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SHORT COMMUNICATION

Six Species of Epiphytic Algae *Phycopeltis* Millardet (Trentepohliaceae, Chlorophyta) Collected from Royal Belum Rainforest, Perak, Malaysia

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Abstrak: Pada kebiasaannya alga dirujuk sebagai alga yang hidup di dalam air dan kewujudan alga yang hidup di darat kurang diperkatakan. Trentepohliales adalah satu klasifikasi alga hijau berfilamen yang hidup di darat. Alga ini boleh menjadi parasit, epifit atau hidup tanpa perumah. Alga selalunya dijumpai di atas bahagian tumbuhan tropikal dan juga batu. Walau bagaimanapun, kajian berkenaan organisma ini masih terhad terutamanya di Malaysia. Oleh yang demikian, objektif kepada kajian ini adalah bagi meninjau taburan alga hijau epifit (*Phycopeltis* Millardet) pada daun tumbuhan di Hutan Simpan Royal Belum, Perak dan juga mengenal pasti genus dan spesis alga berkenaan. Daun tumbuhan hutan yang kebanyakannya tumbuhan semak dengan tompok hijau dan coklat kejinggaan telah dikutip dan diperiksa. Pengecaman bagi genus alga telah dilakukan berdasarkan morfologinya. Dalam kajian ini, alga hijau epifit daripada genus *Phycopeltis* telah dijumpai pada 10 tumbuhan perumah. Antara alga yang dikenal pasti termasuklah *P. amboinensis* (Karsten) Printz, *P. arundinacea* (Mont.) de Toni, *P. epiphyton* Millardet, *P. irregularis* (Schmidle) Wille, *P. flabellata* R.H. Thompson & Wujek dan *P. treubii* Karsten manakala *P. amboinensis* adalah spesis yang paling kerap ditemui.

Kata kunci: Trentepohliales, *Phycopeltis*, Alga Hijau Epifit, Royal Belum Rainforest, Bintik Daun

Abstract: Algae are commonly referred as the aquatic algae and the existence of the terrestrial green algae is less portrayed. Trentepohliales is an order of filamentous green algae living terrestrially. These algae can be parasitic, epiphytic or free living organism. They are frequently found on parts of tropical plants and rocks. However, research regarding these organisms is still limited especially in Malaysia. Hence, the objectives of this study were to survey the distribution of the epiphytic green algae (*Phycopeltis* Millardet) on plant leaves in Royal Belum Rainforest, Perak and to recognise the genus and species of the

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algae. Leaves of forest plants (mainly shrubs) with green or brownish orange spots were collected and examined. Identification of the genus and species of the algae was done morphologically. In the present study, epiphytic green algae from the genus *Phycopeltis* were collected from 10 host plants. Among the identified species were *P. amboinensis* (Karsten) Printz, *P. arundinacea* (Mont.) de Toni, *P. epiphyton* Millardet, *P. irregularis* (Schmidle) Wille, *P. flabellata* R.H. Thompson & Wujek and *P. treubii* Karsten while *P. amboinensis* was the most common species.

Keywords: Trentepohliales, *Phycopeltis*, Epiphytic Green Algae, Royal Belum Rainforest, Leaf Spots

Cephaleuros Kunze (1829), *Stomatochroon* Palm (1934), *Trentepohlia* Martius (1817), *Phycopeltis* Millardet (1870), *Printzina* Thompson & Wujek (1992) and *Physolinum* Printz (1921) are the genera in Tretepohliaceae family (Order: Trentepohliales) (Brooks *et al*. 2015). Nevertheless, existence of the last genus (*Physolinum*) was controversial and in 2017, Zhu *et al*. reported that *Physolinum monilia* was the same as *Trentepohlia rigidula* hence, *Physolinum* was excluded from the order Trentepohliales. Trentepohliacean algae can live as parasitic or epiphytic organism and some are free living (Brooks 2004). *Cephaleuros* is well known for their ability to be parasitic to certain host plants including some economically important plant like guava. According to Sunpapao *et al*. (2016) disease may developed on leaves and even fruits of guava. Infestation on fruits appeared as scab which will decrease the cosmetic value of the guava thus leading to economic loss.

