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THE ANATOMICAL CHARACTERS OF *Ampelocissus* (Vitaceae) IN SUMATRA

Ciri Anatomi *Ampelocissus* (Vitaceae) di Sumatera

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ABSTRACT
The revision of Ampelocissus *was performed by observing the anatomical character of* Ampelocissus *leaves. The leaves were collected from 33 collection numbers of Herbarium Bogoriense (BO) and four collection numbers from Sumatra exploration. The purpose of this study is to update the information of diversity and to support species concept delimitation of* Ampelocissus *based on the anatomical study, especially on the sterile plant. Anatomical characters were observed on nine species and one variety of Sumatran* Ampelocissus*.* Ampelocissus *species is varied by the anatomical characters, i.e.: shape of the anticlinal wall of abaxial and adaxial epidermal cell, number of palisade layers, upper epidermal cell thickness, leaf thickness, presence of papillae on stomata neighboring cell, type, and shape of calcium oxalate crystals, also stomata position. The cluster analysis of* Ampelocissus *in Sumatra based on 16 anatomical characters showed the coefficient similarity in the range of 0.48 - 0.81. The research showed that the leaf anatomical characters can be used as additional characters to distinguish the species of* Ampelocissus*.*

Keywords: *calcium oxalate crystals, diversity, papillae, similarity coefficient, species concept*

ABSTRAK
Revisi *Ampelocissus* dilakukan dengan mengamati ciri anatomi daun dari marga *Ampelocissus*. Sebanyak 33 nomor koleksi Herbarium Bogoriense (BO) dan empat nomor koleksi hasil eksplorasi di Sumatera digunakan. Penelitian ini bertujuan untuk memperbarui informasi mengenai keanekaragaman dan mendukung konsep jenis *Ampelocissus* berdasarkan ciri anatomi, terutama pada tumbuhan steril. Studi anatomi dilakukan pada sembilan jenis dan satu varietas *Ampelocissus* di Sumatera. Jenis-jenis *Ampelocissus* di Sumatera bervariasi pada ciri anatomi yaitu bentuk dinding antilkinal sel epidermis pada bagian abaksial dan adaksial, jumlah lapisan jaringan tiang, ketebalan sel epidermis atas, ketebalan daun, kehadiran papila, jenis dan bentuk kristal kalsium oksalat, serta posisi stomata terhadap sel epidermis pada bagian abaksial daun. Analisis pengelompokan *Ampelocissus* di Sumatera menggunakan 16 ciri antomi dan menghasilkan fenogram dengan koefisien kemiripan 0,48 - 0,81. Ciri anatomi daun dapat digunakan sebagai ciri tambahan yang memiliki nilai taksonomi dalam membedakan jenis-jenis *Ampelocissus* di Sumatera.

Kata Kunci: keanekaragaman, kristal kalsium oksalat**,** koefisien kemiripan, konsep jenis, papila

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INTRODUCTION

Ampelocissus L. is one genus of the Vitaceae family that is distinguished from other Vitaceae by 4-5 merous flower, 5-10 grooved floral disk, tendrils above the inflorescences, white to red hair on the surface, and T-shaped seeds cross-section. *Ampelocissus* grow in the subtropical or tropical forest, along the river banks and open areas of Dipterocarp lowland forest (Wen 2007, Yeo et al. 2013). There are several species also found in the secondary forest.

There are 95 species were distributed in Asia, Australia, Africa and Central America (Wen 2007). Malesia is an important distribution area for *Ampelocissus* with 39 species found (Wen et al. 2013). Previously, seven species of *Ampelocissus* were discovered in Sumatra (Miquel 1863, Planchon 1887, Merrill 1938, Latiff 1982).

The latest study reported that nine species and a variety of *Ampelocissus* were found in Sumatra. The morphological study has clustered *Ampelocissus* of Sumatra into three groups, namely Group I (*A. arachnoidea*), II (*A. elegans*, *A. filipes, A. korthalsii, A. thyrsiflora, A. imperialis, A. ochracea*, *A. ochracea* var. *trilobata*) and III (*A. gracilis, A. polythyrsa, A. rubiginosa*), based on its habitat, tendril, leaf, indumentum, inflorescence and flower characteristics (Dalimunthe et al. 2016).

Morphologically, *Ampelocissus* can be distinguished from other Vitaceae members from the floral characters (Sussenguth 1953), but that identification seems to be difficult to be done because of the small, inconspicuous and ephemeral flowers (Gerrath et al. 2004). The identification based on the generative part, particularly the sterile plant is difficult to be investigated since the floral characters have an important role to identify the species. Therefore, to strengthen the identification of *Ampelocissus* based on morphological characters, and species delimitation concept of *Ampelocissus* in Sumatra, additional studies such as the anatomy of leaves or seeds are needed.

Anatomically, the stem central cylinder of Vitaceae has broad and linear intrafascicular rays. These intrafascicular rays type could define whether the habit of Vitaceae ancestor was a shrubby or arboreal plant (Adkinson 1913). The anatomical study of *Cayratia* and *Tetrastigma* in Thailand suggested that anatomical characters could be used as the diagnostic characters for specific genera (Kochaipat et al. 2013). *Tetrastigma* has druse calcium oxalate crystals on their leaves (Aishah 2016). In the comparative anatomical study of Vitaceae and closely related family, Leeaceae, it was proved that starch grains and trichomes are only found in *Cayratia mollissima* and *Pterisanthes caudigera,* not in *Leea indica* (Najmaddin et al. 2011, 2013). Ickert-Bond and Wen (2014) have presented leaf anatomical characters of *Vitis*–*Ampelocissus* clade which correlates with a transition from tropical to temperate climate, which inform that stomata on toothed leaves margin, mesophyll differentiation, restricted trichomes distribution and lack number of raphides and oxalate crystals are more abundant and distinct on temperate species, compare to tropical species.

