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Research Article

The Identification of Euglenids (Euglenophyceae, Euglenophyta) from the Peat Waters of Palangka Raya, Indonesia

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Abstract
The aim of this study was to identify and describe the Euglenids (Euglenophyceae, Euglenophyta) from the peat waters of Palangka Raya, Central Kalimantan, Indonesia. This study revealed that 8 species of Euglenids were found in the study sites which belong to 3 genera, namely *Euglena, Lepocinclis,* and *Phacus*. The Euglenid species identified include *Euglena gracilis* G.A. Klebs 1883*, Euglena mutabilis* F. Schmitz 1884*, Lepocinclis acus* (O.F.Müller) B.Marin & Melkonian 2003*, Lepocinclis ovum* (Ehrenberg) Lemmermann 1901*, Lepocinclis spirogyroides* B.Marin & Melkonian 2003*, Phacus cordatus* (Pochmann) Zakryś & Lukomska 2015*, Phacus helikoides* Pochmann 1942, and *Phacus orbicularis* Hübner 1886. The eight Euglenid species found in this study have never been reported before, thus these findings provide additional new data regarding algae diversity in peat waters of Central Kalimantan, Indonesia.

Keywords: *Euglena*, *Lepocinclis*, microalgae, peat waters, *Phacus*, phytoplankton

1. INTRODUCTION

Research on algae in Central Kalimantan, Indonesia, especially freshwater algae from peat waters has been intensively carried out since the last few years [1][2]. These studies have contributed the scientific data regarding the diversity of algae in the peat waters habitat. Adam [2] reported 10 species of desmids from the peat waters in Palangka Raya, consisting of 5 *Cosmarium* species and 5 *Euastrum* species respectively. The most recent study is related to the morphological study of *Coelastrum cambricum*, a microalgae species commonly found in peat waters. This study revealed the distinctive morphological characteristics of *C. cambricum* where 6 cells were observed in the center of the coenobium forming a pentagonal pattern that has the potential to be studied further as a key feature of *Coelastrum* species identification [1]. Similar studies still need to be done to develop scientific data related to the diversity of algae in Indonesia, especially Central Kalimantan which has extensive peat water habitats.

Euglenophyceae is a group of flagellate green algae found in a variety of freshwater and marine environments [3][4]. Members of the Euglenophyceae are distinctive with red eyespots that are part of the eyespot apparatus [5]. The eyespot apparatus consists of the paraflagellar body, which connects the eyespot to the flagellum. The function of eyespot apparatus is as a photoreceptive organelle [6]–[8]. According to Algaebase [9], there are 1,007 algal species belonging to Euglenophyceae which lack references that provide data on their distribution in Indonesia, especially in Central Kalimantan. Recent studies regarding the diversity of microalgae in Indonesia have revealed several species of Euglenophyceae that are distributed in Bengkulu (Sumatra) [10], South Sumatra [11], East Java [12], Central Lombok [13], and West Kalimantan [14] which belong to the genera *Euglena*, *Trachelomonas*, *Lepocinclis* and *Phacus.*

The aim of this study was to identify and describe the Euglenids (Euglenophyceae, Euglenophyta) from the peat waters of Palangka Raya, Central Kalimantan, Indonesia, to contribute to the development of scientific data on algae diversity.

2. MATERIALS AND METHODS

2.1. Study Site and Sample Collection

Table 1. The list of published works used to assist the identification phase.

No.	Published Works	Ref.
1	AlgaeBase. World-wide electronic publication. National University of Ireland, Galway.	[9]
2	A Short Guide to Common Heterotrophic Flagellates of Freshwater Habitats Based on the Morphology of Living Organisms	[17]
3	Algae identification lab guide: accompanying manual to the algae identification field guide	[18]
4	A Beginner's Guide to Freshwater Algae	[5]
5	Easy Identification of the Most Common Freshwater Algae	[19]
6	How to know the Freshwater Algae	[20]

This research was conducted in shallow peat waters located in Palangka Raya, Central Kalimantan, Indonesia (Figure 1). Water samples were collected directly from peat waters along with sediment from the bottom of the trench, then stored in collection bottles.

