# Cyanobacteria and diatoms in the cyanobacterial mats in a natural salt water hot spring in Coron, Palawan, Philippines

Milagrosa R. Martinez-Goss<sup>\*1,2</sup>, Jean Edward B. Manlapas<sup>1</sup>, and

Eldrin DLR Arguelles<sup>3</sup>

<sup>1</sup>Institute of Biological Sciences, College of Arts and Sciences, University of the Philippines Los Baños, Laguna

<sup>2</sup>Museum of Natural History

<sup>3</sup>Philippine National Collection of Microorganisms, National Institute of Molecular Biology and Biotechnology, University of the Philippines, Los Baños, Philippines

he Maquinit Hot Spring in Coron, Palawan, Philippines is one of the few salt water (35-40 ppt) hot springs (38°C-41°C) in the world. It is one of two of its kind in Southeast Asia. This unique habitat was studied intermittently over a period of 12 years. It is the first study of the taxonomy of the thermo-tolerant cvanobacteria in cvanobacterial mats and their associated diatoms. A total of 18 taxa are reported and identified. Included herewith are 11 species of cyanobacteria (Division Cyanobacteria) and seven species of diatoms (Division Bacillariophyta) that were dominant and significantly observed. There were five homocystous filamentous cyanobacteria, one heterocystous type, and five species in the coccoid and colonial forms. The diatoms were mostly the benthic and marine types. Of these taxa, the following are new records for the country: three species in Division Cyanobacteria (Pleurocapsa minor, Myxosarcina amethystina, and Calothrix thermalis) and two taxa in Division Bacillariophyta (Achnanthes brevipes var. intermedia and Halamphora coffeaeformis). While eight species are new distributional records in the Philippines. Keys are presented to differentiate the different species in Cyanobacteria and in Bacillariophyta. Diagnostic descriptions, photographs, distribution, and habitat records are given for each taxon.

\*Corresponding author Email Address: mmartinezgoss@gmail.com Date received: February 12, 2019 Date revised: May 11, 2019 Date accepted: May 21, 2019

# KEYWORDS

Coron, cyanobacteria, cyanobacterial mat, diatoms, Maquinit Hot Springs, natural salt water hot spring, Palawan, Philippines, thermotolerant cyanobacteria

# INTRODUCTION

Geothermal springs are distributed globally except probably in Antarctica (Ward & Castenholz 2000). In these habitats, several taxonomic studies of thermophilic cyanobacteria (60°C - 65°C) have been reported based on their morphological characteristics (Brock 1978, Castenholz 1969, 1977; Ward *et al* 1987) and their molecular studies (Brock 1978; Castenholz 1969a-b; (Lacap *et al.* 2005; Lacap *et al.* 2007).). However, the cyanobacterial thermophiles are limited to some taxa, such as *Calothrix, Chloroflexus, Chlorogloepsis Fischerella, Mastigocladus, Oscillatoria, Spirulina, Synechococcus,* (Castenholz 1977; Ward & Castenholz 2000).

Earlier reports did not usually associate diatoms with these cyanobacterial thermophiles. Round (1965) generally did not consider diatoms as thermophilic, although a few species can tolerate temperatures from 33 to 45°C (Stockner, 1967). Lately, more reports showed that diatoms have been recorded in hot springs in Asia (Owen, *et al.*, 2008; Fukushima *et al.*, 2002), Europe (Owen *et al.*, 2008; Brock and Brock, 1967), North America (Hobbs *et al.*, 2009; Stockner, 1967, 1968), Africa (Mpawenayo *et al.*, 2005), Australia and New Zealand (Cassie and Cooper, 1989).

# Table 1: List of cyanobacteria observed in hot springs in the Philippines (1950 - May, 2016).

Species	Occurrence	Reference
Anabaena variabilis Kützing	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap et al., 2007
Aphanocapsa feldmannii Fremy	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap et al., 2007
Aphanocapsa incerta (Lemmerman) G. Cronberg & Komárek	LUZON: Palawan, Coron (Maquinit Hot Springs)	Martinez-Goss, <i>et al.</i> , in this issue
Aphanothece sacrum Okada	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap <i>et al.</i> , 2007
Calothrix thermalis (Schwabe) Hansgirg	LUZON: Palawan, Coron (Maquinit Hot Springs)	Martinez-Goss, <i>et al.</i> , in this issue
Chroococcus minutus (Kützing) Nägeli	LUZON: Palawan, Coron (Maquinit Hot Springs)	Martinez-Goss, <i>et al.</i> , in this issue
Chroococcus turgidus (Kützing) Nägeli	LUZON: Palawan, Coron (Maquinit Hot Springs)	Martinez-Goss, <i>et al.</i> , in this issue
Coleodesmium wrangelii Borzi ex Geitler	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap <i>et al.</i> , 2007
<i>Crocosphaera watsonii</i> Unknown Authority nom. inval.	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap et al., 2007
Cyanobacterium stanieri R. Rippka & G. Cohen-Bazire	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap <i>et al.</i> , 2007
<i>Cyanobium gracile</i> R. Rippka & G. Cohen-Bazire	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap <i>et al.</i> , 2007
Cyanothece sp.	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap <i>et al.</i> , 2007
Dactylococcopsis salina G.M. Smith	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap et al., 2007
Euhalothece sp.	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap <i>et al.</i> , 2007
Fischerella major Gomont	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap <i>et al.</i> , 2007
Fischerella muscicola (Thuret) Gomont	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap <i>et al.</i> , 2007
Gloeothece membranacea (Rabenhorst) Bornet	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap <i>et al.</i> , 2007
Gloeocapsa sp.	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap <i>et al.</i> , 2007
Halothece sp.	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap <i>et al.</i> , 2007
Leptolyngbya boryana (Gomont) Anagnostidis & Komarek	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap <i>et al.</i> , 2007
<i>Leptolyngbya treleasii</i> (Gomont) Anagnostidis & Komárek	LUZON: Palawan, Coron (Maquinit Hot Springs)	Martinez-Goss, <i>et al.,</i> in this issue
<i>Lyngbya semiplena</i> (C. Agardh) J. Agardh	LUZON: Palawan, Coron (Maquinit Hot Springs)	Martinez-Goss, <i>et al.</i> , in this issue
<i>Mastigocladus laminosus</i> Cohn ex Kirchner	LUZON: Laguna, Los Baños (Lalakay Hot Spring, 57-74 °C)	Velasquez, 1952, 1962
	LUZON: Albay, Tiwi (Naglagbong, Baño Hot Springs & Cale Drill Hole VII)	Clidoro, 1975
Merismopedia glauca (Ehrenberg) Kützing	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap et al., 2007
Microcystis aeruginosa Kützing	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap <i>et al.</i> , 2007
Microcystis marginata (Meneghini) Kützing	LUZON: Oriental Mindoro, Pto. Galera (Bisayaan Hot Spring)	Drouet & Daily, 1956
Microcystis orissica West	LUZON: Laguna, Los Baños (Lalakay Hot Spring)	Drouet & Daily, 1956 and Velasquez, 1951, 1962
Myxosarcina amethystina J.J. Copeland	LUZON: Palawan, Coron (Maquinit Hot Springs)	Martinez-Goss, <i>et al.</i> , in this issue
Oscillatoria amphigranulata Van Goor	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap <i>et al.</i> , 2007
Oscillatoria formosa Bory ex Gomont	VISAYAS: Negros Occ., San Carlos, (Mainit Hot Spring)	Soriano, 1953
<i>Oscillatoria geminata</i> Schwabe ex Gomont	LUZON: Albay, (Ligao & Tiwi hot springs); Palawan, Coron (Maquinit Hot Spring)	Pantastico 1977 Martinez-Goss, <i>et al.</i> ,
Oscillatoria subbrevis Schmidle	LUZON: Albay, (Tiwi hot spring ; Palawan, Coron (Maquinit Hot	Clidoro, 1975 Martinez-Goss, <i>et al.</i> ,
Phormidium autumnale (Agardh)	Spring) VISAYAS: Negros Occ. (San Carlos Hot	in this issue Soriano, 1953
Gomont	Spring) LUZON: Laguna, Los Baños (Lalakay	Velasquez, 1952

Gomont	Hot Spring)	
	LUZON: Oriental Mindoro, Pto. Galera (Bisayaan Hot Spring)	Velasquez, 1950, 1952, 1962
Phormidium laminosum Gomont	LUZON: Laguna, Los Baños (Lalakay Hot Spring) Albay, Tiwi (Naglagbong, Baño Hot Springs & Cale Drill Hole VII)	Velasquez, 1951, 1952, 1962 Clidoro, 1975
Phormidium muscicola Huber-Pestalozzi Naumann	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap et al., 2007
Phormidium tenue (Meneghini) Gomont	LUZON: Laguna, Los Baños (Lalakay     Velasquez, 19       Hot Spring, 57 - 74° C)     LUZON: Palawan, Pto. Princesa (Pto.	
	Princesa Hot Spring, 60° C) VISAYAS: Negros Occ., Valencia (Palinpinon Hot Spring)	Velasquez, 1955, 1962 Soriano, 1953
Phormidium valderianum Gomont	LUZON: Laguna, Los Baños (Lalakay Hot Spring) LUZON: Palawan, Pto. Princesa (Pto.	Velasquez, 1952 Velasquez, 1952, 1955
Pleurocapsa minor Hansgirg	Princesa Hot Spring) LUZON: Palawan, Coron (Maquinit Hot Springs	Martinez-Goss, <i>et al.,</i> in this issue
Pleurocapsa sp.	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap <i>et al.</i> , 2007
Pseudanabaena sp.	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap et al., 2007
Spirulina major Kützing ex Gomont	LUZON: Palawan, Coron (Maquinit Hot Springs)	Martinez-Goss, <i>et al.</i> , in this issue
Synechococcus elongatus (Nägeli) Elenkin	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap <i>et al.</i> , 2007
Synechococcus lividus Copeland	LUZON: Albay, Tiwi (Naglagbong Hot Spring) LUZON: Albay, Tiwi (Baño Hot Spring)	Clidoro, 1975 Clidoro, 1975
Synechocystis pevalekii Ercegovic	LUZON: Albay, Tiwi (Naglagbong Hot Spring)	Clidoro, 1975
Synechocystis stridiemni Ercegovic	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap <i>et al.</i> , 2007
Thermosynechococcus elongates Katoh, Itoh, Shen & Ikeuchi, nom. inval.	LUZON: Laguna, Los Baños (Tadlak, Wonder Lake)	Lacap et al., 2007

The temperature in thermal spring effluents of Yellowstone National Parks in Montana, USA where diatoms were observed ranged from  $36^{\circ}$ C to  $54^{\circ}$ C (Kullberg, 1968). On the other hand, the temperature in hot springs from Far East Russia, where diatoms were noted, had a maximum temperature of  $60^{\circ}$ C (Nikulina and Kociolek, 2011). Diatoms are commonly found in marine habitats globally (Round, et al., 1990). However, few studies have been reported globally of cyanobacterial mats with diatoms associated together in hot springs and saline waters.

In the Philippines, Velasquez was one of the early workers on thermophilic algae from natural hot springs in Talakay (Lalakay), Los Baños, Laguna (57-74°C) and in Sitio Bisayaan, Puerto Galera, Oriental Mindoro (Velasquez 1950, 1951, 1955, 1962) (Table 1). Later, more works were done by Velasquez' student (Soriano 1953) and his colleagues (Drouet & Daily 1956a-c). Among the identified cyanobacteria were the unicellular to colonial types, such as *Gloeocystis grevillei* (Berkeley) Drouet, *Coccochloris stagnina* Sprengel, and *Microcystis orissica* J. West. Included in the group were the homocystous filamentous types: *Phormidium autumnale* (Agardh) Gomont, *Phormidium laminosum* Gomont, *P. tenue* (Meneghini) Gomont, *P. valderianum* Gomont, *P. inundatum* Gomont; and a heterocystous type, *Hapalosiphon laminosus* (Kützing) Hansgirg.

In 1955, Velasquez did more studies of thermal springs in Palawan and noted *Phormidium tenue* and *P. valderianum*. Clidoro (1975) observed four common cyanobacteria in three thermal springs in Tiwi, Albay, whose water temperatures ranged from 48°C to 60°C, such as *Synechococcus lividus* Copeland, *Synechocystis pevalikii* Erecegovic, *Phormidium laminosum* Gomont and *Mastigocladus laminosus* Cohn.

