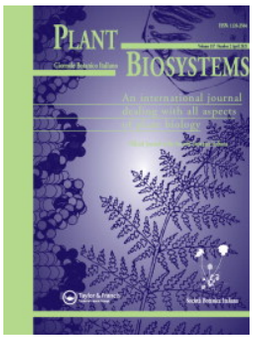

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Leaf morphoanatomical character variation of *Gyrinops* and *Aquilaria* (Thymelaeaceae) in Indonesia region at east Wallace line

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ABSTRACT

There are seven species of *Gyrinops* and two species of *Aquilaria* (Thymelaeaceae), which are distributed in Eastern Indonesia at the east of the Wallace line. The anatomical character of the paradermal tissue, midrib and petiole were the important characteristics possessed by plants that can be used as data for identification. The purpose of this article was to study leaf morphoanatomical character variation of *Gyrinops* and *Aquilaria* in Indonesia Region at East Wallace Line. The *Gyrinops* specimen used in the research was the collection of the Agarwood Study Center, Universitas Mataram, Mataram, Indonesia. The paradermal slide was made with the whole-mount slide method and the slide cross-section of midrib and petiole was made by using a hand-free section. Their preparation process used a permanent slide method and mounted with glycerine jelly. Based on the results of the research conducted, it can be seen that the anatomical characters of paradermal tissues, midrib and petiole of *Gyrinops* and *Aquilaria* have general characteristics such as hypostomatic leaves, stomata type anomocytic, non-glandular and unicellular trichome. While other characters of structure paradermal, midrib and petiole could be used as a taxonomy diagnostic marker to identify at the level genus and species on *Gyrinops* and *Aqilaria.*

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Aquilaria; *Gyrinops*; leaf anatomy; Wallace's line; Indonesia

Introduction

Agarwood-producing plants from the Thymelaeaceae family consist of 58 genera, two of which are *Aquilaria* and *Gyrinops*. The genus of *Gyrinops* is one of the seven aromatic resin producer's genera in Indonesia and is the most widely used genus besides *Aquilaria* and *Gonystylus* (Susilo et al. 2014). *Gyrinops* can generally be found in central and eastern Indonesia, which includes Sulawesi Island, Lesser Sunda Islands, Moluccas Islands and Papua (Hou 1960; Eurlings and Gravendeel 2005; Mulyaningsih and Yamada 2008, 2020; Roemantyo and Partomihardjo 2010; Lee et al. 2018). Central and eastern Indonesia are areas that are at the east of the Wallace line. Those areas are places that have a high level of biodiversity. At the east of the Wallace line, there are islands belonging to the transitional region and the Australis region. The transitional area includes Sulawesi Island, and the Lesser Sunda Islands, while the Australis region includes the Moluccas Islands, Papua Island and its surroundings (Bisjoe 2015).

Differences in morphological characters that are difficult to distinguish among *Gyrinops* species cause logging to be carried out on all species. According to Hou (1960), the grouping between species of *Gyrinops* spp. is very difficult because the characters vary not only within one species but also within one specimen. There are various kinds of characters that can be used in classifying the type of a plant, one of which is anatomical characters such as stomata, midrib (midvein) and petiole.

Leaf anatomy studies such as type, shape, the density of stomata, epidermal cell shape and mesophyll structure can support plant identification on species in Malvaceae (Dorly et al. 2016). Thakur and Patil (2011) used stomata characters to distinguish species in Euphorbiaceae. Anatomical petiole characters could be used to identify the *Cinnamomum* species (Abeysinghe and Scharaschkin 2019), species of Asteraceae (Mobel et al. 2013; Ekeke and Ogazie 2020); Microcos (Malvaceae) (Nurul-Aini et al. 2013), and leaf anatomy of *Bouea, Mangifera* and *Spondias* (Norfaizal and Latiff 2013), Rubiaceae species (Lima et al. 2020). This research aims to study leaf morphoanatomical character variation including leaf paradermal tissue, midrib and petiole of the leaves, of the genus *Gyrinops* and *Aquilaria* (Thymelaeaceae) in the Indonesian region east of the Wallace line.

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Materials and methods

Plant material

The materials used in this study were obtained from different sites in the area of East Wallace Line Indonesia, Lesser Sunda Island, Celebes, Moluccas and Papua (see Table 1). The taxa studied was *Aquilaria cumingiana* (Dence) Ridl. (Ridley 1901), *A. filaria* (Oken) Merr. (Merrill 1950), *Gyrinops versteegii* (Gilg.) Domke, *G. decipiens* D. Hou, *G. moluccana* (Miq.) Baill., *G. podocarpa* (Gilg.) Domke, *G. ledermannii* Domke, *G. caudata* (Gilg.) Domke and *G. salicifolia* Ridl (Hou 1960). Leaf specimens from Mataram University Lombok Herbarium (MUL) were dehydrated in hot water at 100 °C for 5 min, then preserved in alcohol 70%. Three leaves for each species were used.

Light microscopy

Preparation of leaf paradermal tissue preparations were carried out using the whole mount method (Sass 1951). Epidermis and stomata characters were studied from paradermal sections. The leaf paradermal section of the microscope slide was used for the maceration method by the procedure by Kadiri and Olowokudejo (2010), Munir et al. (2011), Chikmawati (2013), and Chinwe (2020). The cutting leaves near the midrib were soaked in 50% HNO_3 solution for 24 h or until the epidermal tissue could be easily separated from the mesophyll tissue.

Preparation of cross-sectional microscope slides of midrib and petiole was done according to the semi-permanent slide method by a standard of freehand section (Lux et al. 2005; Berden 2020). The midrib and petiole samples were sliced cross-section using a sharp (new) razor blade, resulting in a very thin incision. The incision is placed on a glass object that has been dripped with water. The water in the object-glass is absorbed using tissue paper until the object glass is clean. The incisions were stained with double stain (used solution 1% Safranin O in 50% alcohol and solution 1% (1 part aniline blue + 4-part picric acid) in 90% alcohol). Then the incisions were dehydrated with glycerine series: alcohol 50%, glycerine 50% and glycerine 100%. The mounting process used glycerine jelly and the edge of the cover glass was dripped with Entelan.

Paradermal slide and cross-section of midrib and petiole slides were observed using a Zeiss Binocular Microscope Primo star type. Measurement of cells and tissue size (thickness, length and diameter) was carried out using Image J software (Reinking 2007; Rasband 2012; Labno 2021). All stomatal characters refer to abaxial epidermal stomata (Table 1). Stomata index was calculated using the methods by Stace (1965), Kadiri and Olowokudejo (2010), Munir et al. (2011), Ajuziogu et al. (2018).

Characters and character states: The terminology of leaf epidermis is followed by Baranova (1992), Ash et al. (1999), and Carpenter (2005). Thirty leaf epidermal characters of the lamina, structure of midrib and petiole, trichomes, epidermal cells and stomata were coded and scored for each sample (Tables 1, 2, and 5). Character states of each sample were numbered as in Table 5.

Anatomical data were analyzed using a similarity coefficient based on Manhattan distance and the results were used to construct a dendrogram by the unweighted pair method with arithmetic mean (UPGMA) (Sokal and Michener 1958) using the PAST 4.09 program (Hammer et al. 2001). Principal component analysis (PCA) of anatomical characters was performed to identify the characters with the highest contribution.

Result and discussion

Leaf morphology

Leave of *Gyrinops* and *Aquilaria* are simple, linear-lanceolate, coriaceous; base cuneate, acuminate; apex acute with a blunt end, acuminate – caudate, mucronate; margin entire. Lamina pubescent, tomentose or pilous, midrib prominent to abaxial. Petiole semi-terete or dorsiventral flattened (Figure 1).

Leaf anatomy

Stomata

All of the species *Gyrinops* and *Aquilaria* leaves examined have characteristics of a hypostomatic type. The hypostomatic type is one of the adaptations of plants to suppress the rate of transpiration, as found in *Hoya* spp. and nine species of *Nepenthes* from Peninsular Malaysia (Hafiz et al. 2013; Ghazalli et al. 2019). They have anomocytic stoma, but each species of those genus has several types of stomata, such as the genus of Anacardiaceae with anomocytic, anisocytic,

Table 1. Locality, habitat and collector number of examined specimens.

