CULTURING, ISOLATION AND GENETIC IDENTIFICATION OF ENDOLITHIC CYANOBACTERIA FOUND IN THE ARABIAN GULF

BY

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A Thesis Presented to the

DEANSHIP OF GRADUATE STUDIES

KING FAHD UNIVERSITY OF PETROLEUM & MINERALS

DHAHRAN, SAUDI ARABIA

In Partial Fulfillment of the
Requirements for the Degree of

MASTER OF SCIENCE

In

ENVIRONMENTAL SCIENCES

JANUARY 2012

KING FAHD UNIVERSITY OF PETROLEUM AND MINERALS

DHAHRAN, SAUDI ARABIA

DEANSHIP OF GRADUATE STUDIES

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Dedicated to the unsung heroes of our time who dedicated their lives to understanding our environment

ACKNOWLEDGMENT

First of all, I do thank and praise the Almighty Allah, the cherisher and sustainer of the worlds; the most Gracious, the most Merciful and the most High in glory, for giving me an opportunity to pursue knowledge.

I would like to express my profound gratitude to my distinguished thesis committee especially Dr. Assad Al-Thukair (chairman) for his exceptional efforts and support to our program and for the constructive comments throughout the study. Also, great thanks to Dr. Michael Kaminski and Dr. Adly Saafin, (members) who exerted irreplaceable efforts for establishing this thesis in a best scientific and professional manner.

My many thanks to King Fahd University of Petroleum and Minerals, for awarding the master degree in the Environmental Sciences Program of the Earth Sciences Department. I really enjoyed being a graduate student in this important discipline of science in this prestigious educational society. I appreciate the support, encouragement and cooperation from the chairman of Earth Sciences department Dr. Abdulaziz Al-Shaibani in sorting out any difficulty we faced throughout the program.

This work wouldn't have been possible without the unrelenting contributions and moral support of my friends and colleagues of Mr. Mushabab Yahya (SEM LAB) Maruf Hussein, Musa Muyideen, Azeez, Abdulsalam, Abbass, Luqman Atanda, Saravan.

Finally, I would like to thank my family for their continuing unrelenting support and prayers during my study at King Fahd of Petroleum and Minerals University.

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THESIS ABSTRACT

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TILTLE OF THE STUDY: **CULTURING, ISOLATION AND GENETIC IDENTIFICATION OF ENDOLITHIC CYANOBACTERIA**

MAJOR FIELD: **ENVIRONMENTAL SCIENCES**

DATE OF DEGREE: **JANUARY 2012**

Euendoliths are photosynthetic microorganisms that are found to play a considerable role in the biogeochemical cycles, and support a variety of grazing animals. In the last five years they gained attention due to their importance in space research and their adaptive capabilities to survive in extreme environments. Euendolithic cyanobacteria were identified, isolated, cultured from calcareous sediments collected from three locations along the Arabian Gulf (Tarut, Mussalamia and Abu Ali) and one site from Costa Rica, South America. Techniques such as micromanipulation, resin embedding, light and scanning electron microscopy were used to study the morphometric diversities of these organisms. Microbial studies of the Costa Rican samples led to the discovery of three species of endolithic cyanobacteria, which were found to bore shell fragments and other calcareous sediments. Those identified species were classified under genus *Hyella* and compared with known Arabian Gulf assemblages. Their identification was accomplished according to their morphological characteristic.

ملخّص الرسالة

الاسم: أبدلجاميو أولاليكان اماو

عنوان الرسالة : ***استنبات وعزل وتحديد الهوية الوراثية للبكتريا الخضراء المزرقة المجهرية الدقيقة***

الختصص : العلوم البيئية

تأريخ التخّرج: يناير 2012

ألكائنات المجهرية الدقيقة التي لها القابلية على التمثيل الضوئي والتي تعيش داخل الصخور الكلسية، قد وجد أن لها دوراً كبيرا في الدورات البيوجيوكيميائيه، كما انها تدعم انواع متنوعة من الكائنات التي تتغذى عليها. في السنوات الخمس الماضية اكتسبت هذه الكائنات الاهتمام نظراً لأهميتها في أبحاث الفضاء وقدراتها على التكيف للبقاء على قيد الحياة في البيئات القاسية من حرارة والبرودة والملوحة العالية. أن ألكائنات الزرقاء المخضرة المجهرية الدقيقة في هذه الدراسة قد تم تصنيفها وتم عزلها من الرواسب الكلسيه البحرية والتي تم جمعها من ثلاثة مواقع على طول الخليج العربي (خليج تاروت ، المسلمية ،و أبو على)، بالاضافة الى موقع واحد من كوستاريكا، أمريكا الجنوبية. لقد تم من خلال الدراسة استخدام تقنيات متعددة منها العزل المتغير والمتعدد ، استخدام الراتنج للتضمين، المجهر الضوئي والفحص من خلال ألمجهرألالكتروني، والذي تم من خلاله دراسة التنوع والتصنيف الشكلي لهذه الكائنات الدقيقة. أن الدراسة الحالية لعينات موقع كوستاريكا أدت إلى اكتشاف ثلاثة أنواع جديدة من هذة ألكائنات المجهرية الدقيقة والتي تبين أنها تقوم بحفر قطع الأصداف الصغيرة والرواسب الحجرية الصغيرة . لقد تم التعرف على الأنواع المكتشفة الجديدة، حيث تم تصنيفها تحت ألكائنات الزرقاء المخضرة المجهرية الدقيقة، لنوع الهايلا،من خلال الخصائص الشكلية واستخدام القياسات المورفومترية للخلايا المتعددة الاختصاص والوظائف.

CHAPTER ONE

INTRODUCTION

What are Endolithic Cyanobacteria?

Endolithic cyanobacteria are a diverse group of photosynthetic prokaryotes that bore into various carbonate substrates in tropical and subtropical marine environments (Al-Thukair and Golubic, 1991a). They have strong affinity for carbonate materials such as rocks (Wierzchos *et al.,* 2011, 2006a, 2006b), shells (Radtke and Golubic, 2005; Radtke and Mainz, 1993), ooids (Al-Thukair *et al.* 1994; Al-Thukair and Golubic, 1991b) etc. According to currently available literature, the geographic distribution of endolithic cyanobacteria in tropical marine environment, is similar to other diversified group of organisms, increasing towards the lower latitudes (Al-Thukair and Golubic, 1996). Golubic (1975) divided the endolithic group of organisms into three groups; namely chasmoendoliths, cryptoendoliths and euendoliths, depending on their preferred niche. Endoliths can be found in cracks or fractures in rocks (chasmoendolithic), in the pore spaces between grains (crypotendolithic), or bore into shells, bones and carbonate rocks (euendolithic) (Garcia-Pichel 2009). The focus of this research however, is on the euendoliths[1] (true borers) and centers on their identification and diversity.

[1] Euendoliths are euendolithic cyanobacteria

Euendolithic cyanobacteria genera have been described from few locations around the world, which include the *Hyella* group (Al-Thukair and Golubic, 1996, 1991a, 1991b; Al-Thukair *et al.*, 1994) *Solentia* (Golubic *et al.,* 1991b), etc. The *Hyella* group of euendolithic cyanobacteria first described by Bornet and Flahaut (1888) dominate the currently known species identified from various marine environments. The earliest members identified in this group form the bases for current morphological identification techniques. For example *H.caespitosa* remained for several years the most common taxonomic reference for all endolithic taxa of similar morphology (Al-Thukair and Golubic, 1996). The genus *Hyella* belong to the Family *Hyellacea* classified under the Order *Pleurocapsales* and Class *Coccogoneae* (Al-Thukair and Golubic 1991b). Pleurocapsaceans are generally described as egg-shaped cyanobacteria that live in close-packed groups where they sit atop long slender gelatinous stalks that radiate upward from the seafloor in pin-cushion like lumps (Schopf, 2001) while the Hyellaceans are represented in modern settings by *Hyella* and in the early fossil record by *Eohyella*. They derived their name as a group of organism that are endolithic (living within rock), cyanobacteria that etch tiny caves in limestone pebbles, boulders, and stony pavements which they then inhabit, living within the outermost rock rind where sunlight penetrates (Al-Thukair *et al.*, 1994). Other species belonging to the genus, *Cyanosaccus* (Radtke and Golubic, 2005), *Abeliella*, *Fascichnus* (Radtke and Mainz, 1993) and also the Chlorophytes (Lukas and Golubic, 1983, 1981).

Classification and Identification of Euendolithic Cyanobacteria

The classification of the endolithic cyanobacteria has become the subject of taxonomical revisions due to several complicated issues (Al-Thukair and Golubic, 1991). Traditionally, they were classified as blue green algae due to their plant type photosynthetic capabilities.

However, they are not algae; they are prokaryotes that lack a nucleus and are akin to Gram-negative bacteria. Their present classification as cyanobacteria was introduced by Stanier (1977) on the basis of their proven prokaryotic properties (Al-Thukair and Golubic, 1991). Anagnostidis (1986) introduced different taxonomical approaches, and often-conflicting interpretations. The genus *Hyella* was assigned to the order *Chroococcales* by Komarek & Anagnostidis (1986) and to the order *Pleurocapsales* by Geitler (1925).

Recently, morphometric classification and identification techniques consider the bored tunnel architecture, dimension, materials occupied and bored as unique identification tool to better understanding these organisms. Morphometric measurements of apical cells, vegetative cells and reproductive cells (baeocyte mother cells) are frequently used to differentiate species of endolithic characteristics. Also euendolithic cyanobacteria can be visualized in resin-cast preparations of modern ooids and other calcareous materials by embedding and casting in resin, curing, cutting to open calcareous surfaces, and the dissolution of surrounding carbonate by dilute hydrochloric acid. (Golubic, 1970).

The most widely used method of identification today, is the morphometric measurement of the mean cell sizes and dichotomy, which inherently undermines the presences of sub and cryptic[2] species. For example, the species *Hyella inconstans* (Al-Thukair and Golubic, 1991) has four dissimilar members grouped together as a species. Advances in molecular biology in the recent decade have ushered in new tools for fingerprinting organisms based on their encoded DNA. These are unique tools in taxonomy, which are able to separate species based on species unique DNA make up. Based on available literature, there are no references

[2] In biology, a cryptic species complex is a group of species which satisfy the biological definition of species—that is, they are reproductively isolated from each other—not similar- they cannot be separated using traditional morphometric criteria

molecular templates to identify each species. Authors including Gerrath, *et al.* (1995); Wierzchos, *et al.* (2011), worked on the genetic analysis of a crustal calcareous rock materials which resulted in large fragments of DNA of both epilithic and endolithic organism. Their research is a great step towards the understanding and establishment of genetic studies with no taxonomic importance, since they failed to conclusively stamp fingerprints on known endolithic organism.

Why do euendoliths bore into calcareous sediments?

The reason why endolithic cyanobacteria bore into calcareous materials has both evolutionary and ecological significance, but yet, the question remained unanswered. There have been attempts to understand the reasons why and how euendoliths bore into calcareous sediments. Cockell and Herrera (2008) attributed a range of environmental stressors and microbial physiological requirements that might act as selection pressures to encourage boring as reasons. They further stated that the acquisition of nutrients, finding a niche with limited competition, selection owing to adverse conditions on the surface of rocks or the prevention of mineralization are the most promising explanations for the evolution and persistence of boring behavior. These theories are not mutually exclusive, a combination of these pressures might have occurred in different environments. Some pressures are more evolutionarily recent, such as the grazing pressure, which has been placed on borers in the intertidal environment since the Cambrian (Gronstal, 2008; Cockell and Herrera, 2008; Javaux and Marshal, 2006; Friedmann and Friedmann, 1995).

How do euendoliths bore into calcareous materials?

Deposits produced by microbial growth and metabolism have been important components of carbonate sediments since the Archaean (Riding, 2000). The principal organisms involved

in these processes are cyanobacteria. The mechanisms for microbial boring are not yet fully understood however several theories have been developed which include (I) Rock dissolution by the production of acid, (II) Sequestration of calcium ions using a calcium pump, (III) and more recently P-type ATPase-mediated transcellular Ca^{2+} transport which supports earlier claims of calcium pump transport.

Rock dissolution by the production of acid

Gracia-Pichel (2006) gave two possible scenarios in the form of plausible model for carbonate rock dissolution mechanism.

Mechanism 1: Temporal separation of photosynthetic and boring activities during the daily cycle: Here, boring activity would simply be relegated to the night time (typically some 8–12 h), when cyanobacteria turn to the oxidation (or fermentation) of intracellular glycogen accumulated during the daytime. In this scenario, release of products of heterotrophic metabolism, namely CO_2 and/or organic acids (mainly formic and lactic) would promote carbonate dissolution in the same manner proposed for most acid-producing microorganisms, the open space being occupied immediately by the fast expansion of apical cells. This model requires necessarily an internal cell-to-cell transport of carbon so as to concentrate the extrusion of acid equivalents around the apical cell and promote the formation of a tunnel, as opposed to a pit. Chemically, it requires the passive transport by diffusion of carbonate and calcium ions away from the dissolution front, which due to their membrane-impermeability, must occur through the interstitial, extracellular space. This mass transport requirement imposes a time constraint on the effectiveness of such phototrophic/heterotrophic transitions as an excavating mechanism

Mechanism 2: spatial separation of photosynthetic and boring activity: In this scenario the photosynthetic activity of cyanobacteria should be restricted to the cells closer to the opening of the tunnel, while the distal cells would be mostly respiring (See **Figure 1**). This would naturally create acidity at the interstitial space of the leading end of the tunnel and promote localized dissolution. Such mechanism would also require a net intracellular transport of organic carbon down the filament, to sustain the respiratory activity and growth of the apical cells. Diffusion of carbonate and calcium up the interstitial space in the tunnel could in principle account for long-term mass balance.

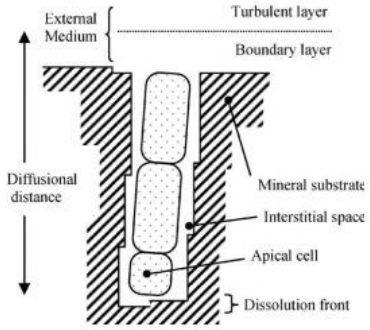


Figure 1 Abstracted cross-section of a multi-cellular microborer/substrate system, depicting physically and biologically important components (*Gracia-Pichel 2006*)

Sequestration of calcium ions using a calcium pump

Another mechanism may be based on active transport of Ca^{2+} through the cyanobacteria filament so that low concentrations of free Ca^{2+} in the interstitial space (**Figure 1**) at the end of the tunnel are created, thus locally decreasing IAP below levels that would make dissolution thermodynamically favorable. In this scenario the released carbonate ions could be (partly) taken up as bicarbonate and used for photosynthesis and partly would diffuse outwards. This mechanism would require necessarily the presence of Ca^{2+}uptake mechanisms

across the apical cell membrane, transport between cells, and export at the trailing end, so that a net mass transport away from the tunnel can occur efficiently.

P-type ATpase-mediated transcellular Ca^{2+} transport

This model (assumes that all P-type Ca^{2+} ATPases pump Ca^{2+} out of the cytoplasm and require a counter transport of protons . Intense extrusion of intracellular Ca^{2+} by P-type ATPases strategically anchored on the plasma membrane of the distal cells, close to the borehole entrance, keep intracellular concentrations within the typical physiological micromolar range and establish an intracellular diffusive mass transport down the concentration gradient that brings Ca^{2+} along the trichome toward this region. Either through active transport with additional ATPases located at the cross walls, or through channels that allow facilitated diffusion of Ca^{2+} and protons, counter-migration propagates transcellularly to the apical cell. Strategically placed Ca^{2+}channels allow the down-gradient entry of extracellular Ca^{2+}into the apical cell, lowering interstitial extracellular concentration below that of calcite saturation, and promoting mineral dissolution. The involvement of channels is consistent with the moderately negative effect of specific inhibitors on boring. Counter transported protons (two per Ca^{2+}) promote the conversion of carbonate ions released from calcite into CO_2, most likely in concert with the carbon concentrating mechanism (Garcia-Pichel, *et al.,* 2010).

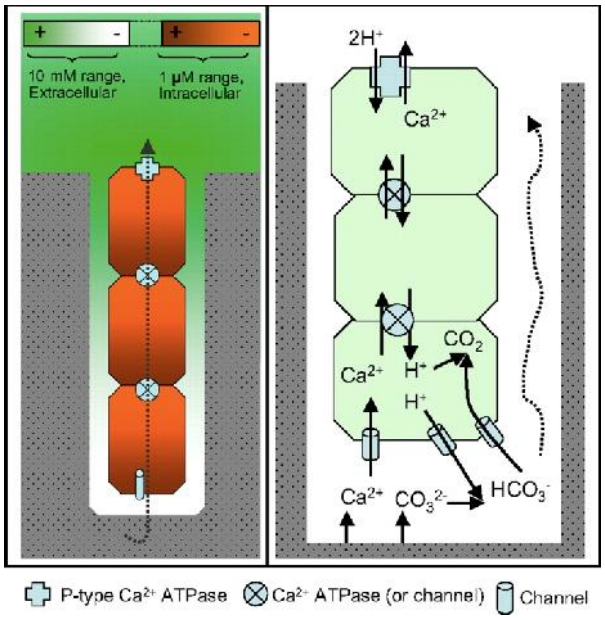


Figure 2 Working model for cyanobacteria excavation of calcium carbonates. Left: Concentrations and ranges of extracellular Ca^{2+} (measured; green intensity) and intracellular Ca2+ (inferred, red intensity) around the boring system, as well as the major direction (Garcia-Pichel *et al.,* 2010)

Importance of Euendolithic Cyanobacteria (Euendoliths)

Micro-borers are relevant as proven agents of geological transformations, as ecological keystone species, as well as for their value as paleoenvironmental indicators. Scientists have expressed keen interest in the euendolithic cyanobacteria due to the host of adaptive capabilities they possess to live within lithified materials. Successful experiments have been conducted with Mars rock analogues found on earth with euendolithic cyanobacteria. It is hoped that future space missions to Mars searching for endolithic forms would rekindle the possibility of life once existed on Mars if they are discovered. Cyanobacteria in general are thought to be the oldest oxygen-producing organisms dating back to the formation of the earth Atmosphere. They profoundly changed the earth's atmosphere and allowed for the evolution of organisms that can use oxygen for respiration.

Euendoliths play an important role in calcium carbonate recycling in aquatic environments by converting un-dissolved calcium carbonate of rocks, shells and other forms of calcium carbonates to dissolving forms for other aquatic organisms such as snails, clams etc. could to build their skeleton. They also support a variety of grazing animals, equipped with hard rock-scraping mouthparts. They produce sheltered microbial habitats with improved local water retention, which, in turn, foster bioerosion[3] and maintain and increase biological diversity in such environment. In addition, endoliths have been used as ecological indicators of water quality and depth (Golubic *et al.*, 1975, Knoll, 1985, Knoll *et al.*, 1986).

The photosynthetic activity of cyanobacteria, their extracellular polymeric substances, ability to penetrate carbonate material and possibly also their adherent heterotrophic bacteria are responsible for the construction of various carbonate structures. Boring activity of euendoliths results in biological corrosion and disintegration of carbonate surfaces. Grazing organisms on carbonate surfaces colonized by epi- and endolithic cyanobacteria produce specific biokarst forms and specific grains which can contribute to near shore sedimentation. Aline (2008), concluded that few living corals show evidence of boring, suggesting that they are able to deter boring microorganisms. However, dead coral is extremely vulnerable to the micro boring processes, which rapidly weaken the structure and break down smaller particles to mud and sand size, suggesting endoliths are agents of bioerosion. Biological corrosion and abrasion together constitute bioerosion. The results of all these processes are calcareous crusts, typical traces and biokarst forms which in many cases have a high fossilization

[3] Bioerosion describes the erosion of hard ocean substrates – and less often terrestrial substrates – by living organisms

potential, and therefore can be powerful ecological, palaeoecological and facies indicators in recent as well as in fossil environments.

The importance of euendolithic cyanobacteria cannot be over emphasized, together with other bacteria, they were present during the early stages of the earth formation, instrumental in the production of the readily available oxygen we all enjoy today and are still present today all around us with significant economic importance. Cloud (1974) nicely summarized the origin, existence and importance of cyanobacteria as "Compared with known living organisms it could hardly have been called anything but a bacterium. Such creatures may not look very clever, but they are extraordinarily inventive biochemically. They have explored every conceivable habitat, and some inconceivable ones - like sulfuric acid and carbolic acid, smoldering coal, and jets of hydrogen sulfide-rich fluids at the crests of deep-oceanic spreading ridges. They outwit mankind's most elaborate efforts to exclude them from their company. And one may be sure that their ancestors were a match for the monotonously anoxic habitats of Archaean history. Bacteria even evolved the ability to repair molecular dislocations in their DNA caused by high-energy ultraviolet radiation - persuasive evidence for their descent from lineages that existed before there was enough O_2 to create a shielding ozone screen.".

Challenges in Study of Euendolithic Cyanobacteria

The main problem in the study of this organism arises from the fact that they live within hard carbonate substrates from which it is difficult to remove them intact. The breakthrough in the understanding of endolithic habit, was partly due to the work of Golubic *et al.*, (1970), an embedding-casting method developed using a slow penetration resin which slowly fill fine boring tunnels and embed the organism within the rock. Customarily, the materials were

fixed, the carbonate matrix dissolved, and the organisms subsequently isolated from the undissolved organic remnants. The undesirable effects of this procedure are damage caused by solvents and collapse of the whole structure after the removal of the hard substrate, and cause the loss of initial space relationships between the filaments. (Golubic *et al.*, 1970; Leznicka *et al.*, 1991; Horath et *al.*, 2006; Javaux and Marshal, 2006). However, it is cautionary to note that both methods are still in use in the extensive morphometric identification of endolithic forms. (Knigshof and Glaub, 2004; Lukas and Golubic, 1981; Lukas and Golubic, 1983; Al-Thukair and Golubic, 1991; Al-Thukair *et al.*, 1994; Radtke and Golubic, 2005).