This study will focus on the genus *Phycopeltis*, an epiphytic algal found on plant foliar, twigs, fruits, stems, and other non living substrates (Thompson & Wujek 1997). They usually colonise the substrate superficially and can be wholly free living. Furthermore, this genus exists as disk or fan like shape thalli (ramuli). The erect structure consists of sporangiate lateral produced on filamentous sporangiophore. Sporangium is found on the terminal part of the sporangiate lateral. In addition, gametangium of *Phycopeltis* is commonly produced in prostrate growth part of all species and sometimes in erect growing structure for the species that borne absolute filamentous sporangiophore.

In Malaysia, there were very few studies done on these Trentepohliacean algae. Previous studies were done on *Trentepohlia* and *Printzina* by Millow and Salleh (2006) focusing on certain substrate such as plant bark and stumps and also rocks. On the other hand, Neustupa (2001) also reported *Trentepohlia* and *Phycopeltis* as the most dominant Trentepohliacean algae from tropical rainforest in Peninsular Malaysia. Other reports of Trentepohliacean algae were on valuable crops such as black pepper and mango infected by the genus *Cephaleuros* (Kueh 1979; Lim & Khoo 1985). In the present study, colonisation on plant leaves is the main focus. Colonies appeared as patches of reddish orange, brownish orange or green filamentous structure of the Trentepohliacean algae. Identification of the algae was done morphologically by referring to the monograph prepared by Thompson and Wujek in 1997 and Zhu *et al*. in 2015.

Samples were collected from Royal Belum Rainforest, Perak on 2 to 4 February 2016. Several streams were visited, namely, Sungai Papan, Sungai Kejar and Sungai Kooi (Fig. 1). Leaves with colonisation of epiphytic green algae were collected randomly from the places mentioned. Herbarium specimens were kept in personal collection of the author while specimen's codes were as stated after the host name in Table 1. The algae colonies were usually found growing on the upper side of the leaves and can exist on the lower leaves side as well.

Fresh specimens were examined for sign and symptoms observations by using stereo microscope (Olympus SZ2-LGB). Algae were removed from the leaves and observed with light microscope (Olympus BX53F-CCD). Structures of thalli (ramuli), sporangia, sporangiophores and gametangia were the main focus for identification. However, other structures such as gametangia, gametes, and zoospores were not found during the studies. Additionally, measurements of the structures were presented in range of length × width.

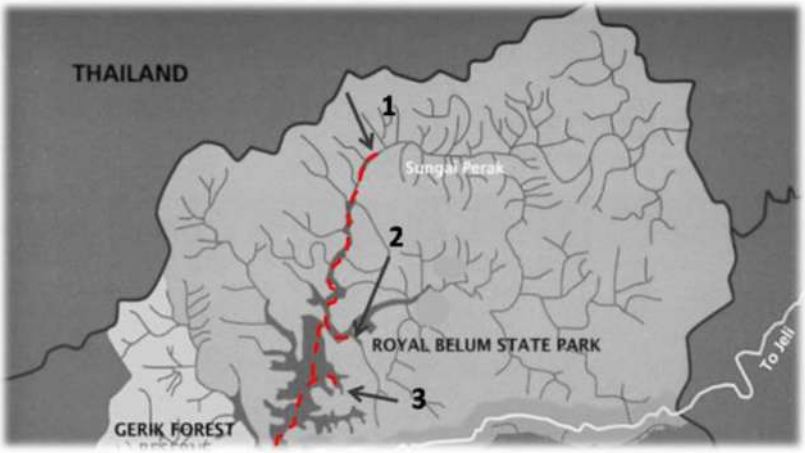

Figure 1: Royal Belum Rainforest Perak, Malaysia. 1 = Sungai Kejar, 2 = Sungai Kooi, 3 = Sungai Papan.