No	Species	Locality	Habitat	Collector number/Herbarium number
1	*Gyrinops salicifolia*	Jayapura hill, Papua	Limestone mountains	48/TM-IY/30/08/2005 MUL 00000091
2	*G. caudata*	Mapi, Papua	Swamp forest	52/TM-IY/03/09/2005 MUL 00000107
3	*G. ledermannii*	Jayawijaya Mts, Papua	Secondary forest Mountain's peak	38/TM-IY/27/08/2005 MUL 00000086
4	*G. ledermannii*	Meja Mt. Sumba	Secondary forest	52/TM-IY/03/09/2005 MUL 00000062
5	*G. moluccana*	Namrole, Buru, Moluccas	Secondary forest	357/ TM-MI/10/02/2017 MUL 00000652
6	*G. podocarpa*	Sorong, Papua	Swamp forest	413/ TM-MI/04/08/2018 MUL 00000908
7	*G. podocarpa*	Bintuni, Papua	Limestone mountains	422/ TM-MI/09/08/2018 MUL 00000939
8	*G. decipiens*	Bantilang, Tomuti Lake, Sulawesi	Secondary forest	60/TM-IY/01/09/2006 MUL 00000115
9	*G. decipiens*	Masamba, Sulawesi	Agroforestry	402/ TM-MI/03/09/2018 MUL 00000866
10	*G. versteegii*	Bima, Sumbawa	Agroforestry	429/ TM-MI/04/11/2018 MUL 00000140
11	*G. versteegii*	West Lombok	Agroforestry	Fresh specimen: TM-Bq. JPS-VF/ 10/04/2020-
12	*Aquilaria cumingiana* (Dence) Ridl.	Sorong, Papua	Agroforestry	417/ TM-MI/09/08/2018 MUL 00000923
13	*Afilaria* (Oken) Merr.	Fak-fak, Papua	Secondary forest	423/ TM-MI/09/08/2018 MUL 00000945

Notes: TM: Tri Mulyaningsih, Bq. JPS: Baiq Jelita Puspita Sari, VF: *via* Febrianti, MI: Michiho Ito, IM: Isamu Yamada

Table 2. Type, shape and size of epidermis and stomata of the genera *Gyrinops* and *Aquilaria* (Thymelaeaceae) in the Indonesian region east of the Wallace line.

Taxon	Epidermis						Stomata				
	Upper			Lower							
	Anti-clinal thickened pattern	Length (µm)	Width (µm)	Anti- clinal thickened, pattern	Length (µm)	Width (µm)	Length (µm)	Width (µm)	Density (mm^{-2})	Index (%)	Type
1. *Gyrinops caudata*	7	21.86 ± 1.45	16.56 ± 044.	7	20.60 ± 1.77	13.07 ± 0.96	19.87 ± 0.67	13.54 ± 0.78	16.13	10	b,c,f,l,p,q,s
2. *G. decipiens*	1	20.74 ± 2.54	15.34 ± 1.73	1	21.45 ± 2.20	16.71 ± 1.69	20.67 ± 1.29	15.60 ± 0.58	21.24	17	b,h,q,r,s,
3. *G. decipiens*	1	20.64 ± 3.52	14.83 ± 3.11	1	22.21 ± 0.55	18.58 ± 1.15	18.85 ± 1.65	15.33 ± 0.95	27.21	27	b,h,q,r,s
4. *G. ledermannii*	5	18.80 ± 0.95	15.92 ± 1.59	5	21.33 ± 0.99	15.82 ± 1.36	19.55 ± 1.39	12.50 ± 0.87	25.52	20	b,q,k,s
5. *G. ledermannii*	5	18.44 ± 0.38	14.87 ± 2.19	5	20.28 ± 0.40	15.27 ± 1.24	19.77 ± 2.49	12.56 ± 0.26	24.66	19	b,q,k,s,
6. *G. moluccana*	2	19.36 ± 1.40	13.02 ± 0.95	2	19.74 ± 1.05	15.96 ± 1.23	20.59 ± 1.52	14.39 ± 1.92	21.39	15	b,q,t
7. *G. podocarpa*	3	19.36 ± 2.24	15.29 ± 3.32	3	19.66 ± 1.96	13.61 ± 1.13	20.55 ± 0.70	11.52 ± 1.11	14.85	12	b,e,i,o,q,s,
8. *G. podocarpa*	3	19.75 ± 1.71	14.63 ± 2.73	3	19.89 ± 0.50	14.14 ± 2.18	19.56 ± 0.66	14.94 ± 1.64	19.26	14	b,d,h,j,s,
9. *G. salicifolia*	6	19.12 ± 1.00	17.57 ± 0.27	6	22.03 ± 0.61	16.83 ± 3.56	19.33 ± 0.86	14.68 ± 1.65	22.51	21	b,e,l,o,s
10. *G. versteegii*	2	21.46 ± 0.15	16.47 ± 2.06	2	22.03 ± 1.75	16.22 ± 2.73	20.48 ± 0.74	13.40 ± 4.72	13.95	13	b,c,m,n,r
11. *G. versteegii*	4	20.72 ± 0.54	18.45 ± 1.15	4	22.94 ± 1.26	13.32 ± 2.12	18.78 ± 0.58	13.70 ± 0.25	11.11	9	a,b,i,q
12. *Aquilaria filaria*	9	9.83 ± 0.49	7.38 ± 0.94	9	20.07 ± 2.30	14.56 ± 1.96	16.73 ± 3.19	12.80 ± 1.52	20.67	14	b,s
13. *A. cumingiana*	8	18.39 ± 1.77	14.82 ± 0.24	8	21.73 ± 3.19	14.06 ± 1.86	17.02 ± 0.87	13.02 ± 1.90	36.69	23	b,q

Notes: Anticlinal thickened pattern of upper & lower epidermis: Rounded (1); Irregularly (2) Beaded (3);Branched (4); Branched and Ring (5); Beaded and ring (6); Buttressed and ring (7); Buttressed (8); Smooth and ring (9); Stomata type: a. Anomocytic stoma, circular; b. Anomocytic stoma (AS), subsidiary cell straight; c. Anisotetracytic, subsidiary cell straight; d. Anisotetracytic stoma, subsidiary cell straight; e. Actinoheptacytic stoma, subsidiary cell rounded (ApSl); f. Anisopentacytic stoma (ApS); g. Anisopentacytic stoma, subsidiary cell straight; h. Anisohexacytic (AhS); i. Anisopentacytic stoma, subsidiary cell rounded; j, Anisoheptacytic stoma, subsidiary cell straight; k. Actinoheptacytic stoma, subsidiary cell tube; l. Straurocytic stoma; m. Paracytic stoma; n. Anomoparahexacytic stoma; o. Actinoparaheptacytic stoma; p. Stephanocytic bicyclic; q.Tetracytic stoma (TS); r. Anomohexacytic stoma, subsidiary cell rounded (HxSl); s. Actinohexacytic stoma, subsidiary cell straight (HxSs); t. Actinoheptacytic stoma, subsidiary cell sinuous (HpSc)

staurocytic and diacytic stoma (Norfaizal and Latiff 2014), the Myrtaceae genus with three types of stomata: anisocytic, paracytic and hemiparacytic (Al-Edany et al. 2012). In some species of the *Mangifera* have anomocytic, anisocytic and staurocytic stoma (Norfaizal and Latiff 2013).

Stomata of the genus *Gyrinops* and *Aquilaria* were arranged solitary and in clusters. Solitary stomata have very varied types, including actinocytic, anisocytic, anomocytic, parasitic, staurocytic, Stephanocytic bicyclic and tetracytic stoma types with several variations in shape and number of subsidiary cells (Table 2 and Figure 2). This condition causes the type of stomata to become complex, complex stomata will be a contributor in resolving an overview of the diversity of stomata structures in different taxa (Timonin 1994).

Most stomata in *Gyrinops* and *Aquilaria* were arranged in clusters, such as the tetracytic- series cluster, anomocytic-series cluster and clustering difference stoma (Figure 3), as in *Terminalia catappa* (Combretaceae), some of the stomata are also arranged in clusters (Ekeke and Agbagwa 2015). According to Hafiz et al. (2013), the presence of single stomata and clustering will affect the level of stomata density possessed by a species. Species that form clusters have a higher density level when compared to species with single stomata. Stomata that have more clustering types than single stomata will affect the level of stomatal density. The distribution of stomata can be influenced by genetic factors and environmental factors, but genetic factors have a greater role when compared to environmental factors. According to Camargo and Merenco (2011), the distribution of stomata on 35 plants in the Amazon Forest is not affected by differences in growing environmental conditions; this indicates that genetic factors have a greater role in determining the character of a plant.

The different variations in the type of stomata found in the *Gyrinops* genus indicate a high level of diversity between *Gyrinops* species. Stomata in the genus *Gyrinops* have variations in the number and shape of subsidiary cells. According to Salisbury and dan Ross (1992), the number and arrangement of subsidiary cells are influenced by the plant species. The arrangement and number of subsidiary cells are indicators used to determine the type of stomata. Subsidiary cells develop from epidermal cells after the formation of guard cells. Subsidiary cells that have been formed will surround the guard cells. This is related to the cell function of subsidiary cells that help guard cells open and close stomata.

Each species of the genus *Gyrinops* and *Aquilaria* has single stomata types between 2 and 7 types. *G. caudata* has the highest stomata types, including anomocytyc, actinocytic, anisocytic, tetracytic, staurocytic and stephanocytic bicyclic stoma with varying shape and number of subsidiary cells, with stomata density (SD)16.13 and stomata index (SI) 10% (Figure 2, Table 2). Stomata density in this species is relatively low because it is composed of relatively more single stomata (Hafiz et al. 2013). Like in *Terminalia catappa* has six types of stomata and one clustering of different stomatal types (Ekeke and Agbagwa 2015). *Coffea rubiaceae* (coffee) has a paracytic stoma (Gray et al. 2020).