Lately, molecular analyses of cyanobacterial mats in geothermal springs or pools (40-70°C) in "Wonder Lake," located near Tadlak Lake (colloquially known as Alligator Lake) in Laguna, Philippines (N 14°10.630', E12° 12.333') revealed four theromphilic phylotypes, among them *Synechococcus, Oscillatoria, Fischerella*, and *Chloroflexus*, although about 25 more species were identified (Lacap *et al.* 2005; Lacap *et al.* 2007). This number of species comprised about 114% increase in number over those cyanobacteria that were identified morphologically in 1950-1975.

On the other hand, 77 species of cyanobacteria have been observed in brackish to marine waters in the Philippines from 1941 to May, 2016 (Table 2, Martinez 1984). Among these species, only *Microcystis aeruginosa* was observed in both thermal and saline habitats, but at different places and at different times (Tables 1 &2; Martinez 1984; Lacap *et al.* 2007).

Furthermore, seven species of diatoms (Class Bacillariophyceae) that were observed associated with the cyanobacterial mats are recorded for the first time in the Philippines. However, these microalgae in the cyanobacterial mats are characterized more as thermo-tolerant species because they occurred at temperatures between 38°C to 41°C, and not at temperatures as high as where the thermophilic species are

	Reference	
	Pantastico <i>et al.</i> , 1976	
VISAYAS: Aklan, Tangalan (Afga)	Vicencio, 1977 Cordero, 1981	
	Drouet & Daily,1956	
	Velasquez, 1962	
	Martinez-Goss, et al.,	
	in this issue	
LUZON: Laguna. Laguna de Bay	Pantastico, 1977	
LUZON: Oriental Mindoro, San Isidro	Velasquez, 1950, 1962	
LUZON: Oriental Mindoro, Pto. Galera (Muelle Bay)	Fortes & Trono, 1979	
LUZON: Cagayan, Babuyan Island, Calayan Island	Velasquez, 1962	
	Saraya &Trono, 1979	
	5	
LUZON: Cagayan, Calayan Is.,	Fan, 1956	
	Como in 8 Valorence 1070	
LUZON: Batangas, Nasugbu LUZON: Ilocos Sur, (Narvacan	Cornejo &Velasquez,1970 Fan, 1956	
Beach)		
LUZON: Or. Mindoro, Pto. Galera, (Ensanada Cove)	Velasquez, 1950	
(Dooñgan cove)	Velasquez, 1962	
LUZON: Or. Mindoro, Pto. Galera,	Velasquez, 1950	
	Fortes & Trono, 1979	
LUZON: Palawan, Coron (Maquinit	Martinez-Goss, <i>et al.</i> ,	
Hot Springs) LUZON: Batangas, Nasugbu	in this issue Cornejo & Velasquez,	
LUZON: Rizal, Malabon	1970 Esguerra, 1951	
LUZON: Bulacan, Bulacan	Boonmee, 1979	
(fishponds)	Boonmee, 1979	
(fishponds)	,	
	Boonmee, 1979	
	Martinez-Goss, et al.,	
	in this issue	
	Boonmee, 1979	
	Boonmee, 1979	
(fishponds);	Boomiee, 1979	
Palawan, Coron (Maquinit Hot Springs)	Martinez-Goss, <i>et al.,</i> in this issue	
	Velasquez, 1955	
(Bongao)	<b>L</b> ,	
LUZON: Bulacan, Bulacan (fishponds)	Boonmee, 1979	
MINDANAO: Sulu, Jolo	Velasquez, 1955	
VISAYAS: Iloilo, Leganes, (Dumangas)	Soriano, 1953	
LUZON: Palawan, Araceli,	Velasquez, 1955, 1962	
(Babuyan)		
LUZON: Batangas, Calatagan	Cornejo & Velasquez, 1970	
LUZON: Rizal, Parañaque, (Dagat-	Drouet & Daily, 1956	
dagatan)	Velasquez, 1962	
	Velasquez, 1950, 1962	
LUZON: Rizal, Malabon	Trono, 1961	
LUZON: Rizal, Malabon	Trono, 1961	
LUZON: Laguna, Laguna de Bay	Pantastico, 1976	
	LUZON: Oriental Mindoro, Pto.         Galera (San Isidro, Big Balatero)         LUZON: Palawan, Coron (Maquinit         Hot Springs)         LUZON: Oriental Mindoro, San         Isidro         LUZON: Cagayan, Babuyan Island,         Calayan Island         LUZON: Cagayan, Babuyan Island,         Calayan Island         LUZON: Cagayan, Calayan Is.,         Babuyan Is.,         LUZON: Batangas, Nasugbu         LUZON: Or. Mindoro, Pto. Galera,         (Ensanada Cove)         LUZON: Or. Mindoro, Pto. Galera,         (Ensanada Cove)         LUZON: Or. Mindoro, Pto. Galera,         (Ensanada Cove)         LUZON: Or. Mindoro, Pto. Galera,         (Paniquian Is.)         LUZON: Or. Mindoro, Pto. Galera,         (Paniquian Is.)         LUZON: Batangas, Nasugbu         LUZON: Batangas, Nasugbu         LUZON: Batangas, Nasugbu         LUZON: Batangas, Nasugbu         LUZON: Bulacan, Bulacan         (fishponds)         LUZON: Bulacan, Bulacan         (fishponds)         LUZON: Bulacan, Bulacan         (fishponds);         Palawan, Coron (Maquinit         Hot Springs)         LUZON: Bulacan, Bulacan	

Gomphosphaeria aponina Kützing	LUZON: Laguna, Laguna de Bay	Al-Saboonchi, 1980
Hormothamnion solutum	LUZON: Palawan, Araceli,	Velasquez, 1955, 1962
Bornet & Grunow ex Bornet & Flahault	(Catadman Sound, Cuyo)	
Hydrocoleum cantharidosum (Montagne) Gomont	LUZON: Camiguin Island	Velasquez, 1950
(noningne) comon	LUZON: Or. Mindoro, Pto. Galera, (Ensañada Cove, Farola Point)	Velasquez, 1962
H. comoides (Harvey)	LUZON: Pangasinan, Alaminos	Velasquez, 1962
Gomont H. lyngbyaceum Kützing	LUZON: Cagayan, Babuyan Is.,	Velasquez, 1962
	Calayan Island	
Leptolyngbya treleasii (Gomont) Anagnostidis & Komárek	LUZON: Palawan, Coron (Maquinit Hot Springs)	Martinez-Goss, <i>et al.,</i> in this issue
Lyngbya aestuarii Liebmann ex Gomont	LUZON: Pangasinan, Dagupan	Velasquez, 1941
	Palawan, Pto. Princesa, Araceli (Cuyo, Iwahig Penal Colony)	Velasquez, 1955
L. confervoides	LUZON: Or. Mindoro, Pto. Galera	Velasquez, 1979
Agardh ex. Gomont		Fortes, 1981
	VISAYAS: Leyte, Tacloban	Velasquez, 1962
I. Commentaria Area 11	Cebu, (Liloan Beach)	Velasquez, 1979
L. ferruginea Agardh	MINDANAO: Zamboanga LUZON: Laguna, Laguna de Bay	Dickie, 1876
L. limnetica Lemmermann L. maior Meneghini ex Gomont	LUZON: Laguna, Laguna de Bay LUZON: Bulacan, Bulacan	Pantastico, 1977 Boonmee, 1979
<i>L. major</i> Meneghini ex Gomont <i>L. majuscula</i> Harvey ex Gomont	LUZON: Bulacan, Bulacan LUZON: Cagayan, Babuyan,	Velasquez, 1979
L. majuscula Harvey ex Gomon	Pangasinan, Bolinao	Chan, 1981
	Bataan, Cabcaban (Lamao)	Velasquez, 1962
	Batangas, Nasugbu	Cornejo & Velasquez,
	Or. Mindoro, Pto. Galera,	1970 Velasquez, 1950, 1955,
	(Little Balatero, Northeast Muelle,	1962
	& First Plateau) Palawan, Araceli & Catadman	Velasquez, 1962
	Sound (floating); (on corals)	Fortes, 1981
	VISAYAS: Iloilo, Miag-ao (Kirayan Norte);	Peralta <i>et al.</i> , 2006
L. martensiana	Camiguin Is. LUZON: Or. Mindoro, Pto. Galera	Velasquez, 1962 Velasquez, 1979
Meneghini ex Gomont L. nordgardhii Wille	LUZON: Laguna, Laguna de Bay,	Pantastico, 1977
	(Talim Island, Malaki Island)	-
<i>L.putealis</i> Montagne ex Gomont	LUZON: Laguna, Laguna de Bay (Talim Is., Balibago, Pantalan Area)	Pantastico, 1977
L. semiplena (C. Agardh) J. Agardh ex Gomont	LUZON: Pangasinan, Lucap Bay (on bancas)	Agor, 1962;
Comont	Rizal, Pasay City;	Velasquez, 1962
	Or. Mindoro, Pto. Galera (on rocky	Velasquez, 1962
	cliffs)	Martinez-Goss, et al.,
	Palawan, Coron (Maquinit Hot Springs)	in this issue Reyes, 1976
	VISAYAS: Siquijor	
L. sordida (Zanardini) Gomont	LUZON: Cagayan, Babuyan Island	Velasquez, 1962
	Or. Mindoro, Pto. Galera	Velasquez, 1962
Microcoleus acutissimus Gardner	LUZON: Or. Mindoro, Pto. Galera	Fortes, 1981
<i>M. chthonoplastes</i> Thuret ex Gomont	LUZON: Pangasinan, Dagupan	Velasquez, 1941
Microcystis aeruginosa Kützing	LUZON: Bulacan, Bulacan (fishponds)	Boonmee, 1979
	Laguna, Laguna de Bay	Martinez & Eakle, 1977
M. pulverea (Wood) Forti	LUZON: Or. Mindoro, Pto. Galera	Drouet & Daily, 1956

Myxosarcina amethystina J.J. Copeland	LUZON: Palawan, Coron (Maquinit	Martinez-Goss, et al.,
×	Hot Springs)	in this issue
<i>Nodularia spumigena</i> Mertens ex Bornet & Flahault	LUZON: Albay, San Lorenzo, (Tiwi) Pantastico <i>et al.</i> , 1	
Nostoc maculiforme Bornet & Flahault	LUZON: Laguna, Laguna de Bay Pantastico, 1977	
Oscillatoria angusta Koppe	LUZON: Laguna, Laguna de Bay Pantastico, 1977	
O. bonnemaisonii Crouan	LUZON: Or. Mindoro, Pto. Galera, (Muelle)	Velasquez, 1979
	VISAYAS: Cebu, Tacloban City	Velasquez, 1979
O. brevis (Kützing) Gomont	VISAYAS: Iloilo, Lleganes, Alimodian	Soriano, 1953
O. corallinae (Kützing) Gomont	LUZON: Bataan, Lamao	Velasquez, 1962
O. curviceps Agardh ex Gomont	LUZON: Or. Mindoro, Pto. Galera,	Fortes, 1981
O. geminata Schwabe ex Gomont	LUZON: Palawan, Coron (Maquinit Hot Springs)	Martinez-Goss, <i>et al.,</i> in this issue
O. margaritifera (Kützing) Gomont	LUZON: Pangasinan, Bolinao	Chan, 1981
O. nigroviridis Thwaites ex Gomont	LUZON: Or. Mindoro, Pto. Galera	Fortes, 1981
O. subbrevis Schmidle	LUZON: Palawan, Coron (Maquinit Hot Springs)	Martinez-Goss, <i>et al.</i> , in this issue
Phormidium ambiguum Gomont	LUZON: Ilocos Sur, (Narvacan Beach)	Velasquez, 1941
P. tenue (Meneghini) Gomont	LUZON: Bulacan, Bulacan	Boonmee, 1979
Pleurocapsa minor Hansgirg	LUZON: Bulacan, Bulacan LUZON: Palawan, Coron (Maquinit	Martinez-Goss. <i>et al.</i>
	Hot Springs) LUZON: Palawan, Pto. Princesa	in this issue
Porphyrosiphon notarisii (Meneghini) Kützing ex Gomont		Velasquez, 1941
<i>Pseudoanabaena nitida</i> Agardh ex Bornet & Flahault	LUZON: Or. Mindoro, Pto. Galera	Fortes, 1981
P. polyotis (Agardh) Bornet & Flahault Spirulina major Kützing ex Gomont	VISAYAS: Siquijor LUZON: Bulacan, Calumpit (on the	Reyes, 1976 Velasquez, 1941
	side of a canal); Rizal, Malabon (culture cans, ponds) Navotas (fishponds, stomach of milkfish)	Trono, 1961 Vicencio, 1977
	Or. Mindoro, Pto. Galera (rare in one pond); on hard corals	Velasquez, 1952a Velasquez, 1979
	Palawan, Coron (Maquinit Hot Springs)	Martinez-Goss, <i>et al.</i> , in this issue
	VISAYAS: Cebu, Liloan (Silut Bay, rare);	Almase, 1970
	Iloilo (on hard corals) Leyte, Laman	Velasquez, 1979 Velasquez, 1962
	Tacloban (on hard corals);	Velasquez, 1979
Spirulina (Arthrospira) sp.	LUZON: Bulacan, Bulacan (fishponds)	Boonmee, 1979
Symploca hydnoides Kützing ex Gomont	LUZON: Pangasinan, Santiago Island	Saraya & Trono, 1979
	Or. Mindoro, Pto. Galera	Velasquez, 1962
	VISAYAS: Cebu, Mactan	Liao & Sotto, 1980
	Negros Oriental, Dumaguete City	Reyes, 1970
	Siguijor	Reyes, 1976
S. laete-viridis Gomont	LUZON: Albay, Catanduanes (Calolbon);	Velasquez, 1962
	Or. Mindoro, Pto. Galera, (Small Balatero)	
Trichodesmium thiebautii Gomont	VISAYAS: Cebu, Liloan (Silut bay)	Almase, 1970

found in brackish to marine habitats (55°C - 85°C) (Miller and Castenholz 2000).

