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Short Communication: Further morphological evidence for separating *Mukia* Arn. from *Cucumis* L.

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Abstract. *Pratami, MP Chikmawati T, Rugayah. 2019. Short Communication: Further morphological evidence for separating* Mukia *Arn. from* Cucumis *L.. Biodiversitas 20: 211-217. Mukia* Arn. is closely related to *Cucumis* L. based on molecular data, nevertheless, they have high morphological differences resulting in different opinion on taxonomical status of the two genera. *Mukia* Arn. has many similarities in pollen and leaf anatomical characters to *Cucumis* L., but both genera differ in seven seed characters, i.e color, shape, size, surface pattern, seed edge, transverse section at seed neck, and the markings of the inner seed coat surface. So, based on seed characteristics, *Mukia* Arn. is separated from *Cucumis* L.

Keywords: *Cucurbitaceae*, leaf anatomy, Malesia, pollen, taxonomic status

INTRODUCTION

Mukia Arn. and *Cucumis* L. are closely related phylogenetically, and based on molecular data of nuclear and plastid DNA, *Mukia* Arn. was nested into *Cucumis* L. (Renner et al. 2007, Schaefer 2007). In their opinion, de Wilde and Duyfjes (2010) treated *Mukia* Arn. and *Cucumis* L. as different genera, based on morphological characters especially male flowers (sepals, petals, thecae, and connective); female flowers (stigma); fruit (size and ripening color); seeds (color, and shape). *Mukia* Arn. has sepal minute long triangular or linear, petals elliptic or obovate, thecae lateral and straight, connective narrow hairy, 1-6 fascicled flowers, stigma 3 elongate or carnose or papillose lobes; red mature fruit; cream to brown seed color; and ovoid, ovate-obovate, and obovate seed shape. However, *Cucumis* L. has linear sepal, petal with entire margin, sinuate thecae 3 plicate/5-shaped, connective considerably produced, solitary flowers, stigma 3 lobulate lobes; green, yellow or orange mature fruit; whitish, yellow or cream seed color, and oblanceolate seed shape (de Wilde and Duyfjes 2010). In our study of the two genera (Pratami 2018), we found further differences in seed morphology which we consider important in clarifying the taxonomic status of *Mukia* Arn. and *Cucumis* L. In the following, the result of this study will be elaborated.

Pollen evidence has been used to reveal many plant taxonomic problems. The data are usually used to identify doubtful taxon, rearrange, merge, and separate taxa as well as reinforcement of other evidence (Davis and Heywood 1973). Jeffrey (2005) divided the family *Cucurbitaceae* into two subfamilies. Subfamily *Nhandiroboideae* representing a single tribe *Zanonieae* (*Zanonioideae*) and subfamily *Cucurbitoideae* is subdivided into ten tribes viz., 1. *Joliffieae* (Subtribe *Telfairiinae*, *Thladianthinae*), 2. *Bryonieae*, 3. *Trichosantheae* Subtribe *Ampelosicyinae*, *Hodgsoniinae*, *Trichosanthinae*), 4. *Herpetospermeae*, 5. *Schizopeponeae*, 6. Tribe *Luffeae*, 7. *Sicyeae*, (Subtribe *Cyclantherinae*, *Sicyinae*), 8. *Coniandreae*, 9. *Benincaseae* (Subtribe *Benincasinae*), 10. *Cucurbiteae*. Cucurbitaceae pollen are classified into 3 types, *Cucumis melo* type, *Momordica charantia* type, dan *Solena amplexicaulis* type (Perveen and Qaiser 2008).

The characteristics of leaf anatomy can also be used to solve taxonomic problems. Anatomical data are useful for characterizing, revealing plant evolution and genetic relationships among species since they are less affected by the environment. The previous study showed that the anatomical characteristics of fruit stalks and tendrils can be used to distinguish among species within *Cucurbitaceae* (Ekeke et al. 2015). Variations of stomatal types among some *Cucurbitaceae* members are paracytic, diacytic, anisocytic, actinocytic, syclocytic, and staurocytic (Abdulrahaman et al. 2011). The stomata type and variation in a stomatal index can be used to distinguish among species within Cucurbitaceae (Jibril and Bello 2016). The aim of this study was to review the taxonomy status of the two genera *Mukia* Arn. and *Cucumis* L. based on morphology of seed, pollen characteristics, and leaf anatomy.