Gyrinops species that have four types of stomata were *G. decipiens*, *G. podocarpa* and *G. versteegii* from Bima Sumbawa. The first two species have anomocytic, actinocytic, anisocytic and tetracytic stoma with variations in the shape and number of subsidiary cells, and *G. versteegii* from West Lombok has anomocytic, actinocytic, anisocytic and paracytic stoma with variation shape and number on subsidiary cells (Figure 2), such as *Catharanthus roseus* has stomata type anomocytyc and anisocytic (Inandar et al. 1975) and *Datura innoxia* (Hameed and Hussain 2011). Species that have a parasitic type of stomata are also found in *Gironniera hirta*, *Styrax benzoides*, *Cratoxylum sumatranum* and *Bridelia gulaca* species (Wulansari et al. 2020).

Table 3. Midrib characters of the genus *Gyrinops* and *Aquilaria* (Thymelaeaceae) in the Indonesian region east of the Wallace line.

Taxon	Vascular bundle number	Vascular bundle shape	crystal shape	Adaxial midrib	Abaxial mi-drib	Lamina thickness (µm)	Midrib thick-ness (µm)	Xylem length (µm)	Sclerenchyma thickness (µm)	Trachea number	Xylem layer number Horizontal direction
1. *Gyrinops caudata*	1	6	1	2	1	145 ± 3.13	330.77 ± 20.43	271.11 ± 16.9	58.67 ± 3.32	45.33 ± 23.16	41 ± 5.57
2. *G. ledermanii*	1	5	1	2	3	144 ± 10.20	561.41 ± 10.33	383.72 ± 14.15	81.19 ± 9.23	141.33 ± 14.84	66 ± 1.73
3. *G. ledermanii*	1	5	1	2	3	139.53 ± 4.96	515.13 ± 30.85	633.09 ± 23.28	61.91 ± 1.28	109.33 ± 20.01	76.67 ± 9.24
4. *G. salicifolia*	1	1	1	2	2	175.01 ± 3.04	389.54 ± 36.15	272.75 ± 41.85	68.88 ± 11.04	122 ± 3	45.33 ± 3.21
5. *G. podocarpa*	1	3	2	2	1	202.14 ± 12.78	641.40 ± 4.88	527.02 ± 5.2	62.70 ± 26.34	106.67 ± 7.77	64.67 ± 3.51
6. *G. podocarpa*	1	3	2	2	1	246.19 ± 15.15	691.91 ± 5.53	597.97 ± 5.1	77.73 ± 12.33	96 ± 17.32	64 ± 7
7. *G. moluccana*	2	2	1	1	2	176.72 ± 2.39	615.23 ± 13.56	449.33 ± 16.09	43.78 ± 6.12	100.33 ± 18.88	83 ± 12.17
8. *G. decipiens*	1	8	3	3	3	177.03 ± 0.86	434.31 ± 2.57	341.01 ± 13.09	90.23 ± 7.73	67 ± 5.29	43.67 ± 0.58
9. *G. decipiens*	1	1	3	2	3	227 ± 14.70	633.4 ± 65.59	395.97 ± 49.25	93.82 ± 8.04	82.33 ± 14.36	42.33 ± 2.89
10. *G. versteegii*	1	4	3	2	2	209.83 ± 10.01	675.2 ± 35.73	381.35 ± 30.78	76.25 ± 16.88	117 ± 5	62.67 ± 0.58
11. *G. versteegii*	1	1	3	2	2	177 ± 17.86	592.2 ± 7.34	461.35 ± 23.15	61.9 ± 10.95	95 ± 11.14	53 ± 3.46
12. *Aquilaria cumingiana*	1	8	1	1	4	127.34 ± 8.21	590.7 ± 15.48	405.41 ± 29.91	83.22 ± 8.9	47 ± 1.73	71 ± 2
13. *A. filaria*	1	7	3	2	4	190.6 ± 21.24	419.78 ± 4.63	186.99 ± 1.93	69.83 ± 6.61	17.33 ± 4.51	45.33 ± 5.51

Notes. Midrib Vascular bundle shape: (1) plano-convex, (2) interupted concave-convex, (3) lip, (4) cordate, (5) semi-circular, (6) triangular, (7) interupted plano-convex, (8) bolf bat; Crystal shape: (1) absent, (2) elongoid, (3) styloid; Adaxial midrib: (1) flat, (2) semi-concave, (3) concave; Abaxial midrib: (1) entire, (2) lobe. (3) crenate, (4) semicrenate.

Table 4. Petiole characters of the genus *Gyrinops* and *Aquilaria* (Thymelaeaceae) in the Indonesian region east of the Wallace line.

Taxon	Outline shape	Vascular bundle shape	Adaxial surface	Abaxial surface	Trichome type	Trichome density	Collenchyma layer	Crystal shape	Petiole thickened (µm)	Xylem width (µm)	Sclerenchyma thickened (µm)	Number of tracheae	Xylem layers number in horizontal direction
1. *Gyrinops caudata*	1	3	2	1	1	1	3	2	1034.53 ± 56.41	436.23 ± 17.2	68.04 ± 10.4	101.67 ± 22.9	37.33 ± 5.25
2. *G. ledermanii*	1	4	2	3	3	3	4	1	1332.23 ± 33.06	447.08 ± 20.2	135.64 ± 4.46	82.67 ± 11.56	42.33 ± 2.87
3. *G. ledermanii*	4	4	2	3	3	3	4	2	1537.52 ± 84.58	508.12 ± 46.24	116.2 ± 17.45	118 ± 16.39	51 ± 2.94
4. *G. salicifolia*	3	5	2	2	1	1	4	2	878.37 ± 31.39	360.17 ± 5.12	82.37 ± 10.83	101.67 ± 7.85	48.33 ± 2.05
5. *G. podocarpa*	2	2	1	1	2	2	4	1	1480.97 ± 27.31	423.80. ± 25.24	138.57 ± 18.42	57.67 ± 2.49	37 ± 4.55
6. *G. podocarpa*	2	2	1	1	2	2	4	1	1580.65 ± 28.41	451.08 ± 27.47	143.57 ± 7.90	109.67 ± 21.25	42 ± 6.48
7. *G. moluccana*	1	1	2	2	1	1	2	3	1967.92 ± 65.54	474.02 ± 13.12	141.01 ± 11.8	111.67 ± 23.7	40 ± 2.94
8. *G. decipiens*	2	6	2	3	1	1	2	3	1601.68 ± 17.57	546.30 ± 2.73	135.20 ± 15.24	134.33 ± 20.76	42 ± 3.74
9. *G. decipiens*	2	6	3	3	1	1	2	3	1729.84 ± 26.15	434.79 ± 42.7	131 ± 4.23	94.33 ± 11.47	35.33 ± 7.59
10. *G. versteegii*	4	6	3	3	2	2	2	2	1961.29 ± 18.5	523.02 ± 14.09	120.17 ± 12.65	88 ± 9.09	44 ± 4.32
11. *G. versteegii*	4	6	1	3	2	2	2	2	2145.88 ± 15.07	659.75 ± 20.33	104.24 ± 16.87	144 ± 11.58	57.33 ± 1.25
12. *Aquilaria cumingiana*	5	7	2	3	3	1	3	3	1470.93 ± 39.95	463.04 ± 37.29	167.2 ± 7.13	187.33 ± 9.1	47 ± 3.56
13. *A. filaria*	6	8	3	3	3	2	1	2	806.29 ± 16.08	198.69 ± 2.59	78.95 ± 2.93	115.33 ± 8.73	49.33 ± 0.94

Notes. Outline petiole shape: (1) Ribosomal shape (2) square, (3) square widen, (4) square narrow, (5) semi-circular, (6) plano-convex; Vascular bundle shape: (1) oval horizontal, (2lip, (3) convex-concave, (4) semi convex-concave, (5) continuously plano-convex, (6) semicircular, (7) interrupted plano-convex, (8) circular; Adaxial midrib: (1) slightly convex, (2) slightly-concave, (3) flat; Abaxial midrib: (1) entire, (2) lobe. (3) crenate; Crystal shape: (1) absent, (2) styloid, (3) styloid & elongoid; Trichome type: (1) pubescent, (2) tomentose, (3) pilous; Trichome density: (1) sparse, (2) moderate, (3) thick.

Table 5. Character and character stated score of leaf anatomy of the studied *Gyrinops* and *Aquilaria* species in Indonesia region east Wallace line for making the dendrogram.