This paper added nine species of cyanobacteria observed in hot springs, namely *Aphanocapsa incerta*, *Calothrix thermalis*, *Chroococcus minutus*, *C. turgidus*, *Leptolyngbya treleasii*, *Lyngbya semiplena*, *Myxosarcina amethystina*, *Pleurocapsa minor*, and *Spirulina major*. This gives a total number of 47 taxa observed in hot springs in the Philippines. While six species of cyanobacteria are added in the number of total taxa found in brackish to marine habitats giving the total number of taxa to 77 (from 1941 to May, 2016). These added taxa are: *Calothrix thermalis, Leptolyngbya treleasii, Myxosarcina amethystina, Oscillatoria geminata, O. subbrevis,* and *Pleurocapsa minor*. Furthermore, there were five new records of microalgae for the Philippines from this extreme ecological habitats. These are three species in the Division Cyanophyta (Cyanobacteria): Calothrix thermalis, Myxosarcina amethystine, and *Pleurocapsa minor,* and two species in the Division Bacillariophyta: Achnanthes brevipes var. intermedia and *Halamphora coffeaeformis.* Hence, this paper presents for the first time, thermo-tolerant cyanobacteria and diatoms in the cyanobacterial mat in a natural salt water hot springs in Maquinit Hot Springs in Coron, Busuanga Is., Palawan, Philippines.

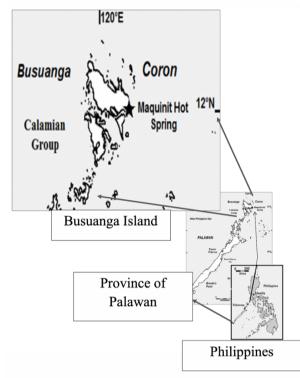


Figure 1: Collecting sites in Maquinit Hot Springs in Coron, Busuanga Is., in the province of Palawan, Philippines.

# MATERIALS AND METHODS

#### **Study Site**

The study sites were the three pools in the Maquinit Hotspring (MHS), located in Coron, in the south eastern side of Busuanga Island (N 11°59'29.4'', E 120°13'43.0''), about 260 km north of Puerto Princesa City in Palawan (Fig. 1). MHS comprise two semi-circular pools (Pool 1 & 2) which were constructed out of stones and concrete cement to impound and collect the salty and hot water from the spring. They have an estimated area of 4 and 5 m<sup>2</sup> for pool 1 &2, respectively and an average water depth of 0.91m. These pools were developed into recreational places by the Jovellanos' family in the late 1970s. Over time, the place became a local tourist spot. Hence, in the 1990s the family decided to build a bigger pool (Pool 3) to accommodate the increasing number of tourists (Fig. 2A). Pool 3 has impounded water from the overflow of waters from Pools 1 &2. It has an estimated area of 80-100 m<sup>2</sup> with an average water depth of 1.22 m. Cyanobacterial mats were observed attached to the rocks (Fig. 2B).

The hot spring outcrops on cracks and fissures in the exposed marblelized gray, metamorphosed limestone discharges hot water of about 38- 41 °C (field temperature), with a heat flow of 174.00 k cal/sec at a flow rate of  $5,115 \text{ L.min}^{-1}$  (Table 3, Sincioco and Bautista 1985). No sulfuric odor was noted within the vicinity. As the hot water springs up from the earth's interior, it brings along some of the weathered materials it passes through and deposits them on the earth's surface. Some of these deposits were noted in Maquinit Hot Springs to be limited to staining of rocks by a brownish material, probably of limonite, an iron ore (Sincioco and Bautista 1985). This is one of few natural salt

water hot springs in the Philippines and is one of two of this kind in Southeast Asia, the other one being in Taitung, Taiwan.



Figure 2: Maquinit Hot Springs pools, (A) showing pools 1, 2 and 3; (B) showing cyanobacterial mat at pool 1, near the hot spring source.

# Sampling and some in situ abiotic parameter readings

Cyanobacterial mats were collected, four times, over a period of 12 years, starting on May 17, 2005 up to August 7, 2017. The first sample was collected on May 17, 2005. The second sample was collected on November 10, 2015 from the two small pools, Pool 1 & 2. Another set of samples were taken on April 25, 2016 that included water samples from the bigger pool (Pool 3), aside from Pools 1 & 2. The fourth samples were collected from the three pools on August 7, 2017. At this time some abiotic parameters were also taken in situ.

The pH of the water in the three pools were taken with the Milwaukee pH 600 pocket-sized pH meter (M-8026, Milwaukee Instrments, Inc., Rocky Mount, North Carolina, USA). Salinity was taken with a hand refractometer (Atago, S/Mill, 8608, Atago Co., Ltd., Japan), while the light intensity was measured with a light meter (Lutron, AD 51634, LX-103, Taiwan), and water temperature was taken with an ordinary field thermometer.

The cyanobacterial mats together with the water samples were collected per pool, kept in an ice-cooled Styrofoam box, and transported by air to Manila. Within four hours after arrival at UPLB, the cyanobacterial mats and the phytoplankton sediments were divided into three lots; one lot was preserved in 4% buffered formalin while the other lot was air-dried, away from direct sunlight serving as voucher specimen, and the third lot was kept in the refrigerator (about 10°C) for microscopic observation and culture.

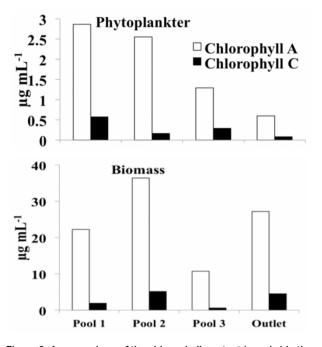


Figure 3: A comparison of the chlorophyll content (a and c) in the cyanobacterial mats and the phytoplankter in the three different pools in Maquinit Hot Springs, Coron, Palawan at noon time on August 7, 2017; outlet is the water sample taken outside the impounded pool no. 3, at the mangrove area, that serves as the control.

#### **Cleaning of diatoms**

Sediments from the water samples, containing diatoms, were collected, concentrated, cleaned and strewn mounts prepared with Canada balsam on slides with cover slips following the procedures of Patrick and Reimer (1966) and Round *et al* (1990).

#### Voucher specimens

Air-dried cyanobacterial mats were mounted on herbarium sheets in triplicates to serve as herbarium specimens. Prepared diatom slides were also in triplicates and kept in a slide box. All herbarium specimens and diatom slides served as voucher specimens and kept at the College of Agriculture Herbarium of the University of the Philippines (CAHUP) of the Museum of Natural History, University of the Philippines Los Baños, College, Laguna, Philippines.

# Microscopy

The cyanobacterial mat and the diatom sediments in each sample were initially examined using a bright-field microscope (American Optical-Spencer, American Optical Co. Scientific Instruments, U.S.A.). Further cyto-morphological characterization of the organisms and photomicrographs were done using a bright-field and phase-contrast microscope (Carl Zeiss Axioscope A1, Carl Zeiss, Göttingen, Germany) with an attached camera (AxioCam ERc5S, Carl Zeiss, Göttingen, Germany). All micrographs were taken under immersion oil objective (100x) unless stated otherwise. Cell dimensions were taken with a calibrated micrometer eyepiece.

#### Identification of the cyanobacteria and diatoms

Identification of the cyanobacteria and diatoms was carried out by characterizing them morpho-cytologically both in fresh and preserved samples with the aid of available taxonomic references. The general references used were: Smith 1950; Prescott 1962; Wehr & Sheath 2003; Lee 2008; Graham *et al.*, 2009. Specific references are indicated in each taxonomic treatment. Current names were based mainly on algaebase (Guiry & Guiry 2005). Synonyms for each taxon were included when some of the cited references used the old name of the taxon. While classification at the division level was based on the combined schemes of Round et al. 1990, Lee 2008, Graham et al. 2009.

# Relative abundance of cyanobacteria and diatoms

The relative abundance of the filamentous cyanobacteria in the cyanobacterial mat was determined by counting the frequency of occurrence of a filament of a taxon per microscopic field (mic field) observation. There were at least ten microscopic field observations done. Likewise, the relative abundance of the different diatom taxa were obtained by getting cell count in ten microscopic fields for each of the prepared strewn mounted diatom slides. The reported numbers are averages of at least ten microscopic field observations.

Chlorophyll analyses were also done to estimate the biomass in the cyanobacterial mats as well as the phtoplankter in the three different pools , and outside the pools, at the mangrove area (that served as control). Extraction of chlorophyll a and c were done using 90% acetone following the procedure of Jeffrey and Humphrey's (1975). Extracted pigments were identified spectrophotometrically at the following wavelengths: 750,664, 647, and 630 nm. Estimation of chlorophylls a and c , in  $\mu$ g.mL<sup>1</sup>, were done following the procedure of Martinez-Goss, et al. (2001). Colorimetric analyses were done in a Shimadzu UV-double beam spectrophotometer (UV 1800, Shimadzu, Japan).

# RESULTS

# I. Distribution and Occurrence

A total of 18 thermo-tolerant taxa of cyanobacteria and diatoms were identified in the cyanobacterial mats in the natural salt water Maquinit hot spring (MHS) (35 - 40 ppt; 38°C - 41°C;) (Table 4). This includes 11 species of cyanobacteria and seven species of diatoms. This is the first time that these species will be reported occurring both in natural salt water and hot spring in the Philippines.

The 11 species of cyanobacteria observed increased the number of total species observed in hot springs to 48 for the country (Table 1), while increasing the number of total species found in marine habitats to 76 (Table 2). The filamentous cyanobacteria were relatively more dominant in the cyanobacterial mats compared to the coccoid forms. Of the filamentous types, Lyngbya semiplena was observed to be the most abundant in all three pools (47 filaments/ microscopic field, or mic field), followed in decreasing order by Oscillatoria subbrevis(41), Spirulina major (39), Leptolyngbya treleasii, (28) and Ôscillatoria geminata (20) All these species were observed to decrease in abundance in pool #3, the pool farthest from the exception to this case was Lyngbya spring source. An semiplena that was consistently in great abundance in all three pools.

Diatoms are reported for the first time in the cyanobacterial mats of natural salt water hot springs in the country, although majority of the diatom species noted here were already observed in different habitats in the country (Mann 1925; Hustedt 1942; Podzorski & Hakansson 1987), except for new country records, such as *Achnanthes brevipes* var. *intermedia* and *Halamphora coffeaeformis*. The diatoms were not as abundant as the cyanobacteria. Their abundance increased with distance from the spring source. The most abundant among the diatoms was *Achnanthes brevipes* var. *intermedia* (17 cells/mic field) , followed in decreasing order by: *Halamphora coffeaeformis* (15), *Nitzschia* cf. *frustulum* (13), *Diploneis didyma* (11), *Gomphonema parvulum* (10), *Cocconeis placentula* var. *euglypta* (9), and *Mastogloia crucicula* (7).