MATERIALS AND METHODS

Area study

As many as 282 specimens representing five species (*M. javanica* (Miq) C. Jeffrey, *M. maderaspatana* (L.) M.Roem., *M. rumphiana* (Scheff.) W.J. de Wilde & Duyfjes, *C. melo* L., and *C. sativu*s L.), have been collected in some areas of Malesia (Java, Madura, West Nusa Tenggara, Mollucas, and Kalimantan) from January to December 2017. The observations of seed morphological characteristics of *Mukia* Arn. and *Cucumis* L. were observed following Abid et al. (2015) and were carried out at the Plant Resource Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Indonesia and Herbarium Bogoriense (BO), Research Center for Biology, Cibinong, Bogor, Indonesia. The observation of leaf anatomy was carried out at the Ecology and Plant Resource Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Indonesia. The observation of pollen morphology was conducted at the Zoology Laboratory, Research Center for Biology, Bogor, Indonesia.

Observations of seed morphology, pollen characteristics, and leaf anatomy

The seed morphological characteristics of *Mukia* Arn. and *Cucumis* L. observed were the color, shape, size, surface pattern, the edge of seed, the shape of seed transversal section, and inner seed surface (Abid et al. 2015).

Pollen grains of *Mukia* Arn. and *Cucumis* L. were examined using a Scanning Electron Microscope (SEM). Pollen was prepared by drying some flowers using *freeze-drying* following Pathan et al. (2010). Parameters observed were unity, polarity, symmetry, aperture type, and exine ornamentation (Erdtman 1952).

Five leaf blades of each observed species were examined to describe their anatomical characters of paradermal and transverse sections. The paradermal sections were prepared following Sass (1951) with modification, while leaf transverse sections were prepared using paraffin method (Johansen 1940).

Data analyses

Seed morphology, pollen, and leaf anatomy data were analyzed descriptively and arranged in matrix data and scored as binary data, then analyzed based on simple matching similarity coefficient, and a dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic Average (UPGMA). All data analyses were performed using the NTSys 2.11a program (Rohlf 2000).

RESULTS AND DISCUSSION

Seed morphology of *Mukia* Arn. and *Cucumis* L. in Malesia

Morphological observations showed that the two genera differ in color, shape, size, surface pattern, edge of seeds, as well as the transverse section at the seed neck, and the markings of the inner seed coat surface (Table 1). *Mukia* Arn. seed has cream-brown color, ovate to broad-ovate shape, smaller size (4.27-6.6 x 3.06-4.47 mm) and grooved edge. *Mukia javanica* (Miq) C. Jeffrey has ovate shape and slightly flat to concave with slightly rough surface borders by a series small cavity, two grooved, and narrow ridge, whereas the other two *M. maderspatana* (L.) M.Roem. and *M. rumphiana* (Scheff.) W.J.de Wilde & Duyfjes have broad ovate shape, papillae texture, two grooved, and no ridge. However, the seed of *Cucumis* L. is yellow-cream (in *C. melo* L.) to white-cream (in *C. sativus* L.), oblanceolate shape, bigger size (9.9-11.89 x 3.79-4.85 mm) and one grooved edge. In *Cucumis* L., however, the two species have similar seed characters, except in its color and surface pattern.

Transverse section as seed neck of *Mukia javanica* (Miq) C. Jeffrey is a narrow ridge, but the other species are not ridge. *Mukia javanica* (Miq) C. Jeffrey, *Mukia maderaspatana* (L.) M. Roem., and *Mukia rumphiana* (Scheff.) W.J.de Wilde & Duyfjes have polygonal inner seed surface, but *Cucumis melo* L. reticulate, and *Cucumis sativus* L. has straight inner surface seed coat marking (Figure 1).