Abbreviation	Variable	Score
PS	Petiole shape	Ribosomal shape (1); Square (2); Square narrow (3); Square widen (4); Ribosomal shape (5); Semi-circular (6); Plano-convex (7)
AdP	Adaxial petiole	Flat (1); Slightly concave (2); Slightly convex (3)
AbP	Abaxial petiole	Entire (1); Crenate (2); Rounded (3)
PCL	Petiole Collenchyma location:	Absent (1); Adaxial (2); Surrounds the vascular bundle, moderate layer (3); Surrounds the vascular bundle, thick layer (4)
PVS	Petiole Vascular bundle shape:	Ovate horizontal (1); lip (2); Convex-concave (3); Semi convex-concave (4); Continuously plano-convex (5); Interrupted plano-convex (6); Semicircular (7); Circular (8)
PTT	Petiole trichome type	Pubescent (1); tomentose (2); pilous (3)
PTD	Petiole trichome density	Sparse (1); Moderate (2); Thick (3)
PCS	Petiole crystal shape	Absent (1); Styloid (2); Styloid & elongoid (3)
MS	Midrib shape	Erect; (2) Flat; (3) Semi-flat
AdM	Adaxial midrib	Flat (1); Semi concave (2); Concave (3)
AbM	Abaxial midrib	Straight (1); Semi crenate (2); Crenate (3); Rounded (4)
EM	Epidermal midrib	Uniseriate (1); Biseriate (3); Triseriate (4)
MCL	Midrib collenchyma location	Absent (1); Present (2).
MVS	Midrib vascular bundle shape:	Horse shoe (1); Triangular (2); Lip (3); Cordate (4); Semi concave-convex (5); Interrupted concave-convex (6); Plano-convex (7); Interrupted plano-convex (8); Bold bat
MVN	Midrib vascular bundle number	One (1); Two (2)
MCoL	Midrib crystal location	Sponge parenchyma & Cortical parenchyma (1); Cortical parenchyma (2); Sponge parenchyma (3); Palisade parenchyma (4); Absent (5)
MCT	Midrib crystal type	Absent (1): Styloid (2): Elongoid (3)
MVT	Midrib vascular bundle thicken	Adaxial & abaxial thin (1); Adaxial thin, abaxial moderate (2); Adaxial thin, abaxial thick (3); Adaxial & abaxial moderate (4)
SCM	Sclereid cell in midrib	Absent (1); Present (2)
ATP	Anticlinal thickened pattern of upper & lower epidermis	Rounded thickened (1); Irregularly thickened (2); Beaded thickened (3); Branched thickened (4); Branched and ring thickened (5); Beaded and ring thickened (6); Smooth and ring thickened (7);Buttressed thickened (8); Buttressed and ring thickened (9)
UEW	Upper epidermal wall	Straight (1); Straight – slightly rounded (2)
LEW	Lower epidermal wall	Straight (1); Straight – slightly rounded (2; Straight –sinuous

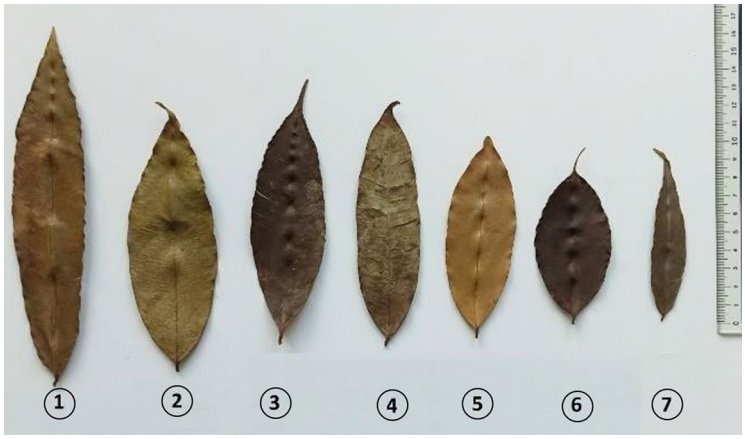

Figure 1. Leaves of *Gyrinops* species. Notes: (1) *G. moluccana* (2) *G. decipiens* (3) *G. caudata* (4) *G. ledermannii* (5) *G. versteegii* (6) *G. podocarpa* (7) *G. salicifolia*.

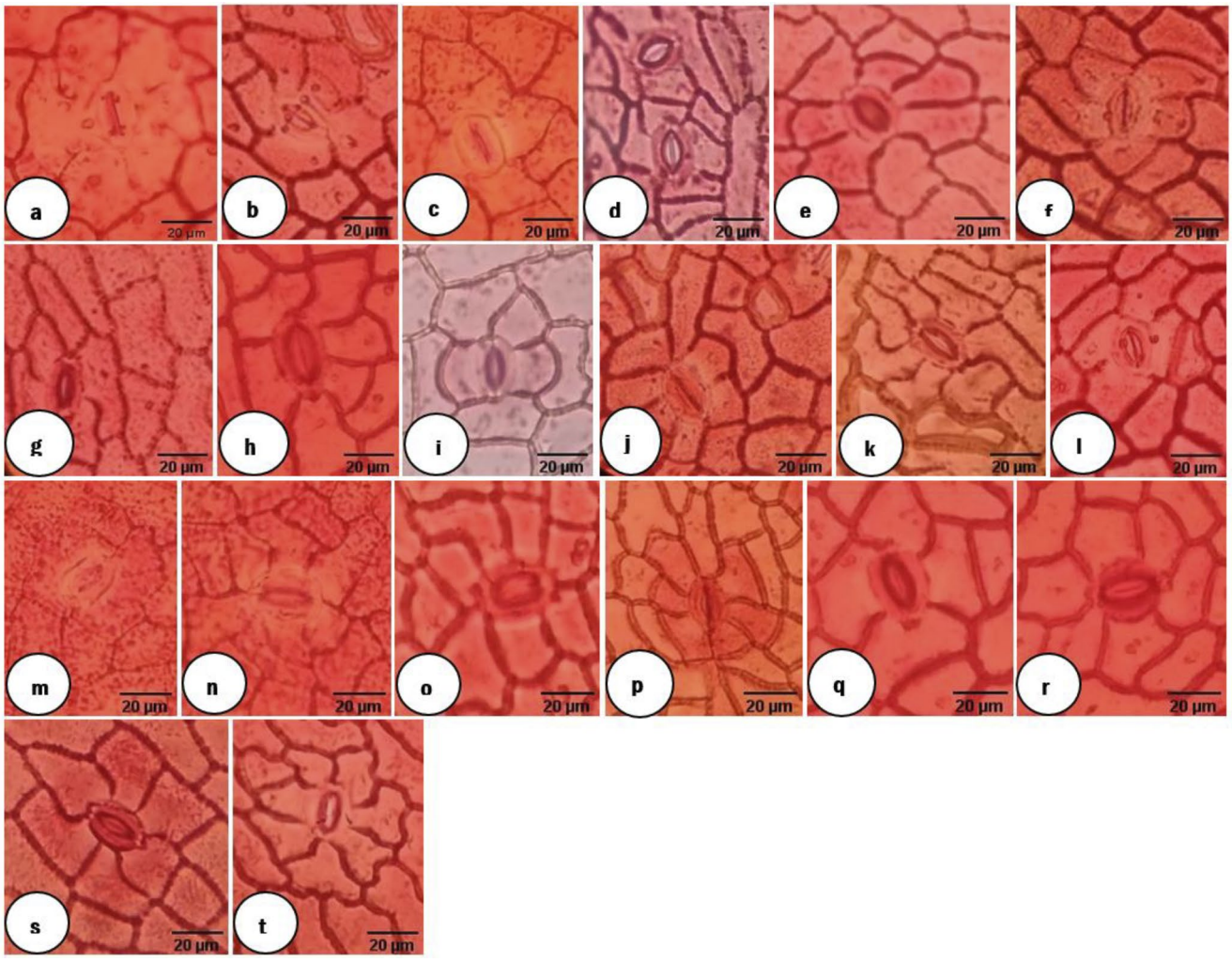

Figure 2. Stomata types on genus *Gyrinops* in Indonesia region eastern Wallace line. **1. Actinocytic stoma** (e. Actinoheptacytic stoma, subsidiary cell rounded (ApSl); k. Actinoheptacytic stoma, subsidiary cell tube; s. Actinohexacytic stoma, subsidiary cell straight (HxSs); t. Actinoheptacytic stoma, subsidiary cell sinuous (HpSc)); **2. Anisocytic stoma** (f. Anisopentacytic stoma (ApS); g. Anisopentacytic stoma, subsidiary cell straight; h. Anisohexacytic (AhS); i. Anisopentacytic stoma, subsidiary cell rounded; j, Anisoheptacytic stoma, subsidiary cell straight; n. Anisoparahexacytic stoma; o. Anisoparaheptacytic stoma); **3. Anomocytic stoma** (a. Anomocytic stoma, circular; b. Anomocytic stoma (AS), subsidiary cell straight; r. Anomohexacytic stoma, subsidiary cell rounded (HxSl)); **4. Paracytic stoma** (m); **5. Stephanocytic bicyclic** (p); **6. Straurocytic stoma (l); 7. Tetracytic stoma** (q. Tetracytic stoma (TS); c. Tetraanisocytic, subsidiary cell straight; d. Tetraanisocytic stoma, subsidiary cell straight) (description based on Ash et al. 1999 and Carpenter 2005).