Table 1: Epiphytic green algae (Phycopeltis) with descriptions (Thompson & Wujek 1997 and Zhu *et al.* 2015) and colonised host plants with decriptions of the findings.

Genus and species	Descriptions (Thompson & Wujek 1997)	Descriptions (Zhu *et al.* 2015)	Findings	Findings (hosts plants/ family/ herbarium code)
Phycopeltis amboinensis (Karsten) Printz (1939)	• Fan like shaped thalli formed with sterile filaments or setae • Thalli cell (22–32 µm × 6–9 µm) • Sporangiophore (11–20 µm × 8–8.7 µm) • Sporangium (22.4–25.7 µm × 13.4–16 µm)	–	• Fan like shaped thalli observed • Setae and sporangiophore holding sporangia were observed • Setae or sporangiophore (9.2–12.3 µm × 5.2–10.7 µm) • Sporangium was ovoid shaped (16.4 × 13.3 µm) • Thalli cell (7.7–17.5 µm × 4.2–9.7 µm) • Pigmentation (pale yellow to reddish orange)	1. *Cynometra malaccensis* Meeuwen (Fabaceae) (FF16005) 2. *Dacryodes rugosa* Blume (Burseraceae) (FF16010) 3. *Ficus chartacea* Wall. (Moraceae) (FF16001) 4. *Zingiber* sp. Boehm. (Zingiberaceae) (FF16006)
P. arundinacea (Mont.) de Toni (1889)	• Largest species of Phycopeltis (large circular disk and long thalli cells) • Thalli cell (28–38 µm × 7–12 µm)	–	• Large fan like thalli were found on the host • Thalli cells were longer than other species • Thalli cell (17.0–23.9 µm × 3.9–10.3 µm) • Pigmentation (green and brownish orange)	1. *Cinnamomum puberulum* Ridl. (Lauraceae) (FF16003) 2. *Symplocos rubiginosa* Wall. (Symplocaceae) (FF16002)
P. epiphyton Millardet (1870)	• Circular disks thalli were formed • Thalli cell (8.4–18 µm × 4.2–6.3 µm) • Sporangiophore were few to several cells long	• Thalli were fan like shaped with crenated margin • Thalli (7.4–23.8 µm × 4.4–8.2 µm) • Sporangium (11.2–15.4 µm × 9.4–12.6 µm) • Pigmentation (yellowish-green or orange)	• Fan like shaped thalli formed with appearance of petite sporangiophore holding the sporangiospore • Thalli cell (10.8–13.5 µm × 4.7–7.6 µm) • Sporangiophore (3–4 cells long) • Sporangia were reniform or ovoid (14.3–15.0 µm × 10.1–11.8 µm) • Pigmentation (yellowish-green)	1. *Garcinia penangiana* var. penangiana Pierre (Guttiferae) (FF16009)

(*continued on next page*)

Table 1: (*continued*)

