

## Diversity of Philippine Photosynthetic Euglenophytes and their Potential Biotechnological Uses: A Review

Eldrin DLR. Arguelles<sup>1</sup> and Milagrosa R. Martinez-Goss<sup>2</sup>

<sup>1</sup>University Researcher II, Philippine National Collection of Microorganisms, National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippine Los Baños, College, Laguna, 4031, Philippines.

<sup>2</sup>Professor Emeritus, Institute of Biological Sciences and Museum of Natural History, University of the Philippines Los Baños, College, Laguna 4031, Philippines.

(Corresponding author: Eldrin DLR. Arguelles)

(Received 27 September 2019, Revised 30 October 2019, Accepted 01 November 2019)

(Published by Research Trend, Website: www.researchtrend.net)

**ABSTRACT:** Photosynthetic euglenoids are group of distant protists along the long evolutionary lineage from prokaryotic organisms to complex multicellular eukaryotes. These photosynthetic protists are found in highly diverse environments and are regarded as a suitable model organism for biotechnological research such as synthesis and production of diverse biologically active compounds (polyunsaturated fatty acids, wax esters, biotin, tyrosine and  $\alpha$ -tocopherol), heavy metal bioremediation, and ecotoxicological risk assessment. In the Philippines, limited studies on the biodiversity and practical importance of photosynthetic euglenoids are being conducted taking into account the latest taxonomic studies and potential use for biotechnology. This paper aims to generalize and analyze the available literature data emphasizing the need for deeper studies concentrating on these valuable protists in order to tap this important genetic resource.

**Keywords:** Biotechnology, Diversity, Photosynthetic Euglenoids, Philippines, Taxonomy.

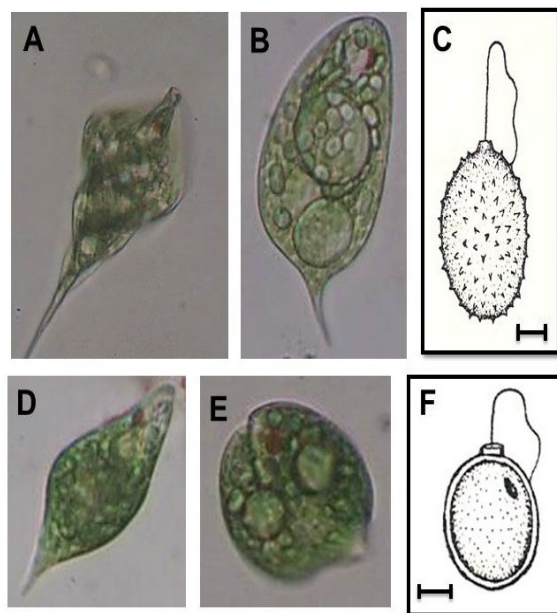
**Abbreviations:** EtOH, ethyl alcohol; PUFAs polyunsaturated fatty acids; SAFAs, saturated fatty acids; mTOR, mechanistic target of rapamycin; MUFAs, monounsaturated fatty acids.

### I. INTRODUCTION

The photosynthetic euglenoids are groups of unicellular, free-living eukaryotic microorganisms (a lone subclade under euglenids) characterized by having a plastid (chloroplast) that are of endosymbiotic in origin, an unusual cytoskeleton which is comprised of proteinaceous pellicle underlain by several microtubules, and a membrane-bound storage structure known as paramylon (paramylum) [1, 2]. These single-celled microorganisms move primarily by swimming using one or two emergent flagellum and are normally limited to brackish and freshwaters [1]. Photosynthetic euglenoids appear bright green under a microscope due to the presence of dominant chlorophyll *a* and *b* existing as disc-shaped chloroplasts scattered throughout the cell (Fig. 1). Other accessory pigment such as xanthophylls and  $\beta$ -carotenes also exists, which causes a brick-red appearance during an algal bloom. Majority of these organisms are flexible (capable of changing shape) because of the pellicle while other species are enclosed by a non-living outer layer that surround the cell known as the lorica. This specialized structure is often characterized with several spines and a short neck or pore from which the flagella come out.

Photosynthetic euglenoids are known for its eyespot found in the anterior part of the organism near the base of the flagella. Contractile vacuoles are also present in photosynthetic euglenoids that help in osmotic regulation within the cell. Reproduction is asexual which occurs by cell division. Cysts formation is observed to some species of euglenoids to withstand periods under unfavorable condition. These organisms are capable of

phototactic circadian rhythm that causes their movement inside the water column in response to the presence and absence of light [3].



**Fig. 1.** Light photomicrograph and taxonomic illustrations of some photosynthetic euglenophytes in the Philippines. (A, B and E) *Phacus* sp. (C and F) *Trachelomonas* sp. and (D) *Euglena* sp. All scale bars = 10  $\mu$ m.

Pigmented euglenophytes include several groups (e.g. *Colacium*, *Trachelomonas*, *Phacus*, *Lepocinclis*, *Euglenaria*, *Strombomonas*, *Euglenaformis*, *Cryptoglana*, and *Monomorphina*, *Discoplastis*, *Eutreptia* and *Eutreptiella*, *Rapaza*, and *Tetraeutreptia*). These groups of organisms together with other phytoplankton form one of the main components in the euphotic zone by being considered as a primary producer of organic matter as well as provision of a nutritional base for zooplankton and subsequently to other invertebrates, shellfish and finfish. Several species of photosynthetic euglenoids are bloom-formers in eutrophic (high organic and inorganic) conditions and are often abundant in ponds, tanks, and puddles, especially those to which livestock have access. The abundance of these organisms under nutrient-rich environment caused them to be considered as a useful biological indicator of aquatic environmental pollution [1, 62].

This paper aims to generalize and analyze the available literature data on the biodiversity, ecology and practical importance of photosynthetic euglenoids in the waters of Philippines, taking into account the latest taxonomic studies and potential use for biotechnology.