Euendoliths Preferred Environment

Endolithic cyanobacteria have been reported from different environments, locations and under different environmental conditions ranging from tropical fresh water to saline habitats such as the Arabian Gulf (Al-Thukair and Golubic, 1991; Al-Thukair *et al.*, 1994), the Bahamas (Lukas and Golubic, 1983; Al-Thukair *et al.*, 1994) Antarctica dry desert (Vestal, 1991; De los Rios, *et al.*, 2004, 2007; Nienow, 2009), Niagara escarpment (Gerrath, *et al.*, 1995; Ferris and Lowson, 1997), Halite Rocks from the Hyperarid Core of the Atacama Desert (Wierzchos, *et al.*, 2006; Cockell, *et al.*, 2008; Wierzchos, *et al.*, 2011). There is however no account of their presence along the coast of Costa Rica.

Statement of Problem

Many studies have documented the importance of cyanobacteria in near-surface environments, and nearly all relied upon morphological descriptions. There is a growing need to establish a genetic basis for the identification of endolithic cyanobacteria due to similarities in morphology usually observed in cultured species as compared with natural

populations. Also, several authors have faced difficulties in establishing euendolithic cyanobacteria in culture. The culturing cyanobacteria is particularly problematic because

- The morphology of laboratory cultures may not always resemble the native form of the cyanobacteria
- Cyanobacteria are typically difficult to establish in vitro
- There is also difficulty to produce unialgal/axenic cultures
- Cyanobacteria may exhibit resistance to culture depending on the choice of solid or liquid media and
- Endolithic Cyanobacteria are slow growers and weak competitors (Sigler *et al.,* 2003; Al-Thukair and Golubic, 1991).

Costa Rica despite it richness in biodiversity and largely undisturbed coastal environment, there are no report of existing endolithic cyanobacteria.

Initial Objectives of the Study

- Identify and isolate endolithic cyanobacteria species found to bore calcium carbonate substrates from samples collected along the Arabian Gulf in Saudi Arabia (Tarut Island and Mussalamia)
- Culture of isolated endolithic cyanobacteria under laboratory conditions.
- Test if isolated strains have the capabilities to bore into calcium by providing carbonate substrates (calcite) during the culturing processes.

- Genetically identify the isolated endolithic cyanobacteria using various molecular techniques including, DNA isolation, PCR, and DGG techniques.

- Establishing a phylogeny tree for the isolated endolithic cyanobacteria of the Arabian Gulf.

Added Objectives

Other objectives were added to the study so as to complement the inability to successful culture the endolithic cyanobacteria in the laboratory and to test if the resistance to culture by the Arabian Gulf Euendoliths is an isolated problem or peculiar to the culturing processes and techniques, the objectives are

- Use morphometric analysis to describe resident endolithic cyanobacteria from a preserved calcareous material collected from Costa Rica.

- Compare the species (if any) to known endolithic cyanobacteria from the Arabian gulf

- Ascertain if the endolithic cyanobacteria can bore into other materials

- Attempt to culture endolithic cyanobacteria from the calcareous materials (store with Sea Water) collected from Costa Rica under laboratory conditions.

- Test if isolated strains have the capabilities to bore into calcium by providing carbonate substrates (calcite) during the culturing processes.

CHAPTER TWO

LITERATURE REVIEW

World Distribution of euendoliths

The existence and distribution of carbonate-boring organisms in marine and continental environments is well known and accounts for substantial rates of sediment and sedimentary rock reworking at the geological scale (Chacun *et al.,* 2006). This unique organisms are ubiquitous in distribution, occupying vast surfaces of shifting sands in shallow tropical seas and have been reported from ooid, shoals of the Bahamas and Arabian Gulf (Al-Thukair and Golubic, 1991a, 1991b, 1996; Al-Thukair *et al.,* 1994) coral rubble and sands on the Pacific atolls (Garcia-Pichel, 2009), North Atlantic ocean (Lukas and Golubic, 1983, 1981), halite evaporite rocks of the Atacama desert (Wierzchos *et al*., 2011, 2006a, 2006b) and the Niagara escarpment (Ferris and Lowson, 1997; Gerrath *et al*., 1995). Such materials may appear macroscopically barren but each calcareous sand grain, if exposed to sufficient light and nutrients, is potentially a cyanobacteria micro-habitat (Lukas and Golubic, 1983, 1981; Vogel *et al*., 2000)

The list of euendolithic cyanobacteria identified from different continents with occasional cross continent finds, is gradually increasing partly due to advances in methods of identification. Al-Thukair and Golubic (1996, 1991a, 1991b) described ten assemblages of endolithic cyanobacteria found in the Arabian Gulf . LeChampion-Alsumard (1985)

compared two species of euendolithic cyanobacteria *H.balani and H. caespitosa* from samples collected from several location along the carbonate coast of the Mediterranean Sea at Marseille (France), Rovinj and Zadar (Yugoslavia), Ancona and Porto Novo (Italy) which now form one of the reference materials for comparative study. Examination of soil gypsum from the Atacama Desert, Chile, the Mojave Desert, United States, and Al-Jafr Basin, Jordan revealed the diversity of endolithic cyanobacteria communities just below the surface of soil gypsum samples (Dong *et al.,* 2011). *Cyanosaccus atticus* was first described by Anagnostidis & Pantazidou (1988) from marine limestone coasts of Attica, Greece; this species, as well as *C. aegaeus* described from marine habitats on the Greek island of Santorini (Anagnostidis & Pantazidou, 1985), are the only representatives of the euendolithic genus Cyanosaccus reported from Europe.

Arabian Gulf Euendoliths

Microbial studies of the Arabian Gulf marine environment have led to the discovery ten (10) species of endolithic cyanobacteria taxonomically classified under Cyanophyta and belonging to *Hyella, Solentia* and *Cyanosacus* genera, members of this assemblage include *Cyanosacus piriformis, Hyella reptans, H.inconsistant, H.arbiscular, H.salutans, H.immanis, H.conferta, H.stella, H.racemus and Solentia sanguinea* (Al-Thukair and Golubic, 1991a, 1991b, Al-Thukair *et al.*, 1994, Al-Thukair and Golubic, 1996, Golubic *et al.*, 1996, Al-Thukair, 1999). Only two species have been reported elsewhere which include *Solentia sanguinea* and *H.racemus* (Al-Thukair *et al.*, 1994). However, there are other species of endolithic cyanobacteria which appear in literature but are not formally described from the Arabian Gulf, these include *H. pyxis, H. gigas, H. kalligrammos, H. maxime, H. balani* (Al-Thukair, 1999).

Costa Rican Euendoliths

The serene and relatively undisturbed coastline of Costa Rica is still virgin in terms of endolithic cyanobacteria. Costa Rica has two coastlines bordering the country, the Pacific Ocean coastline and the Caribbean Sea coastline. There is currently no record of endolithic cyanobacteria from these two locations. However, Al-Thukair *et al.* (1994) described two euendolithic cyanobacteria from the Bahamas *Solentia sanguinea* and *H.racemus. Solentia sanguinea* is characterized by pseudofilamentous cells spreading from the point of entry into the interior of the substrate forming a relatively shallow repeatedly branched thallus. The cells in culture are easily identified by the possession of ring of gelatinous material, remnants of propulsion (jetting) into the calcareous substrate (Golubic *et al.,* 1996). *H.racemus* is characterized by a short distance between cell branches (one cell), cells divide by cleavage alternating in mutually exclusive perpendicular planes, leaving daughter cells closely appressed to each other within a common envelope. The walls separating cells are transverse, longitudinal or occasionally, oblique to the direction of penetration. Branching is dichotomous, originating by longitudinal division of apical cells (Al-Thukair *et al.*, 1994)

While presenting a list of endoliths encountered in the literature consulted so far which are relevant to this research, no attempt is made to claim exhaustive literature search on euendolithic diversity.

Adaptation of euendolithic cyanobacteria to adverse environmental conditions

Euendolithic cyanobacteria are known to possess a host of adaptive capabilities which include endolithic habit (inside calcium and rocks) which compelled them to penetrate and live inside rocks where they can afforded a degree of protection from desiccation, intense sunlight, and extreme temperature fluctuation. (Shashar and Stambler, 1992; Friedmann and

Friedmann, 1995; Herrera and Cockell, 2007; Burns *et al.*, 2009; Garcia-Pichel, 2009; Stivaletta and Barbieri, 2009). Also, they have the capability to avoid UV radiation, by encapsulating their cells with gelatinous sheath and various pigments to avoid UV damage to their DNA (Cabrol and Grin, 1995; Gronstal, 2008; Olsson-Francis and Cockell, 2010).

Nature of Materials Bored

Euendolithic cyanobacteria are known to bore into different kind of substrata including: calcareous rocks, dead corals, oolitic sand grains, glass and shells (Aline, 2008; Cockell and Herrera, 2008; Perkins and Halsey, 1971). Arabian Gulf sediment consists of oolites, shell fragments and other calcareous sediments types (Al-Thukair and Golubic, 1991a, 1991b; Al-Thukair *et al.*, 1994; Al-Thukair and Golubic, 1996; Golubic *et al.*, 1996). Such sediments have been found to be bored by several species of endolithic cyanobacteria, these predominantly members of the *Hyella* species which were discovered along the east coast of Saudi Arabia (Lukas and Golubic, 1980, Al-Thukair *et al.,* 1994). The types of rocks suitable for colonization by endolithic microorganisms are also diverse and include halite, sandstone, quartz, limestone, granite, and dolomite

Boring Rates

Al-Thukair (2011) determined the mean boring rates of seven colonies of Arabian Gulf endoliths and found that it lies between 166 and 510 $\mu m^3\ d^{-1}$ at various growth stages, with a boring rate of 10 $\mu m^3\ d^{-1}$ in calcite measured under light intensity of 20–25 $\mu E\ m^{-2}\ s^{-1}$ at 16:8 h LD cycle, 1 $h\ d^{-1}$ with agitative and no agitative effects. Carbon isotope (^{14}C) measurements reported by Ferris and Lowson (1997) indicated that atmospheric carbon dioxide is used by the endolithic cyanobacteria, rather than dissolved inorganic carbon from the weathering of carbonate minerals in the limestone host rock. In addition, whole rock

multi-element analyses revealed an enrichment of some elements (e .g. phosphorus, barium, lead, and zinc) in the endolithic zone over the host rock, while other elements (e .g. magnesium, calcium, iron and copper) were depleted.

Genetic Studies of Euendoliths

Current investigations of endolithic communities rely on culture independent techniques, which employ standard morphological characteristics to identify community members. Throughout the past decade, culture independent techniques including the direct extraction of DNA has greatly facilitated community analysis of environmental samples. The result of such technique is not sufficient for species-specific identification. If unialgal DNA extraction is achieved, several lines of investigation are possible, including sequencing of clone libraries, and genetic fingerprinting of both whole communities and euendolithic cyanobacteria isolates. Despite the advantages of a culture independent approach, few molecular-based studies of endolithic community structure have been performed. However, the utilization of the molecular approach to investigate cyanobacterial communities has been demonstrated recently in desert soil crusts (Garcia-Pichel *et al.,* 2001;)

Sigler *et al.* (2003) use molecular characterization to establish species diversity of cyanobacteria from Alpine Piora valley (Switzerland) using culture- dependent and -independent approaches on red and green dolomite. Direct microscopy of green dolomite revealed four distinct morphotypes consistent with Chlorophyta genera *Chlorella* and *Stichococcus* and the Cyanobacterial genera *Nostoc* and *Calothrix*, whereas only *Stichococcus* and *Nostoc* were observed in the red dolomite. They concluded that the overall composition of the phototrophic community closely resembles that of endolithic communities located in extreme habitats, suggesting that a cosmopolitan community inhabits the defined niche.

Nubel *et al.* (1997) designed sets cyanobacteria primers[4] CYA359F (forward), CYA781R(a) and CYA781R(b) (reverse) for specific amplification of a 379 bp 16S rRNA gene sequence. CYA781R (a) and CYA781R(b) differ by two polymorphic bases situated at positions 7 and 23 (5' to 3'). These primers have the advantage of giving a PCR product, which corresponds to variable regions V3 and V4, and contains significant information for phylogenetic[5] assignments.. They have been used unmodified and or slightly adapted for numerous Denaturing Gradient Gel Electrophoresis (DGGE)[6] studies investigating cyanobacterial diversity in environmental samples. (Saiz-Jimenez, *et al.,* 1990; Abed *et al.*, 2003; Edwards, *et al.*, 2003; Edwards *et al.*, 2005; Chacun, *et al.*, 2006; Giannantonio, *et al.*, 2009; Grube and Berg, 2009; Horath and Bachofen, 2009; Hong, *et al.,* 2010; Edwards *et al.,* 2011; Ludwig *et al.*, 1998; Abed *et al.*, 2002).

Most common molecular studies focus on biofilm (De lo Rios, 2006) and stromatolites (Foster *et al.*, 2006) using culture independent techniques, since they contain diverse communities of endoliths, attempt to isolate individual species have not been successful.

[4] A primer is a strand of nucleic acid that serves as a starting point for DNA synthesis. They are required for DNA replication because the enzymes that catalyze this process, DNA polymerases, can only add new nucleotides to an existing strand of DNA. The polymerase starts replication at the 3'-end of the primer, and copies the opposite strand. In most cases of natural DNA replication, the primer for DNA synthesis and replication is a short strand of RNA (which can be made de novo)

[5] Relating to phylogeny or phylogenetics or based on evolutionary development or history: a phylogenetic classification of species.

[6] Denaturing gradient gel electrophoresis (DGGE) works by applying a small sample of DNA (or RNA) to an electrophoresis gel that contains a denaturing agent. Researchers have found that certain denaturing gels are capable of inducing DNA to melt at various stages. As a result of this melting, the DNA spreads through the gel and can be analyzed for single components, even those as small as 200-700 base pairs.

CHAPTER THREE

MATERIALS AND METHODS

Sampling Site and Collection of Marine Calcareous Sediments

Samples for this research where collected from two location sampling sites, along the Arabian Gulf (North Tarut Island, Mussalamia and Abu Ali) and one from Playa Concha Costa Rica (**Table 1**). With the exception of Costa Rica, the sites were chosen based on previous knowledge of the existence of those microorganisms within the sediment grains including; shell fragments, oolites and calcium carbonate rock fragments. All samples for this research were provide by Dr. Assad Al-Thukair has part of his on-going research to document the diversity of Euendolithic Cyanobacteria within and outside Saudi Arabia.

Table 1 Sample collection locations from the Arabian Gulf and the Pacific Coast of Costa Rica

Sampling Site	Location	Sampling depth (m)	Salinity
Tarut	26^0 35' 10.41" N 50^0 5' 14.02" E	1-5	-
Mussalima	27^0 24' 34.75" N 49^0 11' 47.12" E	1.5- 4	-
Abu Ali	27^0 21' 16.9" N 49^0 31' 26.08" E	1.5	-
Playa Conchal	10^0 24' 14.52" N 85^0 48' 13.89" W	0.5	33.4 $^0/_{00}$

Tarut (Saudi Arabia): is a tidal delta at Tarut Island, 30km North of Dhahran city. It is a semi-protected environment, sheltered from the open sea by the Island (See **Figure 4**). Calcareous sediment materials are found on the shoaling ooids environment, approximately 100-300m long and 20-30m wide arranged parallel to the island shoreline. Samples were collected from multiple location along the shoal at depth ranging from 1-5m.

Mussalima (Saudi Arabia): Is a semi closed lagoon, located about 120km north of Tarut. Wide tidal channel communicate directly with the open sea at the lagoon's entrance. Samples were collected from1.5 -4m depths. Samples contain considerable amounts of calcareous skeletal materials in addition to ooids characteristic of this location (See **Figure 4**).

Abu Ali (Saudi Arabia): is a hypsographic Island north of Jubail, rising 13m above sea level. It sticks out like a thumb into the Arabian gulf (See **Figure 4**) and has a sensitive marine environment evidenced by the pollution from the 1991 Gulf war. The island played a key role in trapping and blocking the oil flow during the Persian Gulf War so that oil did not move farther south. These land features have functioned in this manner with previous oil spills coming from the northern portions of the Gulf. Samples were collected from a 1.5 m depth on the rocky substrate.

Playa Conchal (Coasta Rica): Playa Conchal (Conchal = spanish for Shell) lies on the Pacific coastline Costa Rica (See **Figure 5**). The 4.5km stretch of coastline is characterised by literally millions of crushed sea shell (See **Figure 6**) and volcanic sand. The beautiful sea front is undisturbed and in a protected area where ecotourism blends smoothly with nature. Samples were scooped from multiple locations at a depth of 0.5m below the clear water surface. At the time the sample were collected surface salinity was estimated at 33.4 $^{0}/_{00}$.

Figure 3 Sample collection sites marked with red dots along the Saudi Arabian Gulf coast

Figure 4 Crushed shell -calcareous materials characteristic of the Playa Conchal, Costa Rica

Figure 5 Sample collection site marked with red dot along the Costa Rican Pacific coast

All the samples were collected using open mouth jars at depths between 1- 6 meters. Several glass jars of 500 ml capacity were filled with approximately 800 g of sediments collected from each site and the samples were divided into two batches, 3% Formaldehyde was added to the first batch for fixation while the other was stored under seawater for nourishment.

Preparation of Salt Nutrient (SN) Solid and Liquid Media.

Three SN media formulations were prepared for the purpose of this research as the need arises for cleaning/micromanipulation and growth purposes according to Waterbury 1989. The formulations include 1% solid SN media, 1.5 % Solid SN media and liquid SN media.

Liquid SN Media

In other to prepare liquid SN media, 750 ml of filtered seawater and 230ml-distilled water were mixed and autoclaved in a 2 liter Erlenmeyer flask and the following nutrient

enrichment which were prepared earlier were added aseptically, and then corked using a locally made cork (cotton, fine linen and aluminum foil).

Table 2 Ingredient formulations for liquid SN medium

Compound and formulation amounts	Quantities Used
$NaNO_3$ (300.0 g/L dH_2O)	2.5 ml
K_2HPO_4 (anhydrous) (6.1 g/L dH_2O)	2.6 ml
Na2EDTA·2H2O (1.0 g/L dH2O)	5.6 ml
Na2CO3 (4.0 g/L dH2O)	2.6 ml
Vitamin B12 (1.0 mg/L dH2O)	1.0 ml
Cyano Trace Metal Solution	1.0 ml
Cyano Trace Metal Solution (Each metal compound was dissolved individually in 100 ml dH_2O and then combined (the six solutions), then the volume was brought up to 1.0 L with dH_2O.	
Citric Acid·H2O	6.25 g
Ferric Ammonium Citrate	6.0 g
MnCl2·4H2O	1.4 g
Na2MoO4·2H2O	0.39 g
$Co(NO3)2·6H_2O$	0.025 g
$ZnSO_4·7H_2O$	0.222 g
Citric Acid·H_2O	6.25 g

*Modified from http://www-cyanosite.bio.purdue.edu/media/table/SN.html

Solid SN media

To prepare 1% and 1.5% solid SN media, 10 and 15 grams of bacteriological grade agar were dissolved in 230ml distilled water (in 500ml Erlenmeyer Flask) and 750 ml of seawater each was dispensed into 1000ml Erlenmeyer Flask. The media and seawater were then autoclaved seperately (VARIOKLAV Steam Sterilizer 25 TC). Also, 10ml each of phosphate and nitrates earlier prepared were measured into screw-capped test tubes and autoclaved. After autoclaving, the SN media were spiked using whole 10ml phosphate and nitrate and thoroughly mixed before pouring in different sizes of media plates depending on weather they were designated for cleaning or growing.

Identification, Isolation and Culturing

Dissecting microscope (Leica EZ4) was used to identify prospective ooids and shell fragments that harbour endolithic cyanobacteria. They were picked up individually by forceps and transferred to solid SN media agar plates. Isolated ooids and shell fragments were

monitored for endolithic growth, as cleansing and transfer continued. Light microscope (Leica CME) was used to monitor baeocytes. Baeocytes of euendolithic microorganisms released after cleansing were streaked on SN agar plates. Various strains of endolithic cyanobacteria were isolated according the procedure described by Al-Thukair and Golubic (1991a). In general baeocytes from bored ooids, or shell fragments were streaked on 1% SN agar plates following their release. Colonies that grew from these baeocytes were transferred into test tubes containing liquid SN medium and kept in an environmentally controlled chamber at 25ºC, 50% relative humidity, and 20-25 $\mu E.m^{-2}.s^{-1}$ of 16:8 -h LD cycle. To assure that the isolated strains maintains its endolithic habits, each petri dishes with 15 ml of SN liquid medium were prepared, and six calcite grains of 3-4 mm were added. These sets were inoculated by baeocytes and agitated by stirring using magnetic stirrer for 1 $h.d^{-1}$. The inoculated petri dishes were placed in environmentally controlled chamber at 25ºC, 50% relative humidity, and 20-25 $\mu E.m^{-2}.s^{-1}$ of light in 16:8h Light/Dark (LD) cycle.

Scanning Electron Microscopy (SEM)

Dehydration of samples for scanning electron microscopy was performed at room temperature in a graded series of Ethanol starting at 50% then going to 70, 95 and 100% for no less than 20-30 minutes each step. By subjecting the calcareous materials to different combinations acetone and spur low viscosity resin, embedding media infiltration was achieved. Curing was done overnight at 60°C (**Figure 6**).

