

Comparative pollen morphology of three *Alternanthera* species (Amaranthaceae)

E.H.S. Chin¹ and A.L. Lim

Institute of Biological Sciences,
Faculty of Science, University of Malaya,
50603 Kuala Lumpur, Malaysia
¹evansee_82@yahoo.co.uk

ABSTRACT. The pollen morphology of three *Alternanthera* species, *A. sessilis* (L.) R.Br. ex DC., *A. bettzickiana* (Regel) G.Nicholson and *A. paronychioides* A.St.-Hil., is reported. Pollen grains of *Alternanthera* are dodecahedral, isopolar and small (12.56–23.57 µm). The pollen morphology of the green and red varieties of *A. sessilis* shows no significant difference in the polar length [$t(98) = -1.35$, $p > 0.05$] and equatorial diameter [$t(98) = 1.32$, $p > 0.05$]. Apertures of *A. sessilis* and *A. bettzickiana* are pantoporate with twelve round pores, whereas the pollen grains of *A. paronychioides* distinctly differ from the other two species in having eighteen oval pores. The pores of all the species are covered by rectangular, sinuous, or elongated ectexinous bodies. The sexine is metareticulate and tectum perforate with unevenly distributed perforations at the top and base of the mesoporia, except in the pollen grains of *A. bettzickiana*, in which the perforations are distributed unevenly at the top of the mesoporia only.

Keywords. *Alternanthera*, Amaranthaceae, pollen morphology

Introduction

Alternanthera Forssk. (Amaranthaceae) consists of 80 species distributed in the tropics and subtropics (Mabberley 2008). Of these, palynological data of 20 species have been reported (Table 1). The majority of these species originated from the New World (Borsch 1998, Eliasson 1988, Müller & Borsch 2005b, Nowicke & Skvarla 1979), India (Kajale 1940, Mittre 1962, Rao & Shukla 1975), Pakistan (Bashir & Khan 2003, Perveen & Qaiser 2002) and China (Li et al. 1993, Liang et al. 1978). Therefore, it would be interesting to study the pollen morphology of the *Alternanthera* species in Malesia particularly in Peninsular Malaysia, and compare these observations with the earlier data.

This is especially meaningful as in recent years, detailed study of pollen morphology has helped to resolve the relationship among members of the Amaranthaceae (Borsch 1998, Müller & Borsch 2005b). Palynological data has also supported the findings in phylogenetic studies of the subfamily Gomphrenoideae (Sánchez del-Pino et al. 2009, Müller & Borsch 2005a).

Though Erdtman (1966) has reported that the pollen grains of *Alternanthera* species possess deeply recessed pores, the apertures are not well studied. The pollen morphology of *Alternanthera* is further defined by Borsch (1998) and described as the

Table 1. References to published palynological studies on *Alternanthera* species.

Species	References
<i>A. albida</i> Griseb.	Borsch 1998
<i>A. bettzickiana</i> (Regel) G.Nicholson	Li et al. 1993
<i>A. caracasana</i> Kunth	Müller & Borsch 2005b
<i>A. costaricensis</i> Kuntze	Borsch 1998
<i>A. filifolia</i> (Hook.f.) Howell J.T.Howell	Borsch 1998
<i>A. flavescens</i> Kunth	Borsch 1998, Nowicke & Skvarla 1979
<i>A. galapagensis</i> (Stewart) J.T.Howell	Eliasson 1988
<i>A. geniculata</i> Urb.	Eliasson 1988
<i>A. gracilis</i> Loes.	Erdtman 1966
<i>A. maritima</i> (Mart.) A.St.-Hil.	Borsch 1998
<i>A. nesioties</i> I.M.Johnst.	Eliasson 1988
<i>A. nodiflora</i> R.Br	Li et al. 1993
<i>A. olivacea</i> Urb.	Borsch 1998
<i>A. paronychioides</i> A.St.-Hil.	Perveen & Qaiser 2002
<i>A. peruviana</i> (Moq.) Suess.	Borsch 1998, Eliasson 1988
<i>A. philoxeroides</i> Griseb.	Li et al. 1993
<i>A. pungens</i> Kunth	Bashir & Khan 2003
<i>A. reineckii</i> Briq.	Eliasson 1988
<i>A. repens</i> Steud.	Mittre 1962
<i>A. sessilis</i> (L.) R.Br. ex DC.	Kajale 1940, Mittre 1962, Rao & Shukla 1975, Li et al. 1993, Liang et al. 1978, Perveen & Qaiser 2002

Pfaffia type. The pollen grains in this category are dodecahedric, metareticulate, tectate perforate with microspines distally arranged in a line. The apertures are pantoporate with pore structure of Type I (Borsch 1998). The diameter of the pores is 3–6 µm and is covered by 20–60 ektexinous bodies. The length of these ektexinous bodies is 1.5–3.0 times its width. These ektexinous bodies are rectangular, sinuous or elongated and are arranged in a mosaic-like pattern, closely adjoined but separated from each other. Each of the ektexinous bodies has one to four distinct microspines attached onto it.