## II. TAXONOMY OF PHOTOSYNTHETIC EUGLENOIDS

The influence of molecular phylogenetics upon euglenoid taxonomy resulted in several taxonomic revisions. And thus, the count of taxonomically accepted species is consistently changing and new taxa of pigmented euglenoids are being reported and described, on the basis of both molecular and morphotaxonomic characteristics [4]. The pigmented euglenoids or euglenophyceae, are protists that are monophyletic composed of the basal monotypic genus *Rapaza*, as well as Euglenales and Eutreptiales [5, 6, 7, 8, 9]. Eutreptiales is composed of two marine genera – *Eutreptiella* and *Eutreptia* [5, 10]. The Euglenales is a group of photosynthetic euglenoid that is divided into two well known clades: Phacaceae, which is composed of three genera that are monophyletic (*Lepocinclis*, *Phacus*, *Discoplastis*) and Euglenaceae with seven monophyletic genera (*Euglenaformis*, *Euglenaria*, *Colacium*, *Cryptoglana*, *Strombomonas*, *Trachelomonas*, *Monomorphina*) and polyphyletic genus *Euglena* [7, 8, 11, 12]. To date, AlgaeBase (<http://www.algaebase.org/>) recorded a total of 2900 species for the pigmented euglenoids, but of these, only around 800 taxa (majority of which in the genus *Euglena*, *Trachelomonas*, *Cryptoglana*, and *Phacus*) are taxonomically accepted and valid [4].

In the Philippines, a few studies have been done on the systematics and ecology of the photosynthetic euglenoid. To date, a total of nine genera and 81 species and varieties of euglenoids (representing about 7% of the total global euglenoids) were documented (Table 1). So far, most of the taxa observed were noted in Luzon, the biggest island of the Philippines. The first data on Philippine photosynthetic euglenoid flora was done by Velasquez, who observed the presence of *Phacus acuminatus* and *Trachelomonas hispida* var. *coronata* Lemmerman on freshwater bodies found in Puerto Galera, Mindoro [13]. Pantastico and Suayan

reported the occurrence of a bloom of *Euglena gracilis* in rice paddy fields found in Los Baños and Bay, Laguna [14]. In addition, several comprehensive studies were also conducted by Pantastico, Martinez, and Zafaralla which added to the diversity of reported species of photosynthetic euglenophyte in the Philippines [15,18]. For example, Pantastico reported 19 species of photosynthetic euglenoid around Laguna de Bay. On the other hand, Martinez [17] added 8 new varieties of euglenophytes [19], in the country among the 28 species she described from the fishponds and fishpens of Los Baños, Laguna. Later on Martinez in 1983 described four more flagellated species belonging to three divisions in the same locality [16]. Zafaralla reported some three photosynthetic euglenoids species in her floristic account of Taal Lake [18]. In 2011, a study made by Boonmee and Martinez-Goss about flagellated algae, described 46 photosynthetic euglenophytes from brackish- and fresh-water fishponds in northern Luzon, Philippines [19]. This study reported two new taxa of photosynthetic euglenoid: *Trachelomonas volvocina* var. *collumae* Boonmee, var. nov. and *T. hispida* var. *minima* Boonmee, var. nov. in the country. A new taxonomic study conducted by Arguelles *et al.*, in Laguna de Bay and its neighboring province [2, 21, 63]. These survey reported 33 species of photosynthetic euglenoids and seventeen species are reported and described for the first time in the euglenoid flora of the Philippines namely: *Cryptoglana skujae* Marin and Melkonian, *Euglena chlamydotheca* Minx, *Euglena ehrenbergii* G. A. Klebs, *Euglena oblonga* Schmitz, *Euglena tristella* Chu, *Euglena viridis* Ehrenberg, *Lepocinclis horridus* Pochmann, *Lepocinclis ovum* (Ehrenberg) Minkiewicz, *Lepocinclis oxyuris* (Schmarda) Marin & Melkonian, *Lepocinclis salina* Fritsch, *Lepocinclis steinii* Lemmerman, *Phacus contortus* (Bourrelly) Starmach, *Phacus elegans* Pochmann, *Phacus pleuronectes* (Ehrenberg) Dujardin, *Phacus tropicalis* Conforti, *Strombomonas acuminata* (Schmarda) Deflandre, and *Trachelomonas similis* Stokes [2, 21].

**Table 1: Number of species and varieties in nine genera of Euglenophyceae observed from the Philippines, as of September 2019.**

Genus	Number of Species and Varieties
<i>Colacium</i>	1
<i>Cryptoglana</i>	2
<i>Euglena</i>	18
<i>Euglenaformis</i>	3
<i>Lepocinclis</i>	9
<i>Monomorphina</i>	1
<i>Phacus</i>	21
<i>Strombomonas</i>	4
<i>Trachelomonas</i>	22
<b>Total</b>	<b>81</b>

The most number of species and varieties observed in the Philippine photosynthetic euglenophytes was noted in *Trachelomonas* (22), followed by the following genera in decreasing order: *Phacus* (21), *Euglena* (18), *Lepocinclis* (9), *Strombomonas* (4), *Euglenaformis* (3), and one species each for the genera *Colacium*, *Cryptoglana*, and *Monomorphina*. All the taxa

enumerated were observed in freshwater habitats, and all are flagellated and free-swimming in their dominant life cycle except for *Colacium*. Some of the probable reasons for low accountability of these algae are their difficulty in preservation, cultivation, and recognition unless they occur in bloom or were immobilized [20].

### III. ECOLOGY AND DISTRIBUTION OF PHOTOSYNTHETIC EUGLENOIDS

The photosynthetic euglenoids are considered cosmopolitan in terms of their habitat and distribution. However, there is limited report on the worldwide prevalence and ecology of the individual species. Approximately, 70% of all accounted taxa were reported in the European countries and about 50% of all known taxonomic group were observed and described in the continents of Asia and the Americas. On the other hand, lowest number of reported studies of euglenoid species is observed from Africa and Australia [4]. This distribution pattern of photosynthetic euglenoids do not reveal the current geographic distribution of these important group of microorganisms, but instead reflect the current taxonomic studies of phycologist who are studying this group of microorganisms in several areas of the world [4]. Based on taxonomic and ecological studies, it can be noted that some species of photosynthetic euglenoids would favor specific habitats where they will occur in abundance, while others are limited and are considered rare.