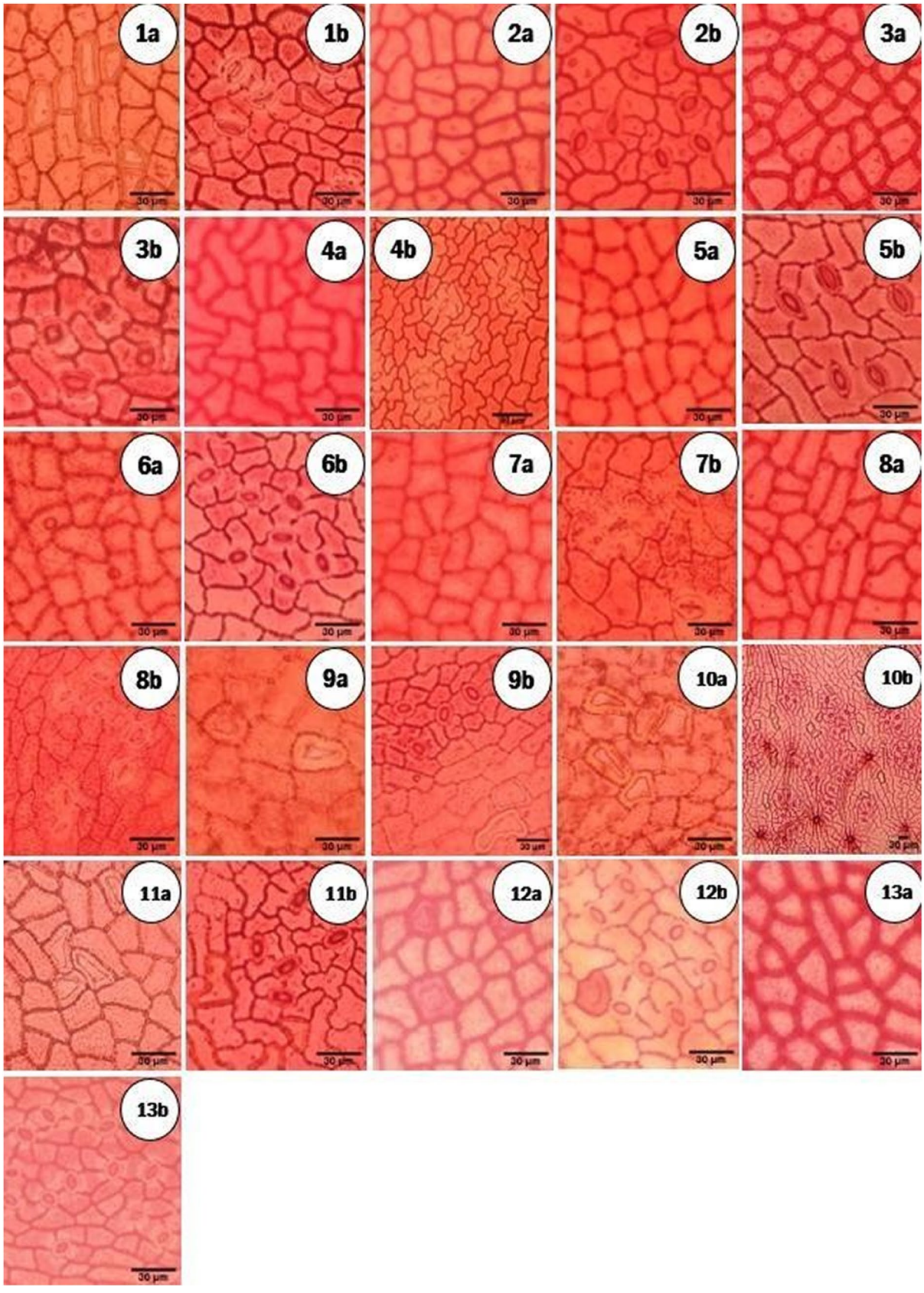

Figure 3. Epidermal leaves on genus *Gyrinops* and *Aquilaria* in Indonesia region eastern Wallace line. Notes: a. Upper epidermis, b. Lower epidermis; (1–13) Species name; 1b. Clustering of same stomata type (HxSl-series) *G. caudata*; 2b. Clustering of different stomatal types *G. decipiens* Masamba, Sulawesi; 3b. Clustering of different stomatal types *G. decipiens* Towuti Lake Sulawesi; 4b. Clustering of different stomatal types *G. moluccana* Buru, Moluccas; 5b. Clustering of different stomatal types *G. podocarpa,* Sorong, Papua; 6b. Clustering of same stomata type (HxSs-series) *G. podocarpa,* Bintuni, Papua; 7b. Clustering of different stomatal types *G. versteegii,* Bima, Sumbawa; 8b. Clustering of different stomatal types *G. versteegii,* West Lombok; 9b. Clustering of different stomatal types *G. salicifolia*; 10b. Clustering of different stomatal types *G. Ledermannii* Meja Mt.; 11b epidermis *G. ledermannii* Jayawijaya Mts. Papua; 12b. Clustering of different stomatal types *Aquilaria filaria*; 13a. Clustering of different stomatal types *A. cumingiana.*

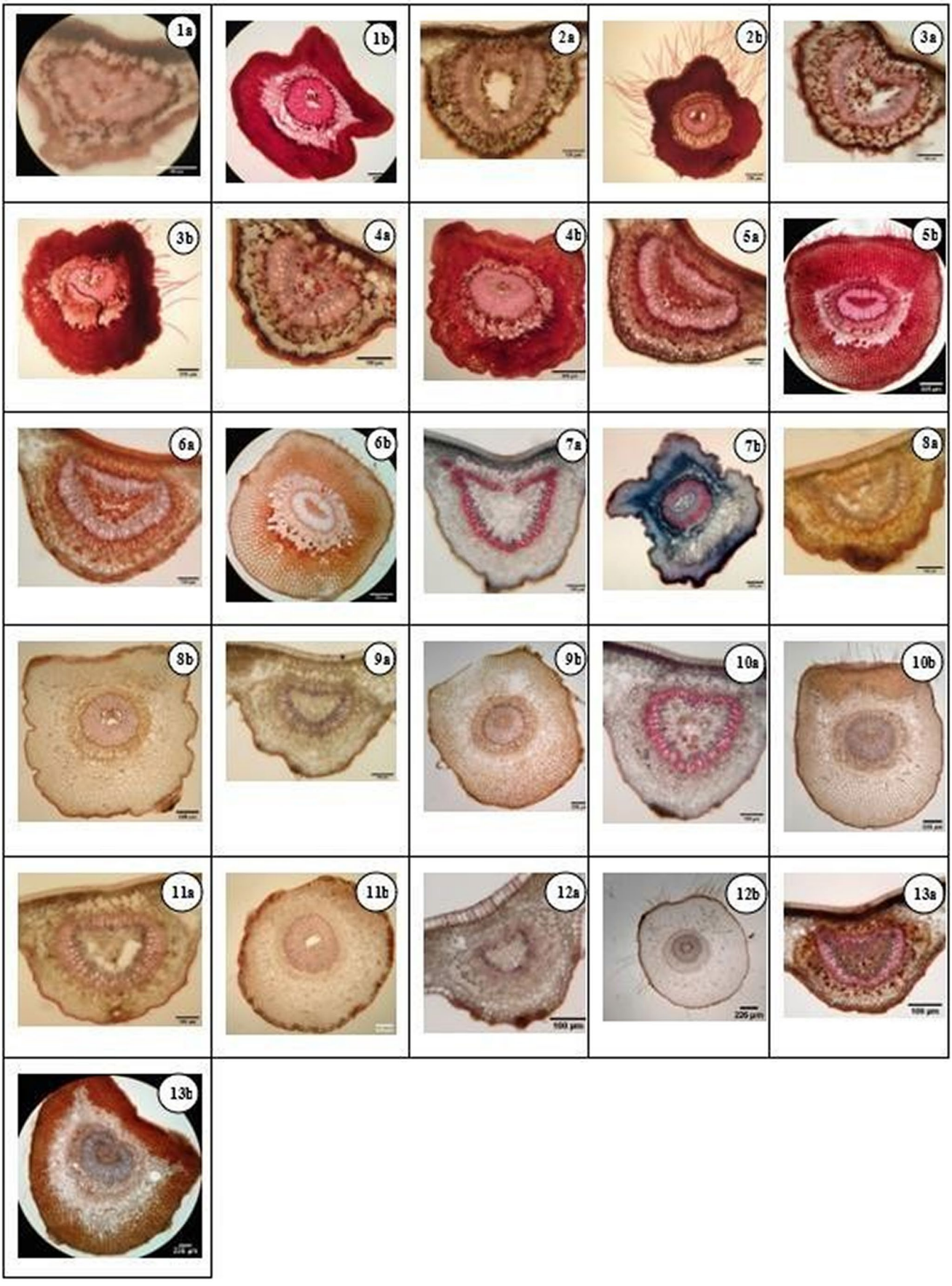

Figure 4. Variations of petiole and midrib shapes in the genera *Gyrinops* and *Aquilaria*. Notes: (1) *G. caudata*; (2) *G. ledermannii*, Jaya Wijaya Mts. Papua; (3) *G. ledermannii*, Meja. Mt. Sumba; (4) *G. salicifolia*, Jayapura Papua; (5) *G. podocarpa,* Sorong Papua; (6) *G. podocarpa*, Bintuni Papua; (7) *G. moluccana*, Buru Mollucas; (8) *G. decipiens*, Tomuti Lake Sulawesi; (9) *G. decipiens*, Masamba Sulawesi; (10) *G. versteegii*, West Lombok; (11) *G. versteegii*, Bima Seumbawa; (12) *A. cumingiana,* Aibrat Papua (13) *A. filaria* Fak-fak Papua.

Table 6. Principal component analysis of 22 leaf anatomical characters of *Gyrinops* and *Aquilaria* species in Indonesia region east Wallace line.