Studies of *Alternanthera* in Malaysia date back to the 18th century when Ridley (1924) reported *A. sessilis* in the Malay Peninsula. *Alternanthera sessilis* (L.) R.Br. ex DC., *A. sessilis* var *tenuissima* (Suess.) Backer, *A. repens* Kuntze, *A. bettzickiana* (Regel) G.Nicholson, *A. philoxeroides* Griseb., *A. brasiliiana* (L.) Kuntze

and *A. porrigens* Kuntze from Malesia were subsequently described (Backer 1949). *Alternanthera triandra* Lamk. has been recorded as a common weed in Malaya. However, in Turner's catalogue of Malayan Plants (1995), only three species (*A. sessilis*, *A. philoxeroides* and *A. bettzickiana*) were recognised. Of these, the pollen grains of three species were investigated in the present study, i.e., *A. sessilis* (both the red variety as well as the green, the later sometimes considered as a distinct species, *A. triandra* Lamk.), *A. bettzickiana* and *A. paronychioides*.

In Peninsular Malaysia, *A. sessilis* occurs in two leaf colours; green and red. Both the green and red varieties of *A. sessilis* are perennial creeping herbs. The red variety is commonly cultivated, whereas the green variety is a common weed found growing in various types of habitat from moist areas near drains, ditches and ponds to dry open wastelands. Similarly, *A. bettzickiana* is usually found in dry open wastelands or moist areas near ditches. However, these two species can be easily identified by the morphology of stem, leaf and flower (Table 2). Lastly, *A. paronychioides* could be found on dry sandy ground such as along canals or roads. This species forms a dense mat with numerous prostrate branches with roots at the nodes.

Alternanthera sessilis is regarded by some communities as a useful medicinal plant indigenous to Malesia. The red variety is usually used as a herb to treat heart disease and hypertension. Scientifically, *A. sessilis* has been reported to have antibacterial activities (Kumaresan et al. 2001, Jalalpure et al. 2008). Free radical scavenging properties have also been found in *A. sessilis* indicating the presence of antioxidant properties in the plant (Balasuriya & Dharmaratne 2007, Bhaskar et al. 2007, Shyamala et al. 2004). Other reported pharmacological activities of *A. sessilis* include reduction of hypertension (Goh et al. 1995), having an hepatoprotective effect against liver injuries induced by hepatotoxins (Song et al. 2006) as well as functioning as a diuretic (Goh et al. 1995). As the two varieties of *A. sessilis* are similar except in leaf colour, this study also aims to elucidate their pollen morphology to determine whether they differ.

Materials and methods

Pollen samples for the green and red varieties of *A. sessilis* were collected from Varsity Lake and the Rimba Ilmu Botanic Garden, respectively, in the University of Malaya, Kuala Lumpur. The pollen grains of *A. bettzickiana* were collected from the Forest Research Institute Malaysia (FRIM) and *A. paronychioides* from the Kuala Selangor Nature Park, Selangor, Peninsular Malaysia. Voucher specimens were deposited in the herbarium of the Institute of Biological Sciences, University of Malaya (KLU).

Anthers of 15 flowers of each species were collected after anthesis. The pollen samples were then acetolysed according to the standard procedure of Erdtman (1960). Slides for light microscopy (LM) study were prepared with glycerine jelly and sealed with wax. The measurements of equatorial diameter (E) and polar length (P) were taken with a Leica DM100 microscope. Then, the pollen shape and size were determined. Acetolysed pollen grains for scanning electron microscopy (SEM) study

Table 2. Morphological characters of *Alternanthera sessilis* (green and red varieties), *A. paronychioides* and *A. bettzickiana*.