According to Abid et al. (2015), *Cucumis melo* L. and *C. sativus* L. can be further distinguished from *M. maderaspatana* (L.) M.Roem. and three other species, *Corallocarpus epigaeus* L., *C. shimperi* (Naudin) Hook.f., *Cucumis prophetarum* L. by having different shape and surface patterns of seed. Thus, morphological characteristics of seed can be used to delimit a taxon and estimate its phylogenetic relationships. Seed morphological data have also been used to assess the phylogenetic relationship among different taxa, and the seed characters were well correlated with morphological and palynological data. Previously, the seed morphology could also be used to distinguish among species of *Trichosanthes* L. (*Cucurbitaceae*) (Rugayah 1999).

The previous study reported that seed morphology was helpful in distinguishing various species and could be used to confirm the tribe and subtribe classifications. Seed character analysis also offered many useful data for evaluating the taxonomy of Cucurbitaceae on both intrageneric and tribal levels (Heneidak 2014). The result indicated that seed morphology of *Mukia* Arn. and *Cucumis* L. have different characters and can be used to distinguish the two genera.

Pollen grains of *Mukia* Arn. and *Cucumis* L. in Malesia

The characteristic variations of *Mukia* Arn. and *Cucumis* L. pollen grains were found in its size, P / E index, and shape. However, a previous research has reported that the pollen unit, shape, and size may be affected by the maturity of the pollen (Erdtman 1952).

Table 1. Comparison of seed morphological characteristics of *Mukia* Arn. and *Cucumis* L. in Malesia

Species	Size (mm)	Shape	Color	Surface pattern	Edge of seed	Transverse section at the seed neck	Markings of inner seed coat surface
M. javanica	5.07-5.09 x 3.06-3.07	Ovate	Cream-brown	Slightly flat to concave with slightly rough surface	2 grooved	Narrow ridge	Polygonal
M. maderaspatana	4.27-5.21 x 3.16-3.72	Broad ovate	Cream-brown	Convex and irregular papillae	2 grooved	No ridge	Polygonal
M. rumphiana	5.01-6.6 x 3.75-4.47 x	Broad ovate	Cream-brown	Convex and rough papillae	2 grooved	No ridge	Polygonal
C. melo	11,65-11,89 x 4.7-4.85	Oblanceolate	Yellow-Cream	Slightly concave and Smooth	1 grooved	No ridge	Reticulate
C. sativus	9.9-11 x 3.58-3.79	Oblanceolate	White-Cream	Flat and Smooth	1 grooved	No ridge	Straight

Table 2. Pollen grain characteristics of *Mukia* Arn. and *Cucumis* L. in Malesia

Species	Size		Index P/E	Shape	Symmetry	Aperture	Polarity	Ornamen-tation
	Polar (P) (µm)	Equatorial (E) (µm)						
M. javanica	48.98	49.28	0.99	Oblate	Radial	Triporate	Isopolar	Reticulate
M. maderaspatana	32.03	44.20	0.72	Oblate	Radial	Triporate	Isopolar	Reticulate
C. melo	39.32	49.50	0.79	Suboblate	Radial	Triporate	Isopolar	Reticulate
C. sativus	43.60	50.70	0.85	Suboblate	Radial	Triporate	Isopolar	Reticulate

Table 3. Comparison anatomical characters of leaf paradermal section of *Mukia* Arn.and *Cucumis* L. in Malesia

Species	Stomata type		Epidermal cell wall pattern		Epidermal shape	
	Ad	Ab	Ad	Ab	Ad	Ab
M. javanica	Staurocytic	Anomocytic	Straight	Wavy with wide U-shaped curve	Polygonal	Irregular
M. maderaspatana	Staurocytic	Anomocytic	Straight	Wavy with very deep curve	Polygonal	Irregular
M. rumphiana	-	Anomocytic	Straight	Wavy with wide U-shaped curve	Polygonal	Irregular
C. melo	Staurocytic	Anomocytic	Straight	Wavy with very deep curve	Polygonal	Irregular
C. sativus	Staurocytic	Anomocytic	Straight	Wavy with very deep curve	Polygonal	Irregular

Note : ad= adaxial side (upper), ab= abaxial side (lower)