Variable	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10	PC 11	PC 12
PS	0.3079	0.2388	0.0083	−0.1916	0.0099	−0.1340	0.0206	0.1129	0.2441	0.0736	0.7078	−0.1939
AdP	0.2690	−0.1364	−0.1784	0.0411	0.0827	0.0375	0.0607	−0.6394	0.3911	−0.1181	−0.0092	0.1145
AbP	0.3390	0.0055	−0.2325	0.0619	−0.1449	−0.0841	0.1784	0.0420	−0.2438	0.3574	−0.2074	0.1669
PCL	−0.2855	0.2244	−0.0985	−0.0630	0.1748	0.3084	−0.0635	−0.0183	−0.1260	0.4529	0.0595	−0.0581
PVS	0.3794	0.0580	−0.0543	−0.2192	−0.0002	0.1270	0.1494	−0.0161	−0.0412	0.1423	−0.2039	−0.2572
PTT	0.0947	0.4257	−0.0399	0.2790	0.0319	0.0831	−0.0504	−0.0529	0.0403	−0.0053	−0.0046	−0.5041
PTD	−0.0687	0.3308	−0.2307	0.1046	−0.4002	−0.0303	0.0728	−0.0488	0.0485	−0.0205	0.1168	0.1257
PCS	0.2606	−0.3097	0.0291	0.1102	0.2273	−0.0757	−0.0626	0.1013	−0.2332	0.2381	0.4686	0.2719
MS	0.0465	−0.0999	0.4113	−0.2161	0.2646	0.2168	0.0654	−0.1130	0.1564	−0.0944	−0.0278	0.0812
AdM	−0.0228	−0.0796	−0.0423	−0.1458	−0.3151	0.4935	0.5189	0.1131	0.0791	0.0231	0.1191	0.0639
AbM	0.3667	0.1071	−0.0945	0.2061	0.1051	0.0295	0.1675	−0.0323	−0.1688	0.1136	−0.1670	0.1893
EM	−0.1144	0.1974	0.3268	0.2482	0.2867	0.1674	−0.0318	−0.0981	−0.0092	0.3136	−0.0044	0.0980
MCL	0.1633	−0.0917	0.3952	0.0021	−0.2686	−0.2433	−0.0385	0.0104	0.1268	0.2683	−0.1610	−0.3191
MVS	0.1963	0.0951	0.0023	0.2324	0.0567	0.4580	−0.2124	0.3341	0.4565	0.0222	−0.0998	0.1979
MVN	0.0190	−0.3280	0.0781	0.3379	−0.0069	−0.0772	0.2054	0.5048	0.1458	−0.0670	−0.0619	−0.1824
MCoL	0.2051	0.1928	0.3992	−0.0699	−0.0512	0.0102	−0.0303	−0.0660	−0.2808	−0.0278	−0.0704	0.0236
MCT	−0.0909	0.0907	0.4189	−0.0698	−0.3181	0.0917	0.2675	−0.0874	−0.1158	−0.0403	0.1310	0.1730
MVT	0.0737	0.0976	0.1850	0.4945	0.0993	−0.1098	0.3420	−0.1685	0.0087	−0.2229	0.0649	−0.0008
SCM	−0.0872	−0.0054	−0.1110	−0.2820	0.4673	−0.1037	0.5009	0.0607	0.0770	0.0701	−0.1033	−0.2232
ATP	0.1874	0.3378	−0.0253	−0.1493	0.2163	0.0324	−0.0020	0.2797	−0.2751	−0.5294	−0.0455	0.1271
UEW	−0.3106	0.1590	−0.0628	0.2322	0.1090	−0.2325	0.3055	−0.0009	0.0446	0.0801	0.0966	0.1294
LEW	0.0072	0.3130	0.1037	−0.2338	0.0068	−0.4100	0.0293	0.1885	0.4137	0.1704	−0.2156	0.4055
Eigen-value	5.4877	4.0384	3.4588	2.4935	1.9229	1.7121	1.0735	0.7371	0.5733	0.3412	0.1615	0.0000
% variance	24.9440	18.3560	15.7220	11.3340	8.7406	7.7823	4.8794	3.3506	2.6057	1.5510	0.7339	0.0000

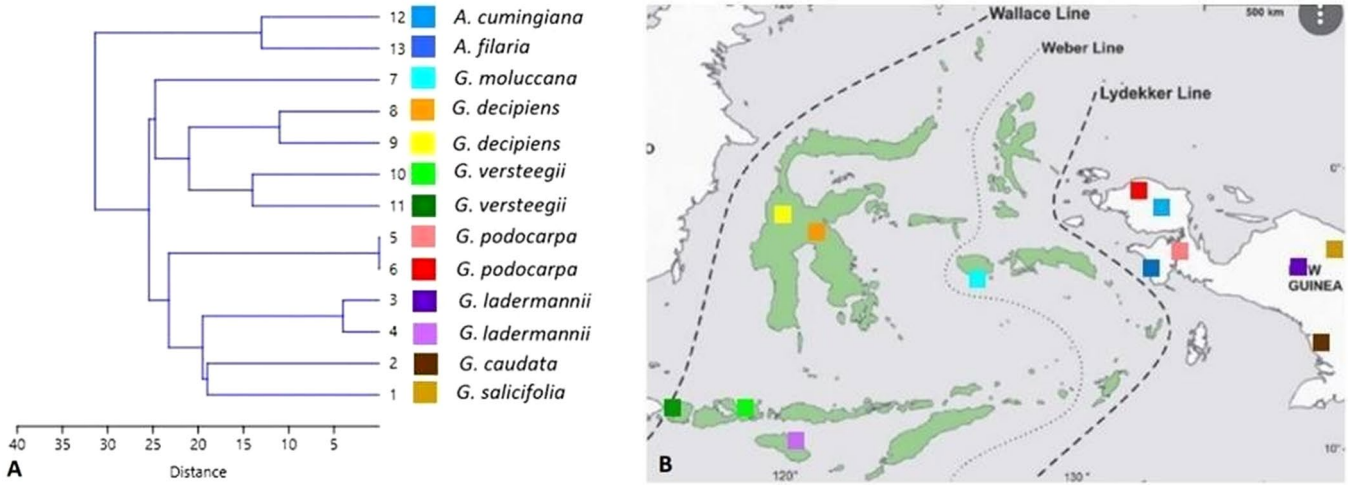

Figure 5. Dendrogram based on leaf anatomy characters of *Gyrinops* and *Aquilaria* in Indonesia region east Wallace line (A). Map of distribution of *Gyrinops* and *Aquilaria* species in Indonesia region east Wallace line (B).

There are differences in stomata types, density and index between *G. versteegii* from West Lombok and *G. versteegii* from Bima Sumbawa, which has anomocytyc, anisocytic and tetracytic stoma, SD 11.11 mm^{-2} and SI 9%, meanwhile *G. versteegii* from west Lombok has anomocytic, actinocytic, anisocytic and paracytic stoma, SD 13.9511 mm^{-2} and SI 13%. Stomata size and stomata index is important in taxonomy and as evidence for the genetic relationship and evolution of plants to some extent, justification for their taxonomic grouping (Ajuziogu et al. 2018). According to Camargo and Marenco (2011), the distribution of stomata on 35 plants in the Amazon Forest is not affected by differences in growing environmental conditions, this indicates that genetic factors have a greater role in determining the character of a plant. Tetracytic stomata type is also found in *Sansevieria trifasciata* which is characterized by four subsidiary cells that are perpendicular and parallel to the stomata (Megia et al. 2015), some species in the Rubiaceae family (Bahadur et al. 1971), and in *Randia* (Judkevich et al. 2020).

Epidermal lamina

Lamina of *Gyrinops* has irregular and polygonal-shaped epidermis, and has various anticlinal thickness cell wall types, depending on species, anticlinal type: smooth and ring thickened on *G. caudata*, rounded thickened on *G. decipiens*, branched and ring thickened on *G. ledermannii*, beaded thickened on *G. podocarpa*, beaded and ring thickened *G. salicifolia*, irregularly thickened *G. versteegii* from west Lombok and branched thickened from Bima Sumbawa. Christophel et al. (1996) stated that anticlinal walls on Lauraceae described as smooth, irregularly thickened, regularly uneven (beaded) or sinuous in outline (buttressed), and Nishida and Christophel (1999) complement the anticlinal type of cell wall on

Beilschmiedia (Lauraceae) into seven types of anticlinal thickening: (a): smooth and angular, (b): irregularly thickened, (c): beaded, (d): buttressed, (e): branched, (f): rounded, (g): undulate, (h): sinuous (Nishida and Christophel 1999). Anticlinal wall thickening type supports a close relationship between *Sinopora* and *Syndiclis*. *Sinopora hongkongensis* is close to *Syndiclis pingbienensis* (Yang et al. 2012). The general leaf cuticular character states of Lauraceae recorded in *Hypodaphnis zenkeri* have two anticlinal types: beaded anticlinal walls on the adaxial surface and buttressed anticlinal walls on the abaxial surface (Kadiri et al. 2019).

