The Anatomy and Morphology of *Taenitis* Willd. ex Sprengel (Monilophytes) in Peninsular Malaysia

(Anatomi dan Morfologi *Taenitis* Willd. ex Sprengel (Monilofit) di Semenanjung Malaysia)

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ABSTRACT

The present study investigates the anatomical and morphological characteristics of four *Taenitis* species sampled from several localities in Peninsular Malaysia, namely *Taenitis blechnoides, T. interrupta, T. dimorpha* and *Taenitis* sp. The morphological and anatomical characters were observered with compound and dissecting microscopes, and sliding microtome. The species exhibited varying frond sizes (length and width) and positions of sori. Conversely, all the species showed common short creeping rhizomes with black hairy scales, scales at the base of stipes, U-shaped steles, simple trichome, sclerenchyma cells under the epidermis on stipes and rhizomes, polocytic stomata, and sinuous anticlinal walls. Morphologically, the frond form of *Taenitis* sp. is distinct from the other *Taenitis*. Thus, the species could be a new record for ferns in Peninsular Malaysia. However, molecular studies are required to confirm this.

Keywords: Endemic; Monilophytes; Pteridaceae; Taenitis

ABSTRAK

Kajian ini menentukan ciri anatomi dan morfologi empat spesies *Taenitis* yang diambil dari beberapa lokaliti di Semenanjung Malaysia, iaitu *Taenitis blechnoides, T. interrupta, T. dimorpha* dan *Taenitis* sp. Penelitian ciri morfologi dan anatomi dilakukan menggunakan mikroskop majmuk, mikroskop pembedahan dan mikrotom gelongsor. Spesies yang dikaji menunjukkan pelbagai saiz fron (panjang dan lebar) dan kedudukan sorus. Sebaliknya, kesemua spesies yang dikaji menunjukkan ciri rizom menjalar pendek dengan berwarna kehitaman di bahagian pangkal stip, stel berbentuk 'U', trikom jenis ringkas, sel skelerenkima di bawah lapisan epidermis pada stip dan rizom, stomata jenis polositik dan dinding antiklin jenis sinuos yang berliku. Bentuk fron *Taenitis* sp. berbeza daripada spesies *Taenitis* yang lain. Oleh itu, spesies ini berkemungkinan merupakan rekod baharu paku pakis di Semenanjung Malaysia. Namun kajian lanjutan dalam molekul diperlukan untuk mengesahkannya.

Kata kunci: Endemik; Monilofit; Pteridaceae; Taenitis

INTRODUCTION

Ferns and fern allies are an ancient group of plants that have existed since the Devonian and Carboniferous periods, 350 million years ago. Ferns are the second largest group of vascular plants and belong to the Monilophyta. The plants are distinguished from angiosperms and gymnosperms as they reproduce via spores that are dispersed via wind, and they do not produce seeds, flowers or fruits. Worldwide, approximately 12,000 species and 319 genera have been recorded (Sharpe, Mehltreter & Walker 2010), while Malaysia documented ca. 1165 taxa with ca. 647 species in Peninsular Malaysia (Maideen, Salleh & Khaduwi 2020; Maideen et al. 2019; Parris & Latiff 1997).

Taenitis is derived from the Greek word 'Taenia', which means ribbon (Bostock 1998), and this genus was introduced by Sprengel (Tryon, Tryon & Kramer 1990). The genus was classified under Pteridaceae. It is

recognizable from its various sporangia forms, which were linear, narrow longitudinal bands between midribs and margins or irregularly along the veins (Holttum 1968b). *Taenitis* is distributed in Ceylon, northern India, Malaysia and Fiji (Holttum 1968a). Currently, 10 species of *Taenitis* have been reported in Malaysia, including three species in Peninsular Malaysia. Species recorded in Peninsular Malaysia are *T. blechnoides*, *T. interrupta* and *T. dimorpha*, while the others are recorded in Sabah and Sarawak (Parris & Latiff 1997).

According to Christenhusz and Chase (2014), the anatomical study of ferns was introduced in the 16th century. Since the invention of microscopes, including SEM, anatomical research has been popular in plant systematic study. Previous research proved that fern anatomical structures, such as the stele shape of rhizomes and stipes, the number of sclerenchyma cell layers in stipes, venation of lamina, stomata structure, and anticlinal wall shapes are essential in classifying ferns (Ogura 1972). Anatomical reports on the ferns from Peninsular Malaysia include the Adiantum (Bidin & Anita 1995), Taenitis alliance (Walker 1968), Polystichum (Roux & Van-Wyk 2000), Selaginella (Maideen et al. 2013), Davallia (Noraini et al. 2012), Blechnum (Noraini et al. 2014) and Pleocnemia (Maideen et al. 2018) species. The present study aimed to examine the morphological and anatomical variations in Taenitis reported for Peninsular Malaysia including an unknown species (Taenitis sp.) was collected from Fraser's Hill recently.