2.2. Microscopic Observation and Microphotography

The samples were observed microscopically using Olympus CX21 at 400× (10× ocular; 40× objective) magnification in the laboratory of Biology Education Program, University of Palangka Raya. Microphotography method explained as follows: specimen objects found that are suspected to be Euglenid species are immediately photographed using 64megapixel cellphone camera directly to the eyepiece of the microscope for further identification.

2.3. Image Processing

The photographed specimen image was then processed for color and detail enhancement using Adobe Photoshop CC. Scale bar was added using ImageJ software version 1.53g [15] to estimate the object length. Scaling on ImageJ uses the method of estimating cell size by knowing the diameter of the field of view of the microscope [2][16], which in this study used a 400× magnification with a diameter of 0.4 mm or 400 µm.

2.4. Euglenid Identification

The identification of Euglenid was carried out by analyzing the observed morphological characteristics (e.g., the presence of red eyespot & flagella; shape of the cells, and cell size) both qualitatively and quantitatively. Morphotaxonomic identification was done up to the species level using all available information. The following published works were used to assist the identification phase (Table 1).

3. RESULTS AND DISCUSSIONS

This study revealed 8 euglenid microalgae species found in the peat waters of Palangka Raya, Indonesia, consisting of 3 genera, e.g., *Euglena* (2 species), *Lepocinclis* (3 species), and *Phacus* (3 species) as presented in the Table 2. All Euglenid species were found in shallow peat waters with the depth ranges from 30-50 cm and pH of 5. These characteristics of peat waters are in accordance with several references which describe that the color of peat water usually appears in blackish brown color, has acidity range between pH 3-5 and the organic matter content is relatively high [21][22].

3.1. Euglena Ehrenberg, 1830

Euglena is spindle-shaped unicellular flagellate green algae with cylindrical or oval body; has a single flagellum embedded at the anterior end of the cell; red eyespots are clearly visible on the anterior area of the cells [5][9][20]. Currently, *Euglena* consists of 156 taxonomically accepted species names and 52 accepted varieties [9].

Taxonomic Enumeration
Euglenozoa
Class Euglenophyceae
Order Euglenida
Family Euglenidae
Genus ***Euglena*** **Ehrenberg 1830**

3.1.1. Euglena gracilis G.A. Klebs 1883

Taxonomy: E. gracilis G.A. Klebs 1883 is currently taxonomically accepted species name according to Algaebase. First published in a paper entitled "*Über die Organisation einiger Flagellatengruppen und ihre Beziehungen zu Algen und Infusorien*" by Klebs in 1883 [9].

Description: E. gracilis were observed to have bright green color; a clearly visible red eyespot; a single flagellum; and known to be the largest species of Euglena found in this study. The shapes of the cell are dynamic due to the metabolic movement, can vary from oval or spherical to highly elongated shape. Cell length from anterior to posterior end ranged from 93-267 µm.

Localities: Shallow peat waters in Palangka Raya, Central Kalimantan, Indonesia. Found in Site 1 (2°13'03.7"S 113°53'38.9"E) and Site 2 (2° 12'55.2"S 113°53'46.9"E).

3.1.2. Euglena mutabilis F. Schmitz 1884

Taxonomy: E. mutabilis F. Schmitz 1884 is currently taxonomically accepted species name according to Algaebase. First published in a paper entitled "*Beitrage zur Kenntniss der Chromatophoren*" by Schmitz in 1884 [9].

Description: E. mutabilis were observed as small *Euglena* cells with a single flagellum, narrow ends in the posterior and anterior areas of the cell, and clearly visible red eyespot. Cell length from anterior to posterior end ranged from 73-89 µm. Previously published work described *E. mutabilis* cells 57–65 µm long and 10–11 µm wide; cylindrical with narrowed ends; cells may be attached to substrate at posterior end; cells bend while swimming [23].

Localities: Shallow peat waters in Palangka Raya, Central Kalimantan, Indonesia. Found in Site 1 (2°13'03.7"S 113°53'38.9"E) and Site 3 (2° 12'58.0"S 113°53'48.8"E).