The majority of photosynthetic euglenoids mainly thrive in brackish- and freshwater environments characterized by either having low or high pH waters, and decaying organic matter. Only a few species inhabit the marine environment (those belonging in the genus, *Eutreptiella*, *Rapaza*, *Eutreptia*, together with some known strains of the genus *Euglena* such as *E. rustica* and *E. obtusa*) [1, 4]. These organisms are infrequent in oligotrophic and mesotrophic waters and generally not observed in large and deep lakes, springs, and fast flowing rivers. Some genera such as *Euglena*, *Trachelomonas*, *Lepocinclis*, and *Phacus* are able to grow abundantly in waters with high concentrations of organic compounds such as municipal wastewater, sewage ponds and dairy wastewater. Photosynthetic euglenoids are also found in habitats with extreme conditions such as acidic tundra ponds (pH 1.8-2.0) and hot and acid mud pool [22]. Occurrence of toxic blooms of pigmented euglenophytes such as *E. sanguinea* and *E. granulata* which are known to produce ichthyotoxins that can cause a significant commercial losses in aquaculture ponds in different areas in the world [23, 24].

Majority of the pigmented euglenoids are observe to be associated with other phytoplankton. Large and worm-like form taxa with short flagella are often observed in bodies of water between surface of the water column and sediment [43, 44]. On the other hand, pigmented euglenophytes (such as *E. mutabilis* F. Schmitz and *E. adhaerens* Matvienko) with small flagella or without flagella exists in the bottom surface of sediments in a body of water. Toxic bloom-forming species, such as *E. sanguinea*, are capable of forming surface films (neuston) in bodies of water caused by producing high

amounts of toxic mucus. In addition, the genus *Colacium* are observed attaching to small planktonic animals since these euglenoids are capable of producing large amounts of mucus which is being used to form mucilaginous stalks and dendroid colonies [1,25].

### IV. ISOLATION AND CULTURE OF PHOTOSYNTHETIC EUGLENOIDS

The popular method for the isolation and culture of photosynthetic euglenoids is single-cell isolation by micropipette using a glass capillary or a pasteur pipette. The objective of isolation using micropipette technique is to single out an algal cell from water sample, placing the algal cell into a new sterile media droplet, picking up the cell once more, and placing it to a second droplet of a new sterile media. The procedure is repeated several times until a unialgal cell (without contaminating protists) is obtained and placed into a fresh culture medium. This technique is known for limiting cell damage by repeated and excessive handling as well as clean single cell isolation. A microscope is necessary for observing and isolation of the cell. The sample consisting of the desired algal species are then transferred in a new plastic or glass dish or in a multi-well plate, or in similar containers [26].

Photosynthetic euglenoids can be cultivated (photoheterotrophically, photoautotrophically, or heterotrophically) in synthetic culture media utilizing several kinds of sources of carbon including lactate, glucose, succinate, galactose, glycerol, fructose, ethanol, methanol, propionate, acetate and pyruvate as the ultimate carbon source critical for growth and proliferation [27, 28, 29, 30, 31, 32, 33, 34]. Mass cultivation of some euglenoids such as *Euglena* can be grown using natural media like potato liquor (a waste product derived from potatoes used in starch production) as the only source of inorganic and carbon substance needed for proliferation and growth generating high algal biomass concentration of 20 g/L in batch cultivation [31]. Synthetic medium such as Modified AF6 medium is considered as one of the common culture medium being used for cultivation of photosynthetic euglenoids. It has been used to culture euglenoids such as *Phacus agilis* Skuja, *Euglena clara* Skuja and *E. mutabilis* Schmitz, the volvocalean algae (e.g., *Carteria*, *Gonium*, *Chlorogonium*, *Pandorina*, *Paulschulzia*, *Platydorina*, *Pleodorina*, *Pteromonas*, *Pseudocarteria*, and some species of *Volvox*), the xanthophytes *Botrydiopsis arrhiza* Borzi and *Botrydium granulatum* (L.) Greville, the dinoflagellate *Peridinium bipes* Stein f. *globosum* Lindermann, and the synurophyte *Synura sphagnicola* (Korsh.) Korsh [26]. Majority of the photosynthetic euglenoids grows over a broad pH range (about pH 3.0–9.0) therefore control of pH during mass cultivation of biotechnology important species (such as *E. gracilis*) poses a small risk [31, 34]. Also, the capability of these organisms to grow under acidic or basic conditions significantly reduces the occurrence of microbial contamination problems usually observed in open pond systems [34].

## V. SPECIMEN PRESERVATION OF PHOTOSYNTHETIC EUGLENOIDS

Preservation of algal specimens is important in taxonomic studies in order to protect the algal sample to biological deterioration. Specimens of photosynthetic euglenoids are usually preserved using common fluid preservatives such as formalin (formaldehyde), ethyl alcohol, Lugol's solutions or a combination of any of these chemicals. Formalin, or probably its effect on low pH, denatures DNA in the specimen that makes it difficult to do molecular studies in the future. Formalin is an aqueous mixture of formaldehyde gas in water, with small amount of methyl alcohol to prevent the formaldehyde from forming a solid mass (polymerization). It is probably the best preservative for freshwater algae [35]. Bouin's solution is a preservative made up of picric acid, formalin, and glacial acetic acid [36]. This preservative rapidly penetrates tissues, and is an excellent preservative of nuclei and chromosomes. This preservative is commonly being used in preserving plant specimens but a study made by Macusi showed that strong Bouin's solution can also be used in preserving specimens of photosynthetic euglenoids. Different combination of strong Bouin's with buffered formalin preserved the shape and other physical features of the cell but not the color of the euglenoids [36].

