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Paludicola turfosa (Batrachospermales, Rhodophyta), a new record from Sebangau National Park, Central Kalimantan, Indonesia

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Abstract. *Adam C, Segah H, Kawamura K, Pitoyo D, Adiwijaya S, Damanik Z, Sustiyah, Pidjath C, Rafsanjani MA. 2023.* Paludicola turfosa *(Batrachospermales, Rhodophyta), a new record from Sebangau National Park, Central Kalimantan, Indonesia*. *Intl J Bonorowo Wetlands 13: 66-72. Paludicola* is a genus of red algae that inhabits freshwater environments. Based on initial observations, this genus was found in peat waters associated with the Rasau plant (*Pandanus helicopus* Kurz ex Miq.) in the peat swamp forest, Sebangau National Park. Of the many studies related to biodiversity in Sebangau National Park, none have reported the presence and description of algae, including members of the freshwater red algae *Paludicola*. The current status of red algae diversity in Indonesia has mostly been reported from marine ecosystems with only two species of freshwater red algae known from Indonesia. In this study, we report the preliminary description of the freshwater red algae *Paludicola turfosa* (Bory) M.L.Vis & Necchi, previously known as *Batrachospermum turfosum* Bory de Saint-Vincent from the peat water of Sebangau River, Sebangau National Park, as a new record for Indonesia. The *P. turfosa* was mainly found attached to the leaves of Rasau (*P. helicopus*) forming hair-like tufts representing its filamentous appearance. The descriptions include the recently updated taxonomy, general ecological macroscopic characteristics, and microscopic appearance of this algae.

Keywords**:** *Batrachospermum*, black water, *Paludicola*, peat swamp forest, Rhodophyta, Sebangau National Park

INTRODUCTION

Sebangau National Park is a national park in Central Kalimantan, Indonesia, established in 2004 and administratively located in three regencies/cities: Palangka Raya City, Katingan District, and Pulang Pisau District. The Sebangau area, since being designated a national park has an area of 568,700 hectares but has undergone recent changes; the area was reduced to 537,126 hectares based on a decree from the Minister of Environment and Forestry of the Republic of Indonesia in 2018 (Ditjen KSDAE 2021). Sebangau National Park is great in biodiversity and is protected by the Indonesian government (Sutrisno 2005; Wicaksono et al. 2015; Husson et al. 2018; Maulidi et al. 2020; Lukas et al. 2021). Husson et al. (2018) reported species diversity in the Sebangau tropical peat swamp forest to be consisting of taxa of 215 trees, 92 non-tree flora, 73 ants, 66 butterflies, 297 spiders, 41 dragon/damselflies, 55 fishes, 11 amphibia, 46 reptiles, 172 birds and 65 mammals. Sutrisno (2005) reported moth diversity consisting of 278 species from 19 families in the Busang River secondary rainforest, Sebangau National Park. Herpetofauna diversity in Sebangau National Park has been reported until 2019 based on the results of research in Punggualas as many as 38 species consisting of 22 reptile species and 16 amphibian species (Wicaksono et al. 2015; Maulidi et al. 2020). The conservation status of these herpetofauna species is mostly Least Concern category (Maulidi et al. 2020).

Various conservation efforts have been made to preserve the biodiversity in Sebangau National Park, including providing preliminary data for undescribed species. One major group of organisms in the peat waters of Sebangau National Park that has not been properly accounted for is algae. Algae have an important role in aquatic ecosystems as primary producers, including peat water ecosystems, by forming the energy base of the food web for all aquatic organisms (Lembi 2003). In addition, algae can also be used as bioindicators to assess the condition of the aquatic environment (Omar 2010; Dell'Aglio et al. 2017; Kadam et al. 2020; Stevenson 2022). Therefore, Algae are important elements of aquatic ecosystems and are significant determinants of the "goods and services" rivers provide (Stevenson 2022). Based on

observations in the field, a red algae (Rhodophyta) of the genus *Paludicola* Necchi & M.L.Vis was found to be associated with the leaves of the Rasau plant (*Pandanus helicopus* Kurz ex Miq.) in the peat swamp forest of Sebangau National Park. Of the many studies related to the biodiversity in Sebangau National Park, none have yet reported the presence and description of algae, especially freshwater red algae and members of *Paludicola*.