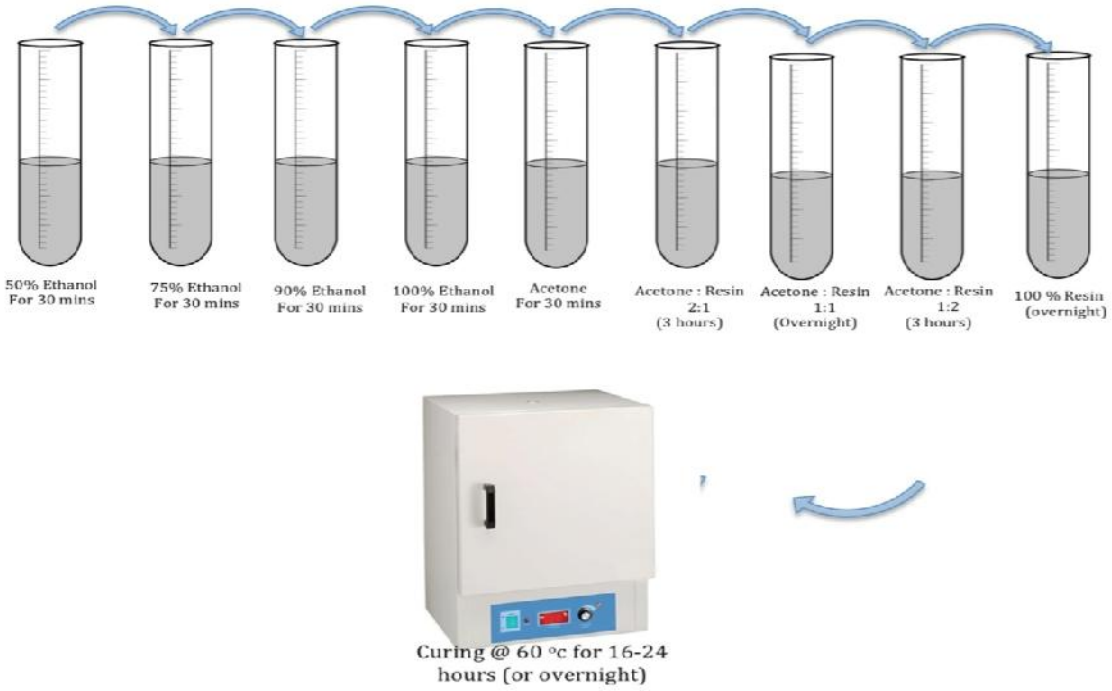


Figure 6 Spurr's Low viscocity resin embedding and dehydration sequence

Table 3 Formulations for Spur Low viscosity medium (Polysciences Inc. Warrington, PA)

Ingredient	Firm	Hard
Diglycidyl ether of polypropylene glycol (D.E.R. 736)	1.43g	0.95g
Nonenyl succinic anhydride (NSA)	5.90g	5.90g
Dimethylaminoethanol (DMAE)	0.1g	0.1g
Cure schedule (hr) at 70°C	8	8
Pot life (days)	3.4	3.4

Selected calcareous sediments grains from (Arabian Gulf and Costa Rica) were embedded and examined using SEM and procedure described by Al-Thukair and Golubic (1991 a, b,) Golubic *et al.* (1970). The blocks with embed calcareous materials were cured and cut open by brushing on an abrasive surface. Calcareous materials in those embedded blocks were dissolve using 3% HCl. Following calcium carbonate dissolution, resin blocks were sputter with gold and examined by Scanning Electron Microscope (Jsm-5900lr Scanning Electron Microscope: JEOL ltd Japan). Three-dimensional displays of bored tunnels and numbers of colonies were photo-micrographed and examined.

CHAPTER FOUR

RESULTS

The results generated from this study are presented in three main sections as presented below; the idea is to address all the component of the experimentation, which were conducted. The first section addresses all the outcome of general analysis of sample and observations, which is considered relevant to this research. The second section, present the finding from the analysis of the Arabian Gulf samples collected from three locations (Tarut, Mussalamia and Abu Ali). Finally, the findings from the investigation of the endolithic cyanobacteria diversity from sample collected from playa Concha: Costa Rica is presented.

- General Sample Analysis
    - Particle Size analysis
    - Surface Boring
- Arabian Gulf Euendolithic cyanobacteria Study
    - Species description
    - Light Micrograph
    - Scanning electron Micrograph
    - Culturing and re-boring techniques
- Costa Rica Euendolithic cyanobacteria Study
    - Cell Measurements

- Species description
- Light Micrograph
- Scanning electron Micrograph

General Sample Analysis

Particle Size (Sieve) Analysis

Modified ASTM C136-01 soil classification system was used in an attempt to distinguish the sizes and preferences of material bored by the euendolithic cyanobacteria (**Figure 7**). Such information will be useful in comparative studies to establish the similarities and differences in the nature of material bored and species-specific preferences for sizes (if any) of materials relative to individual sizes of species of euendolithic cyanobacteria. Carbonate materials collected from four locations Tarut, Mussalamia Abu Ali (Saudi Arabian Gulf) and Costa Rica were subjected to particle size analysis using a standard sieving technique as described in ASTM C136-01 and result presented in **Table 4**. The particle/material sizes were determined with ten selected mess sizes, ranging from 6000 – <50 μm. Carbonate materials from Tarut, Mussalamia and Abu-Ali are predominantly fine particles (73.63 and 63.66 %), which consist of ooids, sand grains, shell fragments and granular carbonate substances (≤850≠0 μm) (**Table 4**). The Coarse particles (26.37%) from these locations are a mixture of carbonate pebble and shell fragments (≤2000≠850 μm). Costa Rica Sediment material consists largely of pellet size (Crushed sea shells) carbonate material (94.10%), which are actually weathered shell, the location where the sample was collected got its name "conchal in spanish = shells " from this sediments and volcanic sand deposited long time ago. The material in its current state appears glossy when dried and largely has no traces of torsion typical of marine shells (≤ 6000>2000 μm).

f Carbonate Sediment sample collected from Mussalamia, Abu-Ali, Tarut and Costa Rica

Mesh Size (µm)	Arabian Gulf (KSA)			Costa Rica
	Abu Ali	**Mussalamia**	**Tarut**	**Playa Concha**
	Mass (g)	**Mass (g)**	**Mass (g)**	**Mass (g)**
5560	-	0.0243	0.0843	2.3484
2000	2.5983	2.1880	1.1880	7.2811
1700	0.4035	0.6788	0.3788	0.3945
1400	0.3845	0.2490	0.5690	0.1057
1180	0.5708	0.0341	1.0341	0.0715
850	1.9415	1.6608	2.7608	0.0342
600	2.1339	1.0016	1.3216	-
425	3.1400	0.9000	0.8003	-
212	1.2939	0.7818	0.5418	-
106	2.1148	0.4800	0.1800	-
≤ 50	0.4246	0.3958	0.0958	-
l Analyzed (g)	**15.0058**	**8.3942**	**8.9545**	**10.2354**

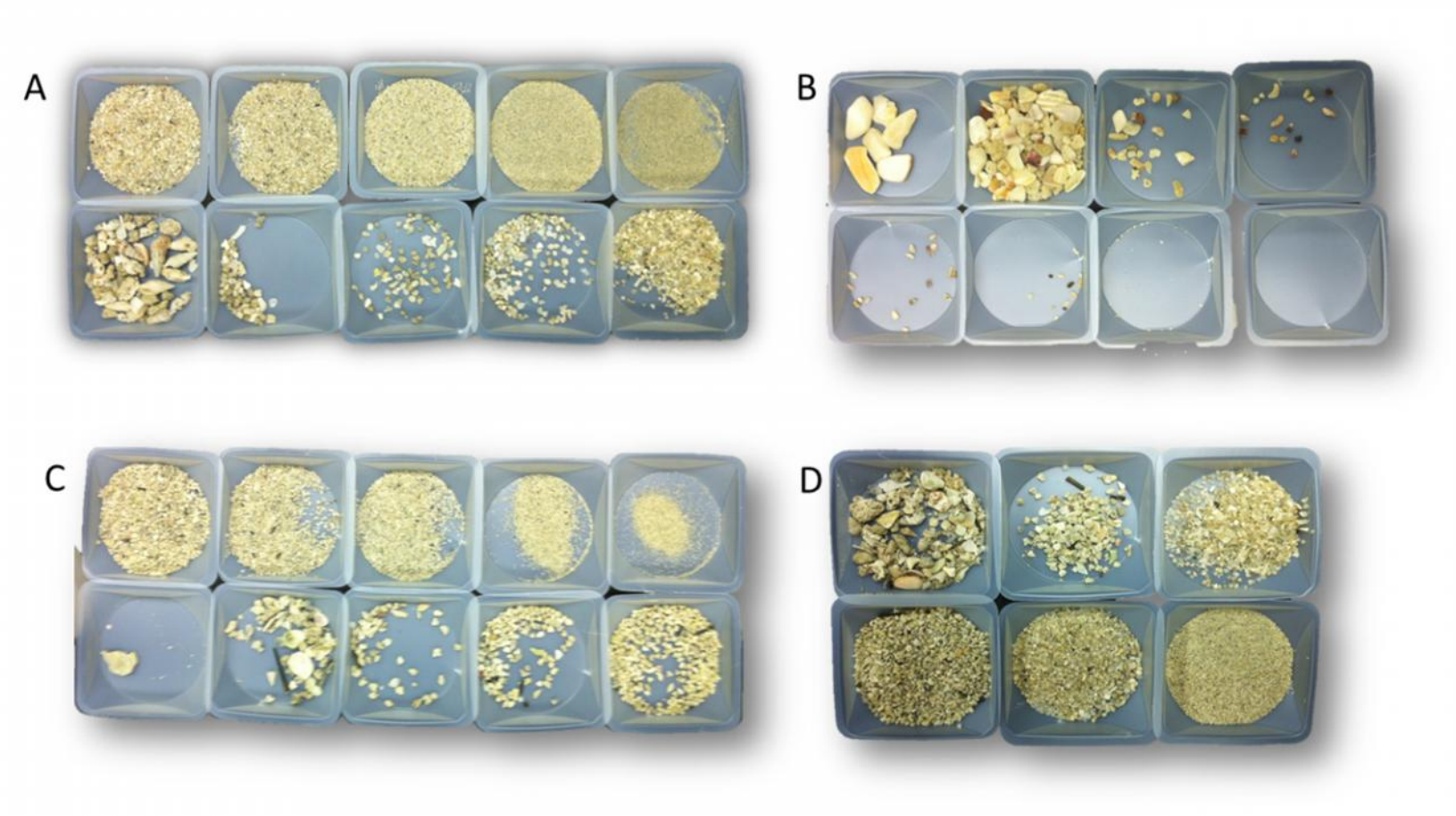


Figure 7 Calcareous sediments sieve fractions from Abu Ali (A), Playa Concha (B), Tarut (C) and Mussalamia (D)

Surface Boring

Light microscope was used to selected calcareous materials heavily bored, the materials were cured in the oven for 5 hours at 100° C to remove all the living life forms from the surface. The cured material was allowed to cool and later spatter with gold for SEM examination. The SEM revealed that materials from the Arabian Gulf are heavily bored (**Figure 8)** also there was the presence of epilithic communities of diatoms with intact frustule (**Figure 9 and 10)**, the epilithic communities are thought to benefit directly from the boring activities of the euendolithic cyanobacteria.

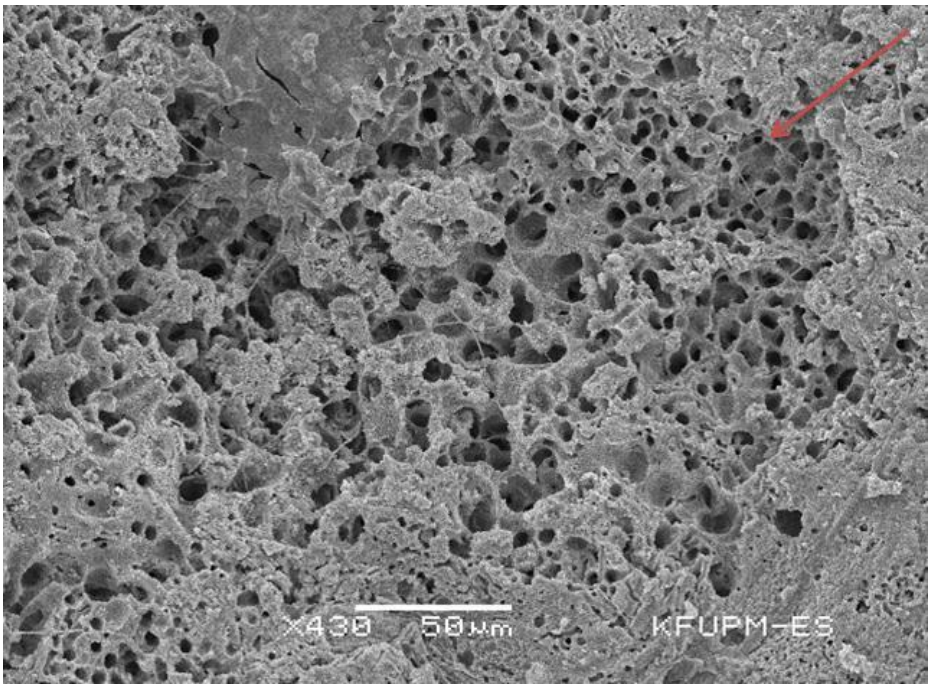


Figure 8 Boring tunnels created by endolithic microflora

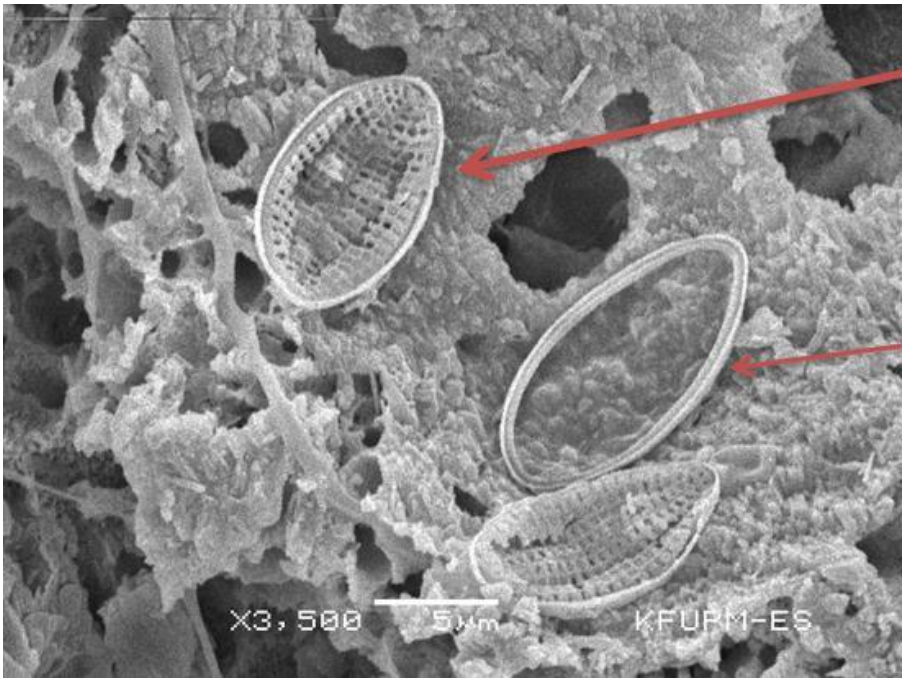


Figure 9 Epilithic diatom community on the surface of a calcareous material with detailed frustule, arrow pointing towards the remains of the diatoms

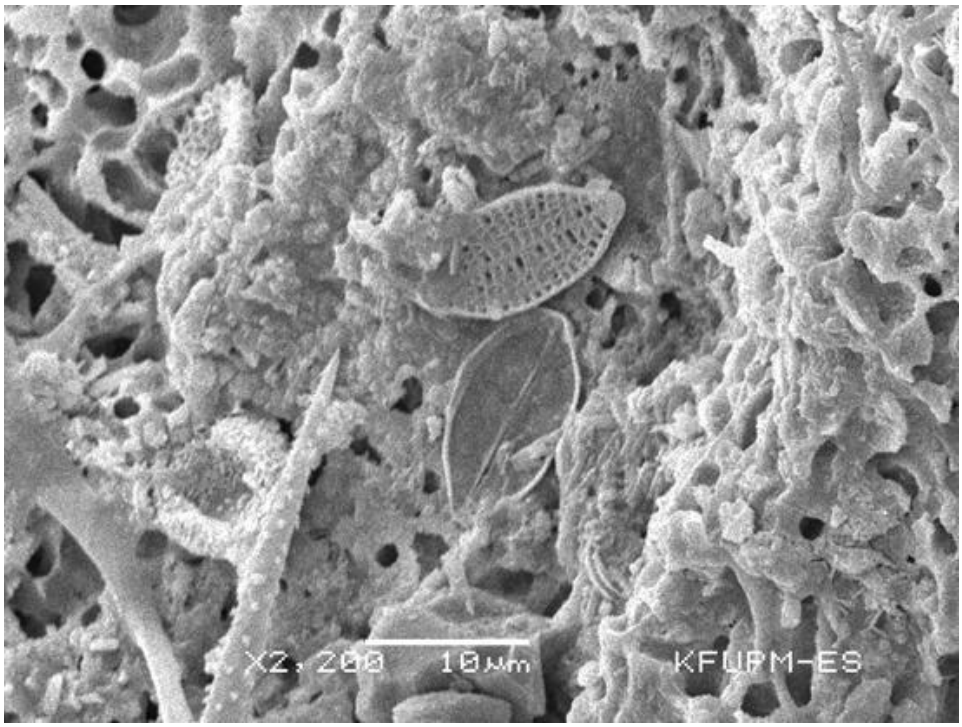


Figure 10 Epilithic diatom community on the surface of a calcareous material with detailed frustule

Arabian Gulf euendoliths

Calcareous material selected for light microscopy where dissolved using 0.1 M HCl on a glass slide, the species of euendolithic cyanobacteria left after the dissolution of the calcium carbonate where observed using a light microscope which resulted in the identification of several species previously described by Al-Thukair and Golubic (1991a). The encountered species include *H.immanis, H.salutan, H.reptans, H.arbuscula* and *H.stella.* Fewer cell measurements were carried out for species of euendolithic cyanobacteria found in the Arabian Gulf, whenever there is noticeable morphological variation from the illustration presented in consulted publications; measurements were performed on natural population of euendolithic cyanobacteria so as to verify with the already available cell measurements.

Class: *Coccogoneae* Thuret 1875

Order: *PleurocapsaIes* Geitler 1925

Family: *Hyellaceae* Borzi 1914

Genus: *Hyella* Bornet & Flahault 1888

Hyella inconstans Al-Thukair *et* Golubic (Al-Thukair and Golubic 1991)

(**Figures 11 and 12**)

The thallus is composed of coccoid cells at the substrate surface and long pseudofilaments, which bore into the substrate, forming prostrate and intertwined, branched networks. Filaments in distal parts uniseriate, in the middle portions biseriate and in parts proximal to the substrate surface rnultiseriate. Branching is frequent and is usually initiated by the protrusion of an intercalary cell, rarely by slippage or by longitudinal division of an intercalary cell. The mucilaginous envelopes are thin, not lamellate and usually colourless but can rarely be yellowish or greenish in color. Intercalary cells are sub spherical, square, oblong or cylindrical. The average distance between branches is 5 cells. Apical cells in young thalli are conical on the average longer than wide, 5.8±1.2 (28) μm (Al-Thukair and Golubic 1991a) wide, and 11.8±2.5 (28) μm (Al-Thukair and Golubic 1991a) long. Vegetative cells are rectangular, square, enclosed in box-like envelopes arranged in uniseriate filaments.

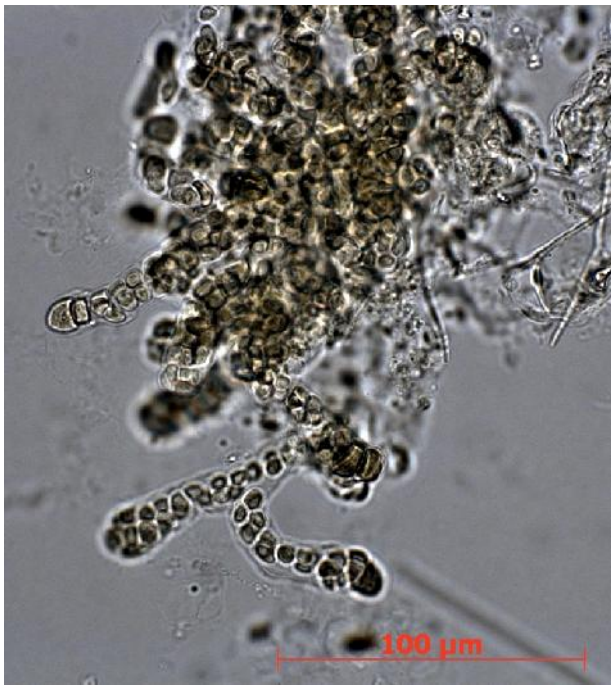


Figure 11 Light micrograph of *Hyella inconstans* showing colony composition (scale = 100 µm)

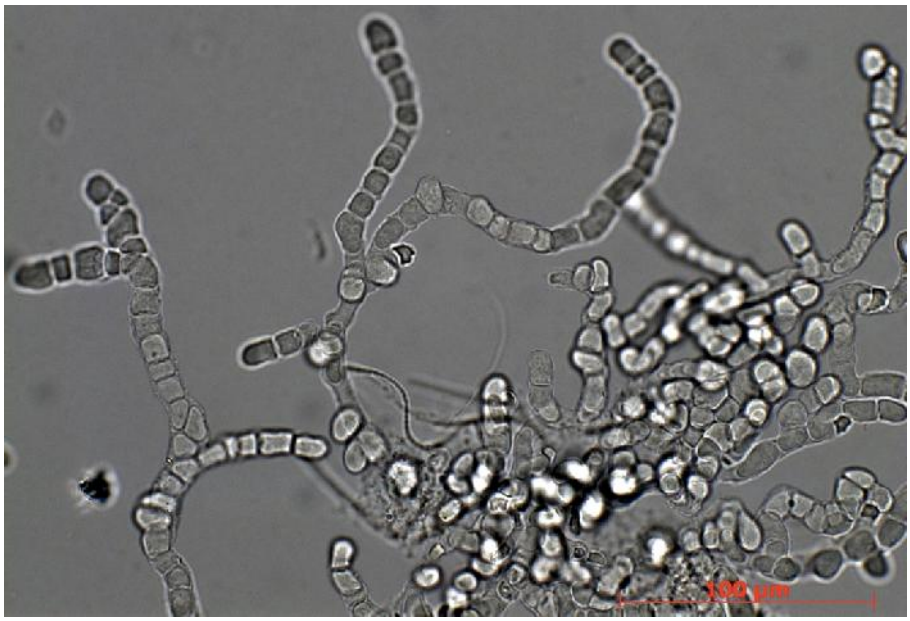


Figure 12 Light micrograph of *Hyella inconstans* showing the tightly packed cells (scale = 100 µm)

Class: *Coccogoneae* Thuret 1875

Order: *PleurocapsaIes* Geitler 1925

Family: *Hyellaceae* Borzi 1914

Genus: *Hyella* Bornet & Flahault 1888

Hyella immanis Al-Thukair *et* Golubic (Al-Thukair and Golubic 1991)

(**Figures 13**)

The Filaments are composed of coccid cells at the substrate surface and short radiating pseudofilaments, which bore into the substrate. Branching is mostly lateral, less commonly dichotomous by longitudinal division of the apical cell. Lateral branching originates by protrusion of the side wall, less commonly by longitudinal division of an intercalary cell, and only rarely by slippage. The average distance between branches is 4-6 cells. Apical cells are rounded or rounded-conical, on the average slightly longer than wide. 13.1± 2.6 (103) μm (Al-Thukair and Golubic, 1991a) wide, and 17.4±3.6 (102) μm (Al-Thukair and Golubic, 1991a) long. Vegetative cells (v) are cylindrical, isodiametric, mostly in uniserial arrangement, 14.9±3.7 (357) μm (Al-Thukair and Golubic 1991a) wide, and 15.6±4.1 (357) μm (Al-Thukair and Golubic, 1991a) long.