Alcohols are good preservatives, especially when used in high strength, because they kill bacteria and molds. However, this preservative can cause dehydration and dissolve certain pigments, protein, and lipids. The most common concentration used as preservative for algae is 70% ethyl alcohol (EtOH). Ethanol is not usually used alone as a preservative. This preservative is usually mixed with other chemical such as formalin for prolonged storage [36]. On the other hand, iodine has long been known as bacteriostatic activity and it is for this reason that iodine-based solutions are good preservatives aside from being useful as a staining solution for the presence of starch (turns blue-black). However, its use can obscure the presence of some cytological features, especially in cells with lots of starch granules. Iodine can also interfere with other stains and fluorescence. The use of chemical preservatives in keeping algal specimen is indeed important in every taxonomic study. However, these chemicals pose a health hazard upon prolonged exposure that could be carcinogenic and may cause infertility in female human beings [36].

## VI. PRACTICAL IMPORTANCE OF PHOTOSYNTHETIC EUGLENOIDS

Photosynthetic euglenoids are regarded as a suitable protist species useful for the synthesis and production of diverse biologically active compounds that are being used in the development of economically important products, such as polyunsaturated fatty acids, wax esters, biotin, tyrosine and  $\alpha$ -tocopherol. Biomass of *Euglena* are being utilized as food supplement for aquaculture and animal feeds. Also, several uses of photosynthetic euglenoids in environmental biotechnology such as bioremediation of heavy metal,

bioremediation of contaminated water and industrial wastewater, as well as ecotoxicological risk assessment. The photosynthetic euglenoids are not recognized to cause diseases in livestock or humans. Uncommon occurrence of parasitism by euglenoids in organisms such as gastrotrichs and tadpoles are reported [37]. Nevertheless, strains of bloom-forming photosynthetic euglenoids are capable of producing notable toxins (e.g. neurotoxins and ichthyotoxins) that can bring about prevalent fish kills in freshwater ponds and aquaculture facilities resulting to economic loss.

### A. Photosynthetic euglenoids as source of biotechnologically- important compounds

In spite of the fact that the main biotechnology subject has been on the utilization of cyanobacteria and microalgae as an alternative source of important medical and industrial products (such as biologically active compounds and biofuels), photosynthetic euglenoids are now currently being utilized as model systems for biotechnological research. Depending on the mode of cultivation, *Euglena gracilis* can produce high amounts  $\beta$ -1, 3-glucan paramylon in heterotrophic culture conditions [38-40] and  $\alpha$ -tocopherol under photoheterotrophic or photoautotrophic growth [40, 41]. Paramylon is a kind of dietary fiber found only in *Euglena*. It is oil-absorbing, indigestible and eliminated from the body without absorption. This dietary fiber functions by carrying fats and cholesterol out of the body and by inhibiting purine absorption in the body resulting to lowering of the level of uric acid in the blood. On the other hand,  $\alpha$ -tocopherol is a bioactive compound derivative of vitamin E that helps in the prevention of damage on cell membranes and proteins during oxidative stress [40, 42]. *E. gracilis* is considered an appropriate organism for the synthesis of other important high-value added products such as essential vitamins (vitamins A, C, and E), amino acids (such as tyrosine), polyunsaturated fatty acids (PUFAs),  $\beta$ -carotene and wax esters [1, 42, 45, 46, 47, 48, 49]. Microalgal toxins from *Euglena sanguinea* such as euglenophycin and ichthyotoxin are being tapped for its therapeutic effects against colorectal cancer [50]. The isolated toxin (49.1-114.6  $\mu$ M) exhibited anti-proliferative, cytotoxic, anti-migration and anti-clonogenic effects opposed to cancer cell lines such as SW620 CRC, HT29 and HCT116. These cancer cell lines are killed and suppressed by the algal toxin by arresting the G1 cell cycle and exhibiting a cell type - dependent modulation of autophagy. In addition, euglenoid toxin was able to cause tumor inhibition that is related to diminishing trend of serum pro-inflammatory cytokines and low expression of autophagy negative regulator mechanistic target of rapamycin (mTOR). The reported findings provide a convincing proof that algal toxin (euglenophycin and ichthyotoxin) can be considered as a potential biologically active substance with cytotoxic activity against colorectal cancer affecting several processes in cancer growth and proliferation. Moreover, this investigation substantiates the growing quest for drug discovery from natural products found in freshwater

habitats as possible source of cytotoxic compounds [50].

Photosynthetic euglenoid are also being tapped as a candidate organism for biomass (for animal feeds) and biodiesel production. A research made by Kings studied the feasibility of utilizing robust algal species *Euglena sanguinea* characterized with high lipid content and productivity for biodiesel production on laboratory and large scale (utilizing a raceway pond) conditions [51, 61]. The total lipid extracted is  $3.8 \text{ mg KOH g}^{-1}$  and is composed of 93% triglycerides. The fatty acid profile analysis using GC-MS of biodiesel produced by *E. sanguinea* displayed the occurrence of known saturated fatty acids (SAFAs): C16:0, C18:0, C22:0, C24:0 as well as monounsaturated fatty acids (MUFAs) C18:1 in amounts suitable to yield good combustion properties and oxidation stability for automotive application. In addition, Mahapatra reported a strain of *Euglena* displaying high growth rate with high algal biomass density ( $1.24 \text{ g L}^{-1}$ ) and highest lipid content of 24.6 % (w/w) at increasing concentrations of ammonium organic carbon (C) and nitrogen ( $\text{NH}_4\text{-N}$ ) [57]. Biomass of *E. gracilis* has a better nutritional quality in comparison to other biomass of different microalgal strains (such as *Scenedesmus*, *Chlorella*, *Haematococcus* and *Spirulina*) [34, 52]. Evaluation of the digestibility of *E. gracilis* showed that it is a little bit higher as compared to that of casein implying that this organism could be utilized as an alternative animal feed ingredients for livestock animals [34, 53]. In addition, comparison of the *Artemia salina*-consuming *Euglena* as feed exhibited an estimated two-fold increase in tocopherol content as compared to treatments fed by traditional microalgal feeds such as *Tetraselmis suecica* and *Dunaliella salina* [34, 54]. A study made by Das *et al.* showed increased resistance of commercially important fish, *Labeo rohita* to infection against *Aeromonas hydrophila* when fed with *Euglena viridis* [55]. The results of these studies explicitly show the potential of pigmented euglenophytes for the development of an effective alternative biomass feedstock for animal feed formulation [34].