Red algae are predominantly distributed in marine ecosystems with only 3% of the total diversity found in freshwater ecosystems (Sheath and Wehr 2015; Fischer et al. 2020; Guiry and Guiry 2021). Freshwater red algae are also mainly restricted to flowing waters (Dodds 2002; Sheath and Vis 2015; Sheath and Wehr 2015). The status of red algae diversity in Indonesia has been mostly reported from marine ecosystems (Ghazali et al. 2018; Zulpikar et al. 2020; Mushlihah et al. 2021). However, Johnston et al. (2014) reported eight freshwater red algae taxa from the order Batrachospermales distributed in Malaysia and Indonesia, and only two of them have thus far been found in Indonesia; namely, *Kumanoa celebes* E.T.Johnston, N. Buhari & ML. Vis and *Kumanoa gibberosa* (Kumano) Necchi & ML. Vis.

Due to the lack of scientific data regarding red algae in Sebangau National Park, it is critical to conduct this preliminary research to understand the algal biodiversity in this bioregion better. This study reports the presence of and describes the general ecological and morphological characteristics of *Paludicola turfosa* (Bory) M.L.Vis & Necchi (Batrachospermales, Rhodophyta) in Peat Swamp Forest, Sebangau National Park, Indonesia.

MATERIALS AND METHODS

Study area and sample collection

This research was conducted in the conservation area of Sebangau National Park, Central Kalimantan, Indonesia, precisely in the Sebangau River (2°29'10.0"S, 114°02'26.7"E), (Figure 1). When selecting the sample, only *Paludicola* specimens associated with the submerged leaves of the Rasau plant (*P. helicopus*) in the peat waters of the Sebangau River were collected for the samples. Samples were stored in collection bottles for further analysis.

Procedures

Collected specimens were observed microscopically using an Olympus CX21 at the 40× (10× ocular; 4× objective), 100× (10× ocular; 10× objective), 400× (10× ocular; 40× objective) magnifications. Specimens were then photographed for further identification. Therefore, to facilitate specimen identification, the photographed images were processed for color enhancement using Adobe Photoshop CC, and a scale bar was added using ImageJ software version 1.53g (Rasband 2020) to estimate filament length. Scaling with ImageJ uses a cell size estimation method that utilizes the known diameter of the microscope's field of view (Armstrong 2021; Adam 2022).