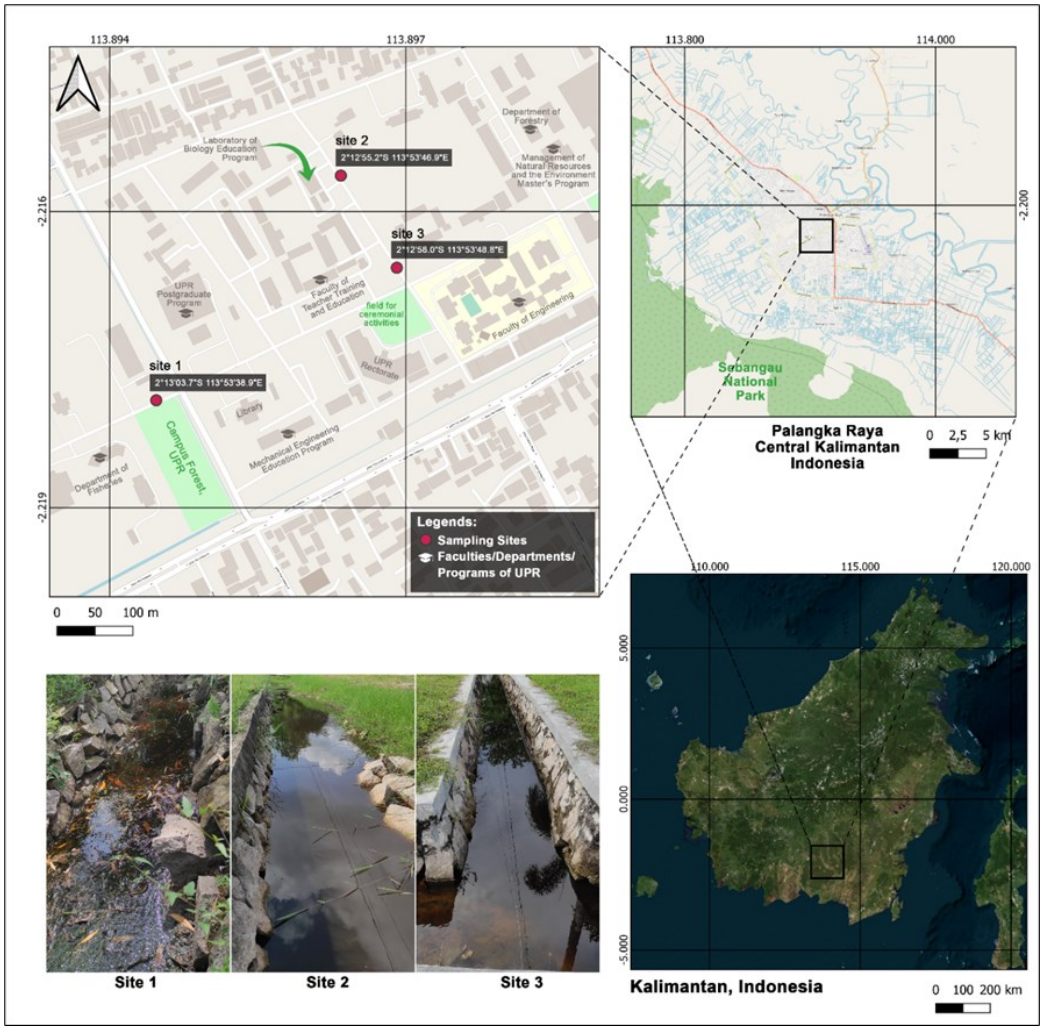

Figure 1. Research Map: Palangka Raya, Central Kalimantan, Indonesia (Map Source: *OpenStreetMap* & *Bing Aerial,* QGis 3.16.3 Hannover).

3.2. Lepocinclis Perty, 1849, nom. cons.

Lepocinclis is photothropic flagellates; ovoid or spindle-shaped; with red eyespots that are clearly visible on the anterior region of the cells as in *Euglena* [4][9][20][24]. The cells are completely rigid and unflattened which distinguishes this genus from the genera *Euglena* and *Phacus* [9]. Currently, *Lepocinclis* consists of 88 taxonomically accepted species names and 66 accepted varieties [9].