Genus and species	Descriptions (Thompson & Wujek 1997)	Descriptions (Zhu *et al.* 2015)	Findings	Findings (hosts plants/ family/ herbarium code)
P. flabellata R.H. Thompson & Wujek (1997)	• Thalli pattern commonly flabellated (narrow to broad fan like shape thalli) • Thalli cell (16.8–22 µm × 5.6–8.8 µm)	• Fan like shaped thalli were formed with loose disk shape • Thalli cell (11–30.6 µm × 5–11.2 µm) • Pigmentation (golden orange)	• Flabellate patterned thalli observed • Thalli cell (10.0–14.1 µm × 4.5–6.4 µm) • Pigmentation (green)	1. *Antidesma velutinosum* Blume (Phyllanthaceae) (FF16007)
P. irregularis (Schmidle) Wille (1909)	• Never form fan like shaped thalli • Thalli were filamentous and branching • Cell shape were cylindrical, barrel shaped and irregular • Thalli cell (13.0–16.2 µm × 5.6–8.8 µm)	–	• Thalli were found as strands of cells (filaments) yet branching • Most cells were barrel shaped • Thalli cell (9.5–14.8 µm × 6.0–8.5 µm) • Pigmentation (brownish-orange)	1. *Leptonychia caudata* Burret (Sterculiaceae) (FF16008)
P. flabellata R.H. Thompson & Wujek (1997)	• Thalli pattern commonly flabellated (narrow to broad fan like shape thalli) • Thalli cell (16.8–22 µm × 5.6–8.8 µm)	• Fan like shaped thalli were formed with loose disk shape • Thalli cell (11–30.6 µm × 5–11.2 µm) • Pigmentation (golden orange)	• Flabellate patterned thalli observed • Thalli cell (10.0–14.1 µm × 4.5–6.4 µm) • Pigmentation (green)	1. *Antidesma velutinosum* Blume (Phyllanthaceae) (FF16007)
P. irregularis (Schmidle) Wille (1909)	• Never form fan like shaped thalli • Thalli were filamentous and branching • Cell shape were cylindrical, barrel shaped and irregular • Thalli cell (13.0–16.2 µm × 5.6–8.8 µm)	–	• Thalli were found as strands of cells (filaments) yet branching • Most cells were barrel shaped • Thalli cell (9.5–14.8 µm × 6.0–8.5 µm) • Pigmentation (brownish orange)	1. *Leptonychia caudata* Burret (Sterculiaceae) (FF16008)

(*continued on next page*)

Table 1: (*continued*)

Genus and species	Descriptions (Thompson & Wujek 1997)	Descriptions (Zhu *et al.* 2015)	Findings	Findings (hosts plants/ family/ herbarium code)
P. treubii Karsten (1891)	• Thalli were fan like shaped • Margin of thalli composed of glandular shaped cells • Thalli cell (12–35 µm × 7–14 µm)	–	• Fan like shaped thalli were formed • Glandular cells or rhizoid liked cells were observed at the thalli margin • Thalli cell (11.2–14.6 µm × 4.3–7.0 µm) • Pigmentation (orange)	1. *Mangifera indica* L. (Anacardiaceae) (FF16004)

Macroscopic View of Thalli

In the present study, *Phycopeltis* usually appeared as patches of colonies on leaves of the forest plants and neither chlorosis nor necrosis was observed upon the colonisation (Fig. 2a). Besides, the thalli were easily removed from the colonised leaves when scratched. *Phycopeltis* are epiphytic algae and are not parasitic or harmful towards the hosts. This genus can colonise any substrates with preferable growing condition and does not withdraw any nutrients from the hosts. Comparing with *Cephaleuros* (parasitic green algae), rhizoid of *Phycopeltis* was absent hence nutrients were not absorbed from the host plant (Thompson & Wujek 1997; Zhu *et al*. 2015). Furthermore, colonies of *Phycopeltis* were easily removed from their substrate as there was no anchor.

Fig. 2b shows the appearance of *Phycopeltis arundinacea* (Mont.) de Toni, 1889 colony on the host leaf in which orange round disk with granulated or protruded complexion were obviously seen on the leaf. Thompson and Wujek (1992) stated that colouration of Trentepohliacean algae was usually yellow to orange in colour. However, in the specimen examined, colonies were usually orange to brown in colour and in some cases, pale green. This phenomenon might be influenced by the less light received by the algae as most of the plants were collected from understory and the forest floor. Green colour was derived from chlorophyll pigments while orange colouration was due to the accumulation of hematochrome (carotenoid pigments and the derivatives) which depended on light intensity received by the algae (King 1954). Furthermore, according to L. Chen *et al*. (2016), low light intensity and nitrogen level resulted in less carotenoid pigments produced. Thus, the algae will not be intensely orange. Moreover, Fig. 2b and Fig. 2c illustrated the differences between colony of *P. arundinacea* and *P. amboinensis* (Karsten) Printz, 1939 in which the former had an obviously rounded thalli patches while the latter possessed a non consistent shapes of patches (arrow). According to Thompson and Wujek (1997) and Zhu *et al*. (2015), *P. arundinacea* and *P. epiphyton* Millardet, 1870 usually appeared as circular disk

colonies on the substrate, whereas *P. treubii* Karsten, 1891 and *P. amboinensis* commonly appeared in open branching and circular flabellated pattern.