# Table 3: Physico-chemical analyses of the pool waters in Maquinit Hotspring, Coron, Palawan in 1985, 2015, 2017.

PARAMETER	1985*	2015**	2016, September 1***	2017(August 7)****
Free CO <sub>2</sub> (ppm)	4.40			
Na (ppm)	8,500.00			
K (ppm)	400			
Mg (ppm)	1,065.00			
Ca (ppm)	850			
S (ppm)	2.3			
As (ppm)	0.05			
SO <sub>4</sub> (ppm)	2,544.80			
Cl (ppm)	18,857.00			
Fe (ppm)	0.005			
$H_2S$	Nil			
Solids (ppm)	14,092.00			
Total acidity	5.00			
Salinity (ppt)		40		35
pH	6.95	7.6 - 7.7	7.6 - 7.7	8.09
Water temperature (°C)	38- 41			40.6
Light intensity (klux)				68.085

\*Sincioco & Bautista, 1985.

\*\*From: Fe Zulaybar, pers. comm.

\*\*\* Range of readings at noon time in the three pools, taken by Lia J. Ramos

\*\*\*\*Average of nine readings in the three different pools at three different time of the day, taken by MRMG and EDLRA.

Table 4: List of cyanobacteria in the cyanobacterial mats and the associated diatoms observed in Maquinit Hot Springs, Coron, Palawan, Philippines in 2005, 2015, and on August 7, 2017.

A. Cyanobacteria

- Unicellular, colonial types Chroococcus minutus (Kützing) Nägeli C. turgidus (Kützing) Nägeli Aphanocapsa incerta (Lemmerman) G. Cronberg & Komárek Pleurocapsa minor Hansgirg Myxosarcina amethystina J.J. Copeland
- Filamentous, homocystous Oscillatoria subbrevis Schmidle
  O. geminata Schwabe ex Gomont Leptolyngbya treleasii (Gomont) Anagnostidis & Komárek Lyngbya semiplena (C. Agardh) J. Agardh Spirulina major Kützing ex Gomont
  Filamentuos, heterocystous type
- Calothrix thermalis (Schwabe) Hansgirg

# B. Bacillariophyta

 Mononraphid Achnanthes brevipes var. intermedia (Kützing) Cleve

Cocconeis placentula var. euglypta (Ehrenberg) Grunow

 Biraphid Halamphora coffeaeformis (C. Agardh) Levkov Mastogloia crucicula (Grunow) Cleve Gomphonema parvulum (Kützing) Kützing Diploneis didyma (Ehrenberg) Ehrenberg Nitzschia cf. frustulum (Kützing) Grunow

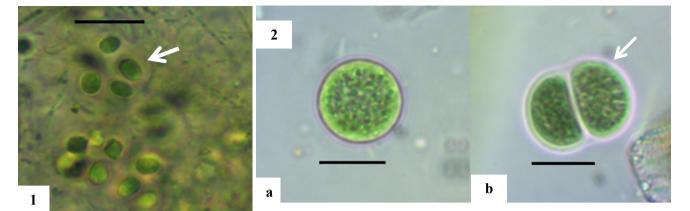
Chlorophyll a is a common chlorophyll component of all photosynthetic algae (Graham, *et al.*, 2009). While chlorophyll c is an accessory pigment of the diatoms but not of the cyanobacteria. Hence, chlorophyll a gives indirectly an indication of the biomass of the total algae of a sample, while chlorophyll c indirectly measures the biomass of the diatoms, if the amount of chlorophyll c was deducted from chlorophyll a, the amount of chlorophyll a is now due to the cyanobacteria that ranged from 10.1  $\mu$ g mL<sup>-1</sup> to 31.165  $\mu$ g mL<sup>-1</sup> (Fig. 3). In our study, chlorophyll a content was higher than the chlorophyll c content ( $\mu$ g mL<sup>-1</sup>) both in the biomass (cyanobacterial mat) and in the phytoplankter in all the three pools, including the outlet (Fig. 3). Likewise, the amount of chlorophyll (a and c) was higher in pools 1 and 2 than in pool 3. The highest chlorophyll a content was noted at 36.3  $\mu$ g mL<sup>-1</sup> in the cyanobacterial mat

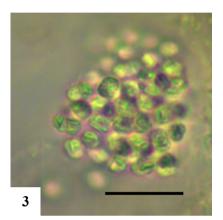
(biomass) in Pool 2, while the lowest value was observed in the phytoplankter in pool 3 at  $1.29 \ \mu g \ mL^{-1}$ . In general, there was a greater percentage of chlorphyll c in relation to chlorophyll a in the phytoplankter (6 - 22%) than in the cyanobacterial mat or biomass (5.8 - 14.2%).

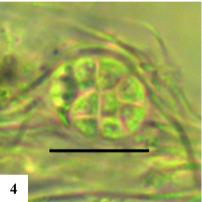
#### **II. Dichotomous keys for the cyanobacteria and the diatoms** Division Cyanobacteria (Cyanophyta)

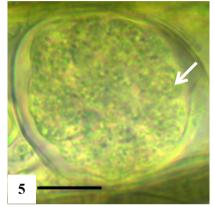
Prokaryotic cells; cell wall made up mostly of peptidoglycan, just like other gram negative bacteria, except that the cyanobacteria have usually thicker peptidoglycan than the latter, in turn their cell walls are usually enclosed by a gelatinous sheath of mucopolysaccharides; the major accessory photosynthetic pigments which include phycobilins, carotenoids, and maybe some amount of chlorophyll d; the phycobilins are the water-soluble pigments such as the phycoerythrin (red), and the blue pigments, as the phycocyanin and allophycocyanin, that are responsible for the blue-green or brownish-blue green color, depending upon the proportion of the red and the blue pigments in the species. Likewise, the thick mucilaginous sheath around the cell walls of many cyanobacteria, such as Scytonema, contains scytonemin, an ultra-violet absorbing compound that gives the organisms a yellow-brown to dark-brown colors (Graham et al., 2009; John et al., 2011); their reserved food product is mainly cyanophycean starch (glycogen); they exhibit vegetative means of reproduction only, such as by cell fission, fragmentation of colonies and filaments, hormogonia formation, or spore formation.

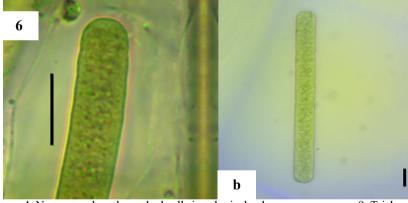
Dichotomous Key for the species in the division Cyanobacteria 1. Cells unicellular or multicellular organized in colonial form
1. Cells multicellular organized in filamentous form
<ol> <li>Solitary or few cells (2-5) in a colony</li></ol>
4. Numerous loosely-packed cells in an irregularly-shaped colony











4. Numerous densely-packed cells in spherical colony

5. Colonial sheath thin, hardly visible

Pleurocapsa minor
5. Colonial sheath thick, hyaline
Myxosarcina amethystina
6.Homocystous filaments8
6.Heterocystous filamentsCalothrix thermalis
7. Trichomes without obvious gelatinous sheath
7. Trichomes enclosed by thick gelatinous sheath
Lyngbya semiplena
8. Trichomes straight, not forming a regular, close spiral
8. Trichomes forming a regular, close spiral
Spirulina major

**Plate I.** Fig. 1. *Chroococcus minutus* (Kützing) Nägeli,  $\downarrow$  colonial sheath; Fig. 2. *Chroococcus turgidus* (Kützing) Nägeli. a. unicellular cell, b. colony of two cells,  $\downarrow$  colonial sheath; Fig. 3. *Aphanocapsa incerta* (Lemmerman) G. Cronberg & Komárek; Fig. 4. *Pleurocapsa minor* Hansgirg; Fig. 5. *Myxosarcina amethystina* J.J. Copeland,  $\downarrow$  showing probably a portion of a colony producing endospores; Fig. 6. *Oscillatoria subbrevis* Schmidle, a. the filament showing the apical cell; b. a hormogonium. all bar scales = 10 µm. All photos are under 1000x magnification.

Division Bacillariophyta

Eukaryotic cells; cell wall is composed of amorphous opaline silica with organic coatings, not usually enclosed by a gelatinous sheath; their major accessory photosynthetic pigments are beta carotene and the xanthophyll called fucoxanthin that results in the golden-brown or brown pigmentation of the diatoms; their reserved food products are chrysolaminarin and lipids; they reproduce vegetatively by means of binary fission; sexual means of reproduction is by oogamy among the centric diatoms but usually have isogamy for the pennate diatoms; they exhibit gametic meiosis, hence, the zygote form or auxospores undergo mitosis, and subsequently generating vegetative cells that are diploid, hence, they have a diplontic life cycle.

Dichotomous key to the species in the division Bacillariophyta
1. Valves monoraphid2
1. Valves biraphid

2. Valves linear to lanceolate or naviculoid

6. Valves constricted in the median portion, true raphe centrally located......Diploneis didyma
6. Valves not constricted at the median portion, true raphe eccentrically located.....Nitzschia cf. frustulum

# **III.Taxonomy**

I. Cyanobacteria

Plate I, Fig. 1

Chroococcus minutus (Kützing) Nägeli

Tilden, Myxophyceae of North America and Adjacent regions, 7, 1910; Desikachary, Cyanophyta, 103-105, pl. 26, fig 15, 1959; John *et al*, The Freshwater Algal Flora of the British Isles, 54, pl. 11, fig F, 2011.

Colony spherical or subspherical, may be unicellular or made up of 2-5 cells,  $5.3 - 18.2 \,\mu$ m dia (diameter), but commonly  $5.3 - 6.1 \,\mu$ m dia; enclosed by a clear or colorless colonial gelatinous sheath ca.  $0.45 - 1.0 \,\mu$ m thick; cells spherical, obovoid or ovoid, homogenous cytoplasm, blue-green,  $2.4-6.6 \,\mu$ m dia; solitary cells,  $3.2 - 3.6 \,\mu$ m dia; enclosing cell sheath colorless,  $0.34 - 0.60 \,\mu$ m thick. Observed several cells in a colony; associated with *Oscillatoria* spp., *Lyngbya* and *Spirulina* in a hot spring pool (ca.  $41^{\circ}$ C) with salt water (40 ppt),

**Distribution:** USA, Marine, (Tilden 1910); Sri Lanka (Ceylon), tycholimnetic, Myanmar (Burma), Pakistan, Lahore (on soil), India, on soil, rice fields, epiphytic (Desikachary 1959); England, Merseyside, marine, supra-littoral or intertidal (Russell 2009).

**Philippines:** LUZON: Bulacan, Bulacan (fishponds, brackish water), Boonmee (1979); Batangas, Taal (Taal Lake), Zafaralla (1998); Palawan, Coron (**this specimen is a new distributional record**), VISAYAS: Iloilo, Cabatuan (moist side of a kiosk in a plaza), Soriano (1953), Drouet & Daily (1956); Leyte, Tacloban City (small pool at air strip), Drouet & Daily (1956), Velasquez (1962), MINDANAO: Sulu, Tawi-Tawi, Bongao (on concrete floor), Drouet & Daily (1956).

**Specimen:** Palawan, Coron (Maquinit Hot Springs, small pool #1, front, November 10, 2015, April 25, 2016), MRMGoss s.n. Photograph prepared from mounted specimen, (Herbarium (Herb.) No. M15-1-a-c, M16-1, a-c, CAHUP).

#### *Chroococcus turgidus* (Kützing) Nägeli

Plate I, Fig. 2a-b

Tilden, Myxophyceae of North America and Adjacent regions, 5-6, 1910; Desikachary, Cyanophyta, 101-102, pl.26, fig 6, 1959; John *et al*, The Freshwater Algal Flora of the British Isles, 54-55, pl. 6, fig D, 2011.

Colony usually spherical, of one to three cells,  $13 - 21 \mu m$  in dia, enclosed by a colorless thick hyaline gelatinous sheath,  $1 - 1.48 \mu m$  thick; cells unicellular or tightly packed in twos or threes together in a colony with homogenous content, blue-green later becoming olive green. Found associated with *Oscillatoria* spp., *Spirulina* and other coccoid cyanobacteria in a salt water (40 ppt), hot spring pool (ca. 41°C).