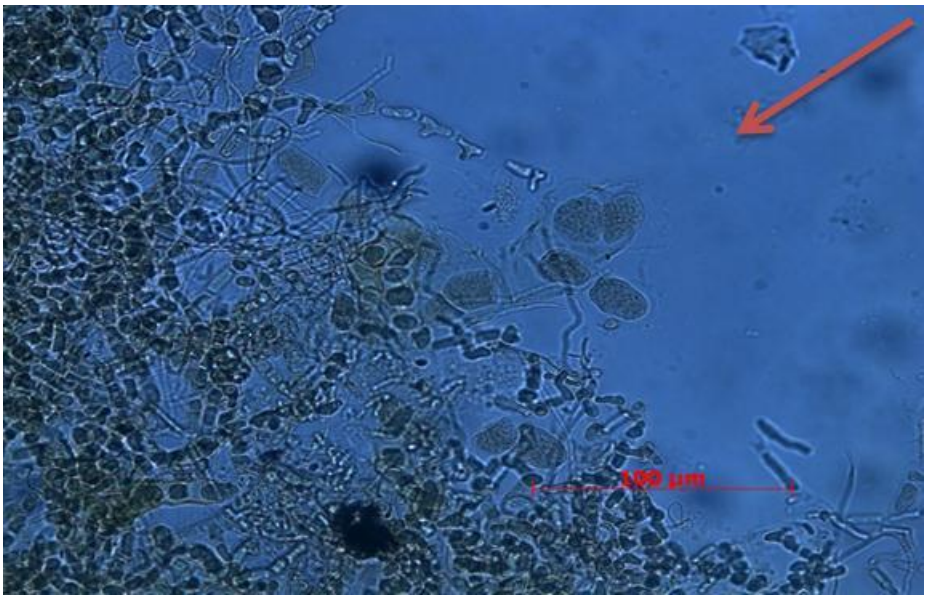


Figure 13 Light micrograph of *Hyella immanis* showing the early stages of colony development (scale = 100 µm)

Class: *Coccogoneae* Thuret 1875

Order: *PleurocapsaIes* Geitler 1925

Family: *Hyellaceae* Borzi 1914

Genus: *Hyella* Bornet & Flahault 1888

Hyella salutans Al-Thukair *et* Golubic (Al-Thukair and Golubic 1991)

(**Figures 14 and 15**)

Thallus endolithic, pseudofilamentous, parallelly branched. Branching is mostly lateral, less commonly dichotomous by longitudinal division of the apical cell. Lateral branching occurs mostly by protrusion of the lateral wall, less commonly by slippage, and rarely by longitudinal division of an intercalary cell. The average distance between branches is 5-7 cells. Apical cells are biconcavely constricted on the average longer than wide, 11.1±1.9(64) μm (Al-Thukair and Golubic 1991a) wide, and 16.3±3.2(64) μm (Al-Thukair and Golubic 1991a) long. Vegetative cells are cylindrical, isodiametric, always uniseriate, 12.1±2.8(375) μm (Al-Thukair and Golubic 1991a) wide, and 11.4±3.3(375) μm (Al-Thukair and Golubic 1991a) long. Vegetative cells are shorter, and slightly wider than apical cells.

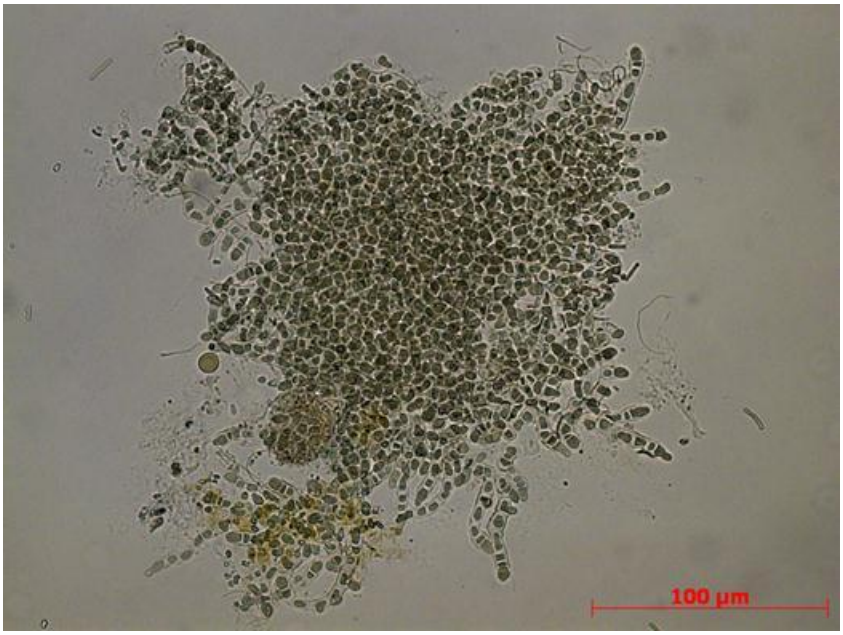


Figure 14 Light micrograph of *Hyella salutans* showing the multi serrated cell and branching (scale = 100 µm)

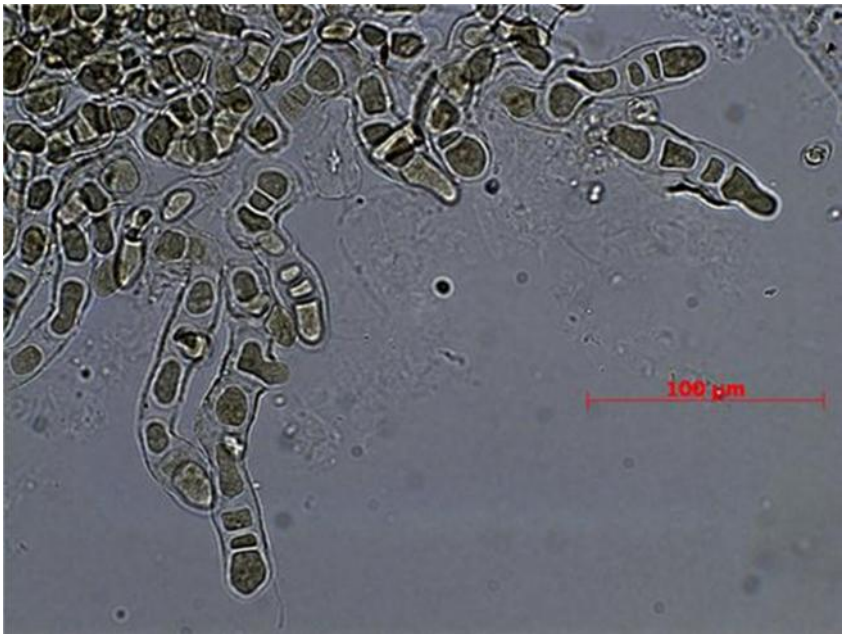


Figure 15 Light micrograph of *Hyella salutans* showing the multi serrated cell and branching (scale = 100 µm)

Class: *Coccogoneae* Thuret 1875

Order: *PleurocapsaIes* Geitler 1925

Family: *Hyellaceae* Borzi 1914

Genus: *Hyella* Bornet & Flahault 1888

Hyella reptans Al-Thukair *et* Golubic (Al-Thukair and Golubic 1991)

(**Figures 16 and 17**)

The thallus is composed of coccoid cells at the substrate surface and long pseudofilaments, which bore into the substrate, sprawling and branched. Branching uniseriate, biseriate. Lateral branching occurs by cell slippage, less commonly by protrusion of the lateral wall of an intercalary cell, or by longitudinal division of an intercalary cell. The average distance between branches is 1 cell. Species identified from Abu Ali sample appear multinucleated. Apical cells are clavate to oval, on the average longer than wide 5.2±1.2 (62) μm (Al-Thukair and Golubic 1991a) wide, and 10.3 ± 3.3 (62) μm (Al-Thukair and Golubic 1991a) long. Vegetative cells are oval, cylindrical with rounded ends, mostly in uniseriate arrangement, 5.5 ± 1.4 (225) μm (Al-Thukair and Golubic 1991a) wide, and 8.9 ± 2.3 (225) μm (Al-Thukair and Golubic 1991a) long.

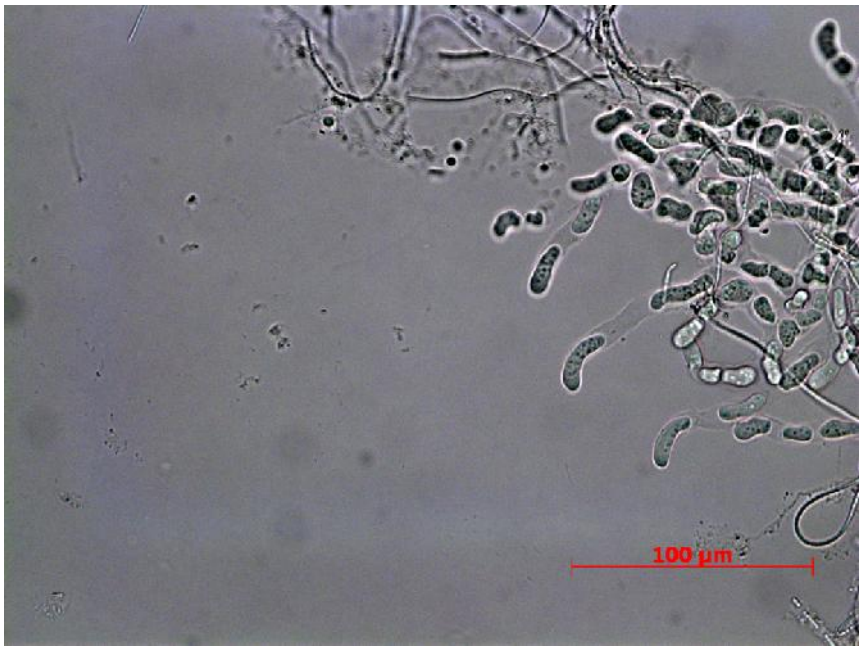


Figure 16 Light micrograph of *Hyella reptans* showing colony composition (scale = 100 µm)

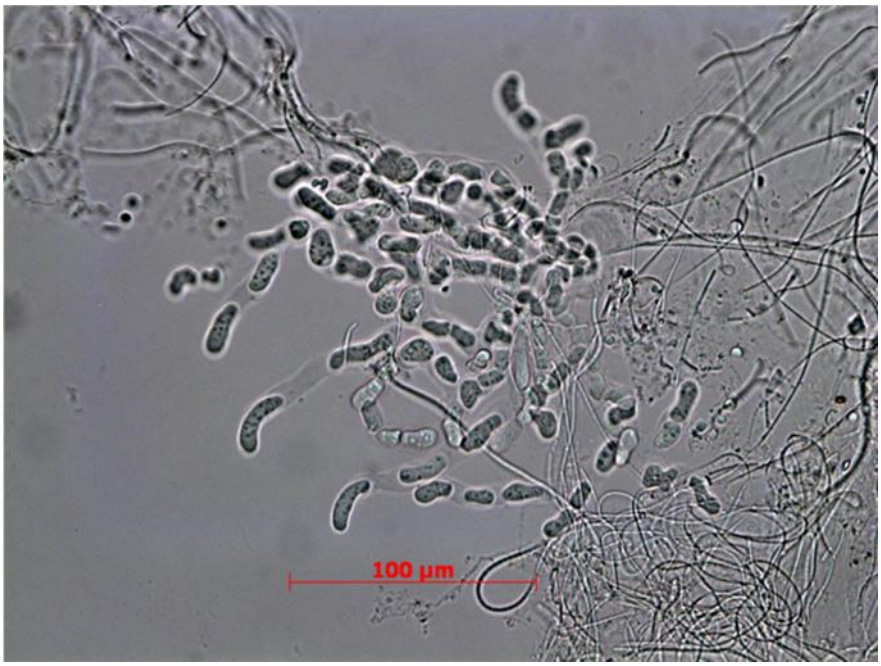


Figure 17 Light micrograph of *Hyella reptans* showing the multi serrated cell and branching (scale = 100 µm)

Class: *Coccogoneae* Thuret 1875

Order: *PleurocapsaIes* Geitler 1925

Family: *Hyellaceae* Borzi 1914

Genus: *Hyella* Bornet & Flahault 1888

Hyella arbuscula. Al-Thukair *et* Golubic (Al-Thukair and Golubic 1991)

(Figures 18 - 21)

The thallus of this taxon is composed of coccoid cells at the substrate subsurface and long radiating pseudofilaments, which bore into the substrate forming dense tree projections. False branching is common, mostly lateral rarely pseudodichotomous. Lateral branches originate by protrusion of the side wall of intercalary vegetative cells. Rare dichotomous branching originates by longitudinal division of apical cells and the distance between cell branches varies between 2 and 6 cells. The growth of the pseudofilament is generated from elongated clubbed shaped apical cells. Cell divides mostly transversely. Apical cells are cylindrical to clavate 4.3 ± 0.7 (27) μm wide (Al-Thukair and Golubic 1991a), and 16.7 ± 3.5 (27) μm long (Al-Thukair and Golubic 1991a). Vegetative cells are uniseriate 4.3 ± 0.7 (27) μm wide (Al-Thukair and Golubic 1991a), and 16.7 ± 3.5 (27) μm long (Al-Thukair and Golubic 1991a)

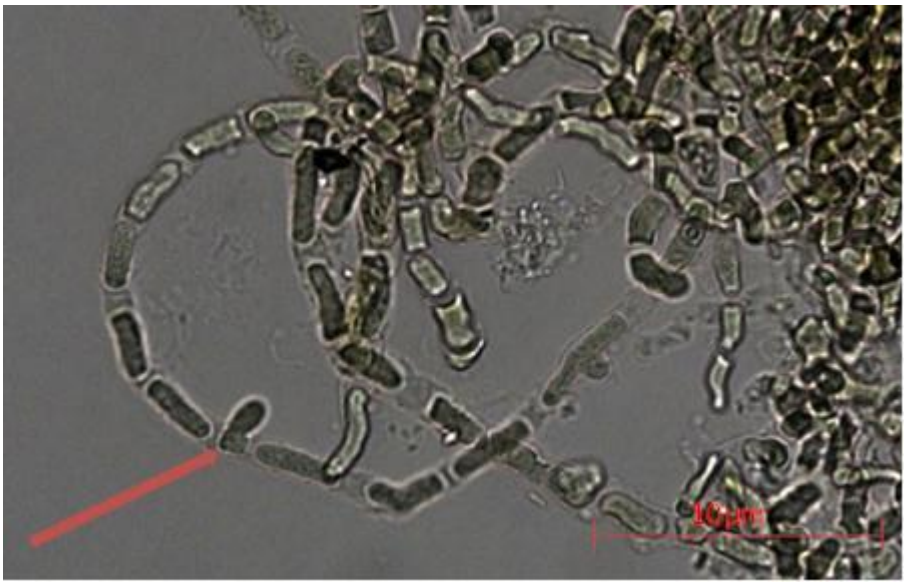


Figure 18 Light micrograph of *Hyella arbuscula* showing elongated and narrow apical and vegetative cell, arrow pointing towards lateral branching (scale = 10 µm)

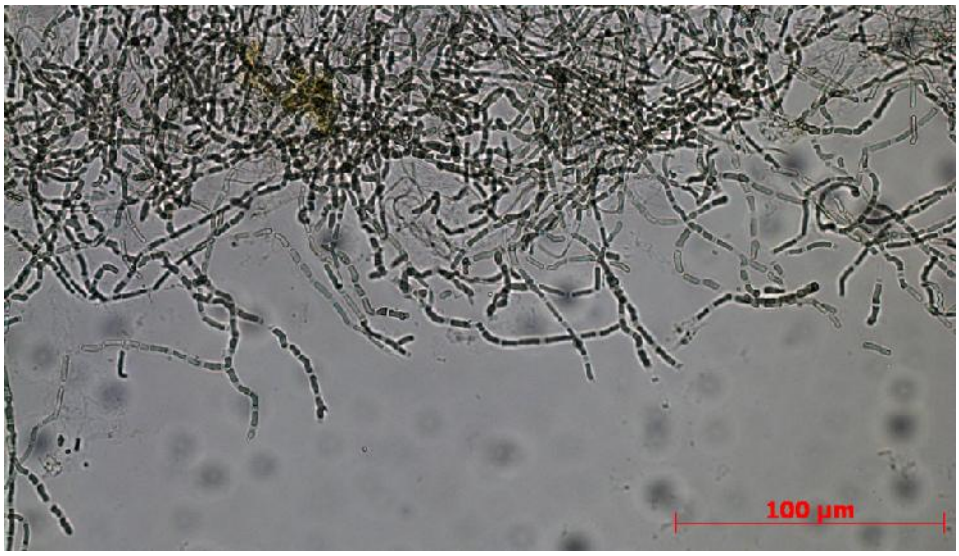


Figure 19 Light micrograph of *Hyella arbuscula* colony showing cells radiating from a common point of enty into the calcareous substrate (scale = 100 µm)

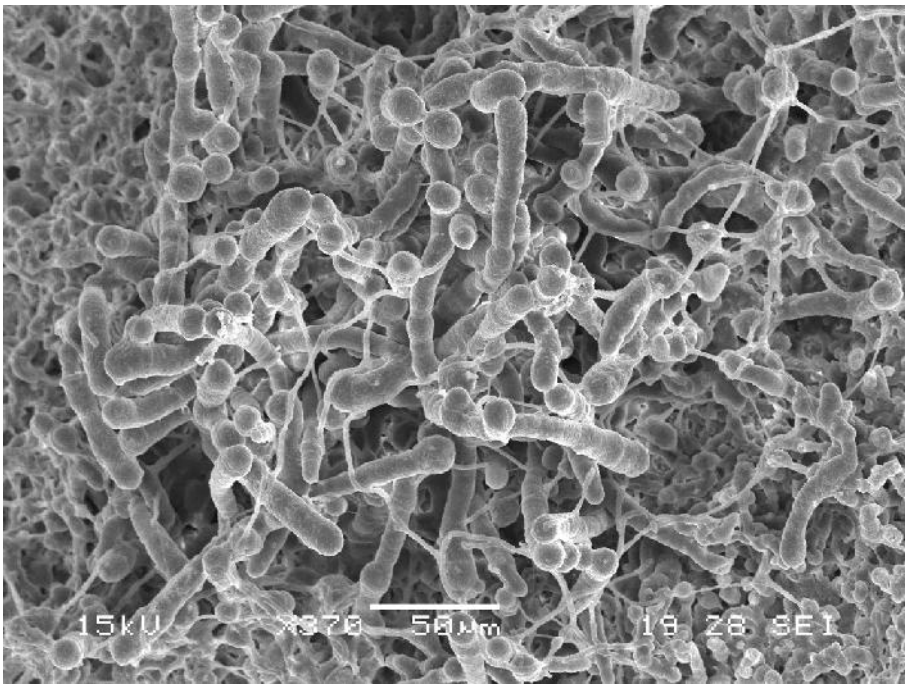


Figure 20 Scanning electron micrograph of *Hyella arbuscula* from samples collect from Tarut island (scale = 50 µm).