Species	Colour, habit and selected morphological traits					
	Stem	Foliage	colour	petals	stamens	ovary
<i>A. sessilis</i> (green variety)	green tinged with purple at the node; ascending, creeping or decumbent	green; simple; opposite; narrowly elliptic or narrowly oblong	creamy white	5, subequal in size	3	compressed
<i>A. sessilis</i> (red variety)	red; decumbent or creeping	red; simple; opposite; variable, mainly narrowly elliptic or oblanceolate	red	5, subequal in size	3	compressed
<i>A. paronychioides</i>	green; prostrate and forming a mat	green; simple; opposite; thick cuticle; elliptic or obovate	creamy white	5, distinctly unequal in size	5	compressed
<i>A. bettzickiana</i>	green tinged with purple at the node; erect or ascending	green; simple; opposite; elliptic	creamy white	5, distinctly unequal in size	5	subconical

were coated with gold, examined and photographed using a JOEL JSM-6400 scanning electron microscope.

Terminology used generally follows that of Fægri & Iversen (1950), Erdtman (1952), Borsch (1998) and Borsch & Barthlott (1998). An independent samples t-test was conducted to compare the polar length, equatorial diameter, diameter of the pores and number of ektexinous bodies between the following: 1) the green and red varieties of *A. sessilis*. 2) the green variety of *A. sessilis* and *A. bettzickiana*. 3) the red variety of *A. sessilis* and *A. bettzickiana*. The analyses were performed using the SPSS version 11.5 for Windows.

Results

The SEM photomicrographs of pollen in polar view, equatorial view, and pore structure are shown in Fig. 1. The pollen grains of *Alternanthera* species are dodecahedral, isopolar and radially symmetrical (Table 3). The pollen grains are small (12.56–23.57 μm in length) with the average polar axis varying between 15.19 and 21.36 μm and the average equatorial axis 17.18–21.85 μm . The t-test (Table 4) indicated there was no significant difference in polar length [$t(98) = -1.35, p > 0.05$] and equatorial diameter [$t(98) = 1.32, p > 0.05$] of the green and red varieties of *A. sessilis*. For the green variety of *A. sessilis* and *A. betzickiana*, the t-test indicated significant difference in the polar length [$t(86.58) = 3.12, p < 0.05$], but no significant difference in equatorial diameter [$t(98) = 1.76, p > 0.05$]. Similarly, the red variety of *A. sessilis* and *A. betzickiana* are significantly different in polar length [$t(98) = 5.24, p < 0.05$] but not in equatorial diameter [$t(98) = 0.46, p > 0.05$].

Under the scanning electron microscope, the apertures of *A. sessilis* and *A. betzickiana* are pantoporate with twelve round pores, whereas pollen grains of *A. paronychioides* have approximately eighteen oval pores. Each of the pores is situated in a pentagonal face which contributes to the dodecahedral body of the pollen grain. The green variety of *A. sessilis* possesses the smallest pore (4.17 μm), followed by the red variety of *A. sessilis* (4.48 μm), *A. betzickiana* (4.83 μm) and *A. paronychioides* (6.06 μm). The result of the t-test shows that there is a significant difference in the pore diameter of both green and red varieties of *A. sessilis* [$t(58) = -3.86, p < 0.05$].

The average number of ektexinous bodies in the red and green varieties of *A. sessilis* is the lowest, 28 and 29 respectively, followed by *A. betzickiana* (33) and *A. paronychioides* (36). The number seems to be related to the size of the pores. For instance, the pollen grains of *A. paronychioides* have the biggest pores (6.06 μm) and highest average number of ektexinous bodies (36). The t-test shows that there is no significant difference in the number of ektexinous bodies of the green and red varieties of *A. sessilis* [$t(17.82) = 1.67, p > 0.05$]. On the other hand, both varieties of *A. sessilis* and *A. betzickiana* are significantly different in the number of ektexinous bodies, i.e., the green variety of *A. sessilis* and *A. betzickiana* [$t(26) = -2.42, p < 0.05$], and the red variety of *A. sessilis* and *A. betzickiana* [$t(16.86) = -4.53, p < 0.05$]. The length of the ektexinous bodies is 4–6 times its width. They are rectangular, sinuous or elongated and each of the ektexinous bodies has 3–4 distinct microspines attached onto it. These ektexinous bodies are arranged in a mosaic-like pattern, closely adjoined but separated from each other.

