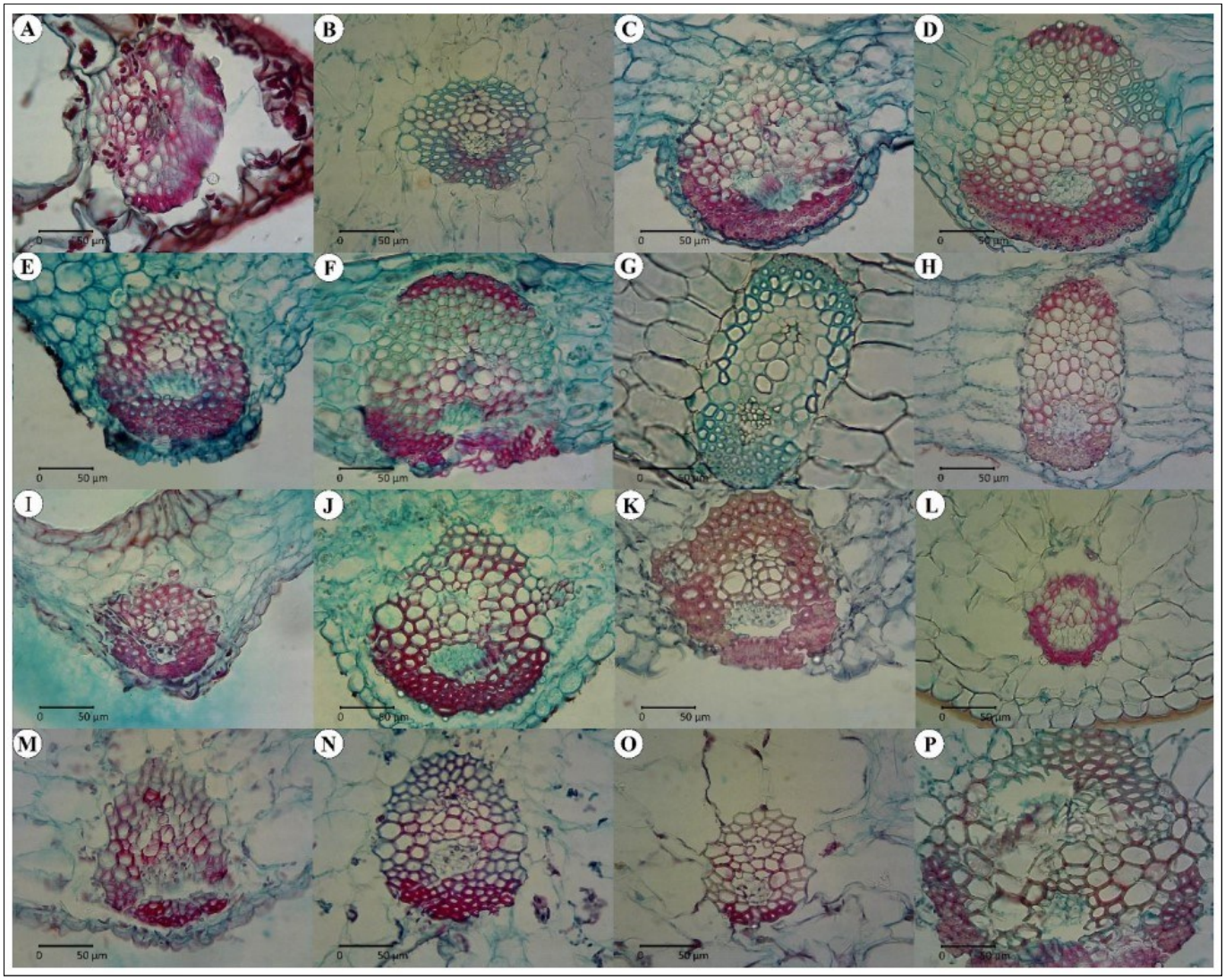

Fig. 2. Variations of major vascular bundles on leaves of *Dendrobium* species. **A.** *D. aloifolium*; **B**. *D. crumenatum*; **C**. *D. derryi;* **D**. *D.cymboglossum*; **E**. *D. calcariferum;* **F**. *D. sanguinolentum*; **G**. *D. flos-wanua 1*; **H**. *D.flos-wanua 2*; **I**. *D. paathii*; *D. compressum*; **K**. *D. oviposteriferum*; **L**. *D. spurium*; **M**. *D. macrostachyum*; **N**. *D. anosmum 1*; **O**. *D. anosmum 2*; **P**. *D. plicatile.*