Three species of *Gyrinops* have the same epidermal shape on the abaxial and adaxial parts, namely *G. salicifolia*, *G. caudata* and *G. decipiens*. *G. caudata* and *G. decipiens* have a polygonal epidermal shape with straight margins on adaxial and abaxial, like epidermis on *Alocasia longiloba* (Erlinawati and Tihurua 2013), and *G. salicifolia* has a polygonal epidermal shape with straight-wavy margin on adaxial and abaxial. Four other species have a different epidermal shape on the abaxial and adaxial parts of the leaf as in *G. ledermannii* and *G. moluccana* which both have an irregular epidermal shape with straight-wavy margin on the adaxial and straight-sinuous on abaxial. *G. podocarpa* has an irregular epidermal shape with a straight-wavy margin on adaxial and straight on abaxial, vice versa on *G. versteegii*.

The size of the epidermal cells in the adaxial and abaxial parts of the leaf has different sizes in the seven *Gyrinops* species that have been observed. Differences in size can be caused by several factors, including leaf size. According to Hidayat (1995), cell size (increase in size) is closely related to the growth of elongated and widened leaves. The enlarged leaf size is caused by the enlargement of the cells that composed it. The range of length × width of the adaxial epidermis is 18–22 m × 13–18 m, while the range of length × width of the abaxial epidermis is 18–25 m × 13–18 m. The size of the adaxial epidermis with the largest length × width is found in the *G. caudata* is 21.86 ± 1.45 m × 16.56 ± 0.44 m (Table 1), while the largest abaxial epidermis size is found in *G. versteegii* from West Lombok with length × width, 22.94 ± 1.26 m × 13.32 ± 2.12 m (Table 1). The adaxial epidermis of most of the *Gyrinops* and *Aquilaria* genera has a smaller size when compared to the size of the abaxial epidermis. This difference is due to the presence of stomata on the abaxial part of the leaf. The presence of stomata causes changes in the structure of the surrounding epidermis.

Trichomes

Lamina trichomes in the genus *Gyrinops* was found only on the abaxial part of the leaves. Gaharu-producing plants from the Thymelaeaceae family have trichomes that are distributed in the abaxial part of the leaves (Widoyanti et al. 2017). Species that have trichomes on the abaxial part of the leaves are also found in *Gmelina arborea* (Haruna & Ashir 2017). Trichomes on *T. catappa* were also found on the underside of the leaves with glandular trichome type (Ekeke & Agbagwa, 2015). The kind of trichome on the lamina and petiole of *Gyrinops* is a unicellular non-glandular. Non-glandular trichomes are trichomes in which there are no cells that function as channels for the secretion of secondary metabolites (Yuliani and Ratnawati 2018). While trichome type on *Gyrinops* leaves is all species that have pubescent trichomes except on *G. ladermanniii* which has pilous trichomes, and tomentose on *G. podocarpa*. Trichome types in the different organs can be used in the delimitation of the genera and species within the Solanaceae family (Adedeji et al. 2007). However the thickness of the indumentum is divided into three kinds, first sparse on *G. caudata, G. salicifolia* and *G. moluccana*, second moderate on *G. podocarpa, G. decipiens* and *G. versteegii*, and thick only on *G. ledermannii*.

Trichomes and stomata indicate an important role for both in drought tolerance in tomatoes and offer a promising way to select for improved water use efficiency of major crops (Galdon-Armero et al. 2018).

The thickness of trichomes in a species can be influenced by environmental factors such as drought. Plants that live in dry places usually have many trichomes. This is related to one of the functions of stomata, namely to reduce evaporation. According to Priscila et al. (2020), trichomes function as a means of protecting plants from predatory animals and reducing evaporation. *Solanum nigrum* lamina has a mesomorphic structure with some xero-heliomorphic adaptations, the high level of habitat illumination, the greater number of hairs being an adaptation by which the plant could reduce transpiration, and the warming of the leaf surface (Krstiã 2002). The location of the trichomes and the presence or absence of trichomes on *Ficus* petiole can be used as characters at the species level (Akinlabi and Oladipo 2021).

Transversal sections of the midrib

Surrounds the vascular bundle, thickness on *Gyrinops caudata, G. ledermannii, G. salicifolia, G. podocarpa,* surrounds the vascular bundle, moderate on *G. decipiens* from Towuti Lake Sulawesi, *G. versteegii* from Bima Sumbawa*, Aquilaria cumingiana,* adaxial thickness *G. decipiens* and *G. versteegii* west Lombok, absent on *G. moluccana,* and *A. filaria.*

The anatomical characteristics of the midrib are presented in Table 2 and Figure 4. The *Gyrinops* midrib protrudes in an abaxial direction. The shape is erect (*G. ladermannii*), semi-flat (*G. moluccana* and *G. versteegii*), flat (*G. caudata, G. podocarpa* and *G. decipiens*) (Figure 4, Table 3). There are three kinds of epidermis, uniseriate (*G. caudata, G. ledermannii, G. moluccana* and *G. decipiens*), biseriate (*G. versteegii*), triseriate (*G. podocarpa, G. salicifolia*) (Figure 4).

The collenchyma layer is below the adaxial epidermis and the abaxial layer is only found in *G. podocarpa, G. moluccana, G. decipiens* and *G. versteegii*. The cortex layer is composed of a layer of cortical parenchyma and is mostly filled with a layer of sclerenchyma (Figure 4).

The thickness of the sclerenchyma layer of each *Gyrinops* species varies, the thinnest layer (43.78 µm) is in *G. moluccana* and the thickest (93.82 µm) is *G. decipiens*. The vascular bundle network in *Gyrinops* is bicollateral in all species, the shape is different for each species: plano-convex (*G. salicifolia* and *G. decipiens* from Mamasa), interrupted concave–convex (*G. moluccana*), lip (*G. podocarpa*), cordate (*G. versteegii* from West Lombok), semi-convex (*G. ledermannii*), circular (*G. ledermannii*), triangular (*G. caudata*), bolf bat (*G. decipiens* from Towuti Lake) (Table 3).

The shape of the vascular bundle shows clear boundaries between species of the genus *Gyrinops*, so the anatomical structure of the midrib provides an additional feature for distinguishing this species. In *Psidium guajava* leaves, some have high taxonomic significance such as the presence of a hypodermis and the presence of isobilateral and dorsiventral leaf types seems to be a good diagnostic, for generic level characteristics (Al-Edany et al. 2012).

Ca-oxalate crystals found in the *Gyrinops* midrib have varied shapes and locations, there are two forms of Ca-oxalate crystals in the midrib, namely elongoid in the sponge parenchyma of *G. podocarpa*, and styloid in the cortical parenchyma of *G. decipiens* from Towuti Lake, Sulawesi, and *G. versteegii* from west Lombok, while *G. decipiens* from Masamba Sulawesi and *G. versteegii* from Bima Sumbawa, is in the parenchymal sponge tissue (Table 3, Figure 4).

Transversal sections of petiole

Petioles transversal section outline on *Gyrinops* varied in shape, that was depending on the species, ribosome shape (*G. caudata, G. ledermannii* from Jayawijaya Mts, Papua), square (*G. podocarpa)*, square widen (*G. salicifolia)*, square narrow (*G. ledermannii* from Meja Mt. Sumba, *G. versteegii*), semi-circular (*Aquilaria cumingiana*), plano-convex (*A. filaria*) (Figure 4, Table 4). Petiole outline shape was specific on each species, like on *Mangifera* (Norfaizal and Latiff 2013). Studies on the petiole shapes of 46 taxa belonging to the family *Cruciferae* have been reported differences in their petiole shapes (Olowokudejo 1987), eight shapes on *Ficus* (Akinlabi and Oladpo 2021). Five shapes of outline petiole on *Microcos* (Nurul-Aini et al. 2013), four shapes on some species of the family Myrtaceae (Al-Edany et al. 2012).

The *Gyrinops* and *Aquilaria* petioles in the eastern Wallace line are covered with a non- glandular unicellular trichome (Figure 4), which is the same type for all species, as the petiole in the Myrtaceae family, it also has the same kinds of trichome as unicellular glandular (Al-Edany et al. 2012). However, the shape and thickness were different on some species, such as pilous and spread thickly on *G. ledermannii*, tomentose and moderate on *G. podocarpa* and *A. filaria* and pubescent and sparse on *G. caudata, G. salicifolia, G. decipiens, A. Cumingiana* and pubescent with moderate distribution on *G. versteegii* (Figure 4, Table 4).

Adaxial petiole shape was slightly convex on *G. podocarpa and G. versteegii* from Bima Sumbawa; slightly-concave on *G. caudata, G. ledermannii, G. salicifolia*, *G. moluccana* and *A. cumingiana*; flat on G. *decipiens, G. versteegii* from West Lombok and *A. filaria*. Abaxial petiole shape was entire on *G. caudata* and *G. podocarpa*; lobed on *G. salicifolia* and *G. moluccana* and crenate on *G. ledermannii,* G. *decipiens, G. versteegii, A. cumingiana* and *A. filaria* (Figure 4, Table 4). While on *Smilax*, the abaxial shape has eleven kinds, and on abaxial 3 kinds (acuminate, slightly acute and round) (Baruah et al. 2016).