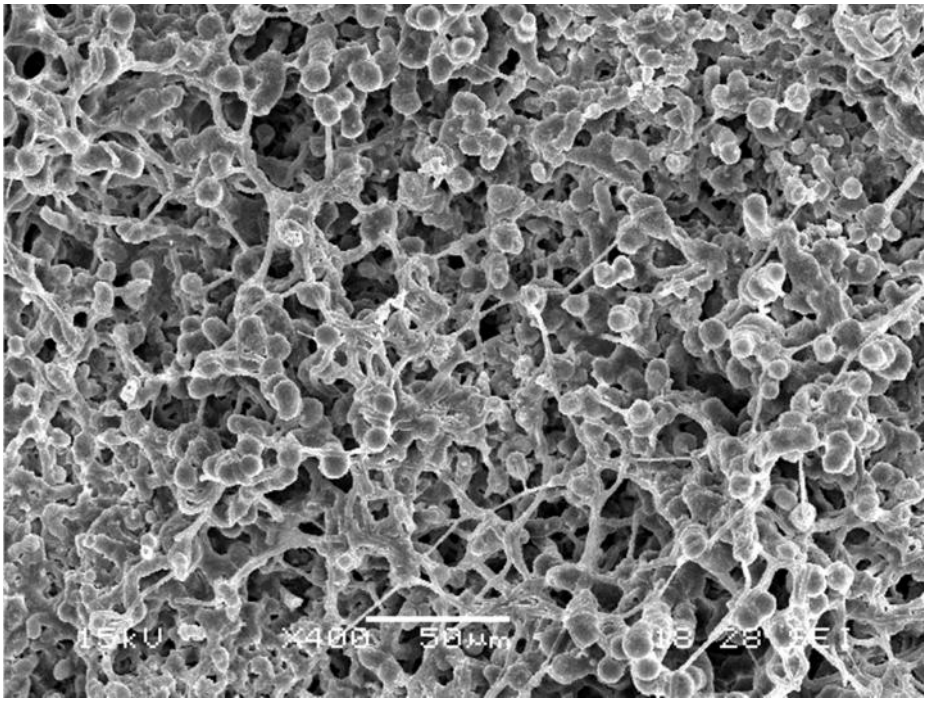


Figure 21 Total colonization of a calcareous material by several species of euendolithic cyanobacteria, prominent among them the *Hyella arbuscula* forming the base matrix and support for other species (scale = 50 µm).

Class: *Coccogoneae* Thuret 1875

Order: *PleurocapsaIes* Geitler 1925

Family: *Hyellaceae* Borzi 1914

Genus: *Hyella* Bornet & Flahault 1888

Hyella stella Al-Thukair *et* Golubic (Al-Thukair and Golubic 1991)

(Figure 22)

Thallus endolithic, pseudofilamentous, divergent. Branching dichotomous or lateral. Lateral branching, originating mostly by slipping of an intercalary cell out of alignment. The average distance between branches is 2-4 cells. Apical cells are cylindrical, isodiametric or slightly longer than wide 10.0±1.8(102) μm wide, and 11.4±2.3(102) μm long. Vegetative cells are spherical to oval, uniseriate, 11.3±2.5(160) μm wide, and 10.1±2.5(160) μm long.

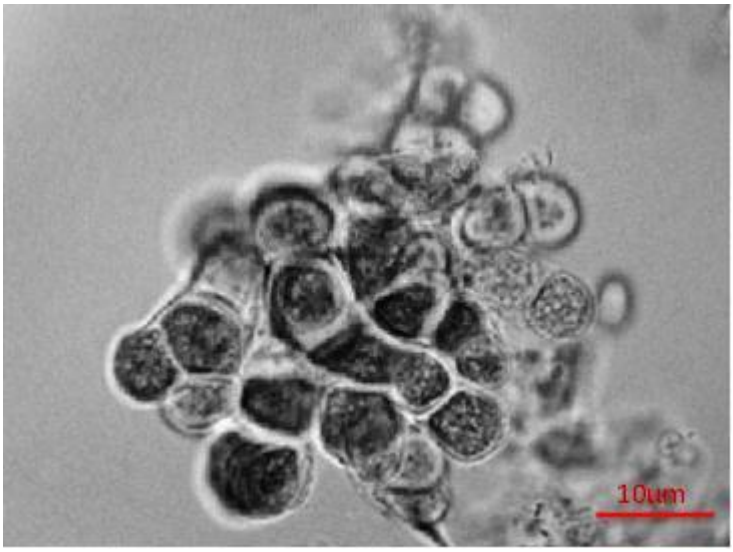


Figure 22 light micrograph of *Hyella stella* showing the prominent dichotomy (scale = 10μm).

Culturing of euendolithic cyanobacteria of the Arabian Gulf

Prospective calcareous carbonate materials with micro boring where selected and clean on solid agar media using micromanipulation techniques. The first baeocyte released were streaked on agar both liquid and solid, some of the cells observed under a light microscope. Calcite grains 2mm by 2mm were provided in the liquid media to test if the euendoliths retain their ability to bore into calcareous substrate. The result is presented in **Figures 23 - 33** and explained in Chapter 5 (Discussions)

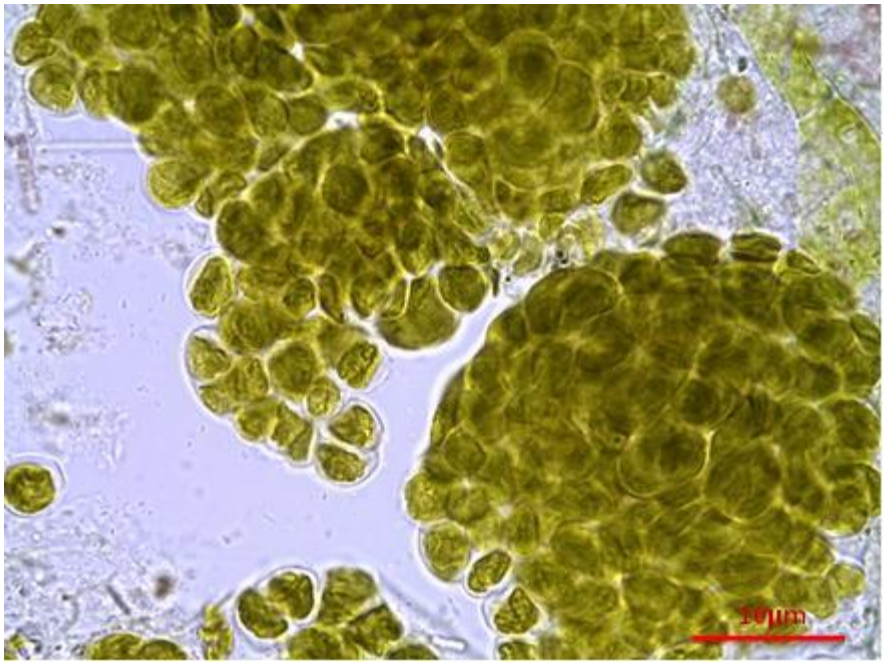


Figure 23 Light micrograph of the release of baeocytes from the culture of Arabia Gulf calcareous sediment (scale = 10µm).

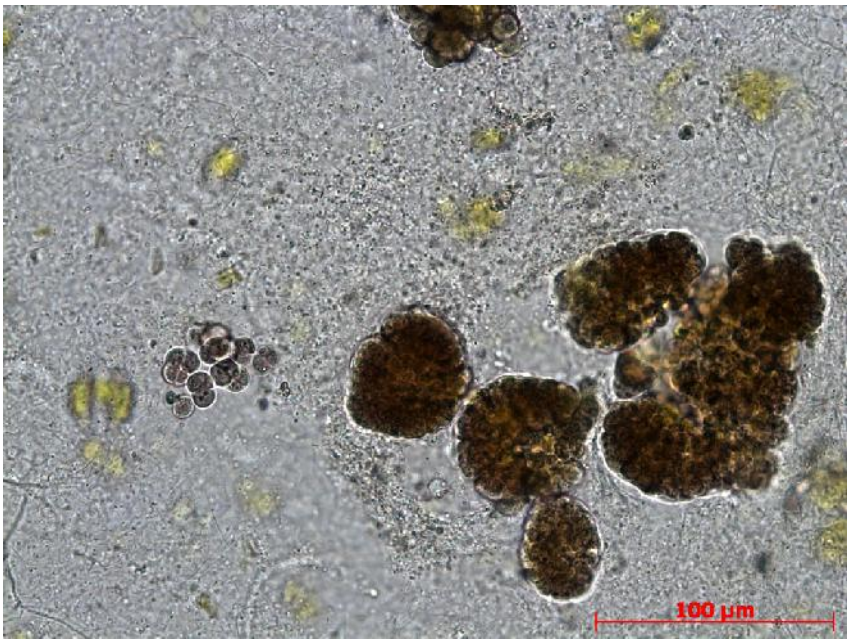


Figure 24 Colony of baeocytes released into media (scale = 100µm).

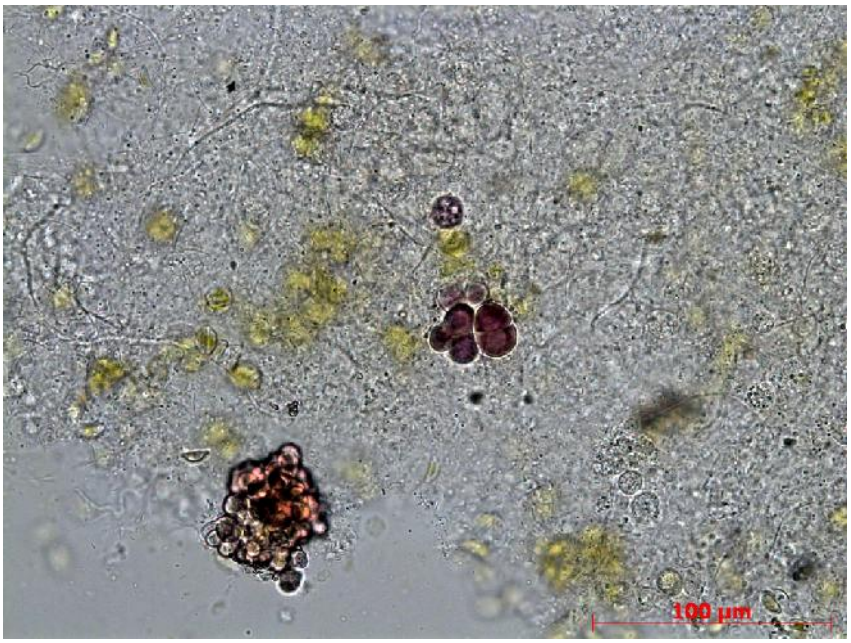


Figure 25 Differentiated baeocyte in solid SN media (scale = 100µm).

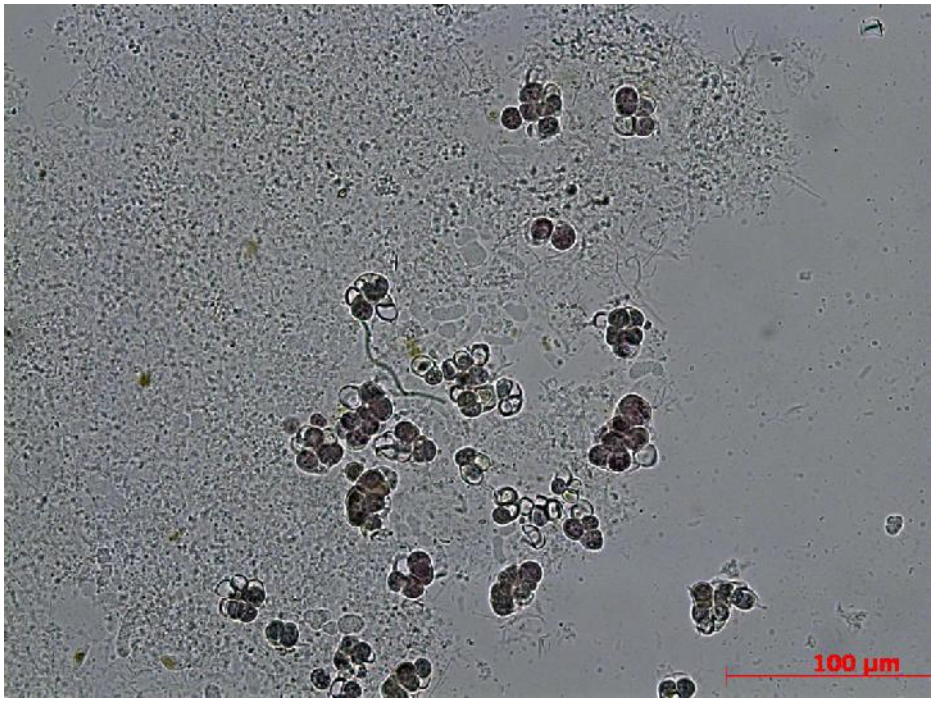


Figure 26 Baeocytes of unidentified euendolithic cyanobacteria in solid SN media (scale = 100µm)

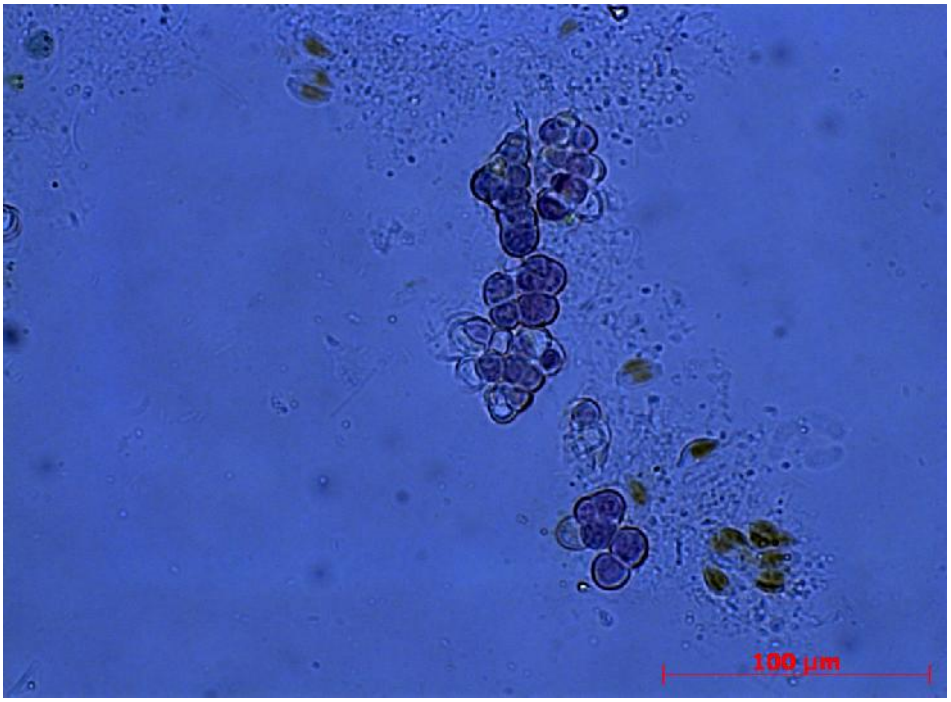


Figure 27 Baeocytes of unidentified euendolithic cyanobacteria in solid SN media (scale = 100µm).

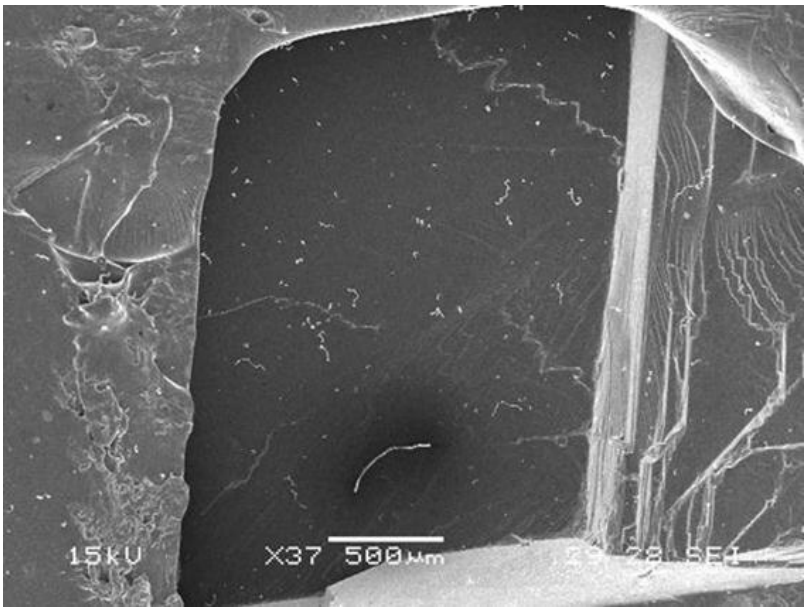


Figure 28 Scanning electron micrograph of resin cast in calcite of euendolithic cyanobacteria initiating boring (scale = 500µm)

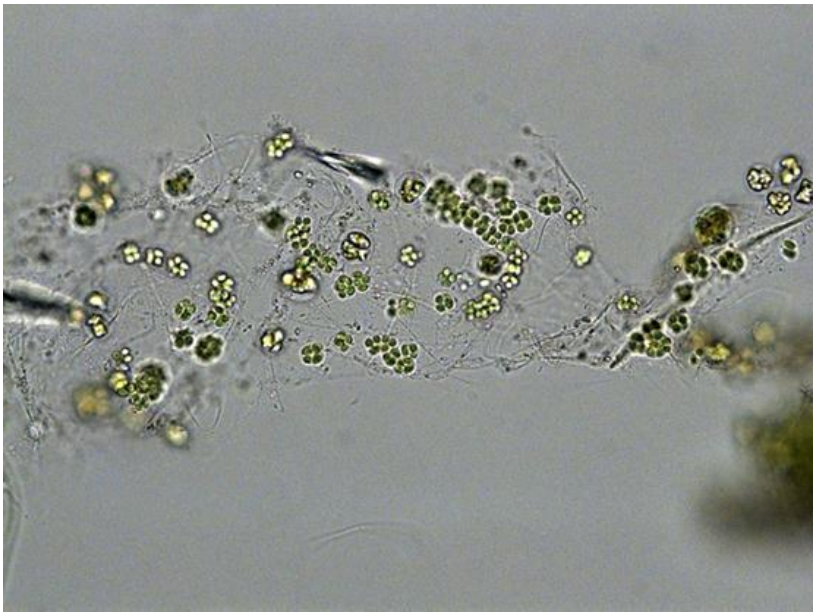


Figure 29 Light micrograph of the release of baeocytes from the culture of Arabia Gulf calcareous sediment (scale = 10μm).

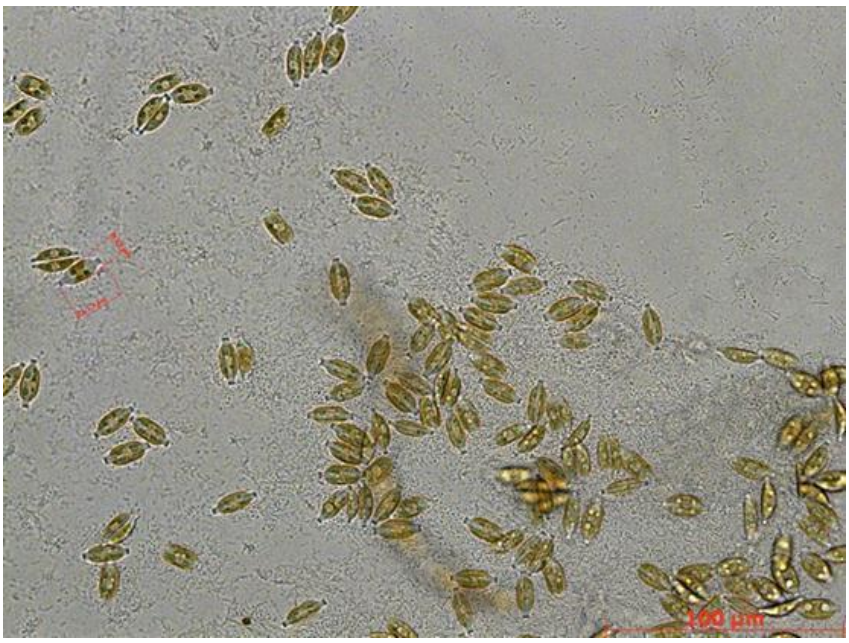


Figure 30 Epilithic diatomic communities contaminating the culture (scale = 100µm).

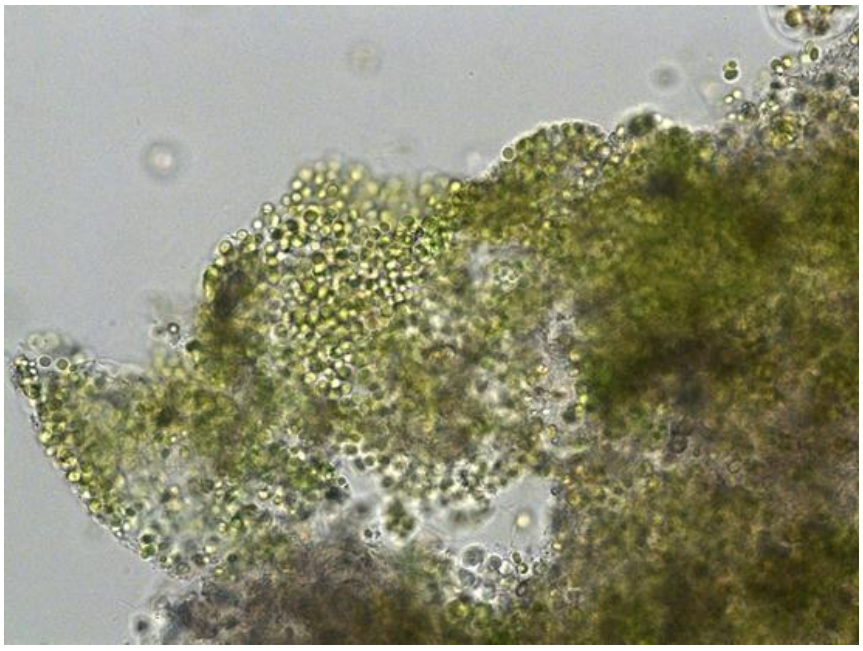


Figure 31 Pronounced green pigmentation in newly released baeocytes from samples collected from Abu Ali (KSA) (scale = 100μm).