Table 4. Stomata, epidermal cell, and trichome sizes of *Mukia* Arn. and *Cucumis* L. in Malesia

Species	Average size of stomata (µm)		Average size of epidermal cell (µm)		Average size of trichome (µm)	
	L	W	L	W	L	W
M. javanica	18.76	12.62	35.64	28.04	160.78	30.76
M. maderaspatana	19.70	11.31	31.89	21.41	174.84	31.26
M. rumphiana	20.48	9.80	42.84	22.42	333.68	40.48
C. melo	22.38	12.32	42.80	26.07	101.82	47.23
C. sativus	26.87	13.59	57.58	37.08	292.88	91.36

Note : L = Length and W = Width

Table 5. Comparison of characters leaf transverse section of *Mukia* Arn. and *Cucumis* L. in Malesia

Species	Leaf thickness (µm)	Epidermal cell size (µm)		Palisade tissue size (µm)		Spongy tissue size (µm)
		Upper	Lower	Palisade 1	Palisade 2	
M. javanica	89.36	7.62-15.21	6.90-11.42	23.45-26.44 x 6.54-11.38	11.4-14.4x 3.1-5.1	0.50-0.65x 0.59-0.71
M. maderaspatana	112.47	13.81-31.07	10.78-22.56	34.26-44.39x 10.05-14.83	-	0.47-0.57x 0.58-0.82
M. rumphiana	124.92	13.52-24.91	7.59-10.69	23.54-31.72x5.87-11.73	3.9-5.2x 2.6-4.0	0.17-0.41x 0.23-0.36
C. melo	136.55	11.72-29.04	13.10-18.30	31.33-35.73x 13.87-20.61	5.3-7.6x 2.8-5.0	0.53-0.78x 0.70-1.13
C. sativus	113.64	9.33-14.48	6.55-11.38	29.65-34.93x 6.9-11.9	6.2-8.5x 3.3-5.1	0.21-0.59x 0.39-0.62