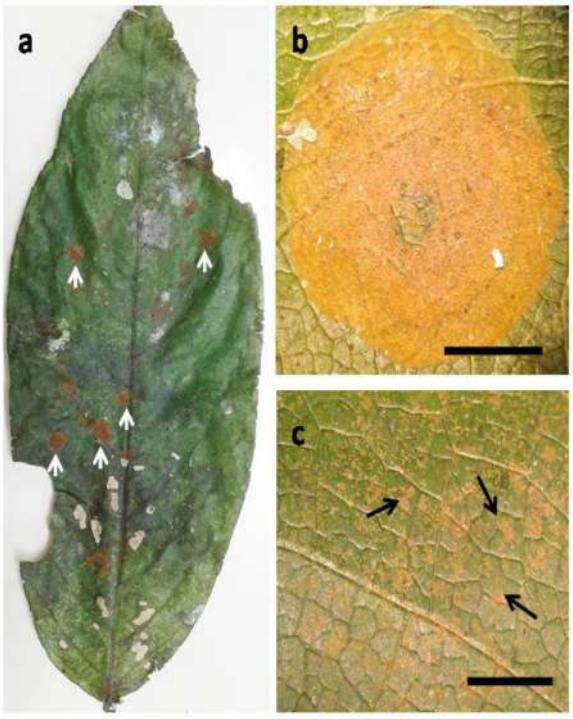

Figure 2: Sign and symptoms observation of *Phycopeltis* spp. on different hosts: (a) Colonisation of *P. arundinacea* on *Symplocos rubiginosa* leaf (arrow); (b) Colony appearance of *P. arundinacea*; (c) Colony appearance of *P. amboinensis* on *Dacryodes rugosa* (arrow). Scale bar = 1 mm.

Morphological Observation

As shown in Table 1, there were six species of *Phycopeltis* identified on 10 collected forest plants. Species of the algae were determined by pattern of the thalli as described by Thompson and Wujek (1997) and Zhu *et al*. (2015).

Phycopeltis amboinensis possess thalli that were usually elongated and had narrow fan like shape with long protruded sterile hairs (Fig. 3a) measured 7.7–17.5 µm × 4.2–9.7 µm which sometimes producing long sporangiophore (9.2–12.3 µm × 5.2–10.7 µm) bearing the sporangium (16.4 × 13.3 µm). The sterile hairs can reach until more than 30 cells long while sporangiophore can be up to 7 cells long. The presence of sterile hairs was a special characteristic that was not observed on other species. On the other hand, *P. arundinacea* had fan like shaped thalli forming obvious round spots or circular disk on the colonised leaf

(Fig. 3b). In case of *P. epiphyton*, the thalli were condensed and merged into a circular disk however, colonisation on *Garcinia penangiana* var. *penangiana* Pierre produces sporangia along with the sporangiophores (3–4 cells long) (Fig. 3c). *P. arundinacea* and *P. epiphyton* were alike. Thalli of both merge into circular disk shape but thalli cells of *P. arundinacea* were longer than *P. epiphyton* which the former were 17.0–23.9 µm × 3.9–10.3 µm and the latter were 10.8–13.5 µm × 4.7–7.6 µm.

Moreover, *Phycopeltis flabellata* R.H. Thompson & Wujek, 1997 had flabellated thalli pattern and merged into almost circular disk colony (10.0–14.1 µm × 4.5–6.4 µm) (Fig. 3d). This make *P. flabellata* almost similar to *P. amboinensis* however, in the present study *P. flabellata* did not produce any sterile hair. Therefore, *P. flabellata* had a similarity with *P. epiphyton* as both can produce sporangiophore and sporangia on the thalli. Nevertheless, during the study, sporangiophore was only observed from *P. epiphyton* plus, thalli of *P. flabellata* were loosely circular while *P. epiphyton* formed perfect disk of thalli.