The sexine of the pollen grain is metareticulate with a row of microspines that are regularly and distally arranged. These microspines are cylindrically elongated and blunt. The average height of these microspines ranges from 0.22–0.45 μm . The pollen grains of all the species studied are tectum perforate with unevenly distributed perforations at the top and base of the mesoporia, except in the pollen grains of *A. betzickiana*, in which the perforations are distributed unevenly at the top of the mesoporia only.

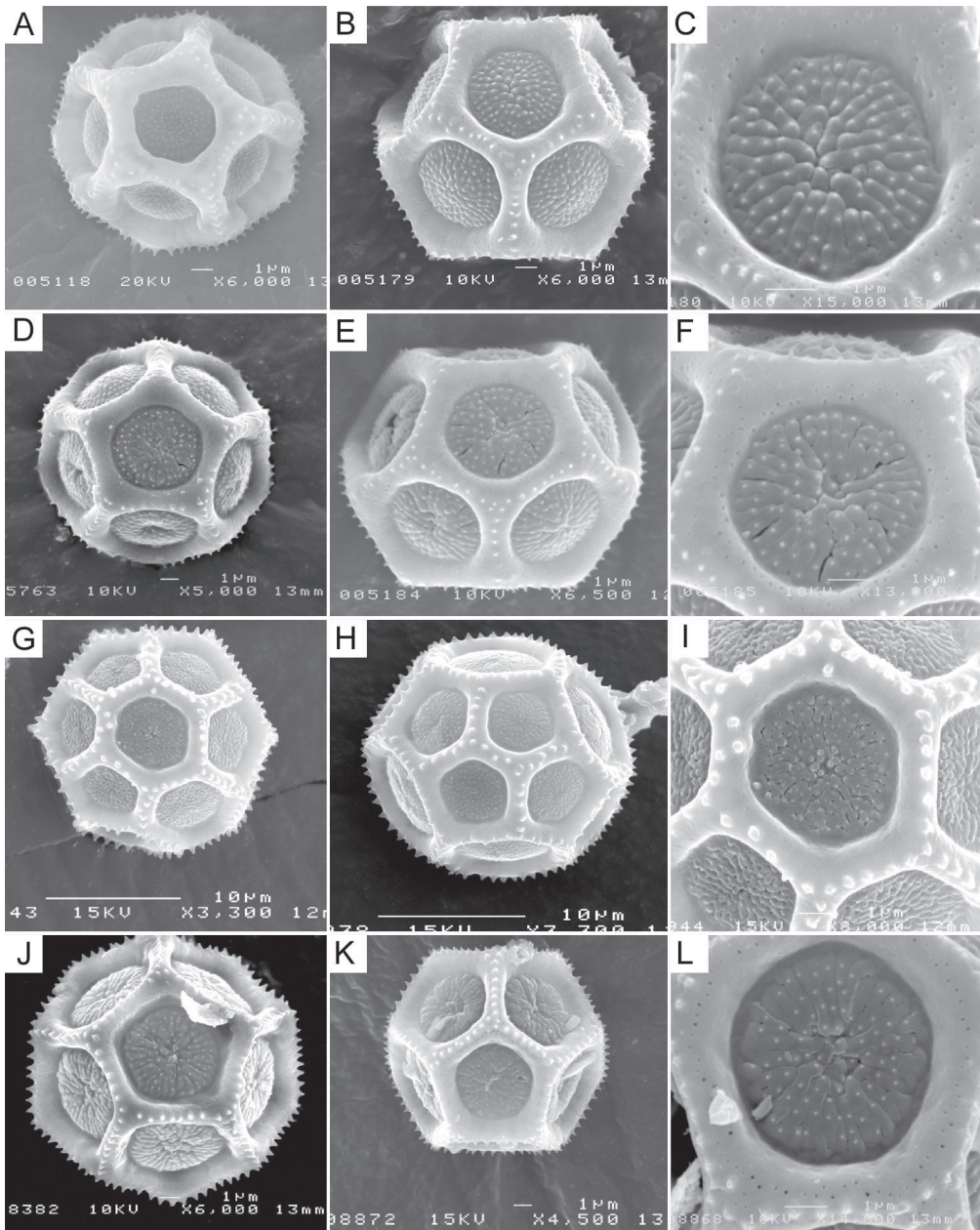


Fig. 1. SEM photomicrographs of pollen in polar view, equatorial view, and pore structure. A–C. *A. sessilis* (green variety); D–F. *A. sessilis* (red variety); G–I. *A. paronychioides* and J–L. *A. betzickiana*.