#### B. Photosynthetic euglenoids as Ecological Indicators and use for Bioremediation

Photosynthetic euglenoids can grow in various culture conditions as well as different carbon sources, over a wide pH range, and even in elevated amounts of toxic substances such as lead, zinc, chromium, cadmium, and mercury [1]. Therefore, it can be used for bioremediation as well as ecological indicators of polluted and eutrophic waters [1, 34]. The use of a photosynthetic euglenoid *Euglena proxima* (isolated from tannery effluents) showed promising result in bioremediation of water contaminated by chromium and lead [56]. The organism caused a significant reduction (76, 80 and 88%, respectively) in the amount of  $\text{Cr}^{6+}$  after 7, 14 and 21 days respectively. *Euglena proxima* could also remove 78%  $\text{Pb}^{2+}$  after 7 days, 82% after 14 days and 90% after 21 days from the culture medium. The acid digestion of *Euglena proxima* showed 84% of  $\text{Cr}^{6+}$  and 88% of  $\text{Pb}^{2+}$  ions accumulated in the organism.

The heavy metal uptake ability of *Euglena proxima* can be further studied for environmental metal detoxification of water contaminated by heavy metals [56]. An investigation made by Mahapatra showed that a strain of *Euglena* displayed fast growth and high biomass density at rising concentrations of organic carbon (C) as well as ammonium nitrogen ( $\text{NH}_4\text{-N}$ ) [57]. This alga when cultivated within a limited span of time (8 days) caused a 98% elimination of  $\text{NH}_4\text{-N}$ , 92% total organic carbon, 85% of ortho-phosphate, 66% of total phosphate and 93% of total nitrogen. Li *et al.*, studied the possibility of using *Euglena gracilis* as bioindicator in analyzing the genotoxicity potentials of different organic pollutants found in Meiliang Bay of Taihu Lake in China [58]. Results showed that organic substances and extracts of Taihu Lake water influenced the enzymatic actions of peroxidase and superoxide dismutase of *E. gracilis*. Also, there is a high rate of DNA fragmentation observed during the assay for genotoxicity when the euglenoid (*E. gracilis*) was subjected to organic pollutants of water gathered from Taihu Lake in four seasons [58].

#### C. Photosynthetic Euglenoids Blooms and Fish Growth

The euglenoids are not recognized to cause diseases in livestock or humans. Uncommon occurrence of parasitism by euglenoids in organisms such as gastrotrichs and tadpoles are reported [37]. Nevertheless, some toxic bloom-forming photosynthetic euglenoids are capable of producing notable toxins (e.g. neurotoxins and ichthyotoxins) that can bring about prevalent fish kills in freshwater ponds and other facilities for aquaculture [23, 24]. Thick layer of euglenophytes (bloom) is considered as a possible threat to several aquaculture ponds since it is capable of utilizing nutrients of the water bodies (causing proliferous growth) and inhibits light penetration that markedly hindered the growth of useful plankton (Chlorophyceae and Bacillariophyceae) resulting in reduced growth planktivorous fish [59]. A study made by Rahman *et al.*, showed that acidic environment in combination with nutrient enrichment increases the possibility of bloom formation caused by euglenophytes. Such bloom is known in hindering the proliferation of other useful microalgae (bacillariophytes and chlorophytes) and causes seasonal occurrence of fish kill [60]. Toxic blooms of photosynthetic euglenoids leads to oxygen deficiency in fishponds, which greatly affects the normal fish growth leading to lower production. Bodies of water with elevated nutrient content and acidic pH is the most conducive to the euglenophyte bloom whereas acidic pH is detrimental for the proliferation of several planktonic algae and fish. During a euglenoid bloom, several species of pigmented euglenoid cells adhere to the gills of fish causing fish to fall into stressful state (difficulty in breathe) and thus drastically affecting their normal developmental growth. In addition, comparison of fishes in ponds with thick algal bloom showed weight values that are lower than those fish cultivated in ponds where algal bloom is absent [60].

## VII. CONCLUSIONS

In the Philippines, taxonomy and biotechnological studies of photosynthetic euglenoids found in different aquatic environment remain poorly understood. This group of photosynthetic protists is considered as an attractive organism for research because of its unique characteristics and practical importance. Photosynthetic euglenoids are currently being used in other countries for bioremediation of heavy metals, ecotoxicological assessment, and synthesis of high-value added compounds such as  $\alpha$ -tocopherol (vitamin E), paramylon bodies, polyunsaturated fatty acids (PUFAs), wax esters, biotin and tyrosine. Also, several studies have reported that photosynthetic euglenoids are suitable candidate organism in cleaning polluted bodies of water via conversion of pollutants from the aquatic environment into a nontoxic form of biomass that can be utilized for aquaculture and animal feed formulation as a nutritional supplement as well as reliable feedstock for production of biofuel. The Philippine archipelago is considered to have a diverse collection of these valuable protists and deeper studies concentrating not only on the taxonomy but also its biotechnological applications should be conducted in order to tap this important genetic resource.

## VIII. FUTURE SCOPE

Studies concerning diversity and potential use of photosynthetic euglenoids for biotechnological application are often overlooked in the Philippines. This paper emphasizes the need for deeper studies concentrating on these valuable protists by doing several taxonomic survey (from different aquatic ecosystem) and biotechnological studies (such as screening for bioactive compounds and other functionally-important bioproducts) in order to fully utilize this microbial resource.