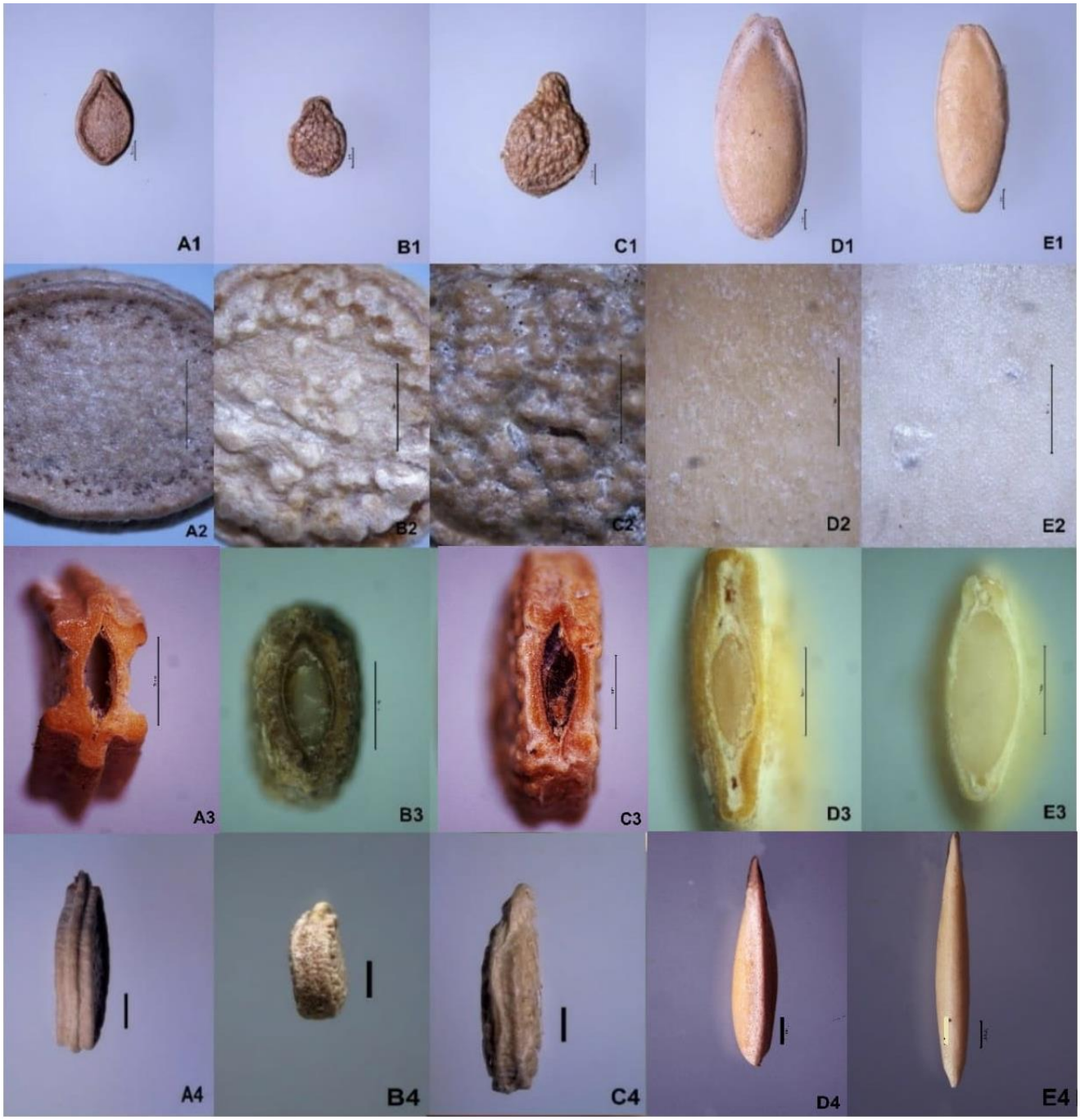

Figure 1. Seed morphology of five species *Cucurbitaceae*: A. *Mukia javanica* (Miq) C. Jeffrey; B. *Mukia maderaspatana* (L.) M.Roem.; C. *Mukia rumphiana* (Scheff.) W.J.de Wilde & Duyfjes; D. *Cucumis melo* L.; and E. *Cucumis sativus* L. 1=seed shape, 2= seed surface texture, 3= inner seed, 4= seed edge. Scale bar =1 mm

Mukia maderaspatana (L.) M.Roem. and *M. javanica* (Miq) C. Jeffrey have polar size of 32.03-48.98 µm and equatorial size of 44.20-49.28 µm, index P/E of 0.72-0.99, oblate shape, whereas *Cucumis melo* forma *agrestis* (Naudin) W.J.de Wilde & Duyfjes and *C. sativus* L. have polar size of 39.32-43.60 µm; and equatorial size of 49.50-50.70 µm, index P/E of 0.79-0.85, suboblate shape. *Mukia* Arn. and *Cucumis* L. also have four similar characters of pollen grains which are polen unit, symmetry, polarity, and aperture type (Table 2). Their pollen grains have radial symmetry, isopolar, and triporate aperture, having 3 porous shaped apertures. Both genera have the same pollen group, *Cucumis melo* type. *Cucumis melo* type has P/E ratio 72-79.0, sub-oblate to oblate, pore circular, sexine thicker than nexine, and coarsely reticulate or reticulate-rugulate (Perveen and Qaiser 2008).

The exine of all observed species has reticulate ornamentation (Figure 2). Exine ornamentation and pollen shape can be used for taxonomic identification (Esau 1953). Exine ornamentation can also be considered as

pollen characteristic of plant species, but the typical ornamentation of Cucurbitaceae is generally reticulate or reticulate-rugulate (Perveen and Qaiser 2008).

Mukia Arn. pollen has many similar characteristics to *Cucumis* L. pollen, i.e. P/E index 72-79, suboblate to oblate shape, pore apertura, and ornamentation coarsely reticulate or reticulate-rugulate.