Because of the above reasons, this anatomical study is expected to be additional characters which can support their species delimitation concept. A study on the seed anatomy of *Ampelocissus*, *Cissus*, and *Leea* has been done by Manchester et al. (2012) to confirm the identification of some *Ampelocissus* and *Cissus*, and also the biogeographical history in South America. According to Carlquist (1957, 1991), Dean and Ashton (2008), anatomical characters of leaves have demonstrated taxonomic utility in numerous vascular plant families. Whereas Munson (1909), Comeaux et al. (1987), Chen et al. (2007), Wen (2007), Ma et al. (2016), Moore and Wen (2016) showed that the micromorphology of leaf trichomes is taxonomically informative, but trichomes alone have been insufficient to resolve taxonomic difficulties in the *Vitis* group. Furthermore, micromorphological and anatomical characters of New World *Vitis* leaves have been studied (Ickert-Bond et al. 2018).

As *Vitis*, the identification of Ampelocissus is also not easy without inflorescence. Therefore, a study on the leaf anatomy of *Ampelocissus* has been carried out. The aim of this study is (1) to provide a supporting character for identification especially when the inflorescence is lacking, (2) to provide the additional characters to *Ampelocissus* in Sumatra for species delimitation.

MATERIALS AND METHODS

Place and time of the research

This research was conducted in April-June 2015. The laboratory work was carried out in the Ecology and Plant Resources Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University. This study was also conducted at the Plant Biosystematic Laboratory and Morphology, Anatomy and Cytology Laboratory of Botany Division, Research Center for Biology, Indonesian Institute of Sciences (LIPI), Cibinong. The transversal section using the freezing method was done in the Laboratory of Wildlife Management and Reproduction of Zoology Division, Research Centre for Biology, Indonesian Institute of Sciences (LIPI), Cibinong, Indonesia.

Table 1. The examined species of *Ampelocissus* in Sumatra

No.	Species	BO Number	Collection Number	Collector	Location, GPS Coordinate [Latitude; Longitude]
1.	*A. arachnoidea*	1617260	5387	Docters van Leeuwen-reijnvaan	Sebesi Isl. (Sunda Strait), [–5.940556; 105.488889]
2.	*A. arachnoidea*	1617289	26	Leg.	Sumatra
3.	*A. elegans*	1618602	34–2	Buddingh	Bangka
4.	*A. elegans*	1618601	s.n.	Teysmann	Riau, Bangka
5.	*A. filipes*	1864859	12221	WJJD de Wilde and BEE de Wilde	Ketambe. Valley of Lau Alas. Near Tributary of Lau Ketambe C 35 Km NW f Kutajane, [–3.677778; 97.661667]
6.	*A. filipes*	1864861	14581	WJJD de Wilde and BEE de Wilde	Ketambe. Valley of Lau Alas. Near tributary of Lau Ketambe c. 35 km NW of Kutajane, [–3.67777; 97.661667]
7.	*A. filipes*	–	18095	WJJD de Wilde and BEE de Wilde	Ketambe. Valley of Lau Alas. Near tributary of Lau Ketambe c. 35 km NW of Kutajane, [–3.67777; 97.661667]
8.	*A. filipes*	1618722	4638	JA Lorzing	Sibolangit, North Sumatra
9.	*A. filipes*	1618725	3539	CGGJ van steenis	Raja Isl., Palembang, [–4.295556; 103.553333]
10.	*A. gracilis*	–	375	K Iwatsuki, G Murata, J Dransfield, D Saerudin	Sikundar Nature Conservation, Tanjung, Bandar Lampung, [–3.815278; 98.025556]
11.	*A. gracilis*	–	19313	WJJD de Wilde and BEE de Wilde	Sekundur Forest Reserve. c. 75 km, WNW of Medan, Besitang River, North Sumatra, [3.916667; 98.083333]
12.	*A. gracilis*	1846657	18282	W Takeuchi, E Sambas	Batangtoru, South Tapanuli, North Sumatra, [1.551111; 99.070556]
13.	*A. imperialis*	1617453	s.n.	Teysmann	Bangka
14.	*A. imperialis*	–	5676	Teysmann	Bangka
15.	*A. ochracea*	1617310	9204	CGGJ van Steenis	Gajolanden near bivak Aer Poetih waterfall, Aceh, [4.019167; 97.151111]

Table 1. The examined species of *Ampelocissus* in Sumatra (continued)