MATERIALS AND METHODS

The samples evaluated in the present study were four *Taenitis* taxa collected from various locations across Peninsular Malaysia (Table 1). All the sample used were fresh except *T. interrupta* which was obtained from herbarium specimens. All voucher specimens examined were deposited in the Universiti Kebangsaan Malaysia Herbarium (UKMB), Forest Research Institute Malaysia Herbarium (KEP) and University Malaya Herbarium (KLU).

The anatomical investigation conducted in the present study followed the protocols reported by Johansen (1940) and Sass (1958) with some modifications. First, the stipes and rhizomes from the voucher specimens were boiled for 15 min. Subsequently, the fresh specimens were fixed in a 3:1 alcohol to acetic acid solution (70% alcohol: 30% acetic acid) for two days before being sectioned with a sliding microtome to a thickness of 10-15 μ m.

For venation evaluation, the leaf lamina were cleared with a basic fuchsin solution and oven-dried at 60 °C for 5-7 days. Subsequently, the lamina were sectioned and stained in Safranin and Alcian Blue. The samples were then dehydrated in a series of alcohol solutions (50%, 70%, 95% and 100%) before being mounted on slides with Canada Balsam. The slides were heated in an oven at 60 °C for two weeks. Finally, the sections were observed and photographed with scientific microscope (Olympus Diaphlan). The image obtained were analysed using Analysis Docu Software.

RESULTS AND DISCUSSION

All the fern species assessed in the present study demonstrated similar features, including short creeping rhizomes with black hairy scales, scales at the base of stipes, U-shaped steles (Figure 6), simple trichome, sclerenchyma cells under the epidermis on stipes and rhizomes, polocytic stomata (Figure 5), and sinuous anticlinal walls (Figure 5). The types of fronds (length and width) and the positions of the sori varied morphologically among the species studied.

The genus evaluated in this study exhibited frond variation, namely simple, trifoliate and pinnate forms. Both the fertile and sterile fronds in *T. interrupta* (Figure 1(c)) and *T. blechnoides* (Figure 1(a)) were pinnate. Pinnae width varies across species, with *T. blechnoides* having broader pinnae than *T. interrupta. Taenitis dimorpha* (Figure 1(b)) showed a fertile frond that was either pinnate or trifoliate, while its sterile frond was simple. The unknown species, *Taenitis* sp., possessed simple sterile, and fertile fronds (Figure 1(d)). The current study also showed that the shapes and sizes of the *Taenitis* sp. fronds differed from the species described by Holttum (1968a).

In the anatomical study, only the upper (2 cm from the first branching of the pinna) and lower (2 cm from the rhizome) sections of the stipes were observed. Figure 3 shows two forms of steles, V-shaped (*T. dimorpha* and *Taenitis* sp.) and U-shaped (*T. blechnoides* and *T. interrupta*), while Figure 2 shows two parallel plates in most species. The sclerenchyma cells were observed in several layers, and the number of layers varies among the species (Table 3).

The vein pattern of all the taxa investigated demonstrated close anastomose veins type without reaching the margin (Figure 4), and this is similar the studies in Walker (1968) and Ogura (1972). All the species also exhibited polocytic stomata (Figure 5) with sinuous anticlinal walls (Figure 4) and possessed longitudinal sori between their margins and midribs (Figure 1). Holttum (1968a) recorded that the space between the sorus and margins of *T. interrupta* was under 1 mm.

In the current study, we document the morphological

features (Table 2) and anatomical characters (Table 3) of the *Taenitis* species, which provide the specific character for further information on this genus. The morphology features obtained from the present study are used in constructing an artificial identification key to the *Taenitis* species, and the key is given herewith.