Taxonomic Enumeration
Euglenozoa
Class Euglenophyceae
Order Euglenida
Family Phacaceae
Genus ***Lepocinclis* Perty, 1849, nom. cons.**

3.2.1. Lepocinclis acus (O.F.Müller) B.Marin & Melkonian 2003

Taxonomy: Lepocinclis acus (O.F.Müller) B.Marin & Melkonian 2003 is currently taxonomically accepted species name according to Algaebase. Published in a paper entitled "*Phylogeny and taxonomic revision of plastid-containing euglenophytes based on SSU rDNA sequence comparisons and synapomorphic signatures in the SSU rRNA secondary structure*" by Marin *et al.* in 2003 [9]. This species has 4 homotypic synonyms and 10 heterotypic synonyms [9].

Description: L. acus were observed to have elongated spindle-shaped cells with narrow ends in the posterior and anterior areas of the cell; cell length around 200 µm. According to Kim *et al.* [24], *L. acus* cells 125-163 µm long and 9-77 µm wide; almost rigid; long-fusiform; the anterior and posterior ends are narrowed.

Localities: Shallow peat waters in Palangka Raya, Central Kalimantan, Indonesia. Found only in Site 2 (2°12'55.2"S 113°53'46.9"E).

3.2.2. Lepocinclis ovum (Ehrenberg) Lemmermann 1901

Taxonomy: L. ovum (Ehrenberg) Lemmermann 1901 is currently taxonomically accepted species name according to Algaebase. First published in a paper entitled "*Beiträge zur Kenntniss der Planktonalgen. XII. Notizen über einige Schwebealgen. XIII. Das Phytoplankton des Ryck und des Greifswalder Boddens*" by Lemmermann in 1901 [9]. This species has 3 homotypic synonyms and 2 heterotypic synonyms [9].

Description: L. ovum were observed in the form of elliptical or spherical cells with clearly visible red eyespot; short caudal process on the posterior. *L. ovum* is the smallest Euglenid species found in this study with up to 25 µm long in diameter. Previously published works described *L. ovum* cells are citriform, fusiform, sub-globose, ovate to elliptical, and elliptical in shape [23].

Localities: Shallow peat waters in Palangka Raya, Central Kalimantan, Indonesia. Found in all three sampling sites: Site 1 (2°13'03.7"S 113° 53'38.9"E), Site 2 (2°12'55.2"S 113°53'46.9"E), and Site 3 (2°12'58.0"S 113°53'48.8"E).

3.2.3. Lepocinclis spirogyroides B.Marin & Melkonian 2003

Taxonomy: L. spirogyroides B.Marin & Melkonian 2003 is currently taxonomically accepted species name according to Algaebase.

Table 2. Euglenid species found in this study.

No	Familia	Genera	Species	Status of Species Name*
1	Euglenidae **Dujardin 1841: 347**	*Euglena* **Ehrenberg, 1830**	*E. gracilis* *E. mutabilis*	accepted taxonomically accepted taxonomically
2	Phacaceae **J.I. Kim, Triemer & W. Shin 2010: 1280**	*Lepocinclis* **Perty, 1849, nom. cons.**	*L. acus* *L. ovum* *L. spirogyroides*	accepted taxonomically accepted taxonomically accepted taxonomically
		Phacus **Dujardin, 1841, nom. et typ. cons.**	*P. cordatus* *P. helikoides* *P. orbicularis*	accepted taxonomically accepted taxonomically accepted taxonomically

**Based on Algaebase data 2022* [9]

Published in a paper entitled "*Phylogeny and taxonomic revision of plastid-containing euglenophytes based on SSU rDNA sequence comparisons and synapomorphic signatures in the SSU rRNA secondary structure*" by Marin *et al.* in 2003 [9]. This species has 4 heterotypic synonyms [9].