No.	Species	BO Number	Collection Number	Collector	Location, GPS Coordinate [Latitude; Longitude]
16.	*A. ochracea var trilobata*	1814796	18550	W Takeuchi, Juprisi Zegar, Kolang Sihotang	Batangtoru, South Tapanuli, North Sumatra, [1.496667; 99.066389]
17.	*A. polythyrsa*	–	4	SH Dalimunthe	Way Kambas NationalPark, Bandar Lampung, [–5.02821; 105.77734]
18.	*A. polythyrsa*	–	15589	WJJD de Wilde and BEE de Wilde	Gunung Leuser Nature Reserve. Climbing Gunung Bandahara; c 6 km NE of Kampung Seldok (Allas Valley). c. 25 km N of Kutacane, Aceh, [3.681111; 97.712778]
19.	*A. rubiginosa*	1622855	251	Soepadmo	Riau Upper, Tenayan Raya, Riau, [0.498889; 101.547778]
20.	*A. rubiginosa*	184397	18328	W Takeuchi and E Sambas	Batangtoru, South Tapanuli, North Sumatra, [1.555278; 99.065833]
21.	*A. thyrsiflora*	1613873	–	JD Kobus	Selambay, Bangka. Maleisch, [–2.288611; 106.064444]
22.	*A. thyrsiflora*	1610383	3	Kostermans	Lobok Besar (SE. part), Bangka
23.	*A. thyrsiflora*	1610382	151	Kostermans	Lobok Besar, Bangka, [–2.568056; 106.626667]
24.	*A. thyrsiflora*	1610381	1025	Kostermans	Lobok Besar. Mt. Pading. Bangka, [–2.688889; 106.556667]
25.	*A. thyrsiflora*	1613876	302	s.n.	Batoe Eialndr, Archipelago Indonesia., [–0.2; 98.499722]
26.	*A. thyrsiflora*	1610374	15595	WJJD de Wilde and BEE de Wilde	Gunung Leuser Nature Reserve. Aceh. Sumut; climbing Mt. Bandahara; c 6 km NE of Kampung Seldok (Allas Valley). c. 25 km N of Kutacane, Aceh, [3.,681111; 97.712778]
27.	*A. thyrsiflora*	1613874	5605	JA Lorzing	Sembahe, Sibolangit, North Sumatra, [3.3725; 98.581944]
28.	*A. thyrsiflora*	1608465–1613880	7195	Bunnemeijer	Singkep Isl., Lingga Archipelag, [–0.472778; 104.425833]
29.	*A. thyrsiflora*	1616389	1602	Achmad	Simaloer, Simeuleu, Tapah Isl., [2.353333; 96.242222]
30.	*A. thyrsiflora*	1610367	1905	HS Yates	Sumatera East Coast
31.	*A. thyrsiflora*	1610368	9320	CGGJ van Steenis	Gajolanden, Aceh, [4.093889; 97.735]
32.	*A. thyrsiflora*	–	50–28	Teysmann	Bangka
33.	*A. thyrsiflora*	–	–	JD Kobus	Bangka
34.	*A. thyrsiflora*	–	6	SH Dalimunthe	Balun Ijuk, Merawang, Bangka, [–2.067222; 106.077778]
35.	*A. thyrsiflora*	–	11	SH Dalimunthe	Bukit Manggis, Gerunggang, Bangka, [–2.104167; 106.095556]
36.	*A. thyrsiflora*	–	7	SH Dalimunthe	Balun Ijuk, Merawang, Bangka, [–2.071389; 106.081667]
37.	*A. ochracea var trilobata*	1814796	18550	W Takeuchi, Juprisi Zegar, Kolang Sihotang	Batangtoru, South Tapanuli, North Sumatra, [1.496667; 99.066389]

Material

The research material is a mature leaf from 33 dry herbarium specimens deposited in the Herbarium Bogoriense (BO) and four wet materials collected in the field. The data was collected from nine species and one variety of *Ampelocissus* in Sumatra, i.e. *A. arachnoidea, A. elegans*, *A. filipes, A. thyrsiflora, A. imperialis, A. ochracea*, *A. ochracea* var. *trilobata*, *A. gracilis, A. polythyrsa, A. rubiginosa* (Table 1).

Method

Anatomy of leaves based on the paradermal and transversal section have been conducted. The paradermal section followed the procedure of Cutler (1978). Base, middle and tip of the leaves were used as the observation points. Each leaf part was cut into 1×1 Cm in size, then soaked in 16.22% nitric acid solution for 5 minutes and boiled with a hotplate in the fume hood until the epidermis was detached. For clearing and staining preparation, the section was immersed in a choral hydrate solution for 30 seconds to remove chlorophyll, washed with distilled water and soaked in a 2% safranin stain solution for 1 minute. The paradermal section was placed on a microscope slide, added with 1 drop of glycerin and covered with a cover glass. The edge of the cover glass was smeared with clear nail polish to make a semi-permanent slide.

The transversal section was done by the freezing method. The middle leaves were cut 1×1 cm and this cutting section will be used for making anatomy preparation. The cutting leaves were prepared on the freezing microtome specimen holder and frozen with CO_2 gas. After freezing, the leaf was sliced using a Yamato RV-240 freezing microtome into 20-25 μm in size. The clearing and staining preparation process followed the same procedure as paradermal section.

There were 16 characters of paradermal and transversal section observed (Table 2). The observation on paradermal and transversal sections was conducted using Nikon e80*i* light microscope series and photos were taken using Nikon camera. The terminology of anatomical characters followed Metcalfe and Chalk (1950), Nishida and Christophel (1999), Ren et al. (2003), Ifrim et al. (2012), Monteiro et al. (2013) and Najmaddin (2014). The description of papillae followed Pandanaceae papillae classification (Tomlinson 1965). The similarity analysis was carried out using NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System) 2.11 software (Rohlf 2000). The similarity coefficient was calculated by SIMQUAL, using a simple matching index and UPGMA (Unweighted Pair Group Method with Arithmetic Mean) grouping method.