KEY TO THE *Taenitis* SPECIES

1.	Fertile and sterile frond pinnate2
	Fertile frond simple or pinnate or trifoliate and sterile frond simple
2.	Pinnae 1-5 pairs, sterile pinnae 2.5-5 cm wide, fertile pinnae 1-3 cm wide,
	thick texture
	Pinnae 6-12 pairs, fertile and sterile pinnae 0.6-1.2 cm wide, thin texture
3.	Fertile frond pinnate or trifoliate, pinnae 2-4 pairs, pinna of fertile frond
	0.8 -1.5 cm wide <i>T. dimorpha</i>
	Fertile frond and sterile frond simple, pinna of fertile frond 1.5-2 cm wide

TABLE 1. The Taenitis specimens used in the study

Species	Collector and collection number	Locality
T. interrupta	Nur Izzaty, Nurul Nadhirah, and Nur Aliah-NI201969 (UKMB)	Gunung Semangkok, Selangor
	Haja Maideen, Farhana Sidek, Nurul Nadhirah, and Nur Aliah-FS201742 (UKMB)	Hutan Simpan Pangkor Utara, Pulau Pangkor, Perak
	Haja Maideen, Nur Fatihah, and Nur Aliah-NF201713(UKMB)	Vale Eco Centre, Teluk Muroh, Lumut, Perak
	Aziz Bidin-AB10266(UKMB)	-
	Anita Shari-AS 38(UKMB)	Ulu Bendul, Kuala Pilah, Negeri Sembilan
	Anita Shari-AS 64(UKMB)	-
	A. Latiff-ALM 6686(UKMB)	-
	Holttum-s.n. (UKMB)	-
T. dimorpha	Nur Izzaty, Nurul Nadhirah, and Nur Aliah-NI 201920, NI 201923, NI 201928, NI 201943, NI201959, NI201966, NI2019108 (UKMB)	Gunung Semangkok, Selangor
	Nurul Nadhirah and Nur Aliah-NN 20191, NN20192, NN20193, NN20194, NN20195, NN20196 (UKMB)	Pine Tree Trail, Bukit Fraser, Pahang
	Razali Jaman-RJ 28(UKMB)	-
	Anita Shari-AS 86(UKMB)	-

	Abd. Samat bin Abdullah andAhmad Shukor-342(KLU)	Penang Hill, Pulau Pinang
	Mohd Shah, Samsuri, and Ahmad Shukor-MS3491(KLU)	Gunung Lawit, Terengganu
	David W. Lee-UL 27(KLU)	Bukit Hitam, Hulu Langat, Selangor
	Abd. Samat bin Abdullah-943(KLU)	Tasik Bera, Pahang
	Benjamin C. Stone-5997(KLU)	Fraser's Hill, Pahang
	Benjamin C. Stone-14581(KLU)	Kota Tinggi Waterfall, Johor
T. blechnoides	Nur Izzaty, Nurul Nadhirah, and Nur Aliah -NI201944, NI2019140, NI 2019144(UKMB)	Gunung Semangkok, Selangor
	Haja Maideen-HM 95, HM 105, HM 220, HM248, HM257(UKMB)	Pulau Redang, Terengganu
	Nurul Nadhirah and Nur Aliah-NN24, NN25, NN26, NN28, NN29(UKMB)	Bukit Bendera, Pulau Pinang
	Anita Shari-AS44(UKMB)	Bukit Pangkor
	Anita Shari-AS39(UKMB)	Pasoh, Negeri Sembilan
	Amir-s.n. (UKMB)	Tembat, Pahang
	Haja Maideen, Nurul Nadhirah, and Samiah-NN38(UKMB)	Hutan Lipur Bukit Wang, Kedah
	Razali Jaman-RJ 3327(UKMB)	Lumut, Perak
	Haja Maideen-HM 248, HM 257(UKMB)	Pulau Kapas, Terengganu
	Willis Litke-W227(UKMB)	-
	Aziz Bidin-AB1026(UKMB)	-
	Haja Maideen and Razali Jaman-RJ3207(UKMB)	Pulau Langkawi, Kedah
	Nur Fatihah, Nur Aliah, and Farahanim-NF 3, NF 18, NF 16(UKMB)	Hutan Simpan Kenaboi, Negeri Sembilan
	Nur Fatihah, Nur Aliah, and Haja Maideen-NF59(UKMB)	Vale Eco Centre, Teluk Muroh, Lumut, Perak