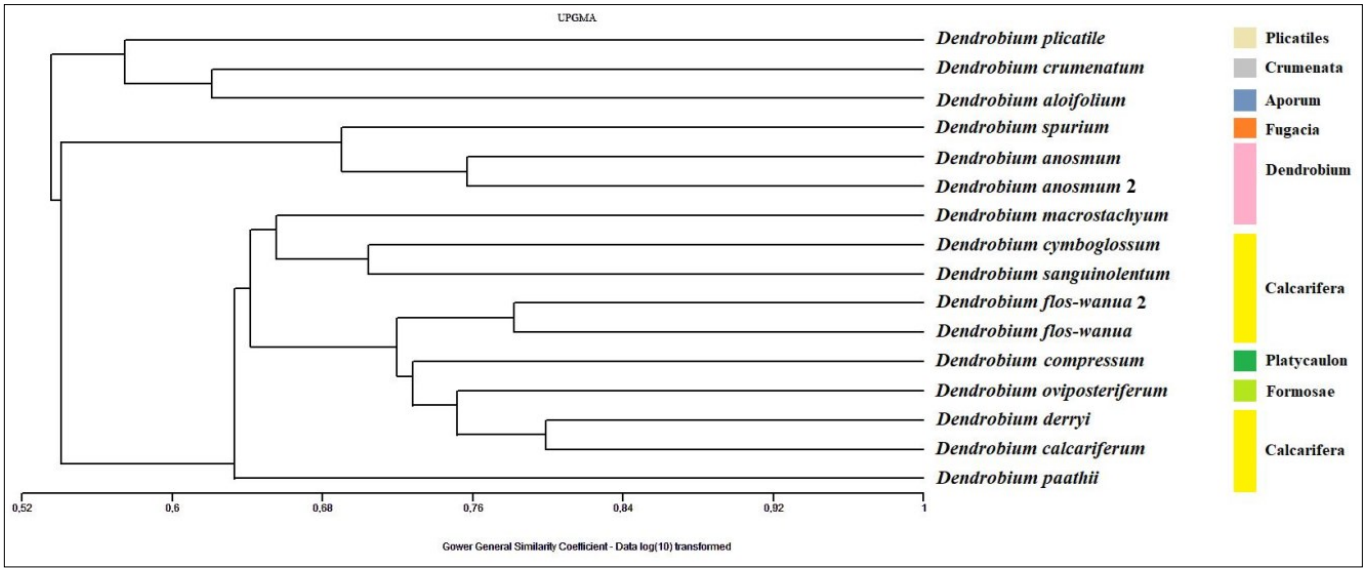

Fig. 3. Dendrogram showing Taxonomic relationships of *Dendrobium* species.

ness of cuticular layer on lower epidermal surface, (c) the length of lower epidermal cells, (d) the existence of hypodermal tissue, (e) the length of fibre cap on xylem of peripheral vascular bundles and (f) the width of fibre cap on xylem of peripheral vascular bundles. Meanwhile, the anatomical features distinguishing cluster II from cluster III were 8 characters which had loadings scores of > 0.2 on the first principal component. These 8 characters were: (a) the arrangement of parenchyma cells in the mesophyll, (b) the shape of parenchyma cells in the mesophyll, c) the length of peripheral vascular bundles, (d) the width