RESULTS AND DISCUSSION

Ampelocissus species varied on anatomical characters. The anatomical variation was observed in both leaf paradermal and transversal sections (Table 2). Variations in the anatomical characters are found in the shape of the anticlinal wall on adaxial and abaxial epidermal cells, the number of palisade layers, the upper epidermal cells and leaf thickness, the presence of papillae on stomata neighboring cell, the type and shape of calcium oxalate crystals and the position of stomata.

In the adaxial part of *Ampelocissus* leaves, there are three shapes of anticlinal walls of epidermal cells. The shape anticlinal walls of epidermal cells are angular (*A. imperialis, A. ochracea, A. polythyrsa*) (Figure 1A), rounded (*A. elegans, A. filipes, A. rubiginosa, A. thyrsiflora*) (Figure 1B), and sinuous (*A. arachnoidea, A. gracilis, A. ochracea* var*. trilobata*) (Figure 1C). Meanwhile, the abaxial leaves has four shapes of the anticlinal walls: rounded (*A. imperialis, A. thyrsiflora*) (Figure 1D), angular (*A. filipes, A. polythyrsa, A. rubiginosa*) (Figure 1E); undulate (*A. arachnoidea, A. elegans, A. gracilis, A. ochracea*) (Figure 1F) and sinuous (*A. ochracea* var*. trilobata*) (Figure 1G).

The types and compositions of unbranched and branched trichomes are the important characters in delimiting a species in Vitaceae (Lombardi 2007), including *Ampelocissus*. The trichome type was observed by the transversal section from the adaxial and abaxial leaf surfaces. Trichome type varies on *Ampelocissus*, namely dense simple uniseriate trichomes (*A.elegans, A. ochracea* var*. trilobata*) (Figure 1H); unicellular glandular trichomes (*A. filipes, A. gracilis, A. imperialis, A. ochracea, A. rubiginosa*) (Figure 1I). *Ampelocissus arachnoidea, A. elegans, A. polythyrsa, A. thyrsiflora* and *A. ochracea* var*. trilobata* have two types of trichomes, simple uniseriate

Table 2. Anatomical variation in leaf paradermal and transversal sections of *Ampelocissus* in Sumatra

No.	Characters	1	2	3	4	5	6	7	8	9	10
1.	Shape of anticlinal wall on adaxial epidermal cell	S	PR	PR	S	AP	AP	S	AP	PR	PR
2.	Shape of anticlinal wall on abaxial epidermal cell	PS	PS	PO	PS	PR	PS	SW	PO	PO	PR
3.	Adaxial simple uniseriate trichomes (SS)	–	+	–	–	–	–	+	–	–	–
4.	Abaxial simple uniseriate trichomes (SS)	+	+	–	–	+	+	+	+	+	+
5.	Abaxial unicellular glandular trichomes (SG)	+	+	+	+	–	–	+	+	+	+
6.	Abaxial multicellular glandular trichomes (SGM)	–	–	–	–	+	–	–	–	–	–
7.	Two types of trichomes on abaxial part	+	+	–	–	+	–	+	+	+	+
8.	Presence of papillae on stomata neighboring cell	–	–	+	–	+	+	+	+	–	+
9.	Shape of papillae	–	–	E	–	E	E	E	FA	–	E
10.	Stomatal position on abaxial leaf epidermis	F	F	EM	F	EM	FP	FP	F	F	FP
11.	Leaf thickness (μm)	120	190	100	220	100	160	126	220	300	290
12.	Upper epidermal cell thickness	TK	TN	TN	TN	TN	TN	TN	TN	TN	TN
13.	Number of palisade layer	1	1	1	1	1	1	1	1	2	1
14.	Raphid solitary calcium oxalate crystals	–	–	–	–	–	+	+	–	–	–
15.	Raphid clustered calcium oxalate crystals	+	+	–	+	+	–	–	+	+	+
16.	Raphid acicular calcium oxalate crystals	–	–	+	+	–	–	–	–	–	–

Information: 1=*A. arachnoidea*, 2=*A. elegans*, 3=*A. filipes*, 4=*A. gracilis*, 5=*A. imperialis*, 6=*A. ochracea*, 7= *A. ochracea var. trilobata*, 8= *A. polythyrsa*, 9=*A. rubiginosa*, 10=*A. thyrsiflora*; (+)=present, (–)=absent, PR= rounded, S=sinuolate, AP=angular, PS= sinuous, PO= undulate, SW=sinuolate wavy, SS=simple uniseriate trichomes, SG=unicellular glandular trichomes, SGM=multicellular glandular trichomes, E=erect, F=flat, EM=emerged, FA= fall, FP=flat papillae, TK=thick, TN=thin, 1=1 layer, 2=2 layer

trichomes and unicellular glandular trichomes (Figure 1J), while *A. imperialis* has dense and multicellular glandular trichomes (Figure 1K).

All *Ampelocissus* species in Sumatra have anomocytic type of stomata with variations of papillae on the stomata structure. *Ampelocissus arachnoidea, A. elegans, A. gracilis,* and *A. rubiginosa* have no papillae (Figure 1L). The presence of papillae is recorded on *A. filipes, A. imperialis, A. ochracea, A. thyrsiflora, A. ochracea* var. *trilobata* with erect papillae (Figure 1M); and *A. polythyrsa* with flat papillae (Figure 1N).