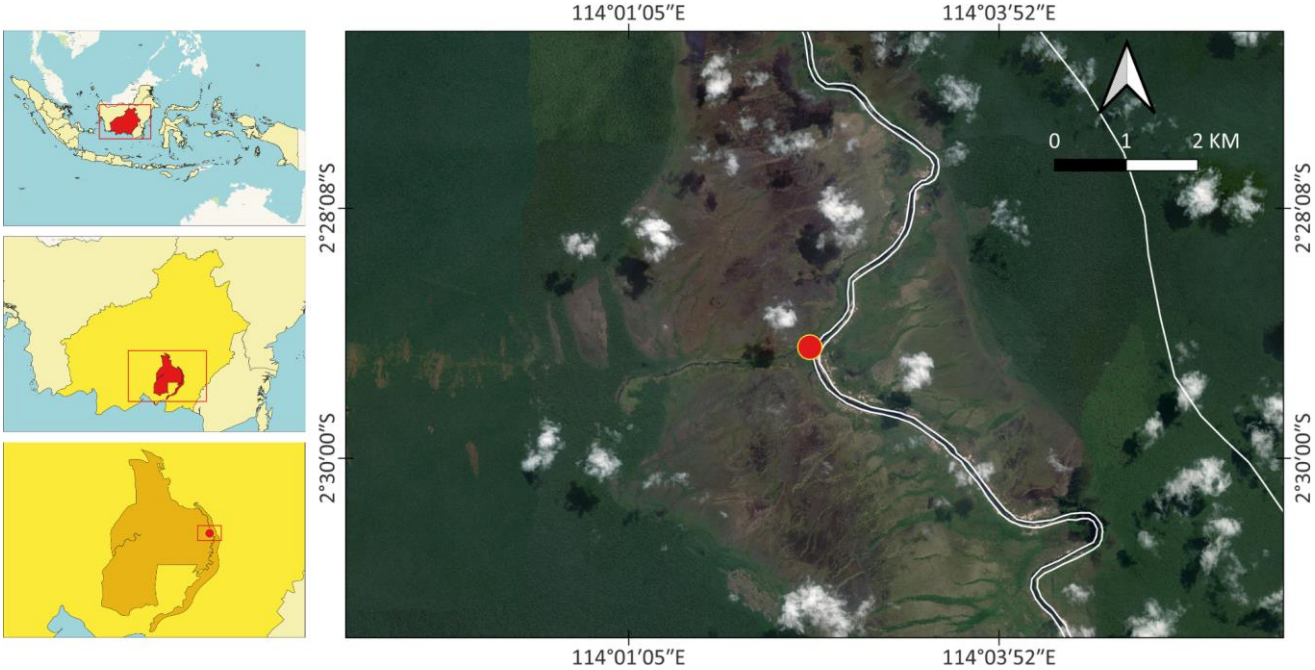

Figure 1. Study area in Sebangau River of Sebangau National Park, Central Kalimantan, Indonesia

Data analysis

This study is a qualitative analysis describing the sampled collections of Paludicola microscopically. The description includes taxonomy, general ecological and morphological characteristics, and the microscopic morphological characteristics (branching pattern, fascicles, carpogonia and spermatangia) using the previously published works of Roeder and Peck (1977), Sheath and Vis (2015), Wehr and Sheath (2015), Vis et al. (2020), Guiry and Guiry (2021), Necchi Jr and Vis (2021).

RESULTS AND DISCUSSION

Taxonomy

The *P. turfosa* has been identified in this study, belonging to Batrachospermales previously known as *Batrachospermum turfosum* Bory de Saint-Vincent. The *B. turfosum* was transferred into a new species *P. turfosa* based on the molecular phylogenetic analysis research using *rbcL* and COI-5P sequences (Vis et al. 2020). Five species of *Paludicola* previously included in the genus *Batrachospermum* and consists of *P. turfosa* (Bory) M.L.Vis & Necchi, *P. keratophyta* (Bory) M.L.Vis & Necchi, *P. orthosticha* (Skuja) Necchi & M.L.Vis, *P. phangiae* (Johnston, Lim & Vis) Vis, Lee, Eloranta, Chapuis, Lam & Necchi, and *P. periploca* (Skuja) Necchi & ML. Vis (Vis et al. 2020). The *P. turfosa* is currently accepted taxonomically and *B. turfosum* is currently regarded as a homotypic synonym (Guiry and Guiry 2021). The taxonomical enumeration in detail is presented below.

Phylum Rhodophyta
Subphylum Eurhodophytina
Class Florideophyceae
Subclass Nemaliophycidae
Order Batrachospermales
Family Batrachospermaceae
Genus *Paludicola* Necchi & ML. Vis, 2020
Species *Paludicola turfosa* (Bory) ML. Vis & Necchi

General ecological and morphological characteristics

The study showed that *P. turfosa* was found in the peat waters of the Sebangau River, Sebangau National Park, and was mainly found in association with the Rasau plant (*P. helicopus*) and was also attached to wooden branches or other macrophytes growing in the water. The *P. helicopus* is a Pandanus species common in peat swamp forests (Keim et al. 2011) and growing in peat waters along the Sebangau River (Figure 2). The *P. turfosa* is one of 3% species of red algae (Rhodophyta) that inhabit freshwater ecosystems such as rivers and streams. It is known that three species of red algae are typical inhabitants of lentic freshwater bodies including *P. turfosa* and two other species, namely *Compsopogon caeruleus* (Balbis ex C.Agardh) Montagne and *Kumanoa mahlacensis* (Kumano et W. A. Bowden-Kerby) M. L. Vis, Necchi, W. B. Chiasson et Entwisle (Sheath and Vis 2015). In addition, *P. turfosa* typically occurs in acid and soft waters (Eloranta and Kwandrans 2007), including peat waters which are similar due to their high acidity levels (Syafalni et al. 2013; Suhendra 2018).