Description: L. spirogyroides were observed to have that makes *L. spirogyroides* appear to have helical striated lines along its cells. According to Dawson *et al.* [25], the ornamentations on the cell wall named pellicular warts, iron-enriched and mineralized structures of the extracellular matrix.

Localities: Shallow peat waters in Palangka Raya, Central Kalimantan, Indonesia. Found in Site 1 (2°13'03.7"S 113°53'38.9"E) and Site 2 (2° 12'55.2"S 113°53'46.9"E).

3.3. Phacus Dujardin, 1841, nom. et typ. Cons

Phacus is a genus of green flagellates with rigid, compressed and flattened cells, and most appear in a leaf-shaped, folds or grooves running helically or longitudinally, giving the cells irregular or triradiate cross-sections; some species twisted into flat corkscrews [5][9]. *Phacus* consists of 167 taxonomically accepted species names and 73 accepted varieties [9].

Taxonomic Enumeration

Euglenozoa

Class Euglenophyceae

Order Euglenida

Family Phacaceae

Genus ***Phacus*** **Dujardin, 1841, nom. et typ. cons.**

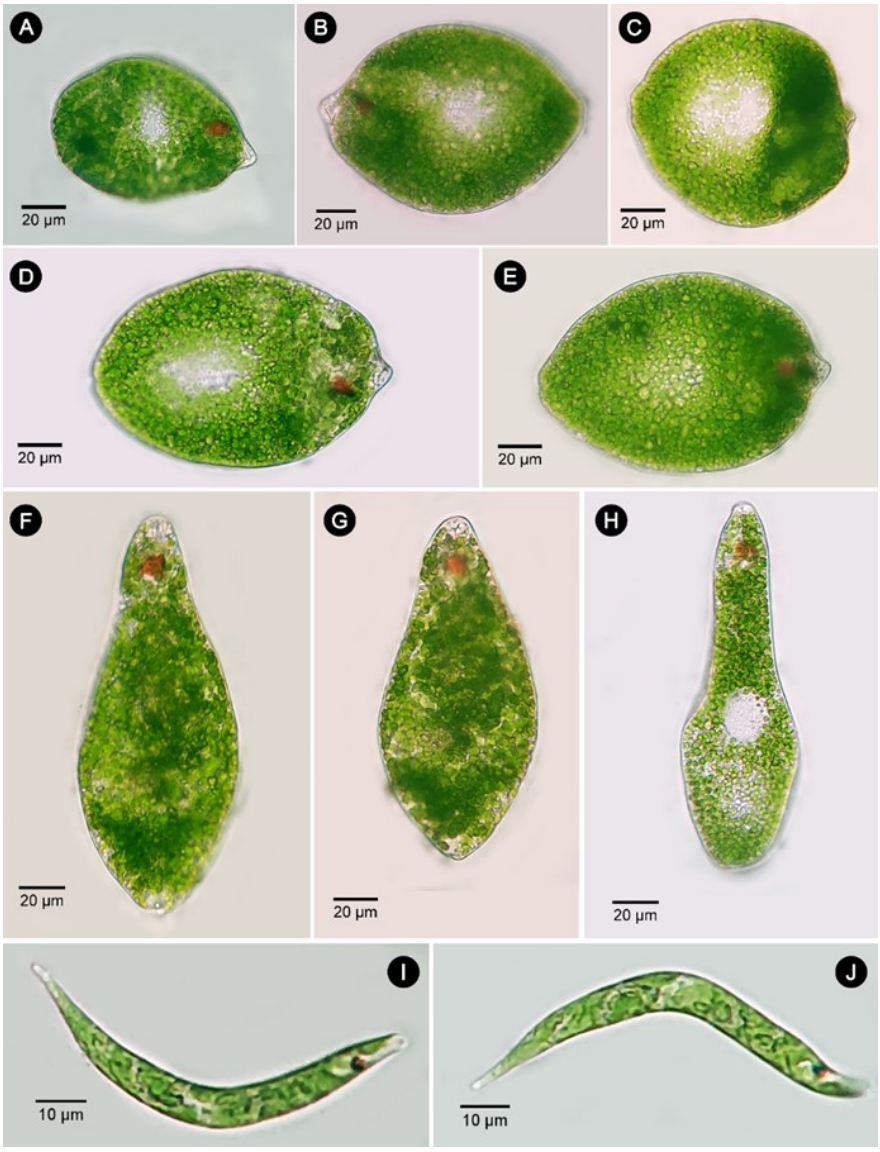

Figure 2. *Euglena*: (A-H) *E. gracilis*; and (I-J) *E. mutabilis.*

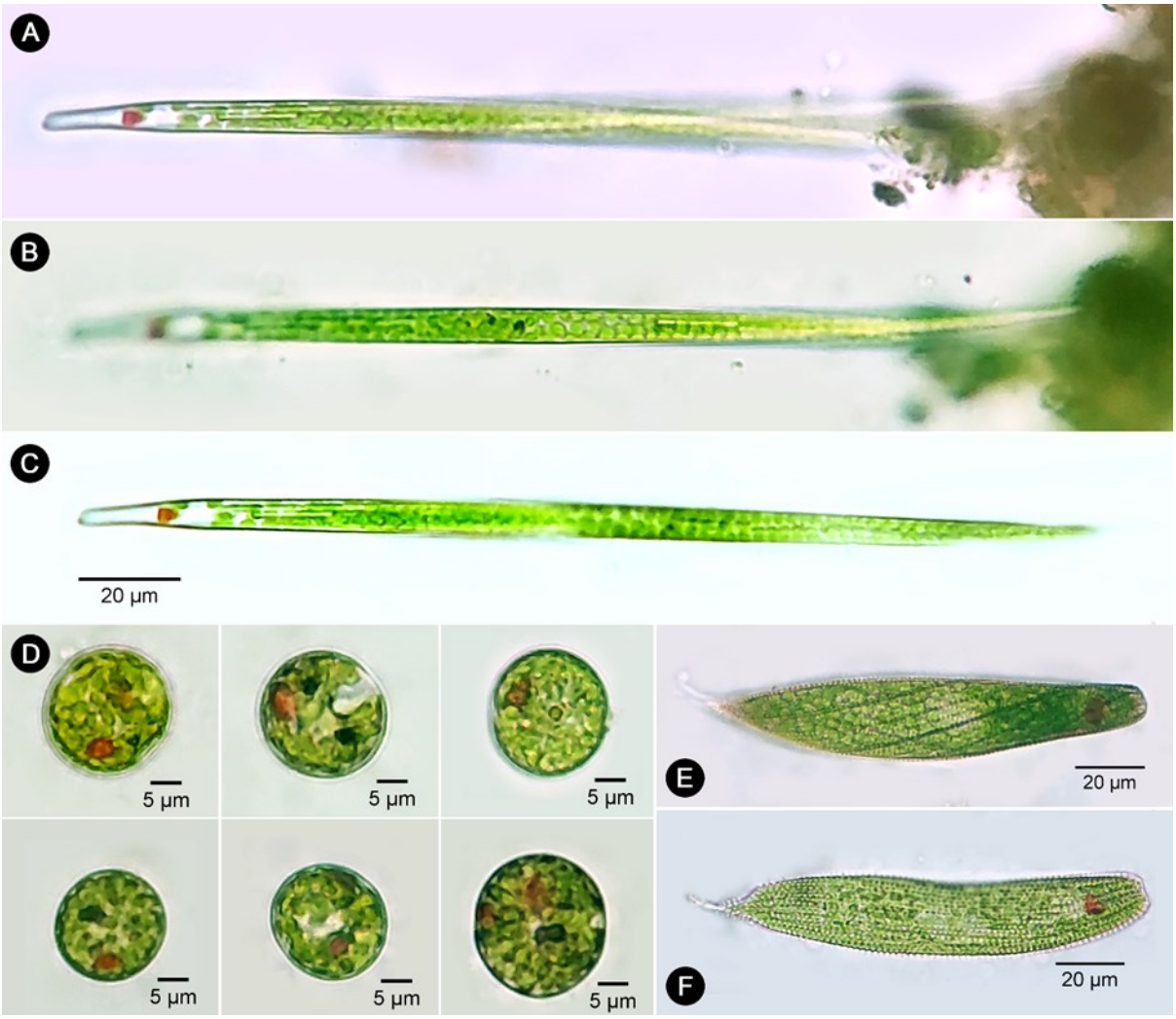

Figure 3. *Lepocinclis*: (A-B) *L. acus*; (D) *L. ovum*; and (D) *L. spirogyroides*.