The papillae on the stomata guard cell are found on species with woolly trichomes on the abaxial part of the leaves, i.e. *A. filipes, A. imperialis, A. ochracea, A. polythyrsa, A. thyrsiflora* and *A. ochracea* var. *trilobata*. On the other hand, it is absent in the species with woolly to strigose trichomes, i.e. *A. arachnoidea* and with scattered villous to tomentose trichomes i.e. *A.elegans* and *A. gracilis* and *A. rubiginosa.*

The presence of papillae on leaves with thick trichomes is possibly caused by adaptation to the environment and being inherited to the next generations. The structure of the epidermis varies, especially papillae on the stomata that functions to reduce transpiration (Tomlinson 1965) since these plants usually grow in dry areas.

In *Ampelocissus* species, papillae on the stomata structure are protruded from neighboring epidermal cells. Variation was seen on species *A. ochracea, A. polythyrsa, A. thyrsiflora, A. ochracea* var. *trilobata, A.*

filipes, A. imperialis, A. gracilis and *A. rubiginosa* with elongated, prominent, curved papillae emerging from the neighboring cells and covering the stomata, made the stomata appear sinking below the epidermis. It suggests that these papillae were formed from the outer epidermal cell walls. Stomatal density and distribution in vine plants especially Vitaceae was influenced by the plant forms and habitat (Tay and Furukawa 2008). But the variation on the stomata is caused by genetic factors and rarely found from the environmental factors (Tomlinson 1965).

There are 6 variations found on the leaf transversal sections, i.e.: leaf thickness, number of palisade layer, the presence and shape of raphid or druse calcium oxalate crystals, and the stomatal position on the abaxial leaves. The adaxial or abaxial leaf surface is covered by a variety of trichomes. On the adaxial leaf surface of *A. elegans* and *A. ochracea* var. *trilobata* has uniseriate simple trichomes (Figure 2A). On the abaxial part, *A. filipes, A. gracilis, A. imperialis, A. ochracea,* and *A. rubiginosa* has uniseriate simple trichomes (Figure 2B). In the