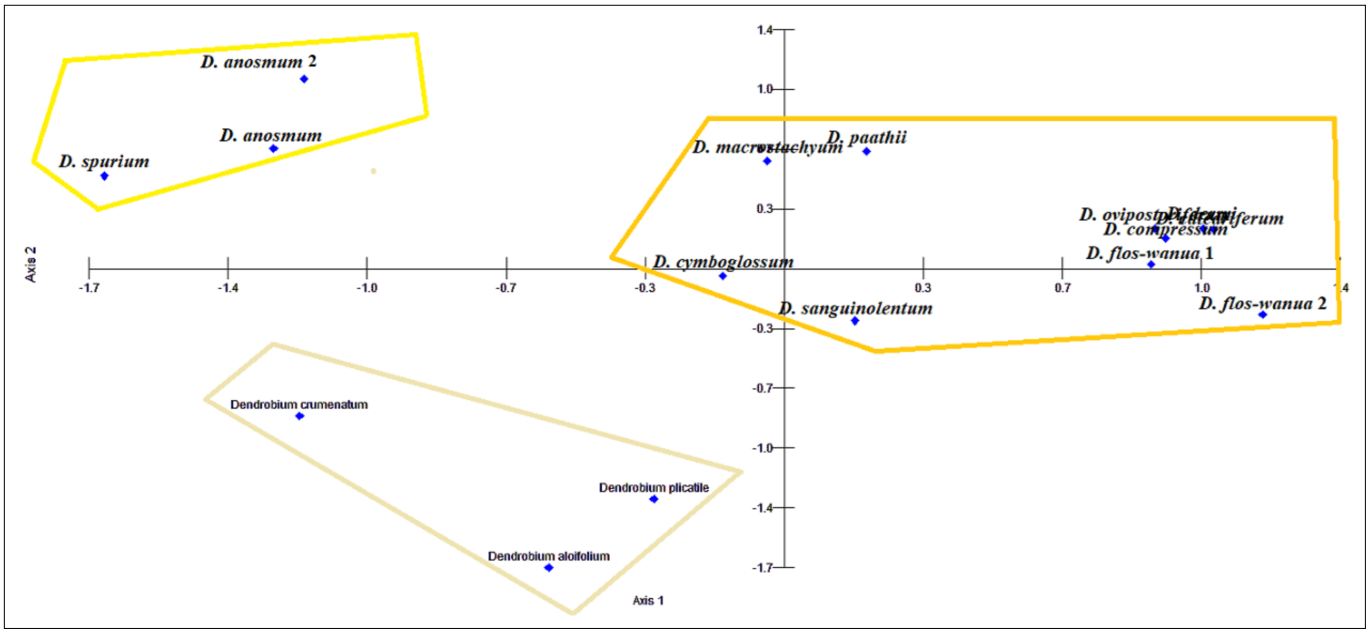

Fig. 4. PCA scatterplot of species.

Table 3. Loading scores of 60 anatomical characters resulted from principal component analysis.

No.	Character code	Axis 1	Axis 2	No.	Character code	Axis 1	Axis 2
1	UEC	0,106	**0,263**	31	VPL	0,082	-0,029
2	UES	0,089	0,049	32	VSN	-0,088	-0,062
3	UEW	0,142	-0,164	33	PVS	0,043	0,094
4	UEL	-0,015	-0,146	34	PVW	0,154	-0,135
5	LEC	0,103	**0,268**	35	PVL	**0,215**	-0,032
6	LES	0,187	0,133	36	PFN	-0,077	0,057
7	LEW	0,122	-0,125	37	PFW	0,133	-0,164
8	LEL	-0,019	**-0,217**	38	PFL	0,097	**-0,226**
9	HYD	0,078	**0,316**	39	PXW	0,131	**-0,238**
10	MCA	**-0,204**	-0,006	40	PXL	0,080	-0,162
11	MCS	**-0,206**	0,093	41	PPN	0,029	-0,126
12	MCO	-0,020	0,053	42	PPW	0,179	-0,025
13	MMR	-0,005	-0,042	43	PPL	0,153	-0,083
14	MVN	0,090	0,178	44	PFW	0,152	-0,110
15	MVD	0,090	0,097	45	PFL	0,140	-0,093
16	MFB	0,030	0,310	46	PSN	-0,023	-0,134
17	VBM	0,024	0,137	47	AVS	-0,016	-0,119
18	VBP	-0,013	-0,020	48	AVW	**0,228**	0,071
19	VBS	-0,106	-0,190	49	AVL	**0,240**	0,150
20	VBW	-0,128	-0,156	50	AFN	0,161	0,071
21	VBL	-0,010	0,081	51	AFW	0,038	-0,024
22	VFN	-0,078	-0,029	52	AFL	**0,209**	-0,029
23	VFW	-0,116	0,020	53	AXW	0,185	0,022
24	VFL	-0,127	-0,040	54	AXL	0,199	-0,033
25	VXW	0,003	-0,076	55	APN	0,163	-0,093
26	VXL	-0,076	-0,087	56	APW	**0,221**	0,079
27	VFP	-0,067	0,061	57	APL	0,173	-0,068
28	VFV	-0,084	0,012	58	APV	**0,219**	-0,067
29	VFH	0,042	-0,108	59	APH	0,196	-0,053
30	VPW	0,046	-0,141	60	ASN	-0,133	-0,062