**Distribution:** USA, marine habitats, Massachusetts, on slimy rocks and piers, Cape Ann, New York Pier, Stapleton, Staten Is., Washington, brackish water, Whidbey Is., California, brackish waters, Hawaii Is. (Tilden 1910); Pakistan, mangroves, India (Desikachary 1959); England, brackish ponds, salt marshes (West & Fritsch 1927), marine supra-littoral and intertidal zone (Russell 2009).

**Philippines:** LUZON: Bulacan, Bulacan (fishponds), Boonmee (1979); Pangasinan, Alaminos, Lucap Bay (on rocks), Agor (1962); Rizal, Malabon, Navotas (fishponds; stomach of milkfish), Vicencio (1977); Rizal, Quezon City, UP (floating & submerged in lowland ricefields), Koh (1964); Laguna, Los Baños, Mayondon (fishponds), Martinez (1976); Or. Mindoro, Pto. Galera, Velasquez (1952), (ponds), Trono (1956); Palawan, Coron (this specimen is a new distributional record), MINDANAO: Sulu, Tawi-Tawi, Bongao (on concrete floor, with *Nostoc* filaments), Velasquez (1955).

**Specimen:** LUZON: Palawan, Coron (Maquinit Hot Springs, small pool #1, front), November 10, 2015, April 25, 2016 MRMGoss s.n. Photograph prepared from mounted specimen, (Herb. No. M15-1-a-c, M16-1-a-c, CAHUP).

#### Aphanocapsa incerta (Lemmerman) G. Plate I, Fig. 3 Cronberg & Komárek

Tilden, The Myxophyceae of North America, 35-36, 1910; Crow, the New Phycologist. 22(2): 59-68, 1923; Drouet & Daily, Butler University Botanical Studies. 12(2): 45,65, fig 16-99, 1956; Desikachary, Cyanophyta, 97, 1959; Prescott, Algae of the Western Great Lakes Area, 457, pl. 102, fig. 5, 1962; Velasquez, Philippine Journal of Science, 91 (3): 278-279, pl. 1, fig. 5, 1962; John *et al*, the Freshwater Algal Flora of the British Isles, 41-42, pl. 15B, 2011.

=*Microcystis incerta* (Lemmerman) Lemmerman =*Microcystis pulverea* var. *incerta* (Lemmerman) Crow =*Anacystis montana* (Lightfoot) Drouet & Daily

*=Microcystis pulverea* (Wood) Forti

=Anacystis montana (Kützing) Drouet & Daily =Anacystis montana f. minor Drouet & Daily

Colony irregular in form, appearing more clathrate, may reach up to 100  $\mu$ m in diameter, made up of more or less loosely arranged cells, enclosed by a thin, usually inconspicuous, gelatinous sheath; cells pale to blue-green, with 2-3 elongated greenish granules measuring about 0.44 - 0.99 x 0.2 - 0.27  $\mu$ m (L x W, length x width); no visible gas vesicles, cells 1.5 - 2.5  $\mu$ m in dia. Associated with *Spirulina major* in hyper saline (ca. 40 ppt) and hot spring waters (ca. 41°C) on limestone.

**Distribution**: USA, Pennsylvania on the bottom of limestone spring, in "Boiling Streams", (Tilden 1910); in hard and soft water lakes, (Prescott 1962); Sri Lanka, Colombo, lake rich in limestone (Desikachary 1959); Israel, Lake Kinneret (Zohary *et* 

*al* 2014); England, frequent in plankton of shallow, moderately nutrient rich standing water (John *et al.*, 2011).

**Philippines:** LUZON: Rizal, Marikina (on aeration tank), Soriano (1952), Navotas, Manila; Or. Mindoro, Pto. Galera; Palawan, Coron, Cuyo, Drouet & Daily (1956), VISAYAS: Iloilo, Passi (on old adobe walls). Buenavista (high cliffs), Leganes (fishponds), Pototan (old stone walls); Iloilo City (on concrete walls); Antique, San Jose (old adobe walls on beach); bark of mango tree), Soriano (1953), MINDANAO: Sulu, Jolo, Busbus (on soil) Velasquez (1955).

**Specimen:** LUZON: Palawan, Coron (Maquinit Hot Springs, small pool #1), MRMGoss s.n. Photograph prepared from mounted specimen, (Herb. No. M16-2-a-c, CAHUP). (An additional new collection).

*Pleurocapsa minor* Hansgirg Plate I, Fig. 4 Tilden, The Myxophyceae of North America, 46-47, 1910; Smith, Freshwater Algae of the United States, 568, fig 477; John *et al*, The Freshwater Algal Flora of the British Isles, 71, pl. 16E, p.73.

Colony an irregular spherical or ellipsoidal form of compactly arranged spherical or polygonal cells (about 12), at least 15  $\mu$ m in diameter; polygonal shape of cells might be due to compaction of cells in the colony, pale green to blue-green with green pigmented granules of varying shapes, cells 2.5 – 3  $\mu$ m dia. Associated with other filamentous cyanobacteria, such as *Oscillatoria* spp. and *Lyngbya* in a hot spring (ca. 41°C) and saline (40 ppt) waters.

**Distribution:** USA, marine, Great Salt Lake (Smith 1950); England, marine on rocks in very nutrient-rich hard water rivers extends up to uppermost part of the tidal zone (John *et al.*, 2011).

# Philippines: (a new country record).

**Specimen:** LUZON: Palawan, Coron (Maquinit Hot Springs, small pool #1, front, November 10, 2015), MRMGoss s.n. Photograph prepared from mounted specimens, (M16-1-a-c, CAHUP).

*Myxosarcina amethystina* J.J. Copeland Plate I, Fig. 5 Copeland. New York Acad. Sci., 36: 69, fig 31, 1936; Smith, Freshwater Algae of the United States, 569, fig 479 A-G, 1950; Brown, Ohio J. of Sci., 65(1): 20-21, fig. 7A,1965.

Colony made up of few to several compactly arranged cells, enclosed by a hyaline but firm colonial gelatinous sheath ca. 3 -4.0  $\mu$ m thick , hemispherical colony 40 - 45 x 23 - 28  $\mu$ m (L x W), rounded or cuboidal colony 28.31  $\mu$ m in diameter; cells spherical, cuboidal, hemispherical to polygonal, maybe due to compaction of cells, 8 - 13 x 4.8 - 8  $\mu$ m (L x W), granulated cytoplasm, usually green-pigmented granules that could be endospores; rare, associated with *Oscillatoria* spp. and *Lyngbya* in a marine (40 ppt) and hot spring pool (ca. 41°C).

**Distribution:** USA, Wyoming, hot spring in Yellowstone National Park (Copeland 1936); California-Nevada, Central Death Valley Desert (travertine pond - a form of limestone deposited by mineral spring especially hot spring, water temperature, ca. 29-49°C) (Brown 1965).

#### Philippines: (a new country record).

**Specimen:** LUZON: Palawan, Coron (Maquinit Hot Springs, small pool #1), MRMGoss s.n. Photograph prepared from mounted specimen (M16-1-a-c, CAHUP).

*Oscillatoria subbrevis* Schmidle Plate I, Fig. 6a-b Prescott, Algae of the Western Great Lakes Area, 491, pl. 107, fig 23, 491, 1962; Desikachary, Cyanophyta, 204, 207, 214, pl. 37, fig 2; pl. 40, fig 1, 1959; Martinez, Taxonomy and Ecology of Algae in Fishponds and Fishpens of Laguna, 53, pl. 5, fig 5, 1976; John *et al*, The Freshwater Algae Flora of the British Isles, 96, 98, 100, pl. 20, fig M, N, pl. 21, fig G, 2011.

Non-branching, straight filament, not constricted at the cross walls; apical cell broadly rounded to convex, not capitate, without calyptra; cell content finely and coarsely granulated, but more coarsely granulated at the center; yellowish blue-green to light blue-green, no separation disks observed, but short filaments (hormogonia) observed that had about 40 cells; cells short, 1.1 - 2.1 x 5.3 – 10  $\mu$ m (L x W) separation disk, about 1 $\mu$ m L.

This specimen is similar to the specimens illustrated by Desikachary (1959), pl. 37, fig 2 and to that of John *et al.*, (2011) pl. 21, fig G. Observed differently from the specimens noted in the fishponds of Mayondon, Los Baños, Laguna where the filaments were observed to have so many separation disks (Martinez 1976), probably our specimen underwent fragmentation, hence, more hormogonia were observed. Observed to be moderately abundant; and relatively less abundant in Pools 2 &3 compared to Pool 1. Associated with other *Oscillatoria* and *Lyngbya* species as a component of the cyanobacterial mat in a natural marine and hot spring pool.

**Distribution**: India, on moist banks of River Ravi Punjab; Mumbai (Bombay), temporary rainwater pools (Desikachary 1959); British Isles, freshwater (John *et al.*, 2001).

**Philippines:** Freshwater habitats: LUZON, Laguna, Los Baños (Mayondon, fishponds), Martinez, 1976; Los Baños (on moist soil), Siniloan (on tin core), Pantastico, 1977; Laguna de Bay, Sta Cruz and Pagsanjan (rivers associated with other *Oscillatoria* species), Al-Saboonchi, 1980; Albay, Ligao and Tiwi hot springs, Pantastico, 1977. Palawan, Coron (**this specimen is a new distributional record**).

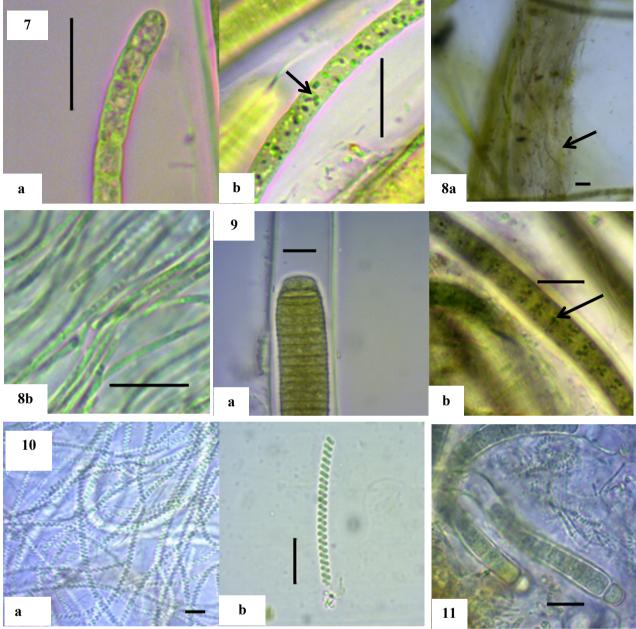
**Specimen**: LUZON: Palawan, Coron (Maquinit Hot Springs, pool # 1, November 10, 2015), MRMGoss. s.n. Photograph prepared from mounted specimen, (M16-2-a-c, CAHUP).

Oscillatoria geminata Schwabe ex Gomont Plate II, Fig. 7a-b

Gomont, Monographie des Oscillarees, 242-243, pl. 7, fig. 6,1893; Tilden, The Myxophyceae of North America, 74-75, 1910; Velasquez, Phil. J. Sci., 295, 377, pl. 2, fig 27, 1962; Cocke, The Myxophyceae of North Carolina, 43, 1967; Setchell & Gardner, The Marine Algae of the Pacific Coast of North America: Myxophyceae, 64, 1967; Dumont *et al*, Limnology and Marine Biology in the Sudan. Developments in Hydrobiology. 2:63, 2012.

Filaments light blue-green, flexuous, sometimes forming loops, usually bent towards the anterior end; apical cell rounded without calyptra; constrictions at cross walls prominent; cytoplasm with few small granules with one to three large refringent granules ( $0.4 - 2.3 \mu m$  diameter) scattered throughout the center of the cell; cells longer than wide,  $2.5 - 5.0 \mu m x 1.0 - 2.9 \mu m (LxW)$ .