Paludicola turfosa was found attached to the leaves of Rasau (*P. helicopus*) forming a hair-like structure that was filamentous in appearance (Figures 3 and 4). These filaments of *P. turfosa* appear green to blue-green. Red algae generally contain chlorophyll-a and phycobilins as the dominant pigments; thus, the cell and filaments can appear reddish brown, blue-green, or violet (Dodds 2002; Sheath and Vis 2015). In addition, the phycobilin content in red algae is similar to that in Cyanobacteria but in different proportions (Dodds 2002). The types of phycobilins contained in *P. turfosa* are phycoerythrin and phycocyanin (Aigner et al. 2017). According to Schagerl and Donabaum (2003), the pigment composition of *P. turfosa* is dominated by chlorophyll *a* and lutein as well as small amounts of zeaxanthin.

This phycocyanin content is thought to be the main pigment that makes *P. turfosa* filaments look blue-green. Phycocyanin is a blue pigment belonging to phycobiliproteins (Eriksen 2008; Buchweitz 2016; Morançais et al. 2018; Zeece 2020; Tripathi et al. 2021) that can be found in the Cyanophyta, Rhodophyta, and Chrysophyta (Eriksen 2008; Morançais et al. 2018). Phycocyanin increases the efficiency of chlorophyll undergoing photosynthesis to produce oxygen under low light conditions (Zeece 2020). This phycocyanin characteristic correlates with *P. turfosa* also found in peat waters in a closed forest area, Sebangau National Park.

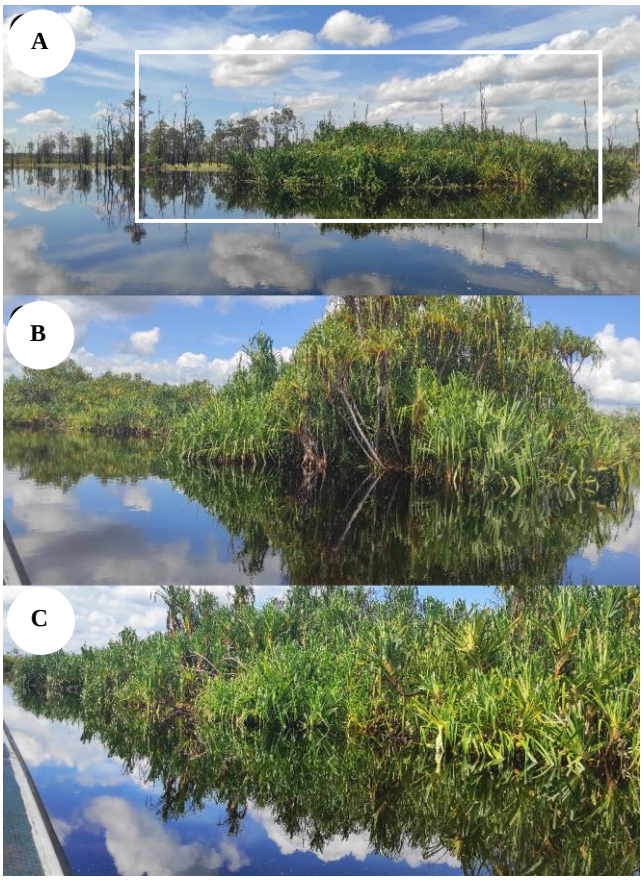

Figure 2. Rasau (*Pandanus helicopus*) (A-C): Plants associated with *Paludicola turfosa*