of accessory vascular bundles, (e) the length of accessory vascular bundles, (f) the length of fiber cap on xylem of accessory vascular bundles, (g) the width of fiber cap on the phloem of accessory vascular bundles and (h) the width of the phloem area on accessory vascular bundles.

Discussion

Dendrobium species from the sections of Aporum, Crumenata and Plicatiles which showed close taxonomic relationships in cluster I can be identified from similarities in anatomical characteristics of their leaves. Two anatomical characteristics shared by the members of this cluster were the presence of hypodermis tissue and the number of fiber bundles in the mesophyll. These characters were related to the leaf morphology of these 3 species which were thicker than the other species. The characteristics of fiber bundles are important taxonomic features for the identification of orchid species that have been reported on *Prosthechea moojenii* and *P. silvana* (12). The role of leaf anatomical characters in classification at the section level has been mentioned in the genus *Croton,* particularly the presence of trichomes and hypodermis for species delimitation (13).

The close taxonomic relationship between the Aporum and Crumenata sections reported in this study was in accordance with the previous study on *Dendrobium.* In this regard, the leaves of species from these 2 sections generally are laterally flattened and the mesophyll consisted of cells with wall thickenings. These characters were related to their adaptation to xerophytic conditions (14). The similarities in leaves of species from sections Aporum and Crumenata provided strong support for the taxonomic significance of leaf anatomy in species identification as well as in the determination of species relationship. Moreover, leaf anatomical structures are related to the ecological adaptation of plant taxa (15).

Taxonomic relationships of species belonging to cluster II was mostly determined by similarities in characteristics of mesophyll and major vascular bundles. The importance of vascular bundles character for the taxonomy of Orchidaceae has been noted as differentiating features between the species of *Grammatophyllum* (16). It is quite interesting that the 3 sections grouped in cluster III (Calcarifera, Formosae, and Platycaulon) which showed similarities in leaf anatomical characters also have similarities in their morphology. In this case, morphological characterization of species belonging to the sections of Calcarifera and Platycaulon showed that these 2 sections had very close relationships (17).

Results of this study indicated that leaf anatomical character shows its taxonomic value for the identification of sterile specimens as well as supporting the classification of *Dendrobium* at the sectional level. A study on 2 closely related *Dendrobium* species, namely *D. capra* and *D. arcuatum*, showed obvious differences in their leaf and root anatomy and these differences were related to adaptation to different habitats (18). The use of anatomical characters in orchid's taxonomy has been reported in previous studies and was proven to play an important role in distinguishing species. A comparison of the anatomical structures of leaves, stems and roots of 4 *Vanda* species showed that the anatomical characteristics of these 3 organs were useful for species identification of sterile specimens (19). In addition, a comparative study on the leaf and root anatomy of 2 *Grammatophyllum* species showed clear differences in their anatomy (16), which supported the use of leaf anatomy in the identification of orchid species.

In general, the results of this study showed that leaf anatomy was useful in confirming the relationships between sections within the genus *Dendrobium*. Accordingly, this study provided validation on the role of leaf anatomy for species identification and supported the classification of *Dendrobium* at the sectional level. The confirmation of the role of anatomy in revealing the taxonomic relationships of species and differentiating sections within the genus *Dendrobium* was recently emphasized (20). Moreover, the results of this study were in line with those found in other genera of Orchidaceae, as reported in the genus *Aechmea* (13), *Microcos* (7) and *Butia* (21).