Table 4. Independent samples t-test comparing the polar length (P), equatorial diameter (E), pore diameter and number of ektexinous bodies between *A. sessilis* and *A. bettzickiana*.

Species	Dependent variable	Levene's test for equality of variances		t-test for equality of means					Lower	Upper
		F	significance value	t	df	significance value (2-tailed)	mean difference	std. error difference		
<i>A. sessilis</i> both varieties	P (µm)	2.63	0.11	-1.35	98.00	0.18	-0.29	0.22	-0.72	0.14
	E (µm)	0.95	0.33	1.32	98.00	0.19	0.25	0.19	-0.12	0.61
	Pore diameter (µm)	1.69	0.20	-3.86	58.00	0.00	-0.32	0.08	-0.48	-0.15
	Number of ektexinous bodies	6.14	0.02	1.67	17.82	0.11	1.64	0.98	-0.42	3.71
<i>A. sessilis</i> (green variety) and <i>A. bettzickiana</i>	P (µm)	5.84	0.02	3.12	86.58	0.00	0.64	0.20	0.23	1.04
	E (µm)	1.45	0.23	1.76	98.00	0.08	0.32	0.18	-0.04	0.68
	Number of ektexinous bodies	0.39	0.54	-2.42	26.00	0.02	-3.29	1.36	-6.07	-0.50
<i>A. sessilis</i> (red variety) and <i>A. bettzickiana</i>	P (µm)	0.53	0.47	5.24	98.00	0.00	0.93	0.18	0.58	1.28
	E (µm)	0.05	0.82	0.46	98.00	0.65	0.07	0.16	-0.24	0.39
	Number of ektexinous bodies	10.68	0.00	-4.53	16.86	0.00	-4.93	1.09	-7.22	-2.63

The pollen morphology of the green and red varieties of *A. sessilis* is remarkably similar despite significant differences in their pore diameter. This difference alone is not sufficient to delimit them into two species. Furthermore, the pollen grains of these two varieties show no significant difference in polar length, equatorial diameter and number of ektexinous bodies.

Interestingly, the present study has shown that pollen grains of *A. betzickiana* are almost identical to those of *A. sessilis*. Still, the pollen grains of these two species could be differentiated by significant difference in their polar length and number of ektexinous bodies. The pollen of *A. betzickiana* is longer (*A. betzickiana*: 15.79 μm ; green and red varieties of *A. sessilis*: 15.19 μm and 15.48 μm , respectively) and the pore is covered by more ektexinous bodies (*A. betzickiana*: 33; green and red varieties of *A. sessilis*: 29 and 28, respectively). Another character that differentiates these two species is the distribution of perforations. The perforations in *A. betzickiana* are at the top of the mesoporia only whereas those from *A. sessilis* are around the whole mesoporia.

Clearly, the pollen of *A. paronychioides* is very different from the pollen of *A. sessilis* and *A. betzickiana* in having around 18 oval pores while the other two species have only 12 round pores. The t-test could not be carried out to compare the polar length, pore diameter and number of ektexinous bodies between *A. paronychioides* and the other two species due to the difficulty in obtaining sufficient number of pollen grains from *A. paronychioides*.

Discussion

On the whole, pollen grains of the three species studied conform well to the *Gomphrena*-type of Erdtman (1966) which corresponds to the *Pfaffia* type of Borsch (1998). The pore structure is similar to the Type I of Borsch (1998). Similar observations have been reported in the pollen grains from the New World (Borsch 1998, Eliasson 1988, Müller & Borsch 2005b, Nowicke & Skvarla 1979) and China (Li et al. 1993, Liang et al. 1978) (Table 1). However, a few exceptions have been reported, i.e., *A. philoxeroides* (Li et al. 1993) and *A. costaricensis* Kuntze (Borsch 1998) are reported to have spheroidal pollen grains.

The present palynological results support existing data which have indicated that *Alternanthera* is stenopalynous in terms of pore number. Most of the species examined (Borsch 1998, Eliasson 1988, Li et al. 1993, Liang et al. 1978, Müller & Borsch 2005b, Nowicke & Skvarla 1979), including *A. sessilis* and *A. betzickiana* in the present study, have 12–14 pores. Only a few exceptions were identified, such as 20–24 pores in *A. philoxeroides* (Li et al. 1993), 25–30 in *A. costaricensis* (Borsch 1998) and 18 in *A. paronychioides* (present study).