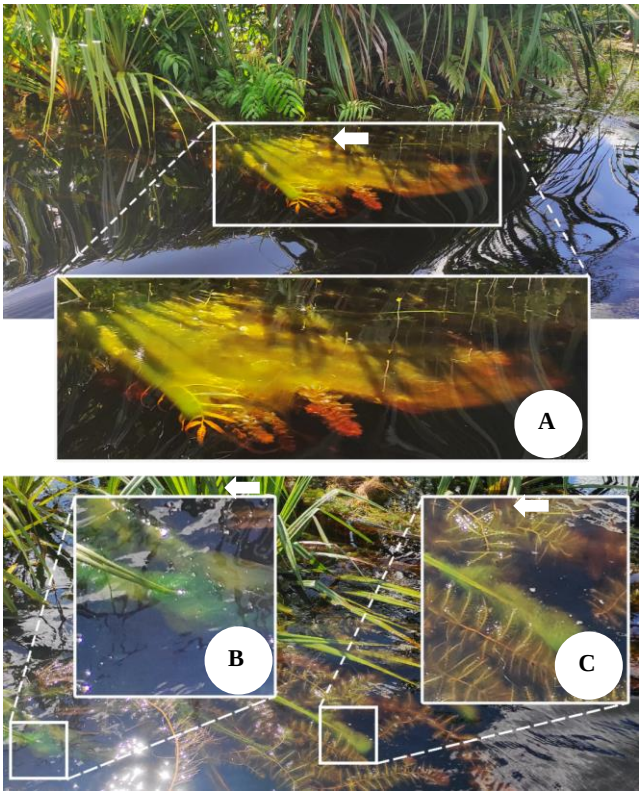

Figure 3. General appearance *Paludicola turfosa* (A-C) attached to the leaves of Rasau (*Pandanus helicopus*)

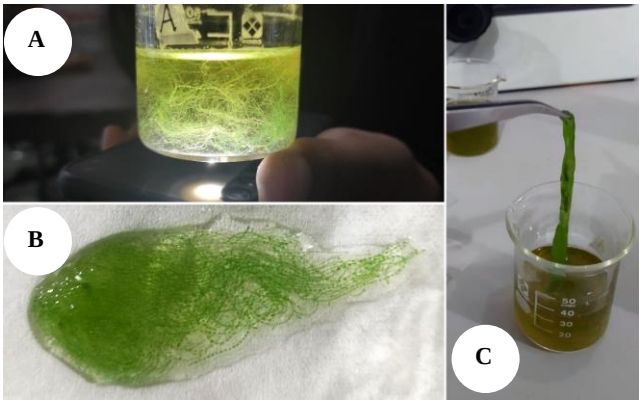

Figure 4. *Paludicola turfosa* filaments: (A, C) in a beaker glass; and (B) on a microscope slide

The filamentous branching pattern is key morphological characteristic of distinguishing red algae species and can be categorized as pseudo-dichotomous or irregular (Vis et al. 2020). Based on morphological observations, our collection of *P. turfosa* generally appears to have a pseudo-dichotomous branching pattern. The bifurcation of thalli was clearly evident, with each branch divided into two derivative branches (Figure 5).

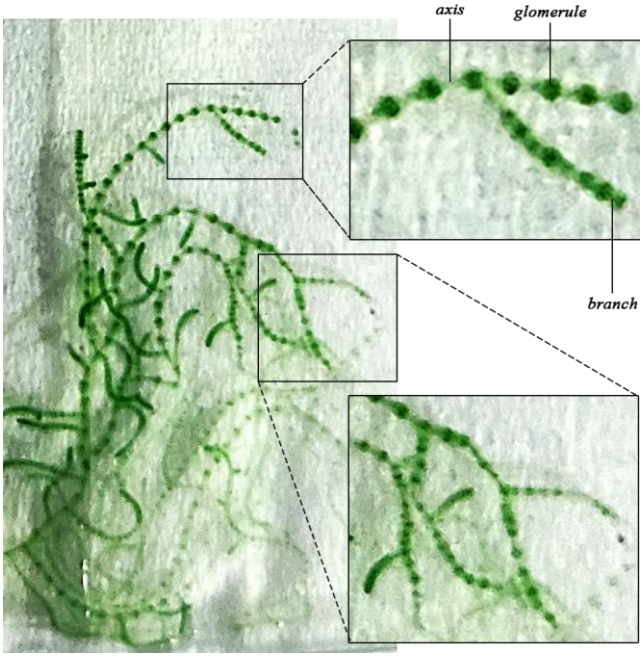

Figure 5. Photograph of *Paludicola turfosa* exhibiting characteristics branching pattern

Microscopic observations

The morphology of *P. turfosa* was also observed at low and higher magnifications (Figures 6, 7 and 8). Figure 6 depicts the crossing filaments, the arrangement of glomerules, and shoot branches of *P. turfosa*. Crossing filaments were clearly visible at 40× magnification (Figures 6.A-C); at this magnification, the *P. turfosa* filaments were often tangled. The arrangement of the glomerules was observed to form different levels of density, ranging from loose to compressed. The densest arrangement of the glomerules is observed in juvenile or newly formed branches. The axis distance (Figure 6.E) between the glomerules was wider away from the branching of the *P. turfosa* filament outgrowths. Shoot branches had a pattern with two derivative branches emerging from each branch known as a pseudo-dichotomous branching pattern (Figures 6.B-C; red arrows).