3.3.1. Phacus cordatus (Pochmann) Zakryś & Lukomska 2015

Taxonomy: P. cordatus (Pochmann) Zakryś & Lukomska 2015 is currently taxonomically accepted species name according to Algaebase. Published in a paper entitled "*Delimiting species in the Phacus longicauda complex (Euglenida) through morphological and molecular analyses*" by Lukomska-Kowalczyk *et al.* in 2015 [9]. This species has 2 homotypic synonyms that are *Phacus longicauda* subsp. cordatus Pochmann 1942 and *Phacus longicauda* f. *cordatus* (Pochmann) Popova 1955 [9].

Description: P. cordatus were observed to have a leaf-shaped; slightly shaped like a heart; red eyespots are clearly visible on the anterior region of the cells; long tail; cell length from the anterior to the caudal end is 133 µm and around 70 µm long from the anterior to posterior base.

Localities: Shallow peat waters in Palangka Raya, Central Kalimantan, Indonesia. Found in Site 1 (2°13'03.7"S 113°53'38.9"E) and Site 2 (2° 12'55.2"S 113°53'46.9"E).

3.3.2. Phacus helikoides Pochmann 1942

Taxonomy: P. helikoides Pochmann 1942 2015 is currently taxonomically accepted species name according to Algaebase. First published in a paper entitled "*Synopsis der Gattung Phacus*" by Pochmann in 1942 [9]. This species has 1 homotypic synonym (*Phacus tortus* var. *helikoides* (Pochmann) Huber-Pestalozzi 1955) and 2 heterotypic synonyms (*Phacus tortus* var. *tortuosus* Skvortsov 1928 and *Phacus sesquitortus* Pochmann 1942) [9].

Description: P. helikoides were observed to have cells with helical folds; flagella and red eyespots; anterior to posterior (caudal end) length ranges from 62-89 µm. *P. helikoides* is actually helical in shape throughout the entire cell in

contrast to most *Phacus* species which are flattened and leaf-shaped.

Localities: Shallow peat waters in Palangka Raya, Central Kalimantan, Indonesia. Found in Site 1 (2°13'03.7"S 113°53'38.9"E) and Site 2 (2° 12'55.2"S 113°53'46.9"E).

3.3.3. Phacus orbicularis Hübner 1886

Taxonomy: Phacus orbicularis Hübner 1886 is currently taxonomically accepted species name according to Algaebase. First published in a paper entitled "*Euglenaceen-Flora von Stralsund*" by Hübner in 1886 [9]. This species has 14 heterotypic synonyms [9].

Description: P. orbicularis were observed to have ovoid cell shape; short curved caudal process; clearly visible red eyespot; anterior to posterior length ranges from 67-89 µm. Kosmala *et al.* [26], Kim *et al.* [24], and Łukomska-Kowalczyk [27] described that *P. orbicularis* are characterized by wide, ovoid cells ending with a sharp curved tail.

Localities: Shallow peat waters in Palangka Raya, Central Kalimantan, Indonesia. Found in all three sampling sites: Site 1 (2°13'03.7"S 113° 53'38.9"E), Site 2 (2°12'55.2"S 113°53'46.9"E), and Site 3 (2°12'58.0"S 113°53'48.8"E).

4. CONCLUSIONS

This study revealed that in the peat waters of Palangka Raya, Central Kalimantan, Indonesia, 8 species of Euglenids (Euglenophyceae, Euglenophyta) were found which belong to 3 genera. The Euglenid species identified include *Euglena gracilis, Euglena mutabilis, Lepocinclis acus, Lepocinclis ovum, Lepocinclis spirogyroides, Phacus cordatus, Phacus helikoides*, and *Phacus orbicularis*. In this study, there were several other specimens that were suspected to be Euglenid species but could not be identified due to lack of information needed to determine the species name, further research needs to be done using molecular identification methods for more accurate results.