The grouping of *Dendrobium* species resulted from cluster analysis based on leaf anatomical characters (Fig. 4) indicated that the relationships between sections were generally in agreement with those reported from phylogenetic analysis using molecular markers. The sections of Crumenata and Aporum, which were placed in proximity compared to the members of section Plicatiles in cluster I, indicated a close taxonomic relationship between the 2 sections. This is in line with the result of the phylogenetic analysis of *Dendrobium* using sequences of 5 molecular markers, in which sections of Crumenata and Aporum were grouped in the same clade (14). A phylogenetic study of *Dendrobium* based on *rbc*L sequences also showed that the sections of Crumenata and Aporum were closely related as indicated by their placement in a monophyletic clade (22). The close relationship of Crumenata and Aporum sections was also supported by the results of the morphological study on *Dendrobium* (23). In this study, the section of Calcarifera with 6 species was closely related to the section of Platycaulon as shown by their placement in cluster III. These 2 sections were closely related based on molecular phylogenetic analysis using ITS and *mat*K (24).

Conclusion

Based on the analysis of leaf anatomical characters of 16 *Dendrobium* specimens using numerical taxonomy methods in this study, it can be concluded that the leaf anatomical characters are proven to be useful to differentiate between species. Leaf anatomical characters also supported the classification of *Dendrobium* at the sectional level. This study, therefore, confirmed the taxonomic significance of leaf anatomy for the identification of sterile specimens and supports the sectional classification in the genus *Dendrobium*.

Acknowledgements

The authors acknowledge Faculty of Biology, Universitas Gadjah Mada for providing financial support through Research Grant KDM 1163/UN1/FBI.1/KSA/PT.01.03/2022.

Authors' contributions

MIM collected plant materials, performed the examination of anatomical slides and data collection, and participated in data analysis .M performed the interpretation of anatomical data and wrote the manuscript. RS designed the research, performed data analysis and wrote the manuscript .All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