**Conflict of Interest.** The authors declare no conflict of interest.

## ACKNOWLEDGEMENTS

The authors acknowledge the support of the Philippine National Collection of Microorganisms, National Institute of Molecular Biology and Biotechnology (BIOTECH-UPLB) as well as the Plant Biology Division of the Institute of Biological Sciences of the University of the Philippines Los Baños. Also, the constructive comments and suggestions of the blind reviewers for the improvement of the manuscript are acknowledged with gratitude.

## REFERENCES

- [1]. Leander, B. S., G., Lax, A., Karnkowska, & Simpson, A. G. B., (2017). Euglenida. In: Archibald J., Simpson A., Slamovits C. (eds) *Handbook of the Protists*. Springer, Cham [https://doi.org/10.1007/978-3-319-28149-0\\_13](https://doi.org/10.1007/978-3-319-28149-0_13)
- [2]. Arguelles, E. D. L. R., Martinez-Goss, M. R., & Shin W., (2014). Some noteworthy photosynthetic euglenophytes from Laguna and vicinities. *The Philippine Scientist*, Vol. 51: 1-36.

- [3]. Suthers, I. M., & Rissik, D. (2009). *Plankton: A Guide to their and Monitoring for Water Quality*. CSIRO Publishing, Melbourne.
- [4]. Triemer, R. E., & Zakryś, B. (2015). Photosynthetic Euglenoids. In: Wehr, J. D., Sheath, R. G., and Kociolek P. (2nd ed.), *The freshwater algal flora of the British Isles. An identification guide to freshwater and terrestrial algae*, Cambridge: Cambridge University Press.
- [5]. Marin, B., Palm, A., Klingberg, M., & Melkonian, M. (2003). Phylogeny and taxonomic revision of plastid-containing euglenophytes based on SSU rDNA sequence comparisons and synapomorphic signatures in the SSU rRNA secondary structure. *Protist*, Vol. 154: 99-145.
- [6]. Yamaguchi, A., Yubuki, N., & Leander, B. S. (2012). Morphostasis in a novel eukaryote illuminates the evolutionary transition from phagotrophy to phototrophy: Description of *Rapaza viridis* n. gen. et sp. (Euglenozoa, Euglenida). *BMC Evolutionary Biology*, Vol. 12(1): 29.
- [7]. Karnkowska, A., Bennett, M. S., Watza, D., Kim, J. I., Zakryś, B., & Triemer, R. E. (2015). Phylogenetic relationships and morphological character evolution of photosynthetic euglenids (Excavata) inferred from taxon-rich analyses of five genes. *Journal of Eukaryotic Microbiology*, Vol. 62: 362-373.
- [8]. Kim, J. I., Linton, E. W., & Shin, W., (2015). Taxon-rich multigene phylogeny of the photosynthetic euglenoids (Euglenophyceae). *Frontiers in Ecology and Evolution*, Vol. 3: 1-11.
- [9]. Cavalier-Smith, T. (2016). Higher classification and phylogeny of Euglenozoa. *European Journal of Protistology*, Vol. 56: 250-276.
- [10]. Cavalier-Smith, T., Chao, E. E., & Vickerman, K. (2016). New phagotrophic euglenoid species (new genus *Decastava*; *Scytomonas saepesedens*; *Entosiphon oblongum*), Hsp90 introns, and putative euglenoid Hsp90 pre-mRNA insertional editing. *European Journal of Protistology*, Vol. 56: 147-170.
- [11]. Kim, J. I., Shin, W., & Triemer, R. E., (2010). Multigene analyses of photosynthetic euglenoids and new family Phacaceae (Euglenales). *Journal of Phycology*, Vol. 46: 1278-1287.
- [12]. Linton, E. W., Karnkowska-Ishikawa, A., Kim, J. I., Shin, W., Bennett, M., Kwiatowski, J., Zakryś, B., & Triemer, R. E. (2010). Reconstructing euglenoid evolutionary relationships using three genes: Nuclear SSU and LSU, and chloroplast 16S rDNA sequences and the description of *Euglenaria* gen. nov. (Euglenophyta). *Protist*. Vol. 161: 603-619.
- [13]. Velasquez, G. T., (1952). Algal pollution from ponds at Puerto Galera, Oriental Mindoro, Philippines. *Natural and Applied Science Bulletin*, Vol. 12(3): 239-258.
- [14]. Pantastico, J. B., & Suayan, Z. A. (1973). Algal succession in the rice field of College and Bay Laguna, Philippines. *Philippine Agriculturist*, Vol. 57: 313-326.
- [15]. Pantastico, J. B. (1977). Taxonomy of the Freshwater Algae of Laguna de Bay and Vicinity. National Research Council of the Philippines, Bicutan, Taguig, Metro Manila, Philippines, 251p.
- [16]. Martinez, M. R. (1983). Algae in fishponds and fishpens of Laguna de Bay, Philippines II. Chrysophyta,