Epidermal petiole uniseriate on *G. caudata, G. ledermannii, G. salicifolia*, *G. moluccana,* G. *decipiens, G. versteegii* from west Lombok, and *A. filaria*; biseriate on *G. versteegii* from Bima Sumbawa and triseriate on *A. cumingiana*. Petiole collenchyma location was in adaxial on *A. filaria*, surrounds the vascular bundle on *G. moluccana*; moderate layer, and surrounds the vascular bundle on *A. cumingiana*; thick layer on *G. caudata, G. ledermannii, G. salicifolia, G. podocarpa*. The cortical parenchyma cells are collenchymatously thickened on *Gyrinops* from Papua (*G. caudata, G. ledermannii, G. salicifolia, G. podocarpa*), and *A. cumingiana* (Figure 4, Table 4).

Petiole xylem thickness all moderate on *G. caudata, G. ledermannii, G. moluccana, G. decipiens* and *G. versteegii*; adaxial thin abaxial moderate on *G. salicifolia*, *A. cumingiana*; adaxial thin abaxial thick on *G. podocarpa*; adaxial moderate abaxial thick on *A. filaria*.

There are nine petiole vascular bundle shapes on *Gyrinops* and *Aquilaria*, ovate horizontal, lip, convex–concave, semi convex–concave, semi convex–concave, continuously plano-convex, semicircular, interrupted plano-convex, circular (Table 4), eleven shapes on *Microscos* (Nurul-Aini et al. 2013), three shape on Myrtaceae (Al-Edany et al. 2012), five shapes on *Ficus* in Southwest Nigeria (Akinlabi and Oladipo 2021).

Petiole crystal shape: styloid on *G. podocarpa, G. ledermannii* from Jaya Wijaya Mt., Papua, styloid & elongoid on *G. caudata, G. salicifolia* and *G. ledermannii* from Meja Mt. Sumba and absent on *G. moluccana, G. decipiens* and *A. cumingiana* (Table 4). On some species in the Myrtaceae family, druse and prismatic crystal shape (Al-Edany et al. 2012).

Petiole outline shape, vascular bundle shape, adaxial surface, abaxial surface, trichome type and distribution, cortical parenchyma cells collenchymatously thickened, crystal shape were anatomical characteristics that can be used as a taxonomic value for identification and classification of species and genus of *Gyrinops* and *Aquilaria* (Table 4; Figure 4). Petiole anatomical characteristics can be used as a taxonomic value for the identification and classification of species in the genus *Ficus* (Ingle and Kothal 2018), *Microscos* (Nurul-Aini et al. 2013). Asteraceae in southwest Nigeria (Mobel et al. 2013).

The relationship between *Gyrinops* and *Aquilaria* species was studied by analyzing the leaf anatomical characteristics (Table 5). Anatomical data were analyzed using a similarity index based on Manhattan-distance and the results were used to construct a dendrogram by UPGMA using the PAST 4.09 program (Hammer et al. 2001). The results of dendrogram analysis were carried out and resulted in three main clusters (Figure 5).

The first cluster is composed of *Aquilaria filaria* and *A. cumingiana* species which are an outgroup of *Gyrinops* species, the geography of distribution is in the area east of the Lydekker line, precisely in the Bird's Head area of Papua. The second cluster consisted of three species *Gyrinops* in the area between the Wallace line and Lydekker line. First *G. moluccana* is located between the Weber line and the Lydekker line, precisely on the island of Buru Moluccas, as a sister group to the second species *G. versteegii*, in the Lesser Sunda Islands and the third species *G. decipiens* from Sulawesi. The position of those areas was located between the Wallace Line and the Weber line. The third cluster of species was *G. podocarpa*, *G. ledermannii*, *G. salicifolia* and *G. Caudata* (Figure 5). Those four species were located in the area east of the Lydekker line, precisely on the mainland of Papua.

However, *G. ledermannii* was also found outlier on Mount Meja, Sumba. The possibility of tectonic history shows that the island of Sumba was once integrated with the Australian

continent, including the mainland of Papua, or Sumba Island was formerly derived from the fragments of the Gondwana continental plate which could not join the Eurasian Plate, after the formation of the Tethys subduction during the Mesozoic Period (Hamilton 1979; Wessink 1991; Rutherford 2000; Astjario 2006). Or due to changes in species composition because of the migration of new species ('Species change'). These changes in species composition resulted from concurrent extinction of existing species and replacement immigration of new species, it is due to the existence of a species–habitat relationship which resulted in a positive correlation between area and habitat diversity and between habitat diversity and species diversity (Wu and Vankat 1995). The pattern in the distribution of species on islands is influenced by ecological and evolutionary processes related to island characteristics such as isolation and area (Wu and Vankat 1995).

Principal component analysis (PCA) showed seven main characters with eigenvalues > 1 (Table 6). The main component with the highest variance was PC1 with 24.94% followed by PC2: 18.36%, PC3:15.72% and PC4: 11.33%. Values on PC1, PC2, PC3 and PC4 have a high contribution in the grouping of 13 tested species. Characters with the highest values >0.3, in PC1, were found in PS (petiole shape), AbP (abaxial petiole), PCL (collenchyma petiole location) and AdM (adaxial midrib); in PC2 in PVS (petiole vascular bundle shape), PTT (petiole trichome type) and ATP (Anticlinal thickness of upper & lower lamina epidermis); in PC3 in PCS (petiole crystal shape), AbM (abaxial midrib), EM (epidermal midrib), MCL (midrib crystal-Ca-Oxalate) and MCS (Midrib crystal Ca-Oxalate shape); PC4 in MVN (midrib vascular bundle) (Table 5). Haydar et al. (2007) showed the characters that have a high positive value in the principal component vector, which they contributed the maximum to the diversity of genetic material.

Key of indentation

Diagnostic taxonomic key of indentation based on the leaf anatomical character observed from species belonging to the genera *Gyrinops* and *Aquilaria* in the Indonesian region east of the Wallace line.

1.a. Stomata position is protruding with epidermal layer 2
b. Stomata position is parallel with epidermal layer 3

2.a. Petiole outline shape is plano-convex, collenchymatous thickened on the cortical parenchyma cells, sclereid absent in cortical parenchyma..... ***Aquilaria cumingiana.***
b. Petiole outline shape is semi-circular, the cortical parenchyma cells are not thickened, sclereid present in cortical parenchyma..... ***A. filaria.***

3.a The cortical parenchyma cells are collenchymatous thickened 4
b. The cortical parenchyma cells are not thickened 5

4.a. Petiole is covered by pilous trichome, thick..... ***Gyrinops ledermannii.***
b. Petiole pubescent and tomentose, sparse – moderate..... 6

5.a. Petiole vascular bundle shape horizontally oval, Ca-oxalate crystal absent..... ***G. moluccana.***
b. Petiole vascular bundle shape semi-circular, Ca-oxalate crystal present.....7

6.a. Petiole pubescent, sparse, adaxial slightly concave, petiole and midrib bundle shape is not lip form like, Ca-oxalate crystal and collenchyma layer midrib absent 8
b. Petiole tomentose, moderate, adaxial petiole slightly convex, petiole and midrib bundle shape lip form like midrib has styloid and elongoid Ca-oxalate crystal, and collenchyma layer surrounds vascular bundle ..... ***G. podocarpa.***

7.a. Petioles pubescent sparsely, abaxial midrib crenate, lamina lower epidermal wall shape straight..... ***G. decipiens.***
b. Petioles are evently pubescent, abaxial midrib rounded, lamina lower epidermal cell wall shape is rounded..... ***G. versteegii.***

8.a. Outline petiole shape ribosomal form like, abaxial entire, vascular bundle convex-concave, midrib vascular bundle triangular, stephanocytic bicyclic stoma..... ***G. caudata.***
b. Petiole shape square widely form like, abaxially rounded, vascular bundle continuously plano-convex, midrib vascular bundle plano-convex, actinoparaheptacytic, stoma..... ***G. salicifolia.***

Conclusion

Based on the results of the study, it can be concluded that the anatomical characteristics of the paradermal lamina, midrib and petiole have taxonomic value for identification and classification (as anatomical markers) in the species and genera of *Gyrinops* and *Aquilaria* in Indonesia region at the east Wallace Line. These markers were obtained from the midrib crystal type character, the character that separated the *Aquilaria* cluster as an outgroup from the *Gyrinops* cluster, while the petiole shape character separated the *Gyrinops* species cluster in the Papua region from the western part of the Lydekker Line. The abaxial petiole character, the location of the petiole collenchyma, and the shape of the petiole bundle are the distinguishing features of *Gyrinops* species in the Wallace area and the western part of the Wallace Line. *Gyrinops* species originating from Sulawesi and the Lesser Sunda Islands are distinguished based on the characteristics of petiole shape, petiole trichome density, petiole crystal type, midrib shape, abaxial midrib, and epidermal midrib.

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Author contributions

T. Mulyaningsih: wrote the manuscript; A. L. Sunarwidhi: prepared the Table 1, 2, 3, 4; V. Febrianti: prepared the Figures 2 & 3; Bq. P. Sari,: prepared the Figures 4; A. Muspiah: prepared the Figures 1; K. Sukenti: prepared the Figures 5 & Table 5; S. Hadi: wrote the manuscript. M. Ito: wrote the manuscript. I. Yamada: wrote the manuscript. All authors contributed to the article and approved the submitted version.

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