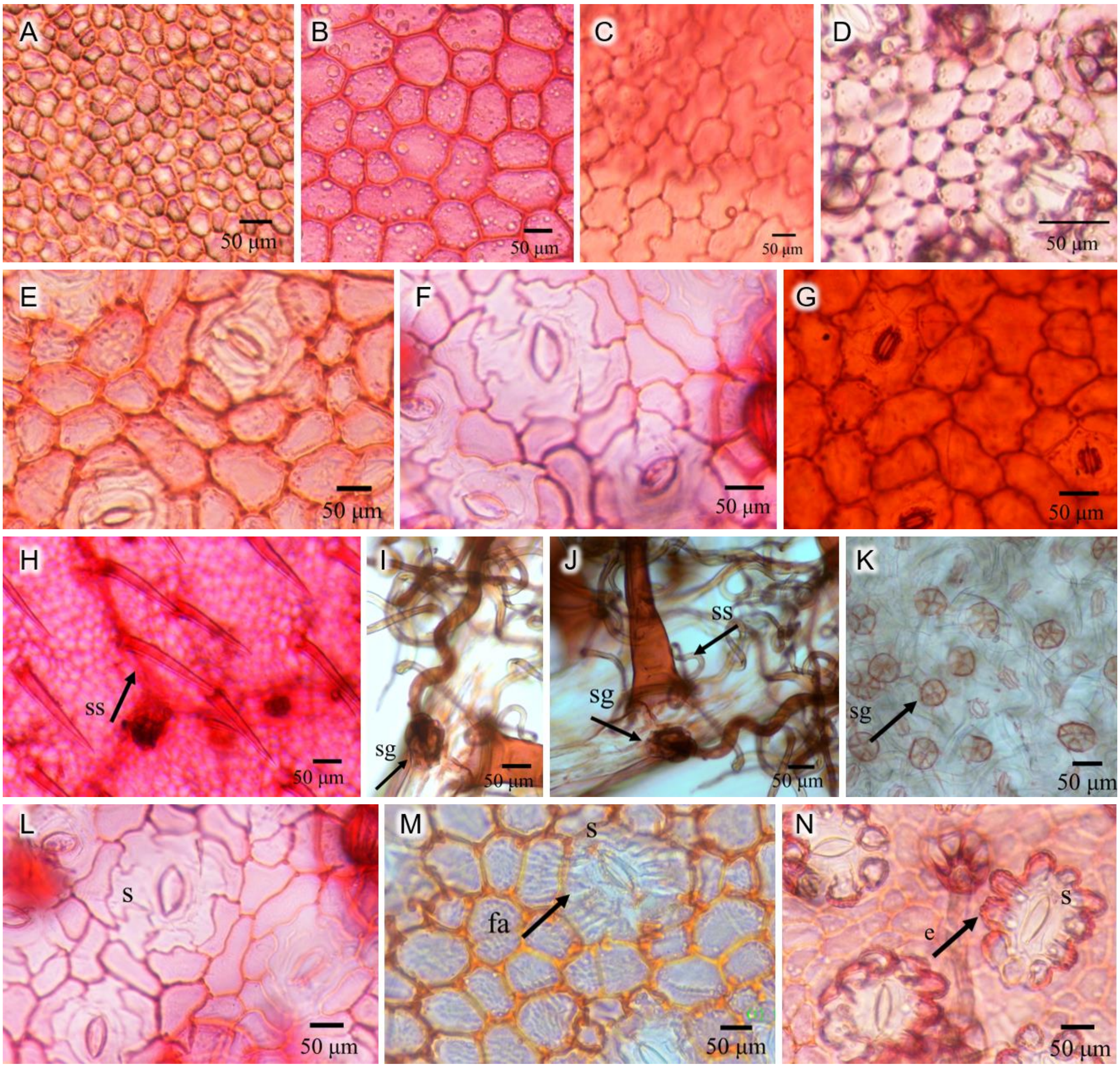

Figure 1. Leaf paradermal sections of *Ampelocissus* in Sumatra (A-C. Shape of anticlinal wall on adaxial epidermal cell: A. angular, B. rounded, C. sinuos; D-G. Shape of anticlinal wall on abaxial epidermal cell: D. rounded, E. angular, F. undulate, G. sinuos; H-K. Leaf trichomes type: H. adaxial, I-K. abaxial, sg= unicellular glandular trichomes, sgm= multicellular glandular trichomes, ss= simple uniseriate trichomes; L-N. Presence of papillae on stomata neighboring cell: L. glabrous, M. fa= fall papillae, N. e= erect papilla, s= stomata)

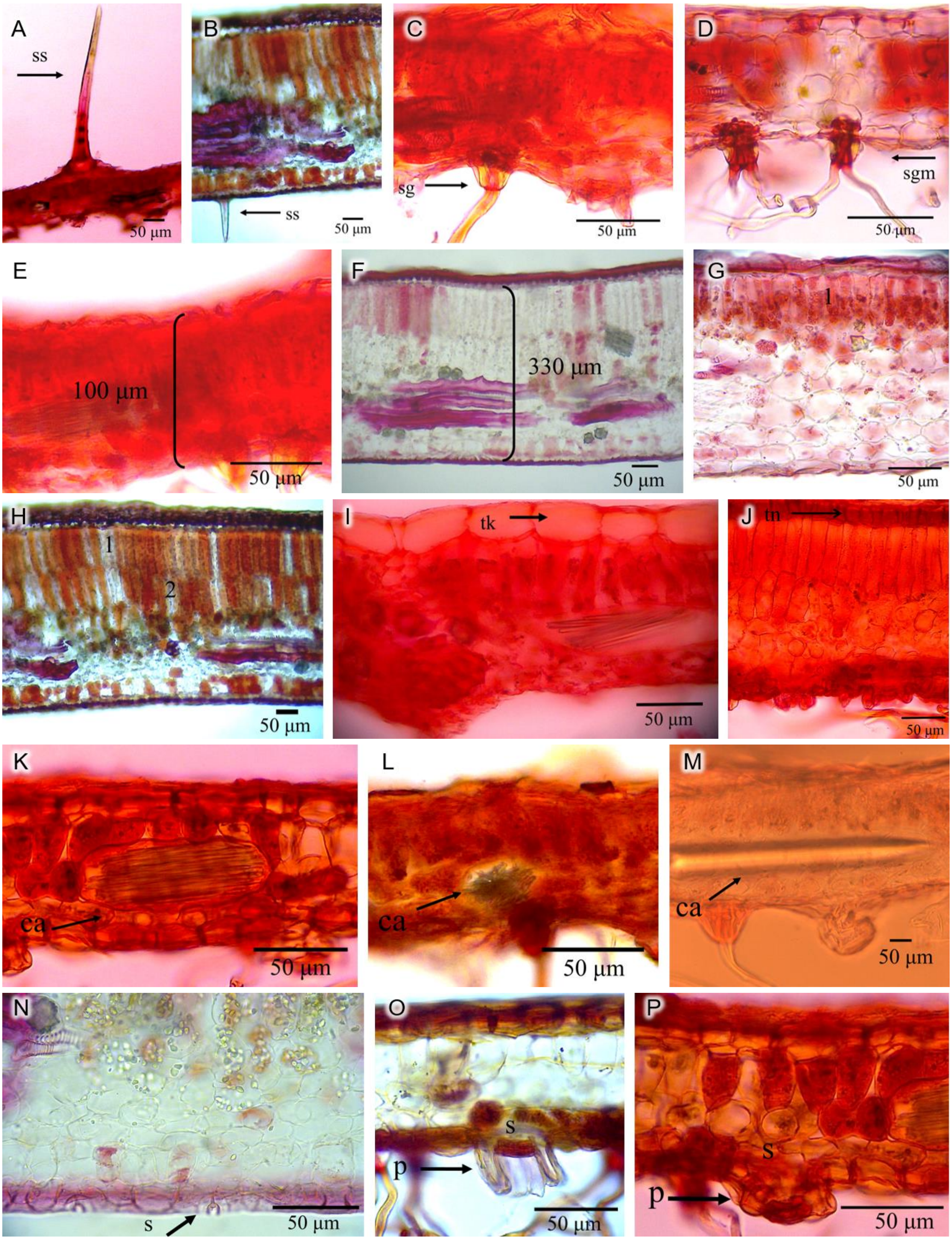

Figure 2. Leaf transversal sections of *Ampelocissus* in Sumatra. (A-D. Leaf trichomes type: A. Adaxial, ss= simple uniserriate trichomes; B-D. Abaxial: B. ss= simple uniseriate trichomes, C. sg= unicellular glandular trichomes, D. sgm= multicellular glandular trichomes. E-F. Leaf thickness: E. thin ≤200 µm, F. thick ≥210 µm; G-H. Number of palisade layer: G. one layer, H. two layer, 1= first palisade layer, 2= second palisade layer. I-J. Upper epidermal cell thickness: I. tn= thin ≤10 µm, J. tk= thick ≥25 µm; K-M. Raphid calcium oxalate crystals shape: K. clustered, L. acicular, M. solitary, ca= calcium oxalate crystals; N-O. Stomatal position on abaxial leaf epidermis: N. flat, O. flat stomata with higher papillae; P. emerged; p=papillae, s=stomata)