- Xanthophyta, Cryptophyta and Pyrrophyta. *Kalikasan, The Philippine Journal of Biology*, Vol. 12(3): 357-365.
- [17]. Martinez, M.R., (1978). Algae in fishponds and fishpens of Laguna de Bay. Philippines. I. Euglenophyta. *Kalikasan Philippine Journal of Biology*, Vol. 7(3): 305-326.
- [18]. Zafaralla, M. T., (1998). *Microalgae of Taal Lake*. National Academy of Science and Technology, Bicutan, Taguig, Metro Manila, Philippines.
- [19]. Boonmee, S., Martinez-Goss, M. R., & Shin, W., (2011). Taxonomy of flagellated algae in brackish-and freshwater fishponds in Central, Luzon, Philippines. *Asia Life Sciences*, Vol. 20(1): 99-141.
- [20]. Martinez-Goss, M. R. (2014). A checklist of photosynthetic euglenophytes (Euglenophyta, Euglenophyceae) from the Philippines. *The Philippine Scientist*, Vol. 51: 37-65.
- [21]. Arguelles, E. D. L. R. (2019). Morphotaxonomic Study of Algal Epiphytes from *Ipomoea aquatica* Forssk.(Convolvulaceae) found in Laguna de Bay (Philippines). *Pertanika Journal of Tropical Agricultural Science*, Vol. 42(2): 817-832.
- [22]. Sittenfeld, A., Vargas, M., Sanchez, E., Mora, M., & Serrano, A. (2004). A new species of *Euglena* (Euglenozoa: Euglenales) isolated from extreme environments in "boiling mudflats" of Rincon de la Vieja volcano, Costa Rica. *Revista de Biología Tropical*, Vol. 52(1): 27-30.
- [23]. Zimba, P. V., Rowan, M., & Triemer, R. E., (2004). Identification of euglenoid algae that produce ichthyotoxin(s). *Journal of Fish Diseases*, Vol. 27: 115-117.
- [24]. Zimba, P. V., Moeller, P. D., Beauchesne, K., Lane, H. E., & Triemer, R. E. (2010). Identification of euglenophycin – A toxin found in certain euglenoids. *Toxicon*, Vol. 55: 100-104.
- [25]. Leedale, G. F., (1967). *Euglenoid Flagellates*. Englewood Cliffs: Prentice Hall.
- [26]. Andersen, R. A., and Kawachi, M., (2005). Traditional Microalgae Isoaltion Techniques. In: R. A. Andersen, (1<sup>st</sup> ed.), *Algal Culturing Techniques*, Amsterdam: Elsevier Academic Press.
- [27]. Chae, S. R., Hwang, E. J., & Shin, H. S., (2006). Single cell protein production of *Euglena gracilis* and carbon dioxide fixation in an innovative photobioreactor. *Bioresource Technology*, Vol. 97: 322-329.
- [28]. Fujita, T., Aoyagi, H., Ogbonna, J. C., & Tanaka, H. (2008). Effect of mixed organic substrate on alpha-tocopherol production by *Euglena gracilis* in photoheterotrophic culture. *Applied Microbiology and Biotechnology*, Vol. 79: 371-378.
- [29]. Kitaya, Y., Azuma, H., & Kiyota, M. (2005). Effects of temperature, CO<sub>2</sub>/O<sub>2</sub> concentrations and light intensity on cellular multiplication of microalgae, *Euglena gracilis*. *Advances in Space Research*, Vol. 35: 1584-1588.
- [30]. Rodriguez-Zavala, J. S., Ortiz-Cruz, M. A., Mendoza-Hernandez, G., & Moreno-Sanchez, R. (2010). Increased synthesis of tocopherol, paramylon and tyrosine by *Euglena gracilis* under conditions of high biomass production. *Journal of Applied Microbiology*, Vol. 109: 2160-2172.
- [31]. Santeck, B., Felski, M., Friehs, K., Lotz, M., & Flaschel, E. (2010). Production of paramylon, a beta-1,3-glucan, by heterotrophic cultivation of *Euglena gracilis* on potato liquor. *Engineering in Life Science*, Vol.10: 165-170.
- [32]. Takeyama, H., Kanamaru, A., Yoshino, Y., Kakuta, H., Kawamura, Y., & Matsunaga, T. (1997). Production of antioxidant vitamins, b-carotene, vitamin C, and vitamin E, by two-step culture of *Euglena gracilis* Z. *Biotechnology and Bioengineering*, Vol. 53: 185-190.
- [33]. Tolivia, A., Conforti, V., Cordoba, O., & Flores, L. (2013). Chemical constituents and biological activity of *Euglena gracilis* extracts. *Journal of Pharmacy Research*, Vol. 7: 209-214.
- [34]. Krajčovič, J., Vesteg, M., & Schwartzbach, S. D. (2015). Euglenoid flagellates: A multifaceted biotechnology platform. *Journal of Biotechnology*, Vol. 202: 135-145.
- [35]. Wehr, J. D., & Sheath, R.G., (2003). *Freshwater algae of North America: ecology and classification*. Amsterdam; Boston: Academic Press.
- [36]. Macusi, E., (2003). Preservation and preparation of permanent mounts of *Euglena* spp. A Special Problem in Cell Biology, under the superiorship of Dr. MR Martinez-Goss, UPLB.
- [37]. Kisiełowska, G., Kolicka, M., & Zawierucha, K. (2015). Prey or parasite? The first observations of live Euglenida in the intestine of Gastrotricha. *European Journal of Protistology*, Vol. 51: 138-141.
- [38]. Calvayrac, R., Laval-Martin, D., Briand, J., & Farineau, J. (1981). Paramylon synthesis by *Euglena gracilis* photoheterotrophically grown under low O<sub>2</sub> pressure. *Planta*, Vol. 153: 6-13. □
- [39]. Sántek, B., Felski, M., Friehs, K., Lotz, M., & Flaschel, E. (2009). Production of paramylon, a -1,3-glucan, by heterotrophic cultivation of *Euglena gracilis* on a synthetic medium. *Engineering in Life Sciences*, Vol. 9: 23-28.
- [40]. Grimm, P., Risse, J. M., Cholewa, D., Müller, J. B., Beshay, U., Friehs, K., & Flaschels, E. (2015). Applicability of *Euglena gracilis* for biorefineries demonstrated by the production of α-tocopherol and paramylon followed by anaerobic digestion. *Journal of Biotechnology*, Vol. 215: 72-79.
- [41]. Ogbonna, J. C. (2009). Microbiological production of tocopherols: current state and prospects. *Applied Microbiology and Biotechnology*, Vol. 84: 217-225.
- [42]. Fujita, T., Ogbonna, J. C., Tanaka, H., & Aoyagi, H. (2009). Effects of reactive oxygen species on α-tocopherol production in mitochondria and chloroplasts of *Euglena gracilis*. *Journal of Applied Phycology*, Vol. 21:185–191.
- [43]. Leander, B. S., & Farmer, M. A. (2000). Epibiotic bacteria and a novel pattern of strip reduction on the pellicle of *Euglena helicoideus* (Bernard) Lemmermann. *European Journal of Protistology*, Vol. 36: 405-413.
- [44]. Esson, H. J., & Leander, B. S. (2008). Novel pellicle surface patterns on *Euglena obtusa* Schmitz (Euglenophyta), a euglenophyte from a benthic marine environment: Implications for pellicle development and evolution. *Journal of Phycology*, Vol. 43: 132-141.
- [45]. Wallis, J. G., and Browse, J. (1999). The Delta 8-desaturase of *Euglena gracilis*: An alternate pathway for