Our specimen's dimensions are within the range of what were reported earlier by the above authors, except that the range of the width of our specimen is narrower  $(1.0 - 2.9 \ \mu\text{m})$  than those observed by them  $(2.3 - 4.0 \ \mu\text{m})$ ; however, our specimen is much shorter than the one described by Gomont (1893), which was as long as 2.3 to 16  $\mu$ m long, but both specimens have prominent



**Plate II.** Fig. 7. Oscillatoria geminata Schwabe ex Gomont, a. showing constriction at the cross walls, b. showing refringent granules in the cytoplasm  $\downarrow$ ; 8. Leptolyngbya treleasii (Gomont) Anagnostidis & Komárek. a. showing a portion of the cyanobacterial mat with *L. treleasii*  $\downarrow$ , b. group of filaments; 9. Lyngbya semiplena (C. Agardh) J. Agardh. a. showing the gelatinous sheath around the trichome; b. showing the granulation at the cross walls  $\downarrow$ ; 10. Spirulina major Kützing ex Gomont a. a group of filaments, b. a filament; 11. Calothrix thermalis (Schwabe) Hansgirg; all bar scales = 10 µm. All photos are under 1000x magnification except 8a & 10a under 400x magnification.

refringent granules. Among the homocystous cyanobacteria, this is the least abundant in the cyanobacterial mat and its abundance decreased successively from Pool 1 up to Pool 3; found associated with *Lyngbya* spp. and *Spirulina major*; attached to the gelatinous sheaths of *Lyngbya semiplena*.

**Distribution:** USA, Montana (Lo Lo Hotspring) hot water, Wyoming, covering bottom of creek in swift current, at 475°C; near upper Geyser basin, Yellowstone National Park (Tilden 1898); hot waters (Gomont 1893); type specimens from the thermal waters of the Euganean springs (Setchell & Gardner 1967).

**Philippines:** Fresh water habitats: LUZON: Rizal, Manila (dried by the side of a canal with *Oscillatoria tenuis, O. anguina, P. uncinatum & Scytonema muscorum*), Velasquez (1941, 1962); Or. Mindoro, Pto. Galera (floating in stagnant water), Velasquez

(1962); Palawan, Coron **(this specimen is a new distributional record).** MINDANAO: Lanao, Mt. Malabong, (freshwater lake), Velasquez (1962).

**Specimen:** LUZON: Palawan, Coron, (Maquinit Hot Springs, small pond #1, November 10,2015), MRMGoss s.n. Photograph prepared from mounted specimen (M115-1-a-c, CAHUP).

# *Leptolyngbya treleasii* (Gomont) Plate II, Anagnostidis & Komárek Fig. 8a-b Tilden, Myxophyceae of North America and Adjacent Regions, 96, 1910; Velasquez, Phil. J. Sci., 91(3): 305, pl. 3, fig 49, 1962; Matula, *et al*, Polish Polar Research , 28(4): 300, 2007; John *et al*, The Freshwater Algal Flora of the British Isles, 92-93, pl, 19, fig I, 2011.

=Phormidium treleasii Gomont

=Lyngbya treleasii (Gomont) Compére

Long, slender, mostly straight filaments; sheaths very thin and usually not visible; pale blue-green; trichomes not constricted at the cross walls; apical cells rounded not capitate; intercalary cells longer than wide,  $2.2 - 2.7 \times 0.8 - 1.05 \mu m$  (L x W). Abundant, found associated with *Oscillatoria* spp. and *Spirulina major* in a salt water (40 ppt), hot spring pool (ca 41°C).

Our specimen shares the same morphology as the specimen described by Matula *et al.*, (2007) and Velasquez (1962). However, the foreign specimens were mostly taken from marine habitats or hot sulphur springs while our Philippine specimen is both from a natural salt water and hot spring on limestone rocks. It was a minor component of the cyanobacterial mat, and its abundance decreased successively from Pool 1 to Pool 3.

**Distribution:** marine, Norway (Matula *et al* (2007); USA, Arkansas (hot springs) (Tilden 1910); Canada, Banff (hot sulphur springs) (Tilden 1910).

**Philippines:** LUZON: Nueva Ecija, Munoz (CLSU-FAC, fishponds) (Boonmee 1979); Or. Mindoro, Naujan (hot spring at Naujan Lake) Velasquez (1962); Palawan, Coron (this specimen is a new distributional record).

**Specimen:** LUZON: Palawan, Coron (Maquinit Hot Springs, small pool #1 front, November 10, 2015), MRMGoss s.n. Photograph prepared from mounted specimen, (Herb. No. M15-2-3a-c, CAHUP).

# Lyngbya semiplena (C. Agardh) J. Agardh Plate II, Fig. 9a-b

Gomont, Monographie des Oscillarees, 158-159, pl. 3, fig. 7-11, 1893; Tilden, Myxophyceae of North America and Adjacent Regions, 4:118-119, pl. 5, fig 38, 1910; Desikachary, Cyanophyta, 315, pl. 49, fig 8 & pl.52, fig 7, 1959; Velasquez, Phil. J. Sci., 91(3): 319, pl. 3, fig 66, 1962; Reyes, Phil. J. Sci., 105(3): 140, pl. 1, fig11-12, 1976; John *et al*, The Freshwater Algal Flora of the British Isles, 92-93, pl, 19, fig I, 2011.

Aggregates of long filaments; mostly dark or dull yellowishgreen, trichomes not constricted at the cross walls, numerous small granules (probably of polyphosphate bodies) at the cross walls (John et al 2011); cells towards the apex getting narrower, apical cells broadly rounded, end cells with weak conical or rounded calyptra (thickening of outer wall of end cell); cells 1/3-1/6 as long as broad, 6.0 - 15  $\mu$ m x 1.5 - 3.5  $\mu$ m (L x W); gelatinous sheath colorless, but sometimes lamellated, 0.9 - 2.39  $\mu$ m thick; separation disk between trichomes could be 10  $\mu$ m long. Our specimen has narrower gelatinous sheath than that described by Desikachary, 1959; Reyes, 1976 and John et al, 2011; relatively, the most abundant among the homocystous cyanobacteria in the cyanobacterial mat in all three pools; found associated with *Oscillatoria* spp. and *Spirulina major* in a natural salt water (40 ppt) and hot spring pool (ca. 41°C).

**Distribution:** marine, Mediterranean; Tahiti, (Gomont 1893); USA, marine (Tilden 1910); India, marine, rocky puddle at seashore (Desikachary 1959); England, marine, especially on rocks in pools in supra-littoral and at upper intertidal zone (Powell 1964).

**Philippines:** marine habitats, LUZON: Pangasinan, Lucap Bay (on bancas), Agor (1962); Rizal, Pasay City (yellowish bluegreen to blue-green growth); Or. Mindoro, Pto. Galera (with *L. confervoides* in a tide pool from rocky cliffs), Velasquez (1962), Palawan, Coron (**this specimen is a new distributional record**); VISAYAS: Siquijor (attached to small dead corals or plants at the upper intertidal zone), Reyes (1976).

Specimen: LUZON: Palawan, Coron (Maquinit Hot Springs, small pond #1 front, November 10, 2015), MRMGoss s.n.

Photograph prepared from mounted specimen, (Herb. No. M16-2-a-c, CAHUP).

*Spirulina major* Kützing ex Gomont Plate II, Fig. 10 a-b Gomont, 1893, Monographie des Oscillarees, 271, pl. 7, fig. 29, 1893; Desikachary, Cyanophyta, 196-197, pl. 36, fig. 13, 1959; Velasquez, Phil. J. Sci., 91(3): 283-284, pl. 1, fig. 12, 1962; Mizuno, Illustration of the Freshwater Plankton of Japan, 114-116, pl. 44, fig. 5, 1979; John *et al.*, The Freshwater Algal Flora of the British Isles, 115, pl. 23F-H, 2011.

Trichomes thin, regularly spiraled or twisted, 1 - 2.0  $\mu$ m wide, distance between coils or spirals 0.5 - 1.03  $\mu$ m; cytoplasm homogenous, blackish-green to bright blue-green.

Our specimen has narrower width and tighter coils/spirals than the one described by Gomont (1893); Desikachary (1959), Velasquez (1962), and John *et al.*, (2011). However, based on the figures presented by the above authors, our specimen looks closely like those in the following figures: Desikachary, p. 194, pl.36, fig 13, 1959; Velasquez, pl. 1, fig 12, 1962; Mizuno, p. 114, pl. 44, fig 5, 1979; John et al, pl 23, fig H, 2011; moderately abundant in the cyanobacterial mat; it was more abundant in Pool 1 than in Pools 2 & 3; observed associated with *Oscillatoria* spp. and *Lyngbya* in a natural salt water (40 ppt) and hot spring pool (ca 41° C).

**Distribution**: Myanmar (Burma), Rangoon: in salt lakes (Desikachary 1959); England, North West county, Merseyside: in upper intertidal or in salt pans or damp sand, in dune slacks, but rare (Powell 1964).

**Philippines:** Marine habitats: LUZON: Or. Mindoro, Pto. Galera (on hard corals), (Velasquez 1979), Palawan, Coron (this specimen is a new distributional record); VISAYAS: Cebu, Silut Bay, rare, Almase (1970), Iloilo, on hard corals, Velasquez (1979); Leyte, Laman, on submerged sticks in a ditch with *Oscillatoria sancta*, Velasquez (1962), Tacloban, on hard corals, Velasquez (1979). Freshwater habitats: LUZON: Bulacan, Calumpit (on the side of a canal with *Oscillatoria princeps, O. tenuis, & O. chlorina*), Velasquez (1941); Rizal, Malabon, AU (culture pond), Trono (1961), Navotas (fishponds; stomach of milkfish), Vicencio (1977); Laguna, Los Baños (rice paddies), Pantastico and Suayan (1973-1974), Martinez (1979), San Pedro, Talim Is., Laguna de Bay (rice paddies), Pantastico (1977), Tadlak Lake, Gonzales (1961), Sta. Cruz and Pagsanjan (rivers), Laguna de Bay, Al-Saboonchi (1980).

**Specimen:** LUZON: Palawan, Coron (Maquinit Hot Springs, small pond #1, front), November 10, 2015, MRMGoss s.n. Photograph prepared from mounted specimen, M15-1-a-c, M16-3-a-c, CAHUP).

Calothrix thermalis (Schwabe) Hansgirg	Plate II,
	Fig. 11
Tilden, The Myxophyceae of North America, 268, pl	l. 18, fig 1,
1910; Desikachary, Cyanophyta, 533-535, pl.114, fig	, 10, 1959.

Filaments usually straight and short, basipetally oriented in growth, with the vegetative cells tapering towards the apical end; heterocyst, one, basally located, sub-spherical, up to 4.52 - 5.03 µm long, 8 µm wide; vegetative cells near the basal heterocyst, wider than long, 3.1 - [6.5] - 8.5 µm wide, but towards the apical end they tend to be longer than wide, ca.  $3.60 \times 3.09$  µm (L x W); cells blue green, homogenous , gelatinous sheath hyaline or colorless thicker towards the base but gets thinner towards the tip. Our specimen differs from those described above by the colors of vegetative cells as blue green and not yellowish or olive colored and the filaments are shorter. Rare, associated with *Oscillatoria* spp., *Lyngbya, Spirulina* and other coccoid

cyanobacteria in a salt water (40 ppt) and hot spring pool (ca 41°C).

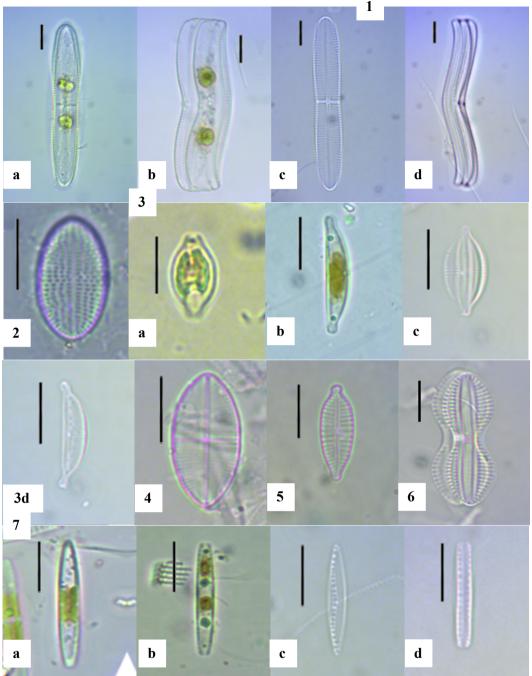


Plate III. Fig 1. Achnanthes brevipes var. intermedia (Kützing) Cleve, a. showing he valve view with two chromoplasts, m b. showing the girdle view with two chromoplasts, c. cleaned valve view, d. cleaned girdle view; 2. Cocconeis placentula var. euglypta (Ehrenberg) Grunow showing the cleaned araphid valve (ARV); 3. Halamphora coffeaeformis (C. Agardh) Levkov a. valve view showing the protoplast, b. girdle view showing the protoplast, c. cleaned valve view; 4. Mastogloia crucicula (Grunow) Cleve, cleaned valve view; 5. Gomphonema parvulum (Kützing) Kützing, cleaned valve view; 6. Diploneis didyma (Ehrenberg) Ehrenberg, cleaned valve view; 7. Nitzschia frustulum (Kützing) Grunow, a. valve view showing the protoplast, b. girdle view showing the protoplast, c. cleaned valve view; d. cleaned girdle view; 4. Mastogloia crucicula (Grunow) Cleve, cleaned valve view; 5. Gomphonema parvulum (Kützing) Kützing, cleaned valve view; 6. Diploneis didyma (Ehrenberg) Ehrenberg, cleaned valve view; 7. Nitzschia frustulum (Kützing) Grunow, a. valve view showing the protoplast, b. girdle view showing the protoplast, c. cleaned valve view, d. cleaned girdle view; all bar scales = 10 µm. All photos are under 1000x magnification.