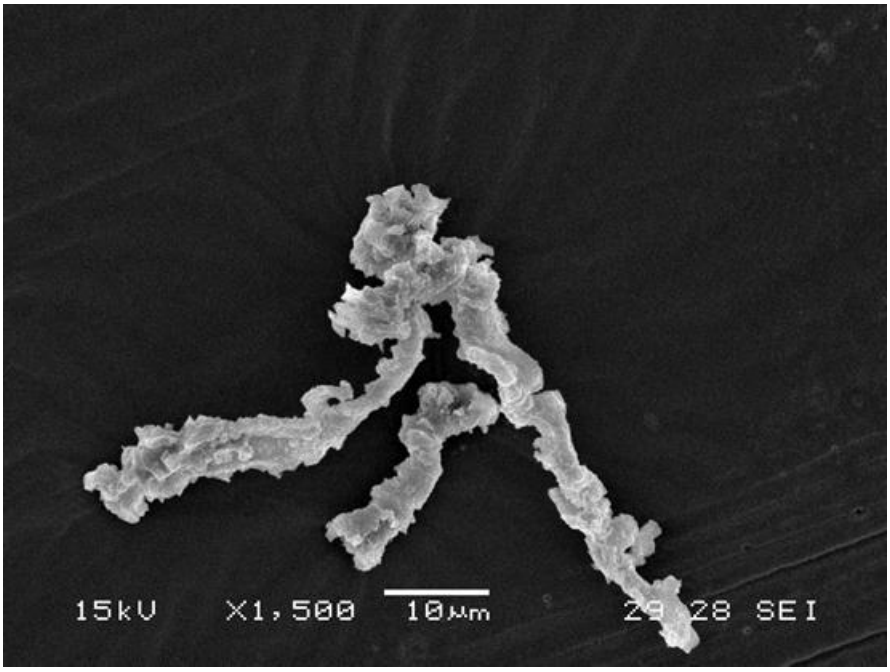


Figure 32 Scanning electron micrograph of resin cast in calcite with a branching endolith species could not be determined (scale = 10µm).

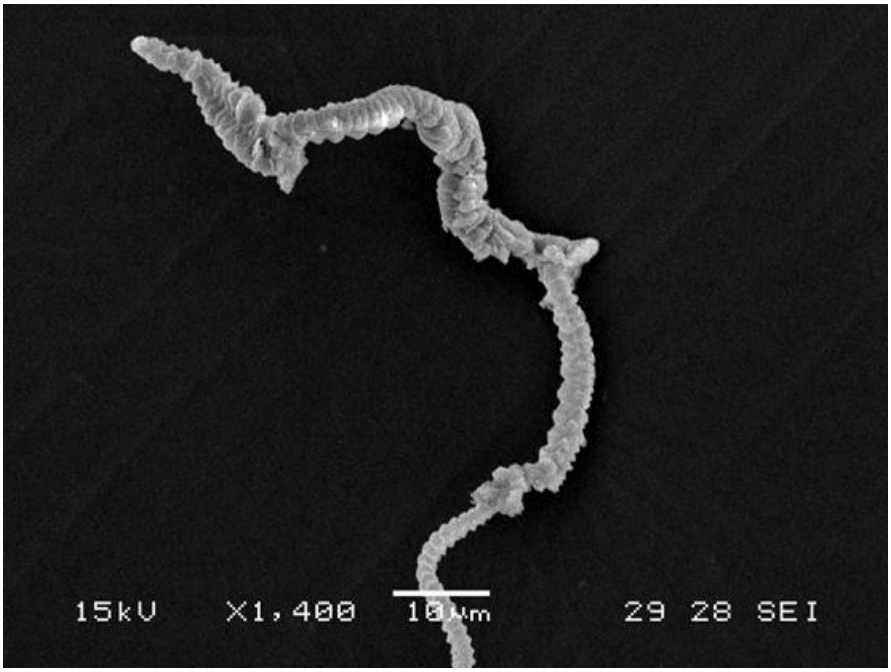


Figure 33 Scanning electron micrograph of resin cast in calcite an endolith (scale = 10µm).

Morphometric Identification of euendolithic cyanobacteria from Costa Rica

Morphometric measurements of different types of cells reveal that the species can be distinguished from one another mainly by the width, length of the apical, vegetative and baeocyte mother cells (Table **5**) . All species considered in this study satisfied the requirements to be included in the Hyella group (bore into substrate) as explained below. Measurements of cell sizes were determined from light microscope photomicrographs using AxioVision 4.8 (Carl Zeiss imaging software) and compared with published illustration *Hyella* and *Solentia* assemblages all around the world. The data is presented as "m ±s.d (n)" where "m" is mean of cell size; "s.d" is the standard deviation and "n" the number of cells measured.

Table 5 Morphometric data for different cell types of three species of Euendolithic Cyanobacteria identified from Costa Rica Samples (m ±s.d (n)) "m" is mean of cell size; "s.d" is the standard deviation and "n" the number of cells measured

Sample	Apical Cells		Vegetative Cells		Baeocyte Mother Cells	
	Width (µm)	Length (µm)	Width (µm)	Length (µm)	Width (µm)	Length (µm)
H. sp 1	11.43 ± 1.9 (182)	16.55 ± 2.8 (182)	9.43 ± 2.3 (182)	12.02 ± 2.8 (182)	15.23 ± 2.2 (16)	27.36 ± 3.8 (16)
H. sp 2	6.50 ± 1.3 (186)	10.14 ± 2.3 (186)	7.11 ± 1.39 (186)	9.78 ± 1.9 (186)	17.27 ± 4.2 (13)	21.87 ± 4.3 (13)
H. sp 3	8.73 ± 1.6 (148)	9.16 ± 2.2 (148)	8.85 ± 1.6 (148)	9.39 ± 2.2 (148)	15.47 ± 5.4 (15)	21.39 ± 4.3 (15)

Class: *Coccogoneae* Thuret 1875

Order: *PleurocapsaIes* Geitler 1925

Family: *Hyellaceae* Borzi 1914

Genus: *Hyella* Bornet & Flahault 1888

Hyella sp 1

(Figures 34 - 42)

Thallus is endolithic, pseudofilamentous, dichotomously branched. Branching is mostly dichotomous (80%), by longitudinal division of the apical cell. Lateral branching of the intercalary cell is very rare. Apical cells are elongated, clubbed shaped on the average longer than wide. Cell measurements: m ± s d (n) = 11.43 ± 1.9 (182) μm wide, and 16.55 ± 2.8 (182) μm long. Vegetative cells are uniseriate and of two types, rectangular and inverted pyramid. The rectangular cells mark early stages of transverse cell division from the apical cell while the inverted pyramid shows late stages and also as a pivotal cell for dichotomous branching. On the average, the vegetative cells are 9.43 ± 2.8 (182) μm wide, and 11.4 ± 3.3 (375) μm long. Vegetative cells are shorter, and slightly wider than apical cells. Cells are enveloped in a thick gelatinous envelope, which are distinctly layered, laterally up to 2 μm thick and 5 μm between cells. The baeocyte mother cell is cylindrical and enclosed in thick sheaths; it is 27.36 μm long and 15.13 μm wide. Morphometric data for SP 1 are displayed graphically in **Figure 42**. Filaments are arranged pentahedrally bearing resemblance to uniseriate macrosporangia. The well-defined symmetrical and multilayered gelatinous envelopes are thinner between cells that divided recently and thicker around paired cells. Dichotomous branching is due to longitudinal division of the apical cells, which occurs prominently, particularly at the early stages of growth, contributing to the initial divergence of the thallus. Subsequent growth is dominated by a series of successive transverse cell

divisions, creating long filaments. SP1 is a large and dominant species in the calcareous sediments examined.

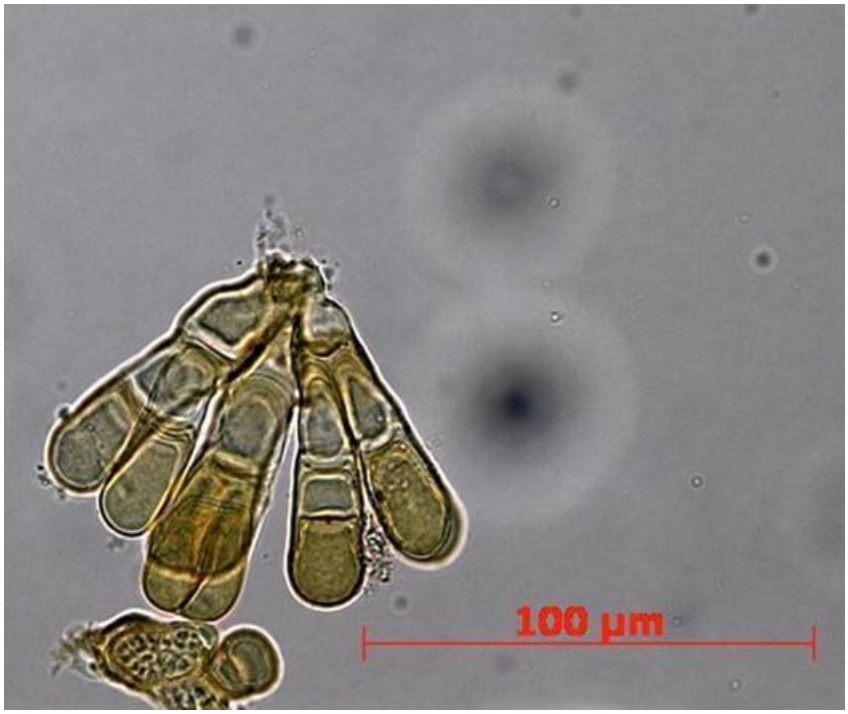


Figure 34 Mature pseudofilaments of sp 1 with dichotomously branched cells (scale = 100µm).

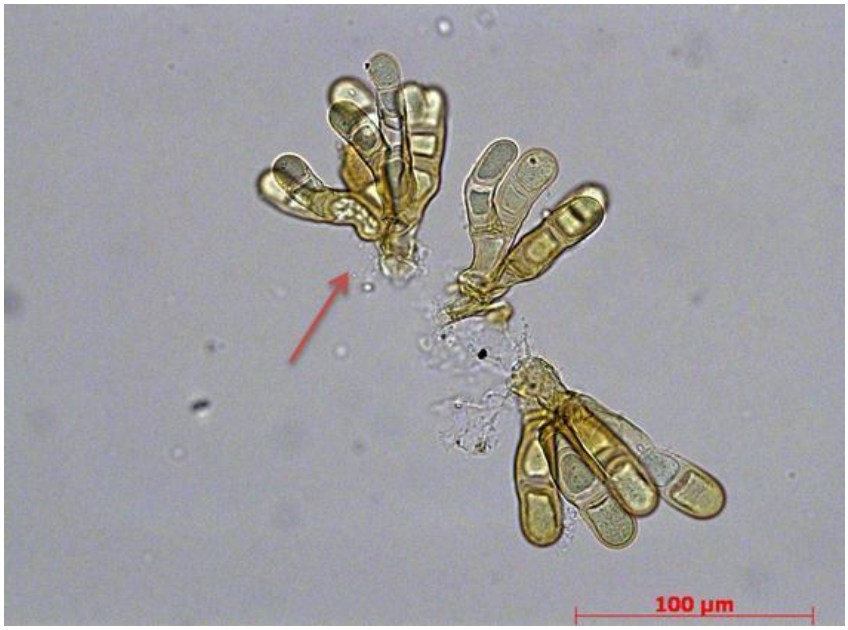


Figure 35 Mature pseudofilaments of sp 1 with dichotomously branched cells and the start of baeocyte production. See insert arrow

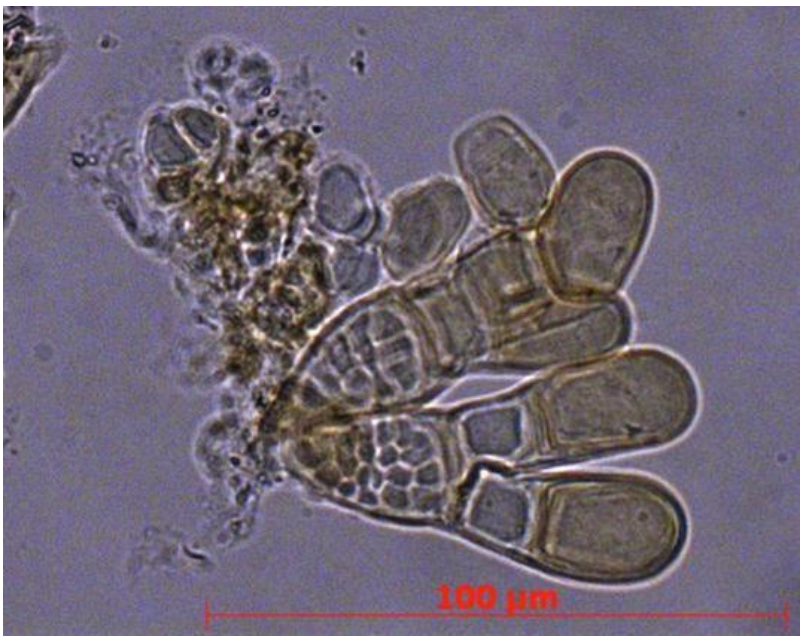


Figure 36 Mature pseudofilaments of sp 1 with dichotomously branched cells and fully formed baeocyte mother cells (scale = 100µm)

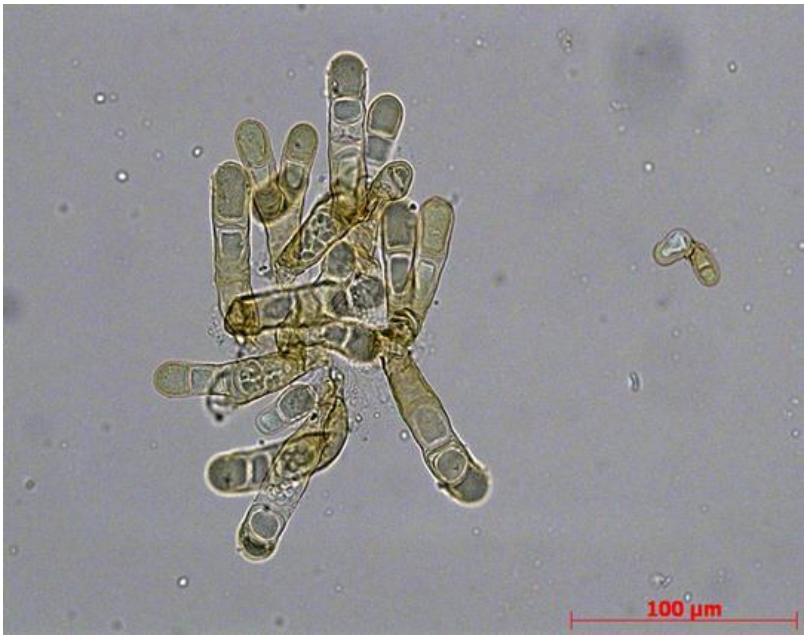


Figure 37 showing dichotomous filament, filaments always appear in pairs (scale = 100µm).

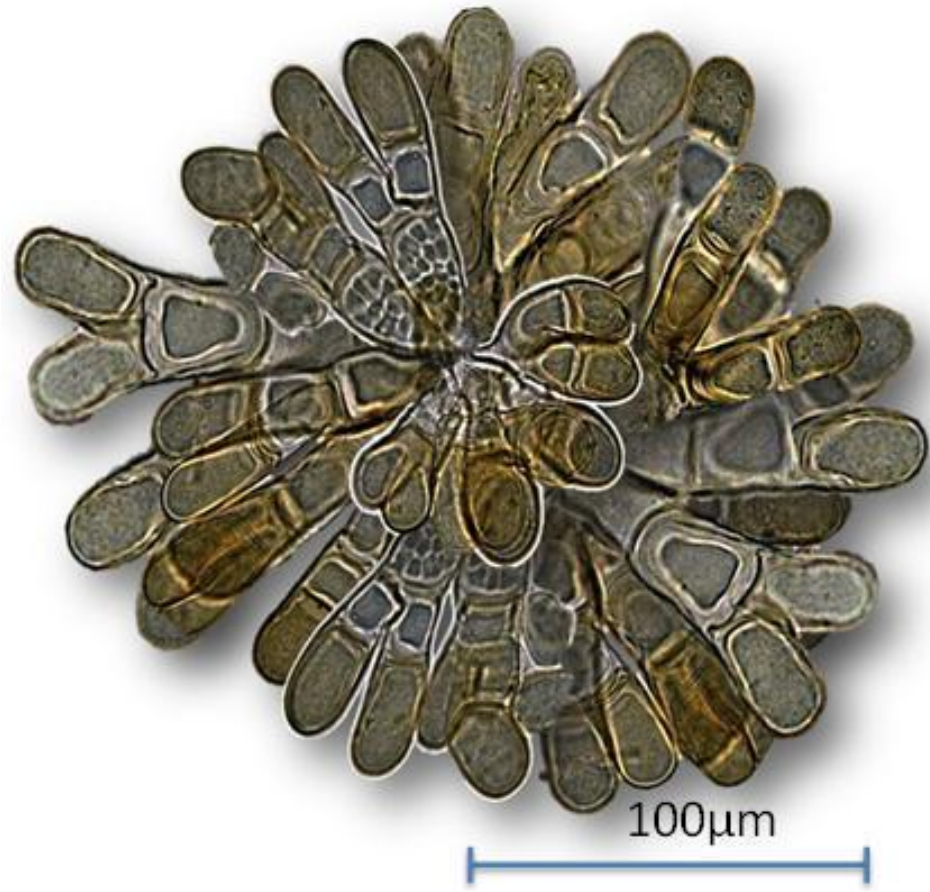


Figure 38 Colony reconstruction of SP1 from Costa Rican Sample (scale = 100µm).

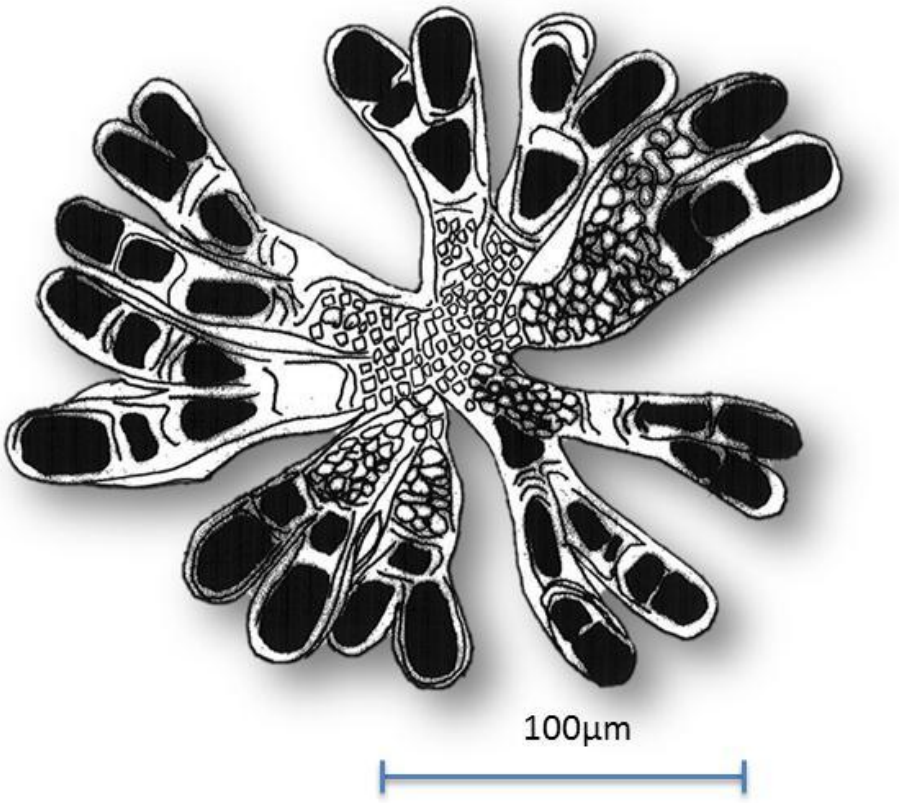


Figure 39 Pencil sketch colony reconstruction of SP1 from Costa Rican Sample (scale = 100µm).