Leaf anatomy of *Mukia* Arn. and *Cucumis* L. in Malesia

Anatomical features of *Mukia* Arn. and *Cucumis* L. leaf paradermal sections varied in the presence of adaxial stomata, epidermal wall pattern types, and epidermal cell shape (Table 3 and Rigure 3). The stomata types of five observed species of Cucurbitaceae are classified into the same types, namely staurocytic on the adaxial and anomocytic on the abaxial surfaces (Figure 3), except on the adaxial surface of *M. rumphiana* (Scheff.) W.J.de Wilde & Duyfjes which has no stomata. Anomocytic stomata type is a diagnostic character of Cucurbitaceae. These results are in accordance with the report of Metcalfe and Chalk (1950) which stated that the Cucurbitaceae has anomocytic stomata type. *Mukia* Arn. has wavy with wide U-shaped curve epidermal cell wall pattern on the abaxial side, except *M. maderaspatana* (L.) M.Roem.; whereas, *Cucumis* L. has wavy with very deep curve epidermal cell wall pattern on the abaxial side.

All observed species have the same type of trichomes, multicellular and non-glandular trichomes found on both adaxial and abaxial surfaces of *Mukia* Arn. and *Cucumis* L. leaves, but their trichomes vary in size. In general, the *Cucurbitaceae* group has non-glandular and glandular trichomes (Metcalfe and Chalk 1957).

The sizes of the stomata and epidermis cells of *Mukia* Arn. and *Cucumis* L. are similar (Table 4). The examination of leaf transverse section of *Mukia* Arn. and *Cucumis* L. in Malesia did not show any clear differences in leaf structure either (Figure 3). It indicated that the leaf anatomy characters cannot be used to distinguish both genera.

Mesophyll tissue of all species observed was divided into two types of tissue, namely palisade, and spongy tissues. The palisade tissue of *Mukia* Arn. and *Cucumis* L. is the dorsiventral type. The results of this study were in accordance with Mulyani's report (2006). The palisade tissue of the studied species consists of two layers, except in *M. maderaspatana* (L.) M.Roem. having only one cell layer (Table 5). The number of palisade tissues of *Trichosanthes* L. indicates a phylogenetic feature, and a layer of palisade tissues indicating primitive features (Rugayah 1999). But, the characteristics of the paradermal and transversal leaf anatomy cannot be used to distinguish between genera *Mukia* Arn. and *Cucumis* L.