**Distribution:** USA: Wyoming, overflow of channel of geyser, 49-54.5°C, temperature 49-50°C. Fountain Hotel Geyser Basin; Yellowstone National Park (Tilden, 1910).

# Philippines: (A new country record).

**Specimen:** LUZON: Palawan, Coron (Maquinit Hot Springs, small pool #1, front), November 10, 2015, MRMGoss s.n. Photograph prepared from mounted specimen, (Herb. No. M15-1-a-c, CAHUP).

#### II. Bacillariophyta,

Achnanthes brevipes var. intermedia Plate III, Fig. 1a-d (Kützing) Cleve

Heurck, A Treatise on the Diatomaceae, 279, pl. 8, fig 325, 1896; Round, *et al*, The Diatoms, 93-96, 1990; Toyoda & Williams, Diatom. 20:159-169, fig 1-18, 2004.

- = Achnanthes intermedia Kützing
- = Achnanthes subsessilis Ehrenberg
- = Achnanthes subsessilis Kützing

Cells heterovalvar, monoraphid; with two chromoplasts, paedogamous; valves more or less elliptical with obtuserounded apices, constricted at the median portion; araphid valve (ARV), with a slightly laterally pseudoraphe that may appear biconvex, striae uniseriate, 10 in 10 $\mu$ m, ARV 62 x 9 $\mu$ m (L x W); raphe valve (RV) with a true raphe extending entire length of the valve face, central area forms distinct and thickened stauros, striae uniseriate, 10 in 10 $\mu$ m, RV 70-76 x 9-16.5  $\mu$ m (L x W); girdle view of frustule geniculate or flexuous where the RV appears concave, the ARV appears convex.

This specimen is similar to *Achnanthes subsessilis* Ehrenberg based on its morphology and habitat (Heurck 1896), but this name has been synonymized by Krammer & Lange-Bertalot, 1988; relatively more abundant in pool 3 than in Pools 1 & 2 (Fig. 3); most of the time found associated with *Lyngbya semiplena*.

**Distribution:** Marine, Holland, England, Scotland, Iceland and throughout the North Sea (Heurck 1896); Germany, Berlin (Toyoda & Williams 2004).

#### Philippines: (A new country record).

**Specimen:** LUZON: Palawan, Coron (Maquinit Hotsprings, abundant in small pool #2, November 10, 2015, and big pool #3, April 25, 2016), MRMGoss s.n. Photograph prepared from mounted specimen, (slide nos. MQ 2015-11-10-A, MQ 2016-04-25, CAHUP).

# Cocconeis placentula var. euglypta Plate III, (Ehrenberg) Grunow Fig. 2 Hustadt Internal Pay

Hustedt, Internal. Rev. der gesamten Hydrobiologie und Hydrographie. 42(1/3), 32, fig. 802c, 1942; Patrick & Reimer, The Diatoms of the United States. Vol. 1, 241-242, pl. 15, fig. 8, 1966; Podzorski & Hakansson, Freshwater and Marine Diatoms of Palawan 45, pl. 14, fig. 4, 1987; Rai, Nepalese J Biosciences, 1: 107, fig. 14, 2011; Romero & Jahn, Diatom Research, 2013,28(2): 175-184.

= Cocconeis placentula var. euglypta (Ehrenberg) Cleve = Cocconeis euglypta Ehrenberg

Cells solitary, usually found in valve view; valves broadly oval, the araphid valve (ARV) was commonly observed; ARV 18 - 20 x 10 - 12  $\mu$ m (L x W); striae conspicuous as "dashes", 18 - 19 in 10  $\mu$ m; the raphe valve (RV) was not observed, but MRMG observed the raphe valve (RV) of this species from earlier collection of epiphytic diatoms on *Sargassum* in Quezon, Plaridel (Tumaggay beach, marine). Based on the latter observation, RV with filiform raphe, striae curved radiate, finely punctate, interrupted near the margin by a hyaline area, another or a second hyaline area encircles the valve at the margin, isolating a short, striate sub-marginal area; striae 20 - 23 in 10  $\mu$ m.

This specimen has the same morphology and habitat (marine habitat on limestones) as that one described from Palawan by Podzorski & Hakansson (1987). However, they identified their specimen as *Cocconeis placentula* Ehrenberg var. *placentula*. Our samples were taken also from Palawan but from hot spring pools with marine waters. However, we used the name *C. placentula* var. *euglypta* because the nominate variety has wider range of length but our specimen had constantly shorter dimensions in apical axis (length). Rare in Pool 3.

**Distribution:** cosmopolitan, freshwater, brackish water, marine, (Heurck 1896); USA, a widespread eurytopous species epiphytic on aquatic plants; apparently salt "indifferent", but not observed in great numbers in slightly brackish waters (Patrick & Reimer 1966); Nepal, Morang (Betana wetland and river, freshwater) (Rai, 2011).

**Philippines**: LUZON: Laguna, (Laguna de Bay), Hustedt, 1942; Palawan (cosmopolitan, freshwater and brackish water) Podzorski & Hakansson, 1987.

**Specimen:** LUZON: Palawan, Coron (Maquinit Hot Springs, pool #3, April 25, 2016), MRMGoss s.n. Photograph of the ARV prepared from mounted specimen, (slide no. MQ 2016-04-25 C, CAHUP). (An additional collection).

Halamphora coffeaeformis (C. Agardh)Plate III,LevkovFig. 3a-dHeurck, A Treatise on Diatomaceae, 125, 132, pl. 1, fig 6, 1896;Smith, Freshwater Algae of the United States, 501-502, 1950;Archibald & Schoeman, S. Afr. J. Bot., 3:83-102, 1984; Roundet al, The Diatoms, 600-603, 1990; Wang et al, Algae, 69, fig6J-M, 2015; Stepanek & Kociolek, Protist, 165: 177-195, 2015.

*=Amphora salina* W. Smith

=Amphora coffeaeformis (C. Agardh) Kützing

=Frustulia coffeaeformis C. Agardh

Cells solitary or maybe in pairs when the cells lie on their valve views seemingly attached on their concave (ventral) sides; most often the single cells are observed in girdle view ; valves cymbiform, slightly constricted near the poles (ends); ends rostrate to slightly capitate; raphe gibbous, lies very close to the concave (ventral), margin of the valve; has single H-shaped chromoplast with single pyrenoid, observed better in girdle view, valves  $12 - 26 \ \mu m \ x \ 3.45 - 5.49 \ \mu m$  (width at widest, middle portion); striae very fine, about 21 in 10 \mum.

Frustules in girdle view broadly elliptical, narrowing towards the poles (ends), with truncate ends;  $12.43 - 19.14 \,\mu\text{m} \ge 6.7 - 7.8 \,\mu\text{m}$  (width at widest, middle portion). Relatively more numerous in Pool 2 compared to Pools 1 &3; associated with other diatoms.

**Distribution:** Marine, common in Belgium (Antwerp), England, Scotland, Ireland, Denmark and throughout the North Sea (Heurck 1896); Korea (Wang et al., 2015); mostly marine but confined in tropical waters (Smith 1950).

# Philippines: (A new country record).

**Specimen:** LUZON: Palawan, Coron (Maquinit Hot Springs, Pool #2, November 10, 2015; Pool #3, April 25, 2016), MRMGoss s.n. Photograph prepared from mounted specimens (slide nos. MQ 2015-11-10-A, MQ 2016-04-25, CAHUP).

*Mastogloia crucicula* (Grunow) Cleve Plate III, Fig. 4 Podzorski & Hakansson, Freshwater and Marine Diatoms of Palawan 68, pl. 25, fig. 5, 1987; Round, et al., The Diatoms, 678, 1990; Louvrou & Economou-Amilli, Nova Hedwigia, 4-5, fig 1-2, 2016.

*= Orthoneis crucicula* Grunow

= Mastogloia quadrinotata Östrup

Cells solitary, usually found in valve view; broadly-spherical; with rounded to slightly truncate ends; valves flat, with a bright hyaline stauros in the middle, ca 0.612  $\mu$ m long; four partecta; striae 20 in 10  $\mu$ m; valve 21 x 10  $\mu$ m (L x W).

This specimen shares the same morphology and marine habitat as the one described by Gaiser (2007) from Florida Bay, USA but it is larger than the one described by Podzorski & Hakansson (1987) from Honda Bay, Palawan. Rare, associated with other diatoms, farther from the source of the natural salt water hot spring (in Pool no. 3).

Distribution: marine, USA, Florida Bay (Gaiser 2007).

Philippines: LUZON: Palawan, Honda Bay (Podzorski & Hakansson 1987.

**Specimen:** LUZON: Palawan, Coron (Maquinit Hot Springs, pool #3, April 25, 2016), MRMGoss s.n. Photograph prepared from mounted specimen, (MQ 2016-04-25 MD, CAHUP). (An additional collection).

#### Gomphonema parvulum (Kützing) Plate III, Fig. 5 Kützing

Heurck, A Treatise on the Diatomaceae, 272, pl. 7, fig 306, 1896; Hustedt, Rev. der gesamten Hydrobiology und Hydrographie, 42, 1942; Patrick & Reimer, The Diatoms of the United States, vol. 2, part I, 122-123, pl. 17, fig. 7, 1975; Podzorski & Hakansson, Bibliotheca Diatomologica, 63, pl.22, fig 6, 1987; Round *et al.*, The Diatoms, 494, 1990.

Cells usually observed solitary, but more of a colonial form; observed attached to the filamentous cyanobacteria and their usual mode of attachment is by means of a stalk , which is a single structure with a certain amount of internal differentiation; stalks are produced from areas of special pores at one or either pole of the organism (Round *et al.*, 1990); girdle view wedge-shaped; valves naviculoid-cuneate, asymmetrical to the transverse axis, one half of the valve (towards the base pole) often narrower than the other pole (head pole), hence, they are heteropolar. Head pole wide, rounded, and capitated; raphe straight, central; a small stigma present towards the middle of valves; striae, uniseriate, radiate, of equal length, except at the median much shorter, striae 13 - 14 in 10 $\mu$ m; valves 17.5 - 18  $\mu$ m long; width at the widest head pole side 6.0 - 6.21  $\mu$ m; at the narrow side, width 5.6- 5.8  $\mu$ m.

Rare, found in the pool farther away from the source of the natural salt water hot spring (41°C.)

**Distribution:** Cosmopolitan, more of a freshwater species (Hustedt 1942; Patrick & Reimer 1975; Podzorski & Hakansson 1987).

Philippines: LUZON: Cagayan, Singuan Lake; Laguna, Los Baños (Bureau of Science), Laguna de Bay; Camarines Sur, Buhi (Lake Buhi, river); Oriental Mindoro, Naujan (Naujan Lake), (Hustedt 1942); Palawan, Pto. Princesa, Roxas (lake, running water) (Podzorski & Hakansson 1987). MINDANAO: Lanao (Lanao Lake); Sulu, Jolo (Seit Lake), (Hustedt 1942).

**Specimen:** LUZON: Palawan, Coron (Maquinit Hotsprings, pool #3, April 25, 2016), MRMGoss s.n. Photograph prepared from mounted specimen, (slide no. MQ 2016-04-25 CG, CAHUP). (An additional collection).

#### Diploneis didyma (Ehrenberg) Ehrenberg

Plate III, Fig. 6

Bailey, Proc. Nat. Sci. Phila., 6: 431-432, 1853; Heurck, A Treatise on the Diatomaceae, 193, pl. 3, fig 147, 1896; Mann, Marine Diatoms of the Philippine Islands, 101, 1925; Patrick & Reimer, The Diatoms of the United States, 417, pl. 38, fig 14, 1966; Round *et al*, The Diatoms, 562-563, 1990.