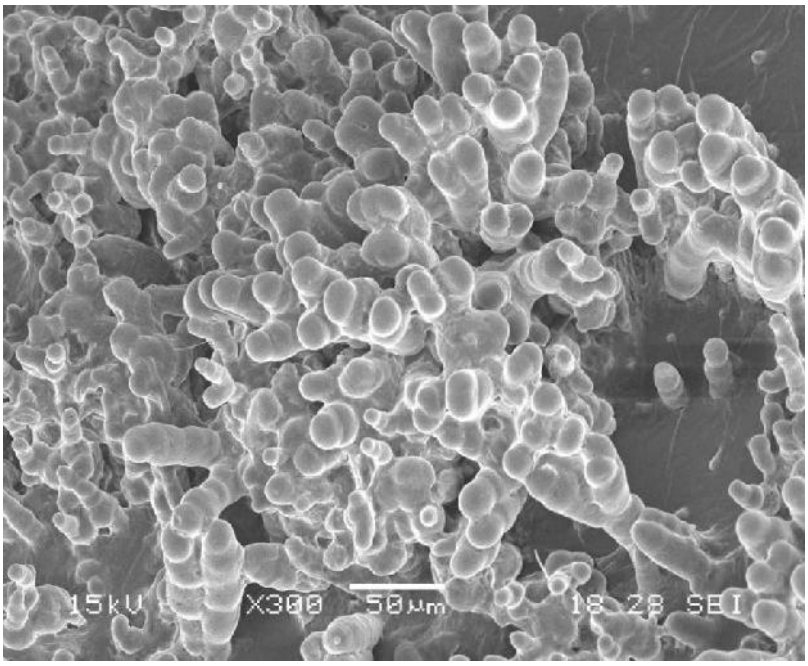


Figure 40 Resin cast Scanning Electron micrograph of SP1, showing the prominent dichotomy (scale = 50 µm)

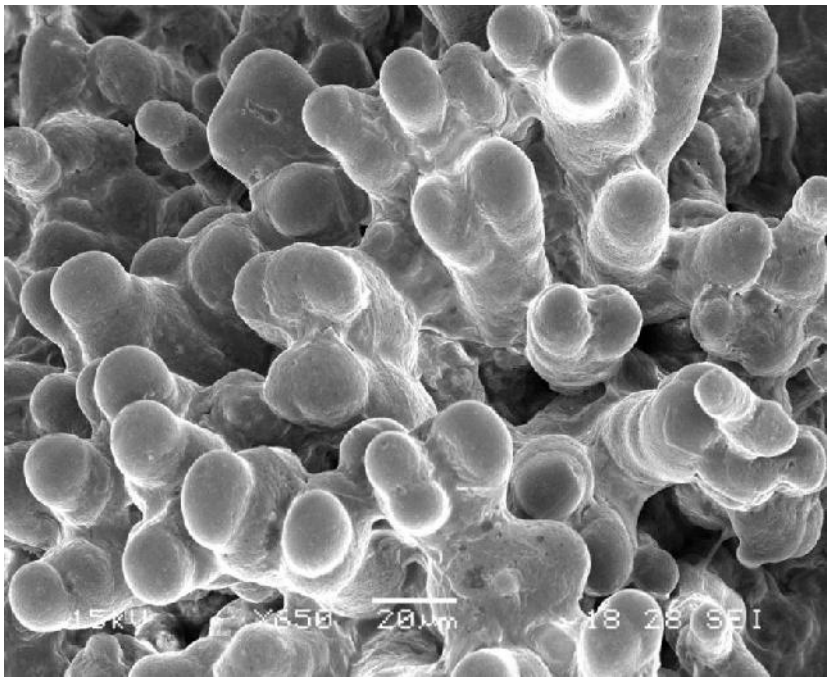


Figure 41 Resin cast Scanning Electron micrograph of SP1, showing the prominent dichotomy (scale = 50 µm)

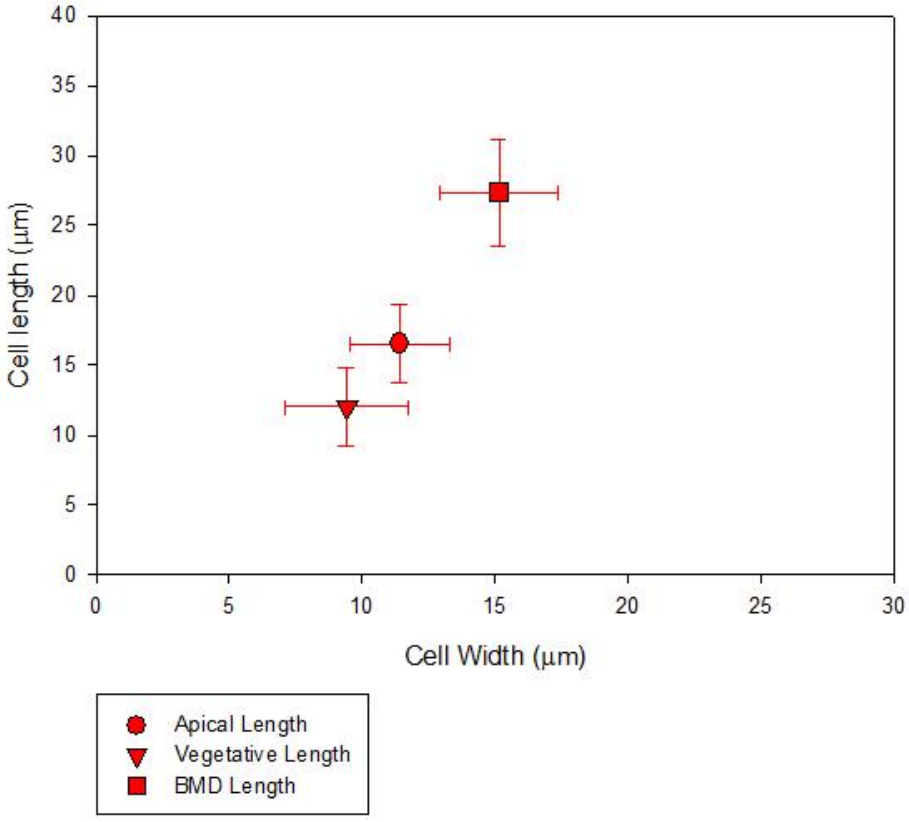


Figure 42 Graphical representation of the morphometric measurements for sp1. apical length=circle, vegetative length=inverted triangle, baeocyte mother cell= square

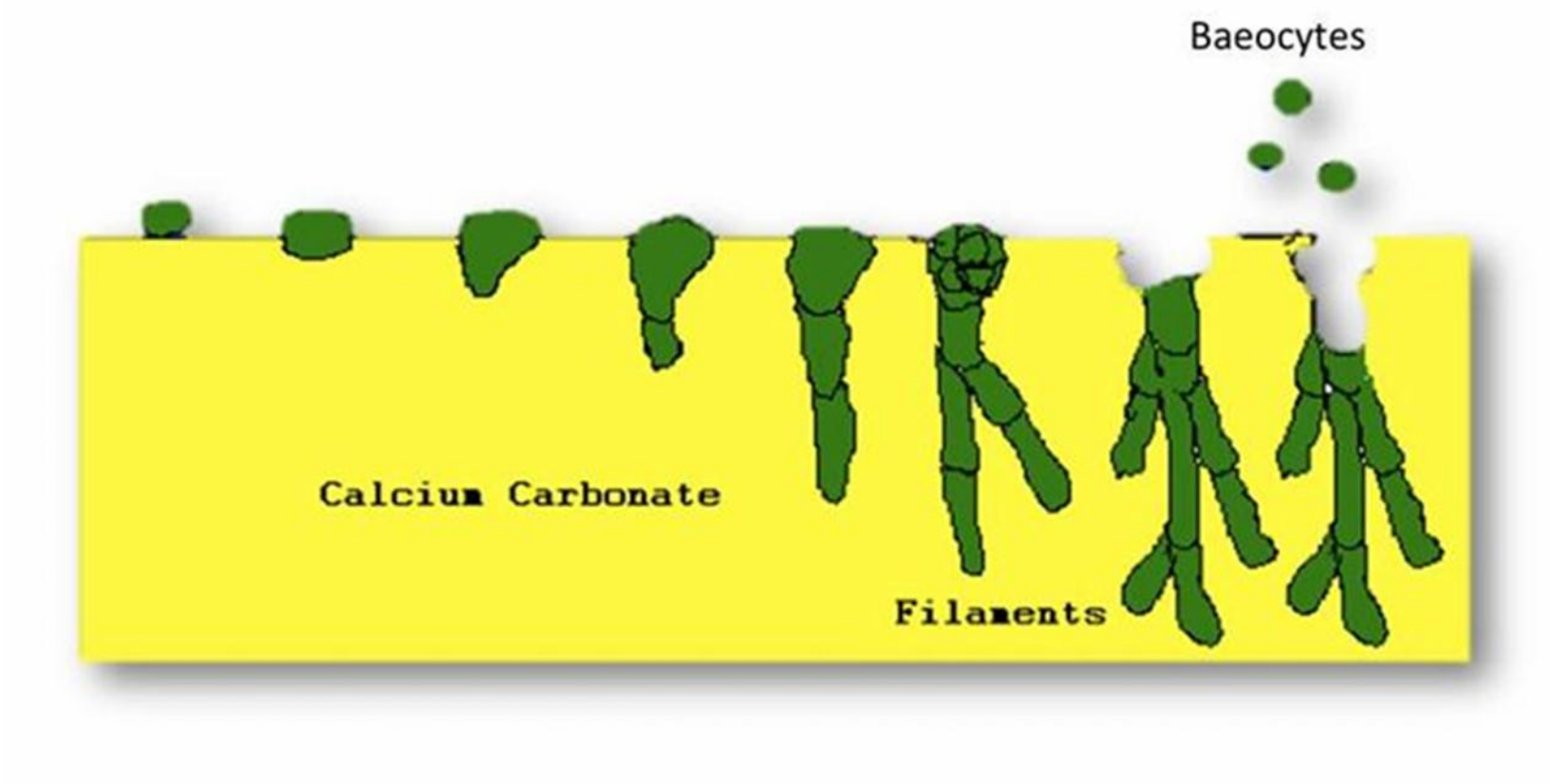


Figure 43 A typical life Cycle sequence of endolithic

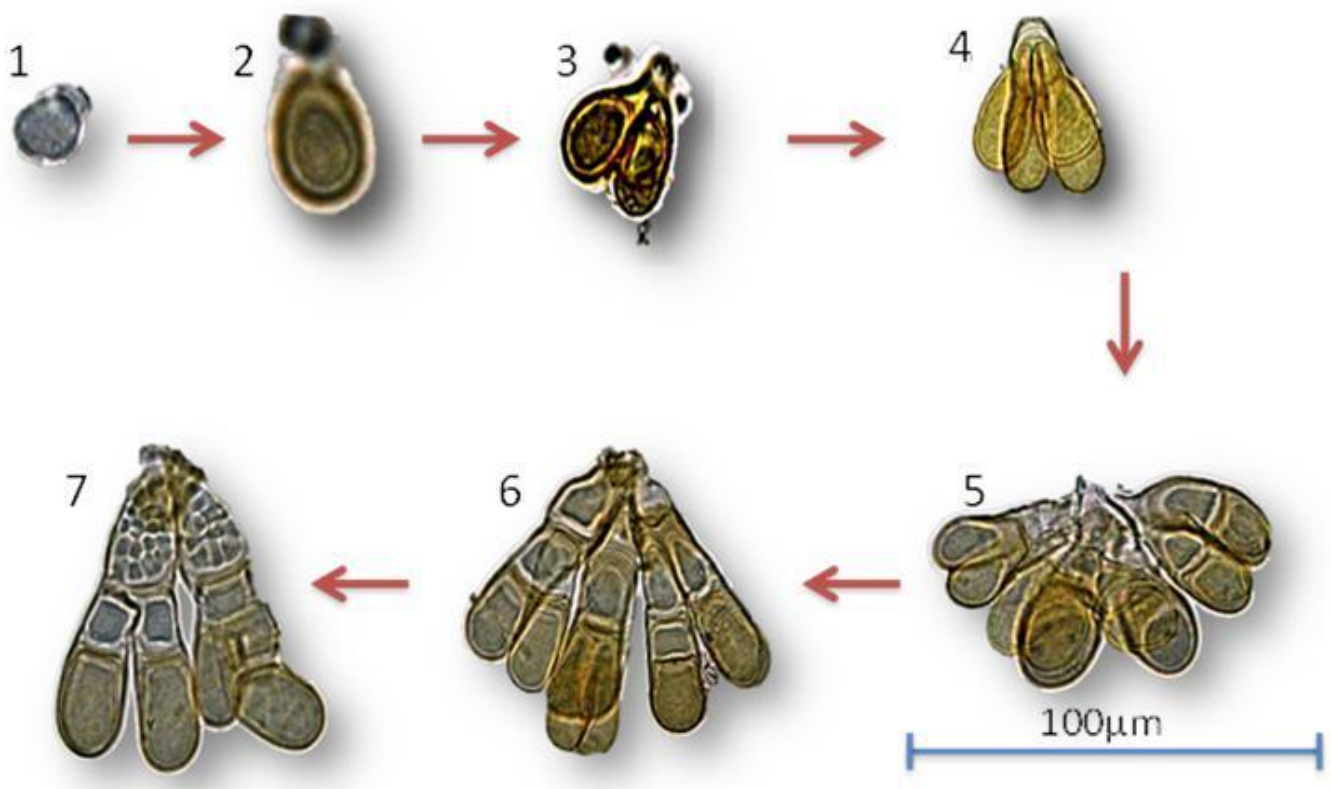


Figure 44 Tentative life Cycle sequence of SP 1. 1- 3 showing early longitudinal development of baeocytes. 4 represent transverse division, dichotomy and growth of apical cell. 5 – 6 showing the formation and ripening of baeocytes mother cell before the release

Typical life cycle of Hyella can be described by starting with the release of small spherical reproductive cells, called baeocytes. Baeocytes settle on calcium carbonate substrates following a brief period of phototactic gliding motility (Waterbury & Stanier 1978). They then penetrate into the substrate by an unknown chemical mechanism. As they enter the substrate, baeocytes elongate and increase in size, divide transversally into two functionally different daughter cells: a distal apical cell, and a proximal (to substrate surface) vegetative cell. The apical cell continues to divide transversally, consecutively delivering a definite number of vegetative cells, in the process positioning itself at the tip of the pseudofilament. The apical cell is the site where most boring activity occurs. Pseudofilaments are initially uniseriate, but they may become biseriate and multiseriate at later stages due to longitudinal or oblique divisions of intercalary (vegetative) cells. Dichotomous branching of pseudofilaments may originate from longitudinal division of the apical cell. Lateral branching may originate from longitudinal division of vegetative cells, their re-orientation by bending or slippage, or by protrusion of their lateral cell wall. Some cells proximal to the substrate surface may increase in size to form baeocyte mother cells, which undergo multiple fission into a large number of small (1-4 μm in diameter) baeocytes, that are released by the dissolution and/or rupture of cell envelopes (Al-Thukair 1991).

Class: *Coccogoneae* Thuret 1875

Order: *PleurocapsaIes* Geitler 1925

Family: *Hyellaceae* Borzi 1914

Genus: *Hyella* Bornet & Flahault 1888

Hyella sp2

(Figures 45 -52)

Thallus: endolithic, pseudofilamentous, sprawling and repeatedly branched. Lateral branching occurs by cell slippage (85%), less commonly by protrusion of the lateral wall of an intercalary cell (15%). The average distance between branches is 2 cells. Apical cells are clavate to oval, on the average longer than wide, rounded at the tip: M ± sd (n) = 6.50 ± 1.3 (186) μm wide, and 10.14 ± 2.3 (186) μm long. Vegetative cells are oval, cylindrical with rounded ends, 7.11 ± 1.4(186) μm wide, and 9.70 ± 1.9 (186) μm long. The Baeocyte mother cell is trilobed with an uneven circumference; it is 21.87 μm long and 17.27 μm wide. The main filaments grow by divisions of an apical cell and produce branches in a monopodia order. Lateral branches may penetrate deeper into the substrate. The branching originates mostly by a rotation of an intercalary vegetative cell, which slips out of alignment into a position perpendicular to the filament direction. The change in orientation of these branch point cells leaves a mark in layering of their envelopes, producing T -shape. Following rotation, these cells act as apical cells of the branches. Morphometric data for different cell types are given in **Table 5** and graphically displayed in **Figure 52.**

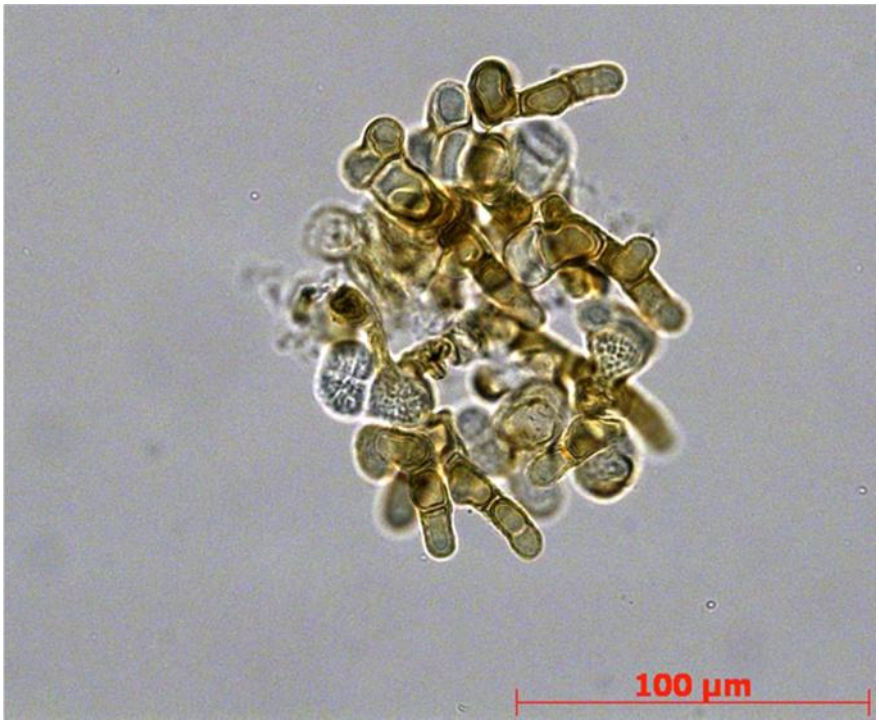


Figure 45 sp 2 with prominent lateral cell division and production of baeocyte mother cell (scale = 100 µm)

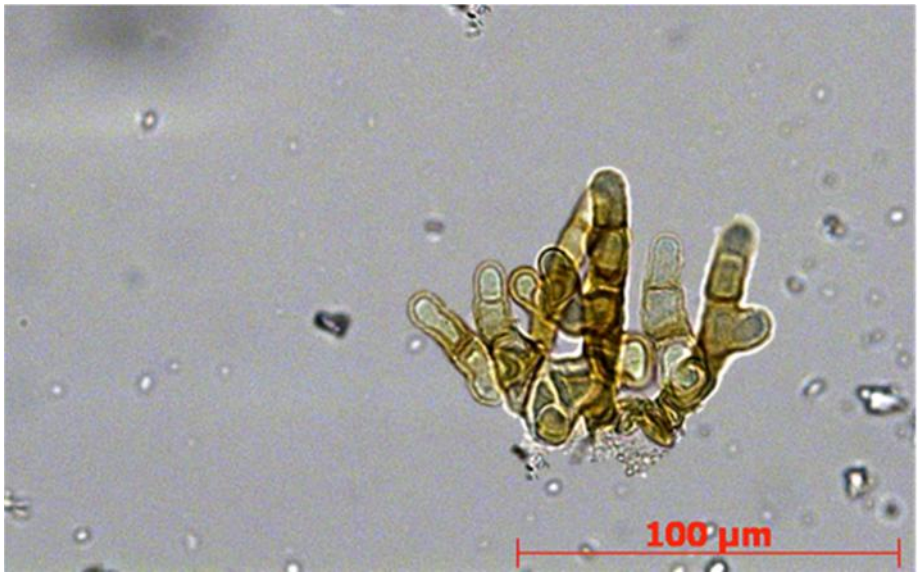


Figure 46 sp 2 lateral cell division by slippage (scale = 100 µm)

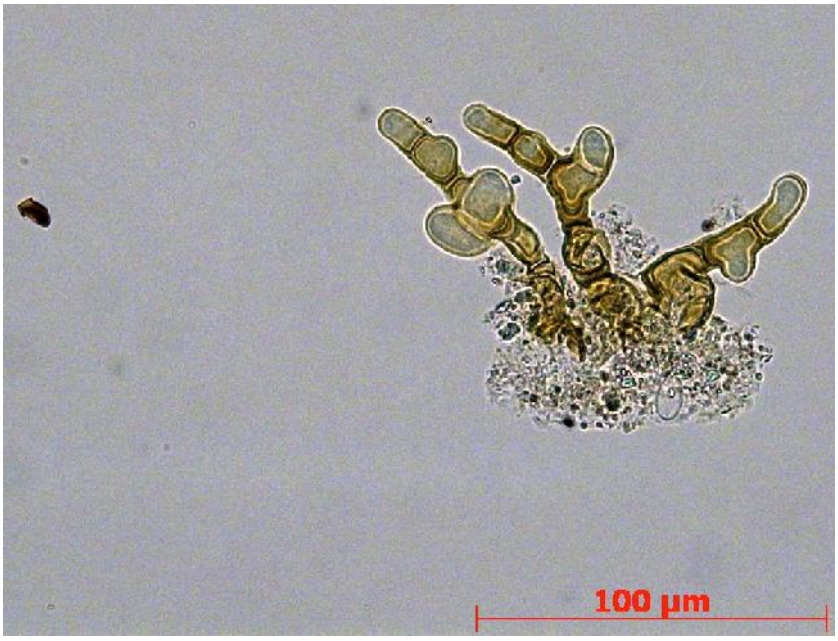


Figure 47 Cell division by protruding cell wall (scale = 100 µm).