	Hafiz Ikhram and Masnoryante-HI 24(UKMB)	Hutan Simpan Pasoh, Negeri Sembilan	
	Razali Jaman-RJ 6772(UKMB)	-	
	Nurul Nadhirah and Nur Aliah-NN 10, NN 16(UKMB)	Pine Tree Trail, Bukit Fraser, Pahang	
	Alia Atierah, Nurul Nadhirah, and Nur Aliah-AA2019207, AA 2019206, AA 2019174(UKMB)	Pintu Rimba ke Seroja, Gunung Korbu, Perak	
Taenitis sp.	Imin, Siti Munirah, Mohd Hairul, Kamarul Hisham-FRI77597(KEP)	Pulau Tioman, Rompin, Pahang	
	Nor Ezzawanis, Angan -FRI58316(KEP)	Gunung Belumut, Kluang, Johor	
	anon s.n. (KEP)	Jalan ke Ladang Tenusu, Bukit Fraser, Pahang	
	Nor Ezzawanis, Bryan, Mohd Fakrullah Haziq, and Wan- FRI89662(KEP)	Gunung Besar Hantu, Jelebu, Negeri Sembilan	
	Kiew, Lindsay, and Middleton-FRI57507(KEP)	Denai Pine Tree, Fraser's Hill, Pahang	
	Nur Izzaty, Nurul Nadhirah, and Nur Aliah-NI 201918, NI 201961, NI 201967, NI 2019105(UKMB)	Gunung Semangkok, Selangor	



FIGURE 1. The *Taenitis* species in their sampling habitats. a and b: *T. blechnoides*, c and d: *T. dimorpha*, e and f: *T. interrupta*, and g and h: *Taenitis* sp.

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Species	Frond type	Number of pinnae in pairs	Width of pinna	Length of stipe	Space between sorus and margin	
	Sterile: Pinnate		Sterile: 2.5–5 cm		2–5 mm	
T. blechnoides	Fertile: Pinnate	1–10	Fertile:2–3 cm	40–60 cm		
	Sterile: Simple		Starilar 2 4 5 am		2 2	
T. dimorpha	Fertile: Pinnate/	1-4	Sterne: 5–4.5 cm	10–20 cm	2–3 mm	
	Trifoliate		Fertile:0.8–15 mm			
	Sterile: Pinnate			Fortilo don storilo:		$\leq 1 \text{ mm}$
T. interrupta	Fertile: Pinnate 6–12	6–12	0.6-1.2 cm	30–50 cm		
Tranitic sp	Sterile: Simple	1	Sterile: 3.5–6 cm		1–2 mm	
iueniiis sp.	Fertile: Simple	1	Fertile: ≥15 mm	10–40 cm		

TABLE 2. The morphological characteristics of the Taenitis species

TABLE 3. The epidermis and sclerenchyma cell layers of the upper and lower stipes of the Taenitis specimens

Species	T. blechnoides	T. dimorpha	T. interrupta	Taenitis sp.
Upper stipe of epidermis cell	1	1	1	1
Lower stipe of epidermis cell	1	1	1	1
Upper stipe of sclerenchyma cell	6–13	5–8	6–10	2–5
Lower stipe of sclerenchyma cell	6–12	5–9	6–10	4–6



FIGURE 2. The cross-sections of the lower stipes of *Taenitis* species. a and b: *T. blechnoides,* d–f: *T. dimorpha*, g–i: *T. interrupta* and j–l: *Taenitis* sp.

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FIGURE 3. The cross-sections of the upper stipes of *Taenitis* species. a and b: *T. blechnoides*, d–f: *T. dimorpha*, g–i: *T. interrupta*, and j–l: *Taenitis* sp.



FIGURE 4. The venations of *Taenitis* species. a: *T. blechnoides*, b: *T. dimorpha*, c: *T. interrupta*, and d: *Taenitis* sp.



FIGURE 5. The abaxial of the *Taenitis* species. a: *T. blechnoides*, b: *T. dimorpha*, c: *T. interrupta*, and d: *Taenitis* sp.



FIGURE 6. The rhizome cross-sections of the *Taenitis* species. a: *T. blechnoides*, b: *T. dimorpha*, c: *T. interrupta*, and d: *Taenitis* sp.

CONCLUSION

In conclusion, morphological characteristics were more significant than anatomical attributes in differentiating the *Taenitis* species. The taxa evaluated in the present study exhibited several similar and varying morphological characteristics applicable to differentiate among the species. Nevertheless, identification with anatomical data is challenging as the different species exhibited similar data. *Taenitis* sp., the unknown species used in this study, showed variation from the other *Taenitis* found in Peninsular Malaysia, and it differed significantly based on the fronds. Consequently, the species might be a novel record or species in Peninsular Malaysia. Nevertheless, molecular investigation is required for further clarification.

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