Thalli of *P. treubii* were flabellated with glandular or rhizoid like shaped cells at the margin (11.2–14.6 µm × 4.3–7.0 µm) (Fig. 3e). Rhizoid like shape cells was an important characteristic to determine this species as the rest usually possess entire margin cells. Besides, thalli of *P. irregularis* (Schmidle) Wille, 1909 were commonly scattered while the other species were usually merged into fan like shape thalli and the cells were barrel shaped (9.5–14.8 µm × 6.0–8.5 µm) (Fig. 3f).

As the findings were compared to the previous records (Thompson & Wujek, 1997; Zhu *et al*. 2015), in terms of thalli appearances on host leaves, all of the identified species were able to produce fan like shaped thalli except for *P. irregularis* where barrel shaped thalli strands were found. On the other hand, thalli appeareance of *P. amboinensis* and *P. flabellata* were almost similar but *P. flabellata* did not produce any sterile hair or long sporangiophore. Despite all that, thalli of *P. epiphyton* and *P. arundinacea* were both formed in circular disk pattern but thalli cells of *P. arundinacea* was much longer than those of *P. epiphyton*. At the same time, *P. treubii* was differed from other fan like thalli forming *Phycopeltis* as it has glandular cells at the margin of the thalli. In addition, pigmentations of the algae were varied.

Moreover, measurements of the algae structures were also varied. For instance, from Thompson and Wujek (1997) records, thalli cells of *P. epiphyton* were measured 8.4–18 µm × 4.2–6.3 µm, while Zhu *et al*. (2015) recorded 7.4–23.8 µm × 4.4–8.2 µm and in the present study, thalli cells were measured 10.8–13.5 µm × 4.7–7.6 µm. These displayed varied measurements of the algae structures. Nonetheless, the measurements were still smaller than those of *P. arundinacea* which appeared almost similar to *P. epiphyton*. According to Thompson and Wujek (1997) thalli cells of *P.arundinacea* were 28–38 µm × 7–12 µm longer yet larger than *P. epiphyton* (8.4–18 µm × 4.2–6.3 µm). The same thing occur in the present study in which *P. arundinacea* possessed larger cells (17.0–23.9 µm × 3.9–10.3 µm) than *P. epiphyton* (10.8–13.5 µm × 4.7–7.6 µm). On the other hand, measurements of sporangia of *P. epiphyton* from the present study

(14.3–15.0 µm × 10.1–11.8 µm) were close to the record from Zhu *et al*. (2015) (11.2–15.4 µm × 9.4–12.6 µm). In case of *P. flabellata*, the present study recorded smaller range of thalli cells size than previous record. However, thalli pattern utterly showed flabellated pattern resembling the monograph by Thompson and Wujek (1997).