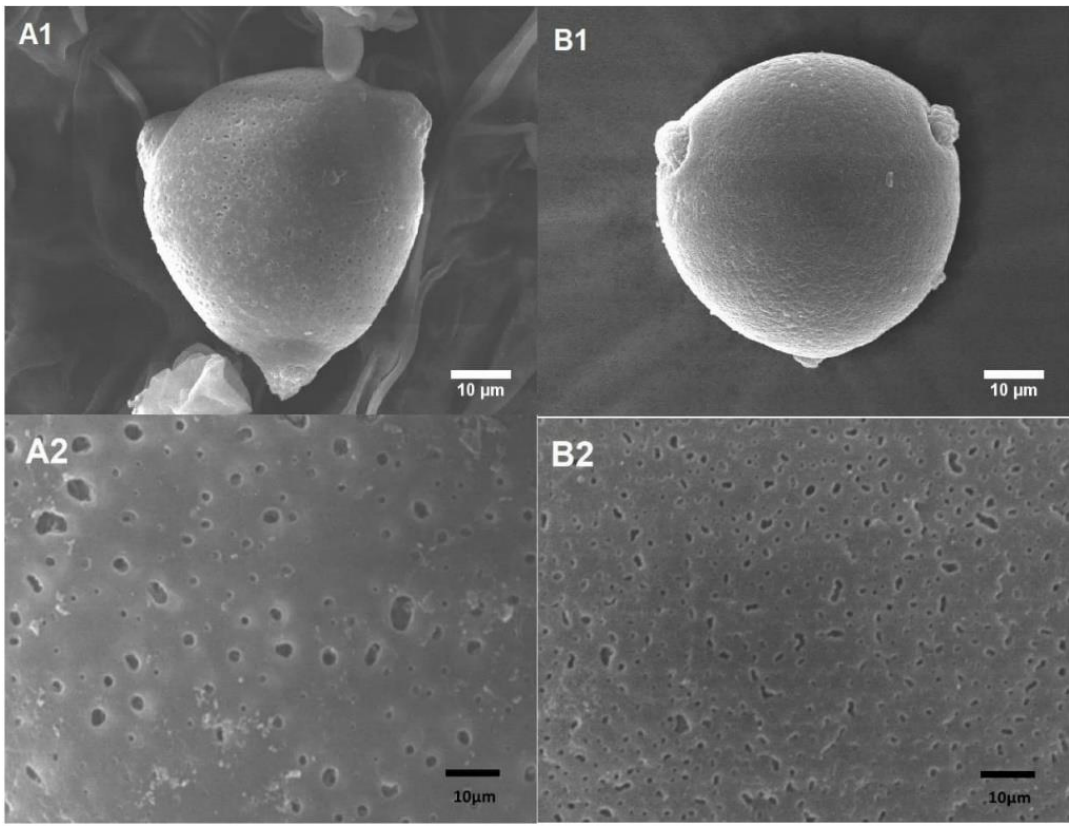

Figure 2. Structure variation of pollen grains: A. *Mukia* javanica (Miq) C. Jeffrey; B. *Cucumis sativus* L. 1=Polar, 2=exine. Scale bar : 10 µm

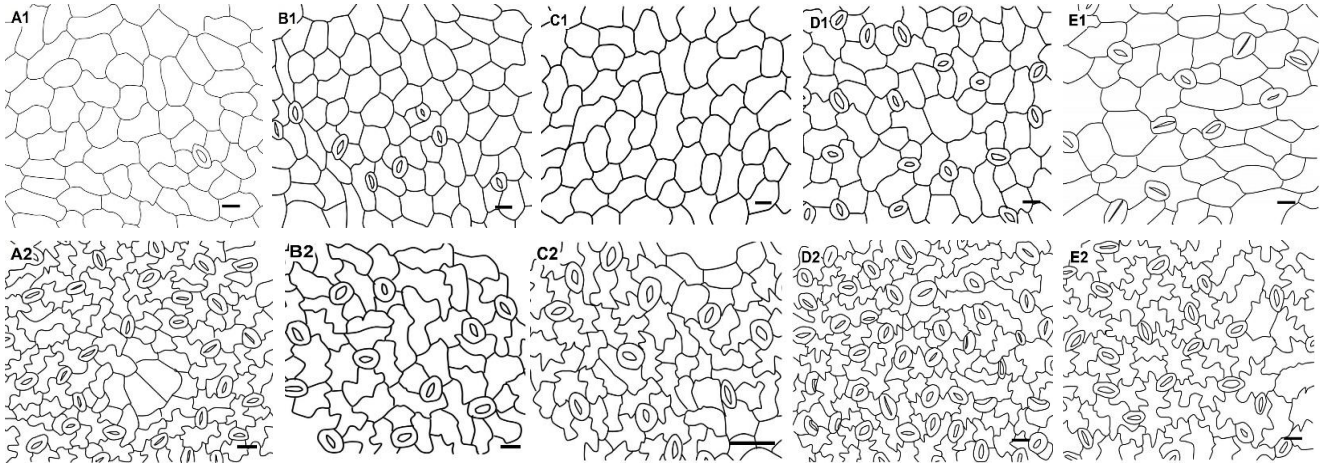

Figure. 3. Paradermal section adaxial and abaxial leaf *Mukia* Arn. and *Cucumis* L. A. *M. javanica* (Miq) C. Jeffrey; B. *M. maderaspatana* (L.) M.Roem; C. *M. rumphiana* (Scheff.) W.J.de Wilde & Duyfjes; D. *Cucumis melo* L.; E. *C. sativus* L.. 1: adaxial side. 2 : abaxial side. Scale bar =20 µm