Compared with previous studies, the pollen grains of *A. sessilis* in the present study are distinctly different from those in Pakistan and India. For instance, the number of pores was reported as six in the grains of *A. sessilis* from the Upper Gangetic plain

(Rao & Shukla 1975) and 3–3.2 from Pakistan (Perveen & Qaiser 2002). In fact, the pollen of *A. sessilis* from India is reported to have granulated sexine ornamentation (Rao & Shukla 1975) and without spinules (Mittre 1962). However, this kind of apparent contradiction, especially the study from India, is difficult to resolve without further confirmatory work because the methodology and voucher specimens of *A. sessilis* were not mentioned by these authors and therefore taxonomic verification could not be carried out.

Further, the pollen grains of *A. bettzickiana* in the present study are different from those reported in China (Li et al. 1993) in having bigger pollen and pore (present study: polar length = 15.79 μm , pore: 4.83 μm) while those from China are smaller (polar length = 10.90 μm , pore = 3.60 μm). Moreover, only a single row of spinules are observed in the present study, whereas 1–2 rows of spinules are observed in the pollen from China.

In addition, data obtained in the previous study of *A. paronychioides* is different from the present findings. For instance, the pollen grains from Pakistan (Perveen & Qaiser 2002) were smaller (15.34 μm) with 6–9 pores while those from this study are bigger (21.85 μm) with around 18 oval pores. Furthermore, the size of the pores in the present study is about twice that of pores from the Pakistan pollen grains, i.e., 6.06 μm and 3.44 μm , respectively.

At the generic level, the pollen morphology of *Alternanthera* species is close to *Pfaffia* Mart. (Borsch 1998, Eliasson 1988), *Tidestromia* Standl. and *Kyphocarpa* Schinz in having dodecahedral pollen grains and metareticulate sexine (Borsch 1998). In fact, the current palynological data is generally in agreement with Borsch (1998) and thus might help in the genus delimitation. For instance, the pollen of *Alternanthera* can be distinguished from *Tidestromia* by the sexine pattern. The pollen of *T. lanuginosa* (Nutt.) Standl. is reported as devoid of spinules (Borsch 1998) and *T. oblongifolia* (S. Watson) Standl. has a very narrow mesoporia which is triangular in cross-section (Eliasson 1988). On the other hand, most of the *Alternanthera* species in the present study have one to two rows of microspines attached on the moderate mesoporia.

The number and shape of apertures appear to be an important key in differentiating the *Pfaffia* and *Alternanthera* species. Instead of having spheroidal grains with more than 20 pores as reported in *Pfaffia* (Borsch 1998), the pollen grains of *Alternanthera* are dodecahedral with less than 20 pores. Most of the *Pfaffia* species conform to the above characters except *P. aurata* (Mart.) T. Borsch, *P. completa* (Uline & W.L. Bray) T. Borsch, *P. costaricensis* (Standl.) T. Borsch and *P. densipellita* T. Borsch (Borsch 1995 & 1998). These species are reported also to have dodecahedral pollen with 12–14 pores and metareticulate sexine. However, the distribution of microspines on the sexine could be used to resolve this problem. The microspines are occasionally arranged in an undulate row or side by side, as seen in *P. aurata* and *P. costaricensis* (Borsch 1995) but distally and usually regularly arranged in most of the *Alternanthera* species.

Conclusion

Pollen grains of *A. sessilis*, *A. bettzickiana* and *A. paronychioides* in Peninsular Malaysia can be differentiated mainly by the number of apertures, number of ectexinous bodies and distribution of perforations at the mesoporia. The apertures of *A. sessilis* and *A. bettzickiana* are pantoporate with 12 round pores whereas the pollen grains of *A. paronychioides* have 18 oval pores. The sexine is metareticulate and tectum perforate with unevenly distributed perforations at the top and base of the mesoporia, except in the pollen grains of *A. bettzickiana*, in which the perforations are distributed unevenly at the top of the mesoporia only.

Although this study has indicated that the palynology of the green and red varieties of *A. sessilis* is remarkably similar, palynological data was only obtained from specimens at a single habitat. As the green-leafed variety of *A. sessilis* could be found from various habitats, e.g., in canals, ditches or wastelands, a survey on the pollen morphology from those habitats should also be carried out. Besides, future work should also include the transmission electron microscopy (TEM) study in order to strengthen the hypothesis that the pollen morphology of the green and red varieties of *A. sessilis* is not significantly different.

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