meanwhile, *A. arachnoidea, A. elegans, A. polythyrsa, A. thyrsiflora* and *A. ochracea* var*. trilobata* has two types of trichomes uniseriate simple trichomes and unicellular glandular trichomes (Figure 2C). *Ampelocissus imperialis* has dense and unique multicellular glandular trichomes (Figure 2D), whereas on one glandular consisted of more than 3 trichome strands. The trichomes type is visible in the leaf transversal section.

The range of *Ampelocissus* leaf thickness is observed on 150-330 μm. The leaf thickness was categorized as thin if it is ≤200 μm (*A. arachnoidea, A. elegans, A. filipes, A. imperialis, A. ochracea, A. ochracea* var*. trilobata*) (Figure 2E) and categorized as thick if it is ≥210 μm *(A. gracilis, A. polythyrsa, A. rubiginosa, A. thyrsiflora*) (Figure 2F). The number of palisade layers varies from single to two-layers. Almost all *Ampelocissus* in Sumatra have a single palisade layer (Figure 2G), except *A. rubiginosa* has two palisade layers (Figure 2H). The upper leaf epidermis varies from thick, (≥25 μm), such as *A. arachnoidea* (Figure 2I) to thin (≤10 μm), as for other species are presented in Figure 2J.

Calcium oxalate crystals in Vitaceae are also an important character and its presence in Vitaceae varies on each species. The presence of calcium oxalate crystals on the leaf tissue is not only influenced by environmental factors, but also by genetic factors (Ifrim et al. 2012). All *Ampelocissus* species have a raphid and druse calcium oxalate crystals types. Also, there is cell sap (mucilage cell) found on empty calcium oxalate crystals sack (Metcalfe and Chalk 1950). Raphid calcium oxalate crystals are found scattered in the palisade, vessels, and sponge tissue. The calcium oxalate crystals and cell sap present in every organ of Vitaceae, but absent on the epidermis (Kannabiran and Pragasam 1994).

Raphid calcium oxalate crystals on *Ampelocissus* are divided into three types of shape. The acicular type was found on the *A. gracilis* and *A. filipes* (Figure 2K). The clustered type was found on the *A. arachnoidea, A. elegans, A. imperialis, A. polythyrsa, A. rubiginosa* and *thyrsiflora* (Figure 2L). The solitary type was found on the *A. ochracea* and *A. ochracea* var*. trilobata* (Figure 2M).

There are 3 variations in the stomata position on abaxial leaf epidermis which can be divided into three types: type I, flat (*A. arachnoidea, A. elegans, A. gracilis, A. rubiginosa, A. polythyrsa*) (Figure 2N).; type II, flat stomata with higher papillae (*A. ochracea, A. thyrsiflora, A. ochracea* var*. trilobata*) (Figure 2O).; type III, emerged, with equal high of papillae and stomata (*A. filipes, A. imperialis*) (Figure 2P).

The similarity coefficient of *Ampelocissus* in Sumatra is determined by the anatomical characters with a coefficient in the range of 0.48 to 0.81. *Ampelocissus* are divided into two groups at coefficients 0.50. Group I consists of *A. elegans, A. Arachnoidea, A*. *gracilis, A. rubiginosa, A. polythyrsa* and *A. thyrsiflora,* and group II consists of *A. filipes, A. imperialis, A. ochracea and A. ochracea* var*. trilobata* (Figure 3).

Group I is clustered six *Ampelocissus* species at coefficient 0.64 by the multicellular glandular trichomes, flattened stomatal position, and clustered calcium oxalate crystals characteristics. *Ampelocissus elegans* and *A. arachnoidea* have the highest leaf anatomy similarity (0.81), but both species can be distinguished by 3 characters, i.e.; the shape of anticlinal wall on the abaxial epidermal cell, the presence of simple uniseriate trichomes on adaxial surface, and shape adaxial epidermis. At the coefficient of 0.71, *A. gracilis* is separated from other species in Group I due to the appearance of simple uniseriate trichomes on the abaxial surface. *Ampelocissus rubiginosa* is separated at a coefficient 0.68 for its angular anticlinal wall on the epidermal cell and two palisade layers characters. *Ampelocissus polythyrsa* and *A. thyrsiflora* are clustered at a coefficient of 0.75 for their presence of papillae on stomata. Both species differ in the trichome type and the stomatal position on abaxial leaf epidermis.

Group II clustered three *Ampelocissus* species at coefficient 0.48 by thin upper leaf epidermis, number of palisade layers, and emerged stomatal position on abaxial leaf epidermis. *Ampelocissus filipes* and A. *imperialis* are clustered at a coefficient of 0.63, both species have differed for their calcium oxalate crystals and glandular trichome type. *Ampelocissus ochracea* and *A. ochracea* var. *trilobata* are clustered at a coefficient of 0.56 for their solitary calcium oxalate crystals, but these two taxa differ in the shape of anticlinal wall: *A. ochracea* has an angular shape on both adaxial and abaxial surfaces, while *A. ochracea* var. *trilobata* has a wavy sinuolate shape on adaxial and abaxial surfaces.