Vis et al. (2020) recently described the revised morphological description of *P. turfosa* (previously *B. turfosum*) in that this species is pseudodichotomously branched, and the whorls are reduced (238-375 µm diameter), obconic, barrel-shaped, and becoming confluent to indistinct in older parts of the thallus. In addition, Sheath and Vis (2015) described the typical morphological characteristics as follows rhizoid-like cortical filaments typically develop from the lower side of the pericentral cells. Cortical filaments grow downward and ensheath axial cells, often producing secondary fascicle branches. Each fascicle cell contains several, ribbon-like, parietal chloroplasts with no pyrenoid.

Figure 7 shows the microscopic appearance of the apical portions of *P. turfosa* thallus at 100× and 400× magnification. The results indicate two different states of the apical portion of *P. turfosa* thallus, i.e., the apex with

dense whorls (Figures 7.A-E) and the apex with loose young whorls (Figures 7.F-H). The apices with dense whorls give the blue-green appearance of the filaments (Figure 3.B), whereas those with loose young whorls give the dominant green appearance of the filaments (Figure 3.C). These conditions indicated that the color of the *P. turfosa* filament depends on pigment concentration resulting from the density of the glomerules which is influenced by the maturation state of the thallus.

The fascicles were microscopically observed at 400× magnification, including the primary and secondary ones (Figure 8). The fascicle is a cluster of filaments or branches with the parts sub-parallel. Figure 8.A shows the microscopic appearance of fascicles at the apical portions of *P. turfosa* thallus; the primary fascicles appear straight. The fascicles are so dense that it is impossible to observe the secondary ones. Vis et al. (2020) describe the fascicles of *P. turfosa* as follows: primary fascicles are straight, cylindrical, ellipsoidal cells becoming obovoidal at tips; secondary fascicles are abundant, covering the entire internode throughout most of the thallus. Figures 8.B-C shows spherical spermatangia at the tips of fascicles with a diameter of about 7 µm. According to Vis et al. (2020), spermatangia of *P. turfosa* is located at the terminal or subterminal on primary and secondary fascicles with a 7-10 µm diameter. The carpogonial filaments were not observed nor were trichogyne due to the limited detail on micrographic images.

Carpogonia and spermatangia are the sexual reproductive organs of *P. turfosa* where carpogonia are female sex organs, and spermatangia are male sex organs. The carpogonium is a club-shaped structure with a lanceolate trichogyn that functions as a receptive organ (Sheath and Vis 2015; Vis et al. 2020). The spermatangium produces a male gamete called spermatium. The spermatium is unicellular, spherical, non-motile, and colorless. In the process of sexual reproduction, when the spermatium is around the carpogonium, it will attach to the trichogyne (Baweja et al. 2016).

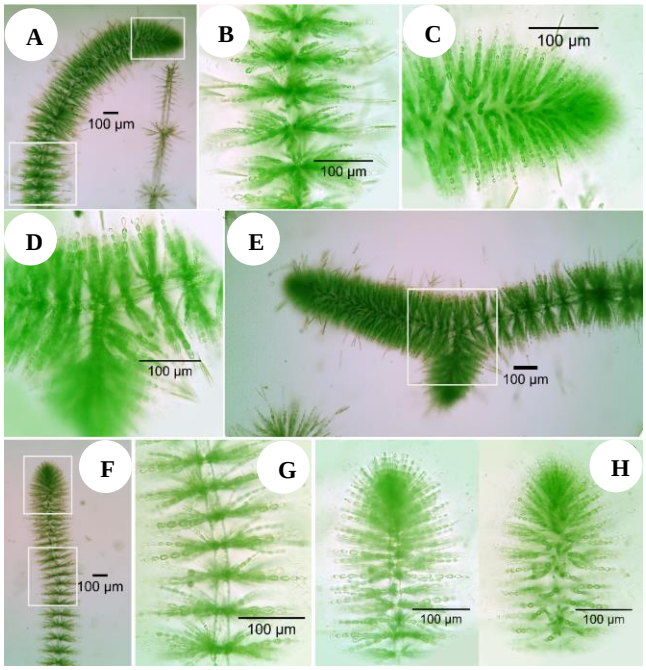

Figure 7. Microphotographs of the apical portions of *Paludicola turfosa* Thallus: (A and E) apex with dense whorls at 100× magnification; (B-D) apex with dense whorls at 400× magnification; (F) apex with loose young whorls at 100× magnification; and (G-H) apex with loose young whorls at 400× magnification