References

1. Alghamdi SA. Influence of mycorrhizal fungi on seed germination and growth in terrestrial and epiphytic orchids. Saudi Journal of Biological Sciences. 2019;26(3):495-502. https://doi.org/10.1016/j.sjbs.2017.10.021
2. Naik SK, Maurya S, Choudhary JS. Growing orchid: An overview. ICAR. 2014.https://doi.org/10.13140/RG.2.1.3764.3761
3. Tang X, Yuan Y, Zhang J. How climate change will alter the distribution of suitable *Dendrobium* habitats. Frontiers in Ecology and Evolution. 2020;8:1-17. https://doi.org/10.3389/fevo.2020.536339
4. Schuiteman A. *Dendrobium* (Orchidaceae): To split or not to split? Gardens' Bulletin Singapore. 2011;63(1 & 2):245-57.
5. Clements MA. Molecular phylogenetic systematics in the Dendrobiinae (Orchidaceae), with emphasis on *Dendrobium* sect. Pedilonum. Telopea. 2003;10(1):247-98. https://doi.org/10.7751/telopea20035619
6. Faria APG, Vieira ACM,Wendt T. Leaf anatomy and its contribution to the systematics of *Aechmea* subgenus *Macrochordion* (de Vriese) Baker (Bromeliaceae). Anais da Academia Brasileira de Ciências. 2012;84(4):961-71. https://doi.org/10.1590/S0001-37652012005000053
7. Shokefun EO, Ayodele AE,Akinloye AJ. Systematic importance of leaf anatomical characters in some species of *Microcos* Linn. section Eumicrocos Burret. in Nigeria. American Journal of Plant Sciences. 2016;7:108-17. http://dx.doi.org/10.4236/ajps.2016.71012
8. Puspitaningtyas DM. Orchid diversity in a logging concession in Tabalongdistrict, South Kalimantan, Indonesia. Biodiversitas. 2020;21(11):5455-64. https://doi.org/10.13057/biodiv/d211154
9. Wood JJ,O'Byrne P. *Dendrobium* of Borneo. Natural History Publications. 2014.
10. Pradhan BM, Bajracharya DM. Anatomical study of *Dendrobium* (Orchidaceae) of Nepal. Annals of Plant Sciences. 2020;9(7):3916-48. https://doi.org/10.21746/aps.2020.9.7.1
11. Kovach WL. Multi-variate statistical package version 3.1. Kovach Computing Services. 2007.
12. Seixas FC, Monteiro SHN, da Silva LB, Leite KRB. Leaf anatomy of *Prosthechea moojenii* (Pabst) W. E. Higgins and *P. silvana* Cath. & V. P. Castro (Orchidaceae) as an approach to taxonomy of species occurring in biomes Cerrado and Atlantic forest of the Bahia. American Journal of Plant Sciences. 2012;3:1752-58. http://dx.doi.org/10.4236/ajps.2012.312214
13. Feio AC, Meira RMSA, Riina R. Leaf anatomical features and their implications for the systematics of dragon's blood, *Croton* section Cyclostigma (Euphorbiaceae). Botanical Journal of the Linnean Society. 2018;187:614-32. https://doi.org/10.1093/botlinnean/boy038
14. XiangX-G, André Schuiteman,LiD-Z, HuangW-C, ChungS-W, Li J-W *et al*. Molecular systematics of *Dendrobium* (Orchidaceae, Dendrobieae) from mainland Asia based on plastid and nuclear sequences. Molecular Phylogenetics and Evolution. 2013;69:950-60. http://dx.doi.org/10.1016/j.ympev.2013.06.009
15. Simpson MG. Plant systematics. 3rd ed. Elsevier Inc., Burlington, USA. 2019.
16. Ishmah S, Metusala D, Nisyawati, Supriatna J. Anatomical comparison of two *Grammatophyllum* spp. (Orchidaceae) species and their specific ecological adaptation. IOP Conf. Series: Earth and Environmental Science. 2021;940:012016. http://dx.doi.org/10.1088/1755-1315/940/1/012016
17. Dauncey EA. A taxonomic revision of *Dendrobium* section Pedilonum (Orchidaceae). Harvard Papers in Botany. 2003;7(2):151-320.
18. Metusala D, Supriatna J, Nisyawati, Sopandie D. Comparative leaf and root anatomy of two *Dendrobium* species (Orchidaceae) from different habitat in relation to their potential adaptation to drought. AIP Conference Proceedings. 2017;1862(030118):1-5. https://doi.org/10.1063/1.4991222
19. Kowsalya A, Rojamala K, Muthukumar T. Comparative vegetative anatomy of South Indian Vandas (Orchidaceae). Flora. 2017;235:59-75. http://dx.doi.org/10.1016/j.flora.2017.09.002
20. Baishnab B, Bora D, Banik B, Paul S, Sarma D, Majumdar K, Datta BK. Comparative morpho anatomical studies of two sections of the genus *Dendrobium* Sw. (Orchidaceae). Vegetos. 2023;36:377-92. https://doi.org/10.1007/s42535-022-00432-0
21. Sant'Anna-Santos BF, Santos SA, Nunes ELP, Francino DMT, Carvalho Júnior WGO. Does leaf anatomy aid in species identification of *Butia* (Arecaceae)? AoB Plants. 2018;10:ply046. http://dx.doi.org/10.1093/aobpla/ply046
22. Moudi M, Yien CYS, Nazre M, Abdullah JO,Go R. Phylogenetic analysis among four sections of genus *Dendrobium* Sw. (Orchidaceae) in Peninsular Malaysia using *rbc*L sequence data. International Journal of Bioassays. 2013;02(06):932-37.
23. MoudiM,Go R. Morphological study of four sections of genus *Dendrobium* Sw. (Orchidaceae) in Peninsular Malaysia. Pakistan Journal of Botany. 2017;49(2):569-77.
24. Takamiya T, Wongsawad P, Sathapattayanon A, Tajima N, Suzuki S, Kitamura S *et al*. Molecular phylogenetics and character evolution of morphologically diverse groups, *Dendrobium* section *Dendrobium* and allies.AoB Plants.2014;6:plu045.http://dx.doi.org/10.1093/aobpla/plu045