The anatomical-based clusters show similarity with morphological-based clusters (Dalimunthe et al. 2016). Both anatomical and morphological characters cluster *A. gracilis, A. polythyrsa,* and *A. rubiginosa* species. They also cluster *A. filipes, A. imperialis, A. ochracea, A. thyrsiflora* and *A. ochracea* var. *trilobata*. However, they also show dissimilarity.

Based on the anatomical characters, *A. arachnoidea* is clustered with *A. elegans*, but based on morphological these two species are differed by the vegetative and generative characters. By morphological characters *A. arachnoidea* has a simple, cordate 3 lobed leaf; panicle-umbellate inflorescence and oblong flower bud, while *A. elegans* have compound obovate-asymmetry leaf, pinnate venation; thyrsi and short inflorescence and rounded flower bud. The difference is also found in *A. thyrsiflora* which is clustered to *A. polythyrsa*. By morphological; *A. polythyrsa* has herb, compound 5-7 leaflets, obovate-asymmetric leaf shape, villous indumentum on abaxial leaf venation, oblong flower bud and basifixed anther attachment; *A. thyrsiflora* has liana, compound 5-7 leaflets, obovate-asymmetric leaf shape, woolly indumentum on leaf abaxial surface, rounded flower bud and dorsifixed anther attachment. Although there are some dissimilarities, the anatomical characters are still important to support the Sumatran *Ampelocissus* clusters.

CONCLUSION

Based on the results and the discussion drawn from this study, it can be concluded that the anatomical characters i.e.: the shape of the anticlinal wall on adaxial epidermal cells, number of palisade layers, upper epidermal cell thickness, leaf thickness, the presence of papillae on stomata, type and shape of calcium oxalate crystals, and position of stomata on abaxial leaves show a high taxonomic value. The clustering analysis based on anatomical characters shows that *A. elegans*, *A. arachnoidea, A. gracilis, A. rubiginosa, A. polythyrsa* and *A. thyrsiflora* cluster together at 0.64 similarity coefficient. Also *A. filipes, A. imperialis, A. ochracea,* and *A. ochracea* var. *trilobata* cluster together at 0.48 similarity coefficient.

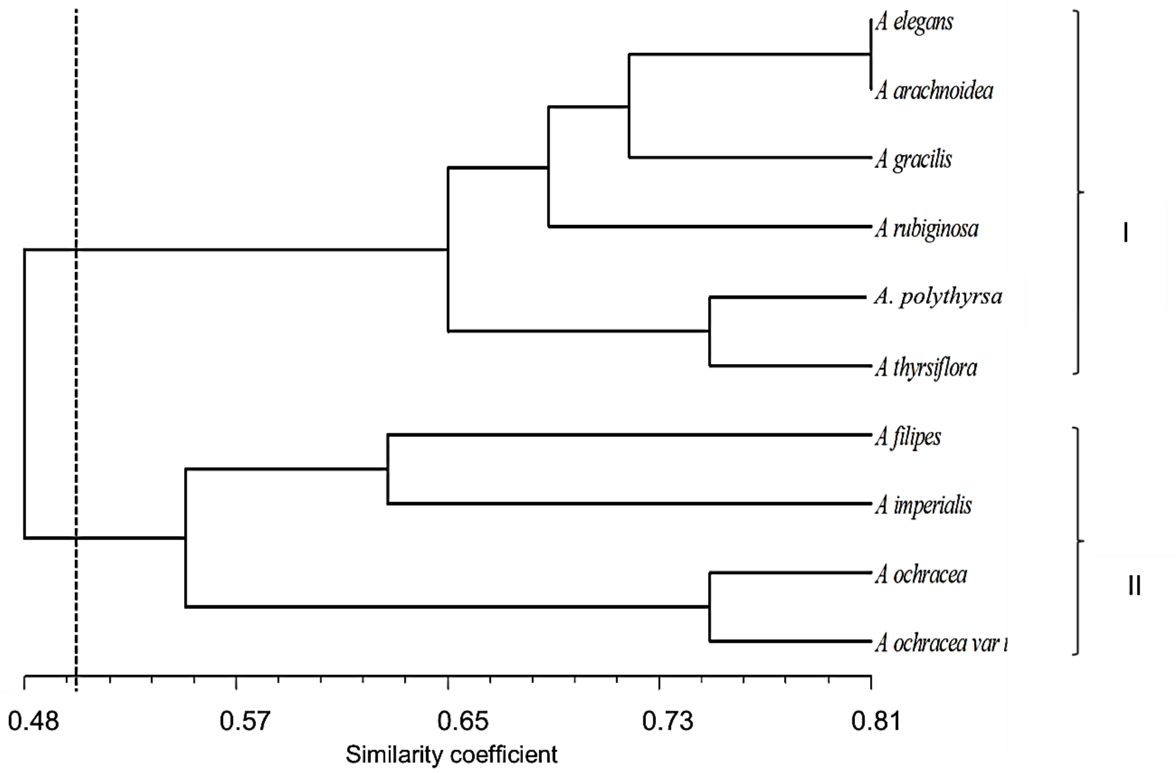

Figure 3. Phenogram of Sumatran *Ampelocissus* by 16 anatomical characters

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