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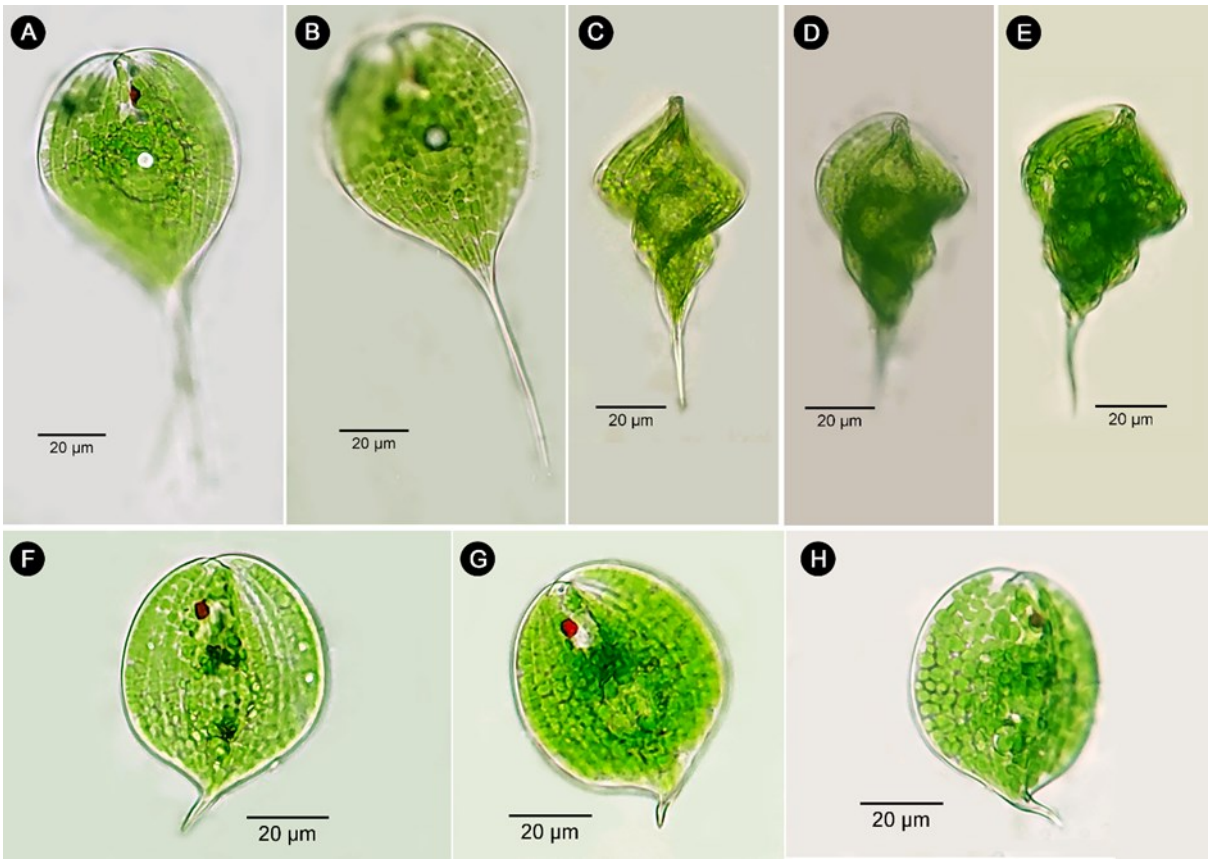

Figure 4. *Phacus*: (A-B) *P. cordatus*; (C-E) *P. helikoides*; and (F-H) *P. orbicularis*.

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Author Contributions

C. A. study conception and design; sample collection, observation, and identification; and draft manuscript preparation. A. H. analysis and interpretation of results. All authors reviewed the results and approved the final version of the manuscript.

Conflicts of Interest

The author(s) declared no conflict of interest

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