= Navicula didyma Ehrenberg

= Navicula (Pinnularia) didyma Ehrenberg

Cells usually unicellular, lying on its valve view, slightly undulating valve face; two chromoplasts one on either side of the apical plane; linear-elliptical, constricted at the center or panduriform, ends broadly rounded; central nodule quadrate; longitudinal canals narrow, slightly wider at the broader portion of the valve face; costae radiate, 11 in 10  $\mu$ m, crossed by undulating ribs; valve 37.5  $\mu$ m long; 10  $\mu$ m wide at the median constriction, 15 - 15.5  $\mu$ m wide at the widest portion. Rare, in a pool (Pool 3) farther from the source of the natural salt water hot spring.

**Distribution:** Marine, Belgium, England and throughout Europe (Heurck 1896).

**Philippines:** all over coastline of the different islands of the Philippines (Mann, 1925); MINDANAO: marine (Bailey, 1853);

**Specimen:** LUZON: Palawan, Coron (Maquinit Hot Springs, pool #3, April 25, 2016), MRMGoss s.n. Photograph prepared from mounted specimen, (MQ 2016-04-25 MD, CAHUP). (An additional collection; a new distributional record)

# *Nitzschia* cf. *frustulum* (Kützing) Plate III, Fig. 7a-d Grunow

Heurck, A Treatise on the Diatomaceae, 403, pl. 17, fig 564-565, 1896; Reimer, 185, fig. 4-F, 1954; McMillan & Rushforth, Pacific Science, 39 (3): 294-301, fig. 2-42, 1985; Krammer & Lange-Bertalot, Bacillariophyceae, 94, pl. 68, fig 1-9, 1988. =Synedra frustulum Kützing

Cells unicellular, usually observed in valve view; valves narrowly lanceolate, linear-lanceolate, valves slightly concave at the middle, and narrowing towards the ends or poles; ends with rounded to wedge-shaped; valves  $22.4 - 26.13 \times 3.0-3.4 \mu m$  (L x W); carinal dots 13 in 10  $\mu m$ ; striae very fine, not easily observed.

This specimen is similar in length to that of Nitzschia frustulum except it has narrower width, about 1/3 the width of N. cf. frustulum. Hence, it is more closely related to N. frustulum var. minutula Grunow in dimensions (Van Heurck 1896) except that the only references we were able to use were that of Van Heurck (1896) and McMillan & Rushforth (1985). Both references elevated the variety to the species level, as N. minutula Grunow. Therefore, it is safer for us to put our specimen under N. cf. frustulum, following the evaluation of N. frustulum by Reimer (1954) and until such time when more specimens and references can be used for referencing. Our specimen shares the same marine habitat as that of N. frustulum (Heurck 1896; Hallfors 2004) and its hot temperature habitat as in the steam vent in Kilauea Volcano Is. in Hawaii, USA but this is cooled over time for N. minutula (McMillan & Rushforth 1985). Rare, observed to be least abundant in Pool 1 but relatively increased in abundance from Pool 2 to Pool 3. Found associated with other diatoms and filamentous cyanobacteria.

**Distribution:** marine, brackish water (Heurck 1896); planktonic in the Baltic sea at the littoral zone (Hallfors, 2004); steam vent in Hawaii, USA (McMillan & Rushforth 1985).

**Philippines**: LUZON: Palawan, Coron (**this specimen is a new distributional record**). MINDANAO: Lanao (Lanao Lake), (Hustedt 1942), however, the specimen observed here may not be the same as our specimen because this is a freshwater lake.

**Specimen:** LUZON: Palawan, Coron (Maquinit Hot Springs, pool #3, April 25, 2016), MRMGoss s.n. Photograph prepared from mounted specimen, (MQ 2016-04-25-N, CAHUP).

# DISCUSSION

This is a taxonomic study on the common cyanobacteria and diatoms in the cyanobacterial mats collected from the Maquinit Hot Springs (MHS), a natural salt water hot spring in Coron, Palawan, Philippines. The cyanobacteria identified in these sites were also recognized in hot springs abroad by Castenholz (1969, 1977); Ward & Castenholz (2000); & Ghozzi et al., (2013).

The cyanobacteria were dominant over the diatoms in the cvanobacterial mats as shown by chlorophyll analyses and microscopic examination. The chlorophyll a values ranged from  $10.117 - 31.229 \,\mu\text{g mL}^{-1}$ . The maximum value obtained at MHS is noted to be six times greater than the value obtained in vitro unialgal culture of a cyanobacterium, Gloeotrichia natans (5.46 µg mL<sup>-1</sup>, at an absorbance of 0.5 at 664 nm) (Martinez et al., 2001). However, when the MHS value is compared with the value obtained in another cyanobacterial mat from a geothermal spring in India, MHS value is only 2/5 of what was obtained in the latter (max =79.79  $\mu$ g mL<sup>-1</sup> of c-phycocyanin using methanol extraction method) Roy et al., 2017). One possible explanation for the difference between the values in the cyanobacterial mats in the two places could be the difference in methodologies used and the limited chlorophyll analyses done in MHS compared to that in India.

The dominance in abundance of the homocystous filamentous cyanobacteria in the mat seems to be largely determined by the water temperature, i.e. in this case, at the lower end of thermophiles, at 40 - 55°C (Lacap et al., 2007; Castenholz 1976; Pentecost 2003; Ghozzi et al., 2013). High sulfur content of hot springs (2,214 mg L<sup>-1</sup>) could also contribute to the dominance of cvanobacteria (Castenholz 1976). However, this could not be a factor in MHS because there was neither a smell of sulfur nor any detectable amount of H<sub>2</sub>S in the water. Although, there was a minmal amount of sulfur in the water at 2.3 ppm. On the other hand, the alkalinity of the place (pH 6.95) favored the growth of the cyanobacteria. The occurrence of Spirulina major in the place is a new distributional record for thermal springs in the Philippines, although it was previously noted in a marine habitat in Tacloban, Leyte (Velasquez 1979). Spirulina was a component of the cyanobacterial mats in hot springs in USA in Hunter's spring in Oregon and Yellowstone Hot Springs (Castenholz 1969), Tunisia (Ghozzi et al., 2013)., Italy (Pentecost 1995), Greece, Israel, Indonesia, and Japan. It seems that Spirulina usually favors temperatures below 51°C and low amount of sulfide or no sulfide at all (Blackenship, et al., 2006). Spirulina was earlier observed to be capable of using small amount of sulphide as a reductant in photosystem II of photosynthesis (Castenholz 1977). Leptolyngbya treleasii or Phormidium, another major component in the mat, is also reported to be sulfide-tolerant and/or sulfide utilizer (Whitford 1956).

Calothrix thermalis was the only heterocystous filamentous type that was observed in MHS. Some specific sites in the cyanobacterial mat might have had lower amounts of NO3-N because it favored the growth of C. thermalis with heterocysts. Furthermore, Castenholz in 1969 noted that there are few analyses available in thermal springs. And of these few materials available, it appears that NO3-N was very low or entirely lacking in many hot spring sources (Brock 1967). Hence, nitrogen-fixing cyanobaceria may not be surprisingly present in this habitat. Another nitrogen-fixing cyanobacterium that has been reported in the Philippines, i.e., Mastigocladus laminosus, but this was observed in places that had higher water temperature (ca 56°C), such as in Lalakay Hot Spring in Laguna and in Tiwi Hot spring in Albay, than that of MHS (41°C). This low temperature in MHS could have eliminated M. laminosus in this habitat.

Among the coccoid/colonial forms noted, such as *Chroococcus, Aphanocapsa, Pleurocapsa, Myxosarcina*, only *Pleurocapsa* were reported in other hot springs such as in Hunter's hotsprings in USA, Oregon (Castenholz 2015). It is obvious from our account that the high temperature-loving cyanobacteria (max. 73 - 74°C) were absent, such as *Synechococcus* spp. Therefore, temperature seems to be one of the limiting factors in the distribution of the cyanobacteria, especially the coccoid forms in cyanobacterial mat of MHS. There could be a number of factors that could have interacted with one another that could have affected the distribution of the cyanobacteria in this habitat. One factor could have been interspecific competition for space (Stockner 1967).

Diatoms have been reported in hot springs, although relatively less documented than the cyanobacteria. They have been reported in six continents and in about 21 countries (Nikulina & Kociolek, 2011). However, this is the first time that diatoms will be reported associated with cyanobacterial mats in natural salt water hot springs in the Philippines. This may not mean that they were not present in these places in the past but it could be that they were not only studied. In our samples, the diatoms were obviously present because of their dominance in number, especially dominance of one kind in great number, such as Achnanthes brevipes var. intermedia. They were usually found associated with the cyanobacterial mats, specifically epipelic to the gelatinous sheath of Lyngbya and other filamentous cvanobacteria. This is true, because all of the seven diatom species are benthic forms (Round et al., 1990). However, some of them can be planktonic, at some point in their life cycle as Nitzschia and Diploneis (Round et al., 1990). Their mode of attachment to the substates can be in two ways: 1) the cells are adnate or closely appressed to the substrates, and the other means is, 2) the cells are pedunculate or attached to the substrates by means of stalks or pads (Round et al., 1990). The former types include Cocconeis placentula var. euglypta, Halamphora coffeaeformis, Mastogloia crucicula and Diploneis didyma (Harper & Harper 1967). While the pedunculate types include Achnanthes brevipes var. intermedia and Gomphonema parvulum. In the case of Achnanthes spp. the mucilage stalk is secreted from the raphe of one of the R-valves while in Gomphonema spp. the mucilage stalks, are single structures with a certain amount of internal differentiation, which are produced from areas of special pores at one or either pole (end of the diatom) (Round et al., 1990).

Diatom species have been observed in water temperatures above 35°C, such as in thermophilic algal communities in Mount Rainier and Yellowstone National Parks (Stockner 1967) and in Montana, U.S.A. (Kullberg 1968). Amphora and Cocconeis species have also been observed in hot springs in Tunisia (Ghozzi et al., 2013), which they attribute to high nitrate content  $(3.1 \text{ mg } \text{L}^{-1})$  and low flow rate of the water  $(1.4 \text{ L sec}^{-1})$ . MHS is a natural salt water hot spring; it has so much salt that could be nutrient source for the oxygenic photosynthetic cyanobacteria and diatoms. Over time, the pH of the pool waters became more alkaline, from pH 6.95 to pH 7.7 (Table 3). The increase in pH indicates that the natural spring waters became generally high in carbonates, bicarbonates, and associated salts (Smith & Smith 1998). Such condition supported more abundant aquatic life than acidic waters, that are generally low in nutrients. The spring water was impounded preventing the water to flow freely, hence, allowing the diatoms to establish themselves in a particular place. Furthermore, of the seven diatoms, all of them are marine or brackish water types except for Gomphonema parvulum, which is common in fresh waters (Heurck 1896). The latter species was found in greater abundance farther away from the source of the hot spring (Pool 3), which is presumably cooler and less saline than the spring water source (Pools 1 &2).

#### CONCLUSION

Maquinit Hot Springs (MHS) in Coron, Palawan, Philippines showed typical cyanobacterial mats common in impounded natural salt water hot springs. The presence of cyanobacteria and the diatoms in the cyanobacterial mats were determined by the physico-chemical characteristics of the waters, such as alkaline pH (pH 7.6 - 7.7), high salinity (40 ppt), low thermophile water temperature (ca 41°C), and no /or low sulfur content. The dominant cyanobacteria in the mats were the homocystous filamentous types, such as Lyngbya semiplena, Oscillatoria subbrevis, Spirulina major, Leptolyngbya treleasii, Oscillatoria geminata. The only heterocystous filamentous type was Calothrix thermalis. Among the coccoid/colonial forms noted were Chroococcus, Aphanocapsa, Pleurocapsa, Myxosarcina. Of these forms only Pleurocapsa has been reported present in other hot springs such as in Hunter's hotsprings in USA, Oregon (Castenholz, 2015). The diatoms that were found associated with cyanobacterial mats were usually the benthic types, such as Achnanthes brevis var. intermedia, Cocconeis placentula var. euglypta. Gomphonema parvulum, and Halamphora coffeaeformis. All the diatoms observed were of marine types, except Gomphonema parvulum. However, G. parvulum was observed farther away from the source of the hot spring, that may be cooler and less salty.

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