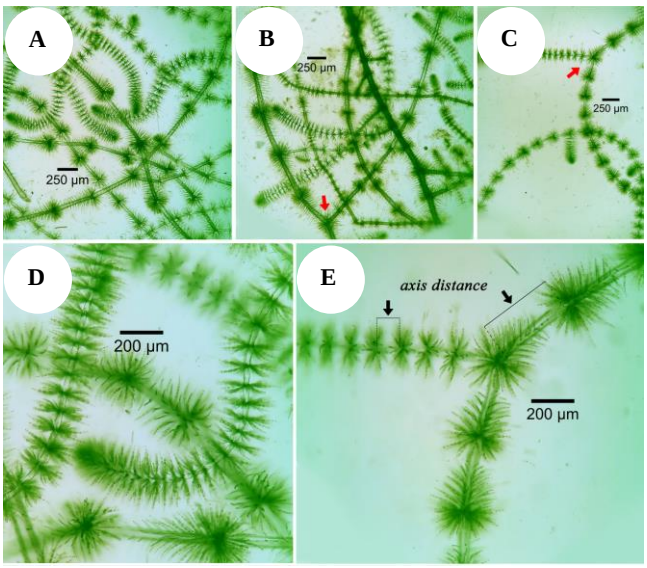

Figure 6. Microscopic appearance of *Paludicola turfosa* at 40× magnification (A-C) and 100× magnification (D-E); Pseudo-dichotomous Branching Pattern (red arrows; B-C); axis distance (black arrows; E)

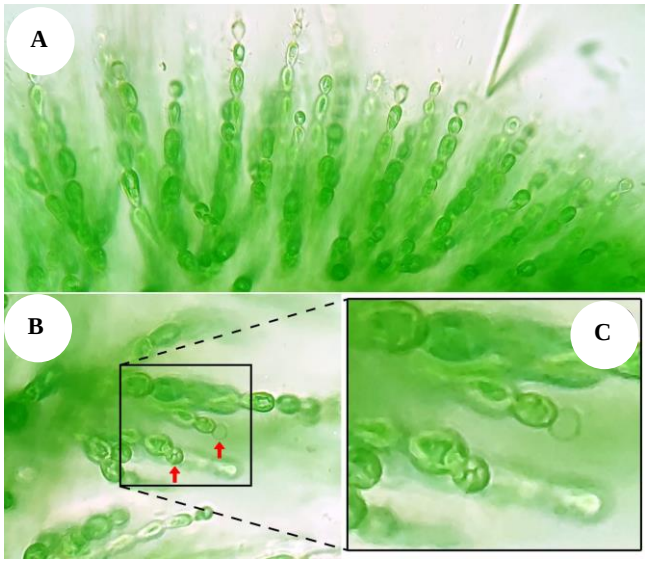

Figure 8. Microphotographs of *Paludicola turfosa* fascicles at 400× magnification (A-C): (A) the fascicles of the apical portions; (B-C) and spermatangia (red arrows)

In this study, we report the preliminary description of the freshwater red algae *P. turfosa*, previously known as *B. turfosum* from the peat waters of Sebangau River, Sebangau National Park, as a new record for Indonesia. The *P. turfosa* was mainly found attached to the leaves of Rasau (*P. helicopus*) forming a hair-like structure representing its filamentous appearance. The *P. turfosa* generally appears to have a pseudo-dichotomous branching pattern. The bifurcation of thalli was clearly observed on the *P. turfosa* filaments, with each branch divided into two derivative branches. The primary fascicles appear straight and so dense that it is impossible to observe the secondary fascicles. The spermatangia at the tips of fascicles were spherical with a diameter of about 7 μm. The carpogonial filaments were not observed nor was the trichogyne due to the limited detail on the microphotographic images.

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