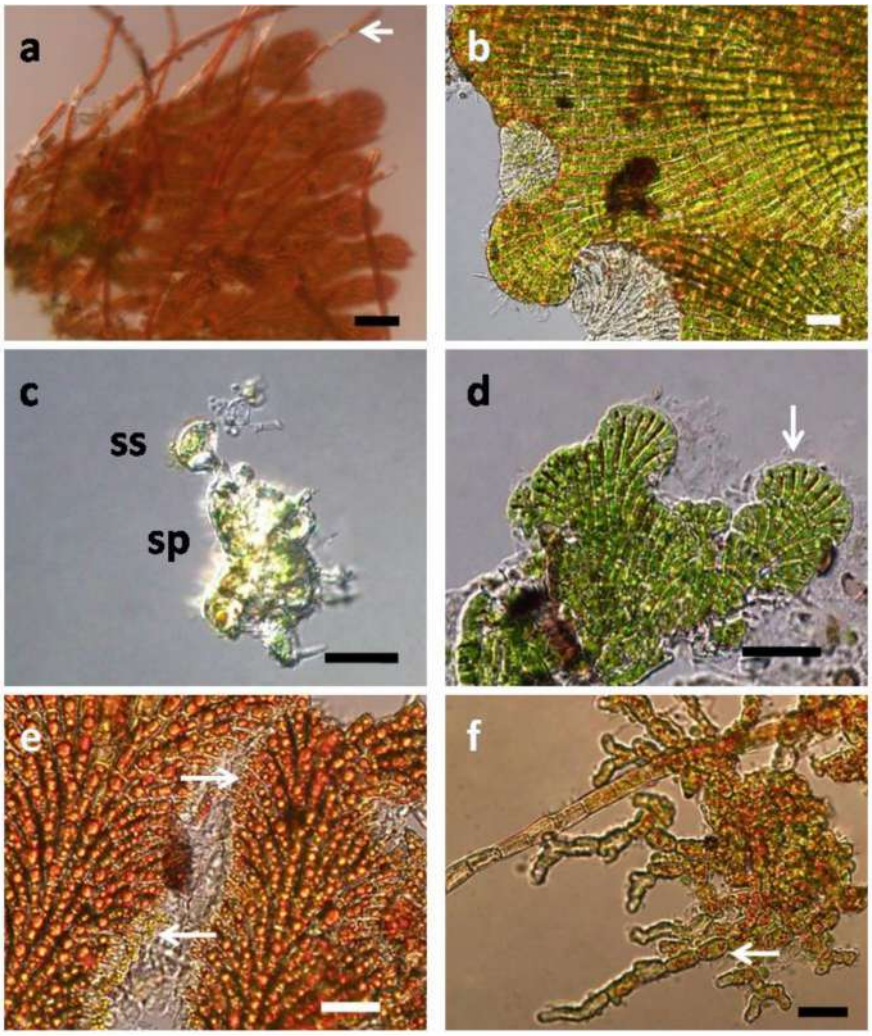

Figure 3: Morphological observation of *Phycopeltis* spp.: (a) *P. amboinensis* thalli (ramuli) with sterile hairs (arrow) on *Cynometra malaccensis*. Scale bar = 50 µm; (b) Green thalli of *P. arundinacea* on *Cinnamomum puberulum*; (c) Sporangiophore (sp.) and sporangiospore (ss) of *P. epiphyton* on *Garcinia penangiana* var. *penangiana*; (d) Thalli of *P. flabellata* (arrow) on *Antidesma venosum*; (e) Reddish orange thalli of *P. treubii* on *Mangifera indica* with rhizoid like shape cells at the margin (arrow); and (f) Scattered and barrel shaped thalli (arrow) of *P. irregularis* on *Leptonychia caudata*. Scale bar for 3b-3f = 20 µm.

Among six species of epiphytic green algae (*Phycopeltis*), the most recorded species was *P. amboinensis* and was found on *Cynometra malaccensis* Meeuwen, *Dacryodes rugosa* Blume, *Ficus chartaceae* Wall. and *Zingiber* sp. Boehm. The secondly frequent species was *P. arundinacea* found on *Cinnamomum puberulum* Ridl. and *Symplocos rubiginosa* Wall. The other species was found on four different hosts. *Phycopeltis epiphyton* on *Garcinia penangiana* var. *penangiana*, *P. flabellata* on *Antidesma venosum* Blume, *P. irregularis* on *Leptonychia caudata* Burret while *P. treubii* on *Mangifera indica* L.

The results indicate the widespread of epiphytic green algae especially *Phycopeltis* in Royal Belum Rainforest Perak. Humid and hot temperature of tropical climate are important factors for the wide distribution of the algae in this area hence deeper survey should be done to discover more about the distribution of this organism. On the other hand, to resolve the difficulties in determining the species, molecular identification should be done.

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