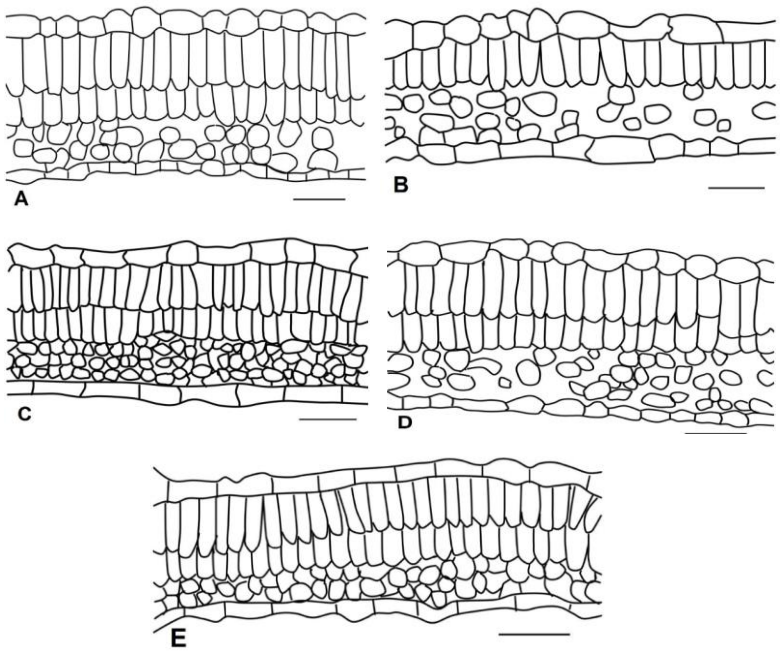

Figure 4. Leaf transverse section A: *M. javanica* (Miq) C. Jeffrey; B: *M. maderaspatana* (L.) M.Roem; C: *M. rumphiana* (Scheff.) W.J.de Wilde & Duyfjes; D: *C. melo* L.; E: C. *sativus* L. Scale bar=50 µm

Cluster analysis of *Mukia* Arn. and *Cucumis* L. in Malesia

Phenetic analysis based on 8 seed characters of *Mukia* Arn. and *Cucumis* L. observed species showed that all species were divided into two major groups with a similarity coefficient of 0.21-0.88 (Figure 5). Group 1 consists of all three *Mukia* Arn. species based on seed size, seed color, and the markings of the inner seed coat surface. *Mukia javanica* (Miq) C. Jeffrey is separated from *M. maderaspatana* (L.) M.Roem. and *M. rumphiana* (Scheff.) W.J.de Wilde & Duyfjes based on three characters, namely ovate shape, slightly flat to concave with slightly rough surface borders by a series small cavity. Group 2 consists of *C. melo* L. and *C. sativus* L. that are united by 4 characters, namely big size, oblanceolate-shaped, smooth texture, and one grooved edge.

In conclusion, the result indicates that seed morphology of *Mukia* Arn. and *Cucumis* L. have different characters, and can be used to distinguish the two genera. Seed morphological evidence can be used to separate *Mukia* Arn. from *Cucumis* L.

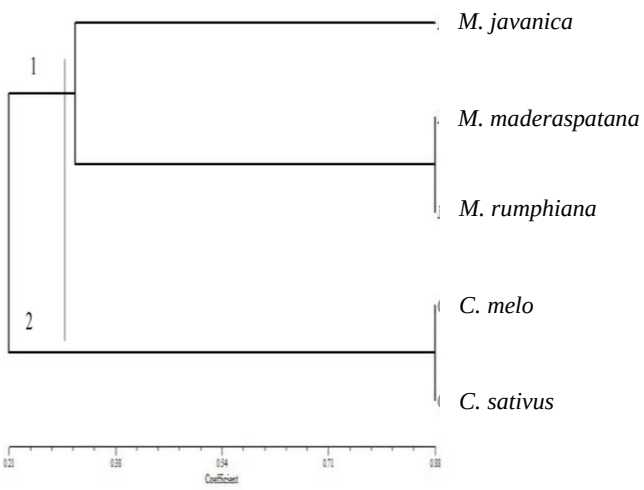

Figure 5. Dendrogram of *Mukia* Arn. and *Cucumis* L. in Malesia based on seed morphology using UPGMA methods

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