- synthesis of 20-carbon polyunsaturated fatty acids. *Archives of Biochemistry and Biophysics*, Vol. 365: 307-316.
- [46]. Teerawanichpan, P., & Qiu, X. (2010). Fatty acyl-CoA reductase and wax synthase from *Euglena gracilis* in the biosynthesis of medium-chain wax esters. *Lipids*, Vol. 45: 263-273.
- [47]. Tucci, S., Vacula, R., Krajcovic, J., Proksch, P., & Martin, W. (2010). Variability of wax-ester fermentation in natural and bleached *Euglena gracilis* strains in response to oxygen and the elongase inhibitor flufenacet. *Journal of Eukaryotic Microbiology*, Vol. 57: 63-69.
- [48]. Dasgupta, S., J., Fang, S. S., Brake, S. T., Hasiotis, & Zhang, L. (2012). Biosynthesis of sterols and wax esters by *Euglena* of acid mine drainage biofilms: Implications for eukaryotic evolution and the early Earth. *Chemical Geology*, Vol. 306: 139-145.
- [49]. Meyer, A., P., Cirpus, C., Ott, R., Schlecker, U., Zähringer, & Heinz, E. (2003). Biosynthesis of docosahexaenoic acid in *Euglena gracilis*: Biochemical and molecular evidence for the involvement of a  $\Delta 4$ -fatty acyl group desaturase. *Biochemistry*, Vol. 42: 9779-9788.
- [50]. Cabang, A. B., Mukhopadhyay, K. D., Meyers, S., Morris, J., Zimba, P. V., & Wargovich, M. J. (2017). Therapeutic effects of the euglenoid ichthyotoxin, euglenophycin, in colon cancer. *Oncotargets*, Vol. 8(61): 104347-104358
- [51]. Kings, A. J., Raj, R. E., Miriam, L. R. M., & Visvanathan, M.A. (2017). Growth studies on microalgae *Euglena sanguinea* in various natural eco-friendly composite media to optimize the lipid productivity. *Bioresource Technology*. Vol. 244(2): 1349-1357.
- [52]. Nakano, Y., Miyatake, K., Yamaji, R., Nishizwa, A., Shigeoka, S., Hosotani, K., Inui, H., Watanabe, F., Enomoto, T., & Takenaka, S. (1995). A protist, *Euglena gracilis* Z, functions as a sole nutrient source in a closed ecosystem. *Japan Journal CELSS*, Vol. 8: 7-12.
- [53]. Miyatake, K., & Nakano, Y. (1998). Effective algal biomass production for multi-utilizable resource from waste carbon dioxide under sunlight. *Japan Journal CELSS*, Vol. 10: 13-23.
- [54]. Vismara, R., Vestri, S., Kusmic, C., Barsanti, L., & Gualtieri, P. (2003). Natural vitamin E enrichment of *Artemia salina* fed freshwater and marine microalgae. *Journal of Applied Phycology*, Vol. 15: 75-80.
- [55]. Das, B. K., Pradhan, J., & Sahu, S. (2009). The effect of *Euglena viridis* on immune response of rohu Labeo rohita (Ham.). *Fish Shellfish Immunology*, 26: 871-876.
- [56]. Rehman, A. (2011). Heavy metals uptake by *Euglena proxima* isolated from tannery effluents and its potential use in wastewater treatment. *Russian Journal of Ecology*, Vol. 42(1): 44-49.
- [57]. Mahapatra, D. M., Chanakya, H. N., & Ramachandra, T. V. (2013). *Euglena* sp. as a suitable source of lipids for potential use as biofuel and sustainable wastewater treatment. *Journal of Applied Phycology*, Vol. 25(3): 855-865. <https://doi.org/10.1007/s10811-013-9979-5>
- [58]. Li, M., Gao, X., Wu, B., Qian, X., Giesy, J. P., & Cui, Y. (2014). Microalga *Euglena* as a bioindicator for testing genotoxic potentials of organic pollutants in Taihu Lake, China. *Ecotoxicology*, Vol. 23: 633-640.
- [59]. Khan, S. 2005. Euglenophytes in aquaculture ponds- their ecology and role in fish production. *Bangladesh Journal of Fisheries Research*, Vol. 9(1): 29-30.
- [60]. Rahman, M. M., Jewel, M. A. S., Khan, S., & Haque, M. M. (2007). Study of euglenophytes bloom and its impact on fish growth in Bangladesh. *Algae*, 22(3): 185-192.
- [61]. Pandey, R., & Kumar, G., (2017). A Comprehensive Review on Generations of Biofuels: Current Trends, Development and Scope. *International Journal on Emerging Technologies*, Vol. 8(1): 561-565.
- [62]. Gupta, K., & Pamposh, (2014). Algal Flora of some Selected Water Bodies of Delhi. *Biological Forum- An International Journal*, Vol. 6(2): 181-188.
- [63]. Arguelles, E. D. L. R. (2019). Systematic Study of Some Epiphytic Algae (Non-diatoms) on the Submerged parts of Water Hyacinth (*Eichhornia crassipes* (Mart.) Solms-Loubach) found in Laguna de Bay (Philippines). *Tropical Life Sciences Research*, Vol. 30(1): 1-21.

**How to cite this article:** Arguelles, E. D. L. R. and Martinez-Goss, M. R. (2019). Diversity of Philippine Photosynthetic Euglenophytes and their Potential Biotechnological Uses: A Review. *International Journal on Emerging Technologies*, 10(4): 24–31.