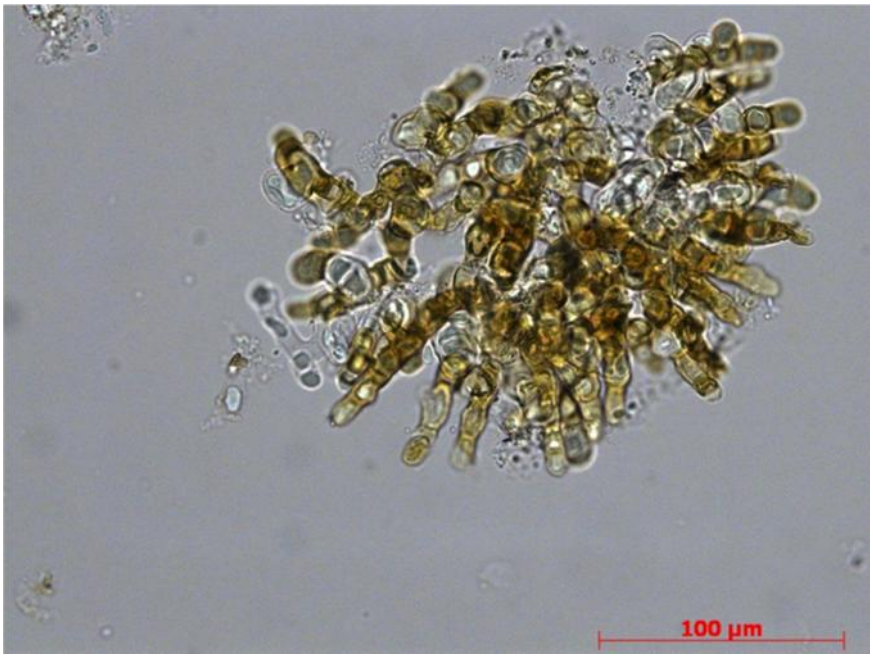


Figure 48 Natural appearance of sp2 colony (scale = 100 µm)

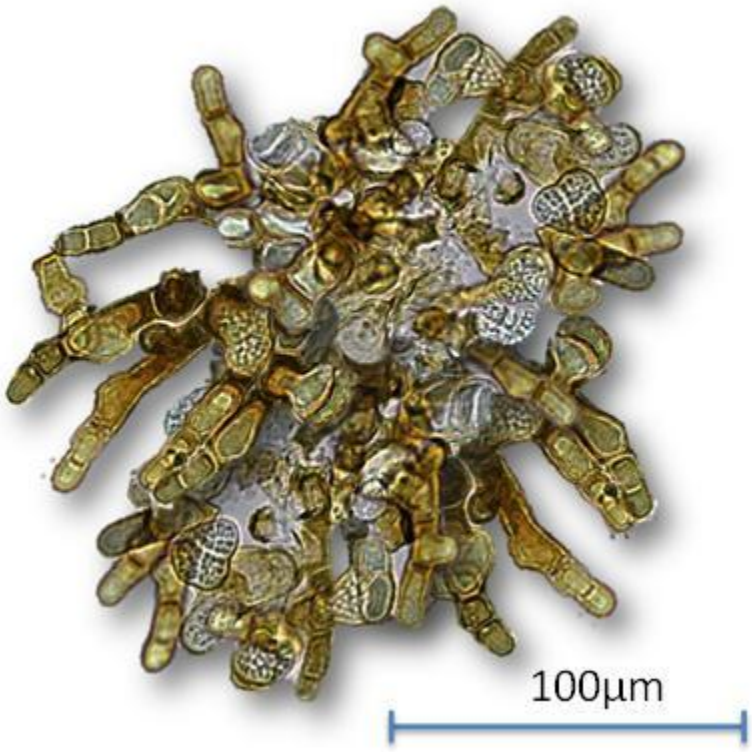


Figure 49 Colony reconstruction of SP 2 from Costa Rican Sample (scale = 100 µm)

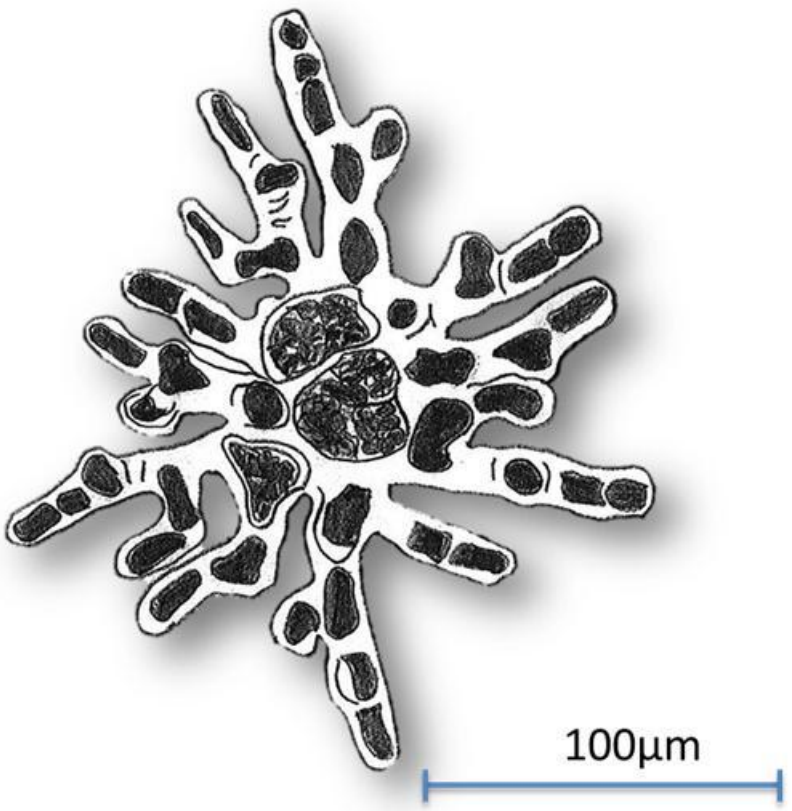


Figure 50 Pencil sketch Colony reconstruction of SP 2 from Costa Rican Sample (scale = 100 µm)

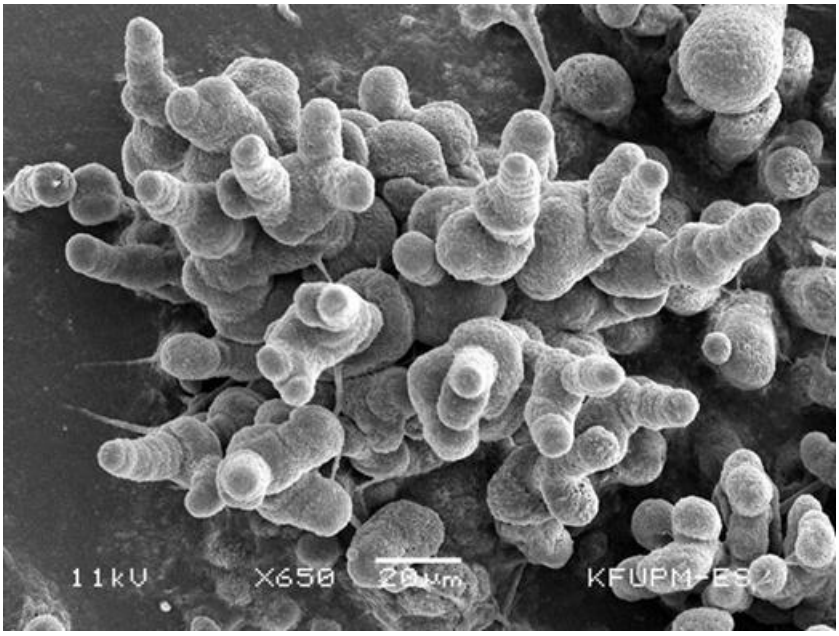


Figure 51 Resin cast Scanning Electron micrograph of SP 2, showing the lateral cell division (scale = 50 μm)

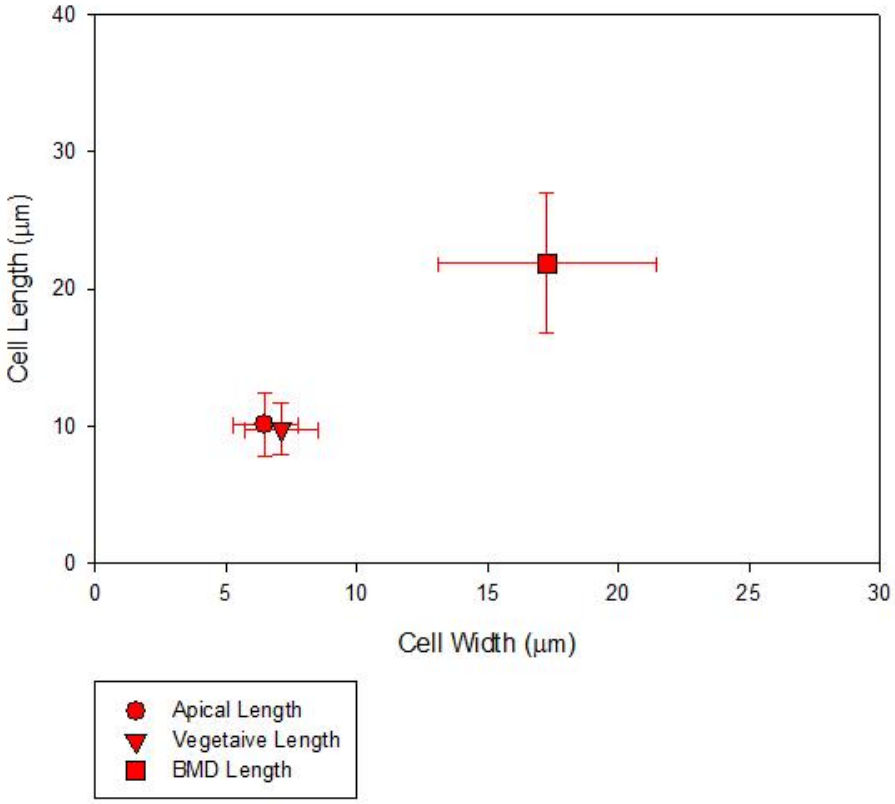


Figure 52 Graphical representation of the morphometric measurements for SP2. apical length=circle, vegetative length=inverted triangle, baeocyte mother cell= square

Class: *Coccogoneae* Thuret 1875

Order: *PleurocapsaIes* Geitler 1925

Family: *Hyellaceae* Borzi 1914

Genus: *Hyella* Bornet & Flahault 1888

Hyella sp3

(Figures 53-59)

Thallus endolithic, pseudofllamentous, divergent. Branching dichotomous (90%) or lateral (10%). Lateral branching, originating mostly by slipping of an intercalary cell out of alignment. Branching relatively dense, with an average distance between branches of 2 cells. Apical cells oval, rectangular and occasionally clubbed shaped, slightly longer than wide, M ± sd (n) = 8.73 ± 1.6 (148) µm wide, and 9.16 ± 2.2 (148) µm long. Vegetative cells are spherical to oval, 8.85 ± 1.6 (148) µm wide, and 9.39 ± 2.2 (148) µm long. Baeocyte mother cells are spherical to oval, 18.8 ± 3.1 (15) µm wide, 20.9 ± 2.8 (15) µm long. The Baeocyte mother cell is bi-lobed and oval, it is 27.36 µm long and 15.13 µm wide. This prominent characteristic is due to its regular dichotomous branching, resulting in almost orbicular divergent thallus and borings. Morphometric data for SP3 are displayed graphically in **Figure. 59.**

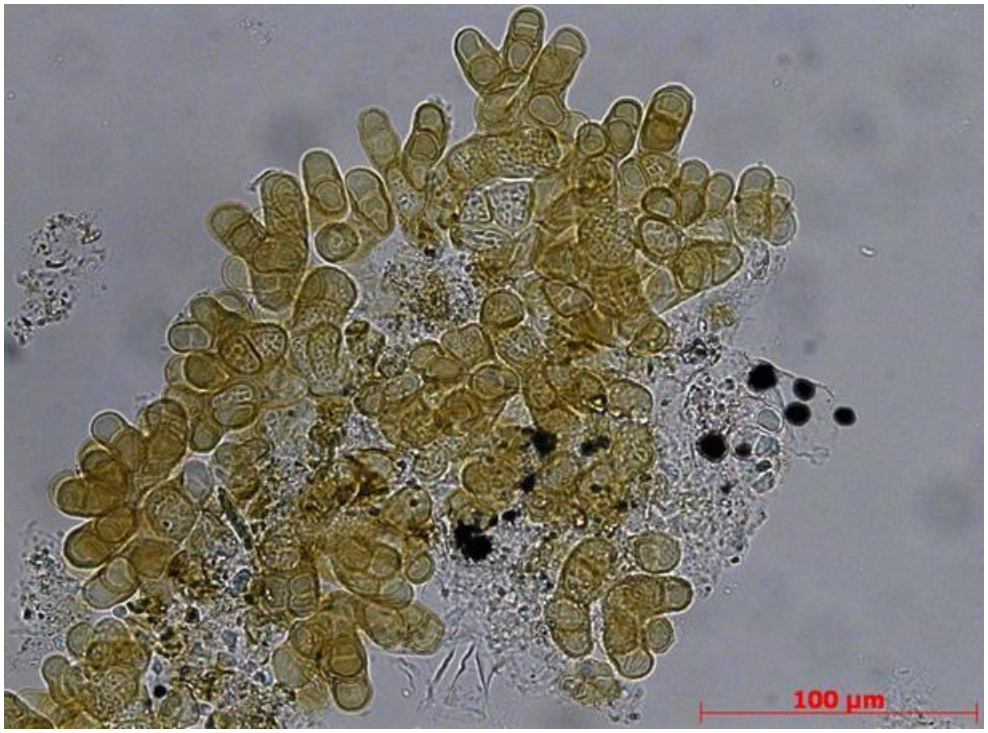


Figure 53 mature colony of sp 2 (scale = 100 µm)

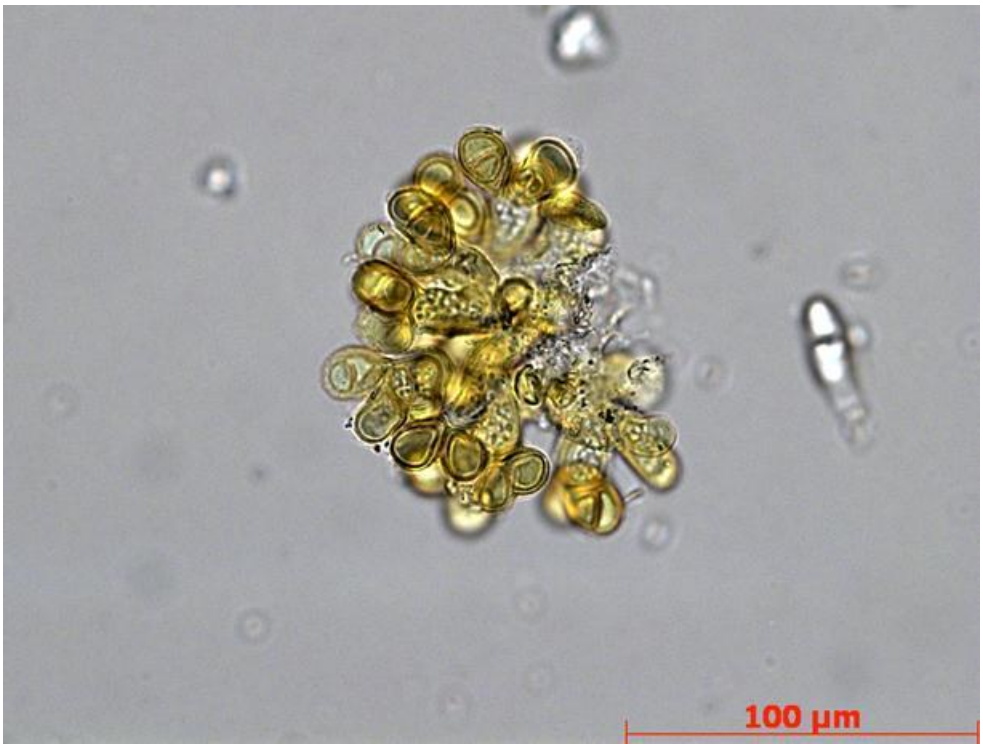


Figure 54 Sp3 showing early stages of the colony formation (scale = 100 µm)

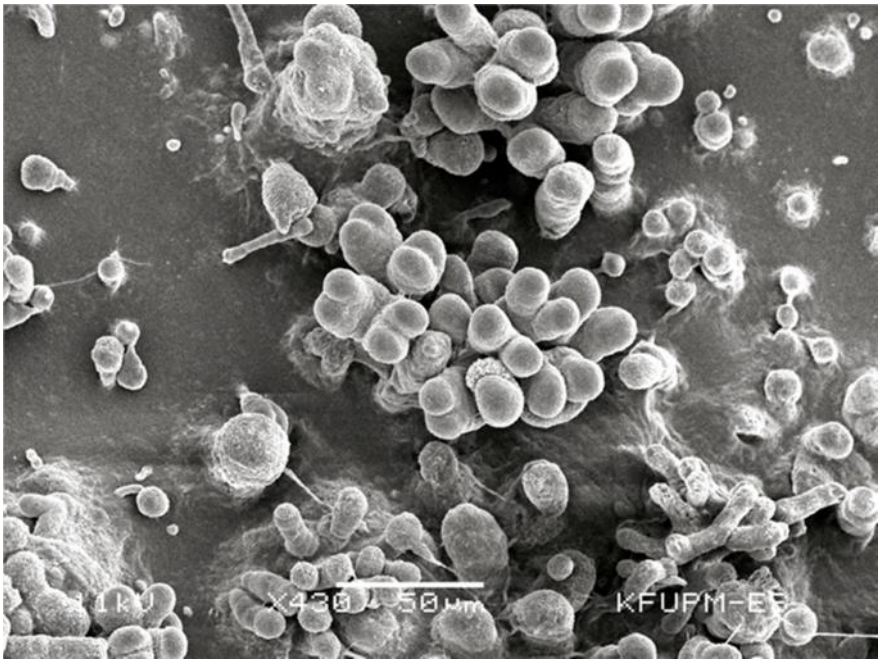


Figure 55 Resin cast scanning electron micrograph of SP 3, showing natural colony dimension and composition (scale = 50 μm)

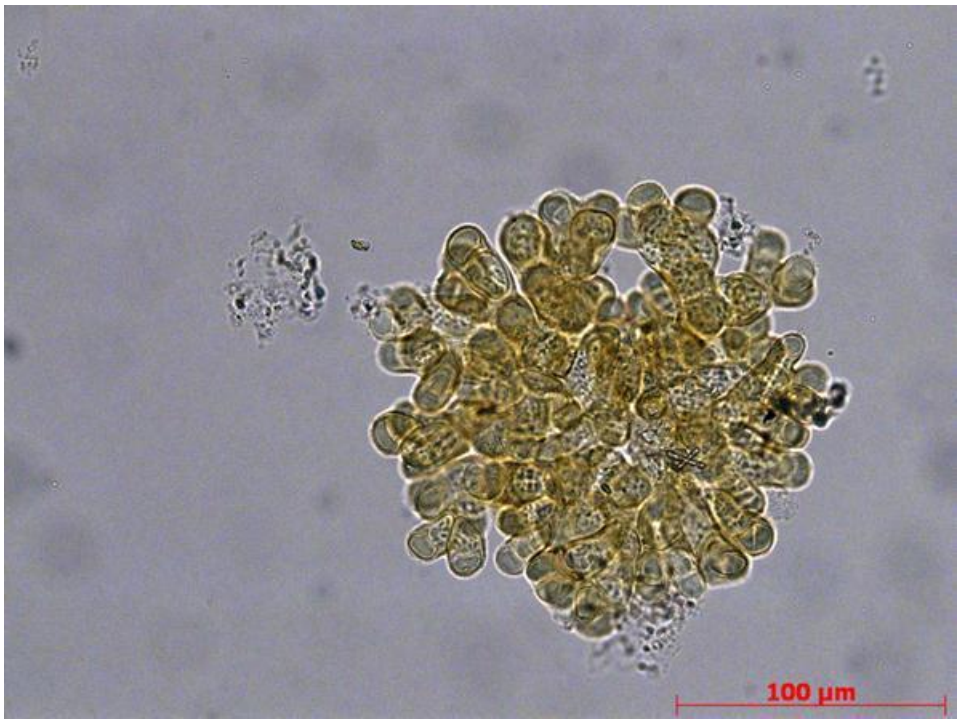


Figure 56 late stages of sp 2 and the onset of baeocyte formation (scale = 100 µm)

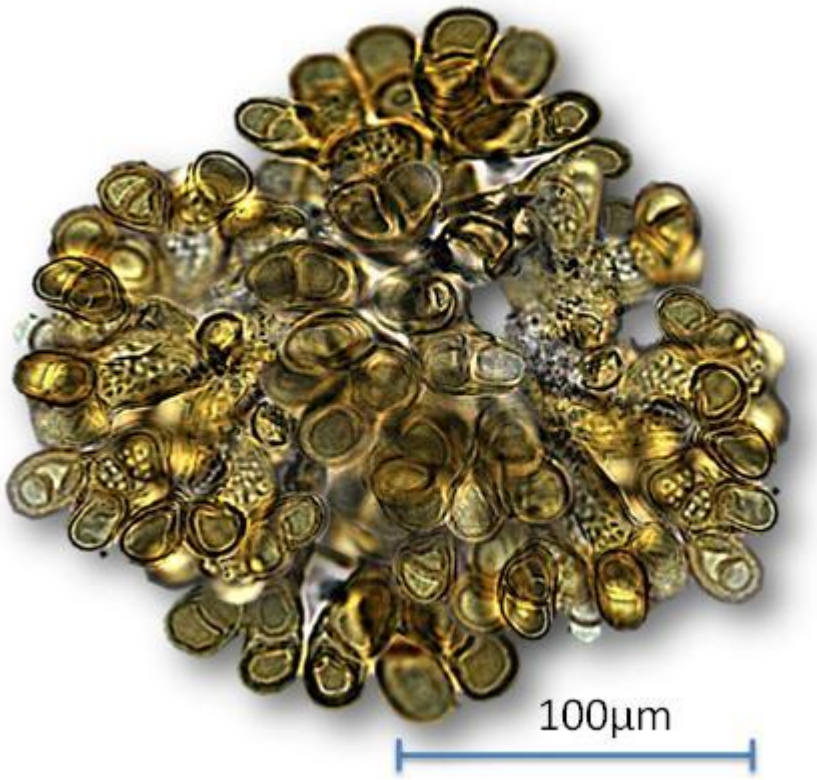


Figure 57 Colony reconstruction of SP 3 from Costa Rican Sample (scale = 100 µm)

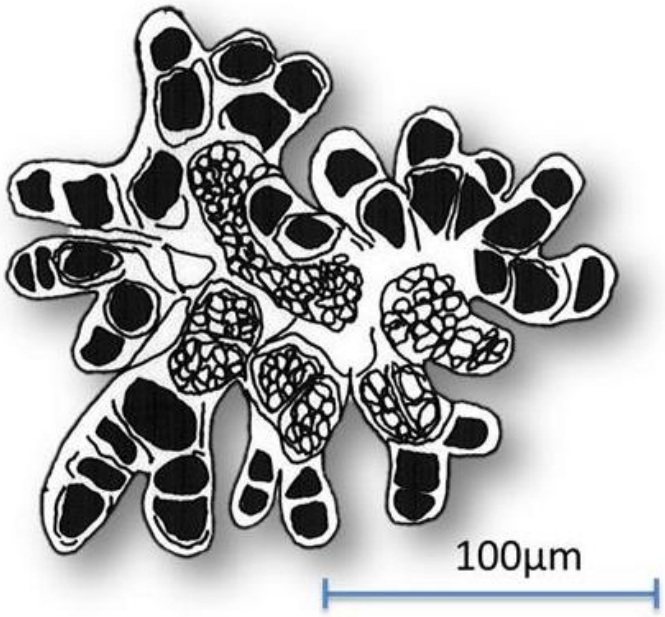


Figure 58 Pencil sketch colony reconstruction of SP 3 from Costa Rican Sample (scale = 100 µm)

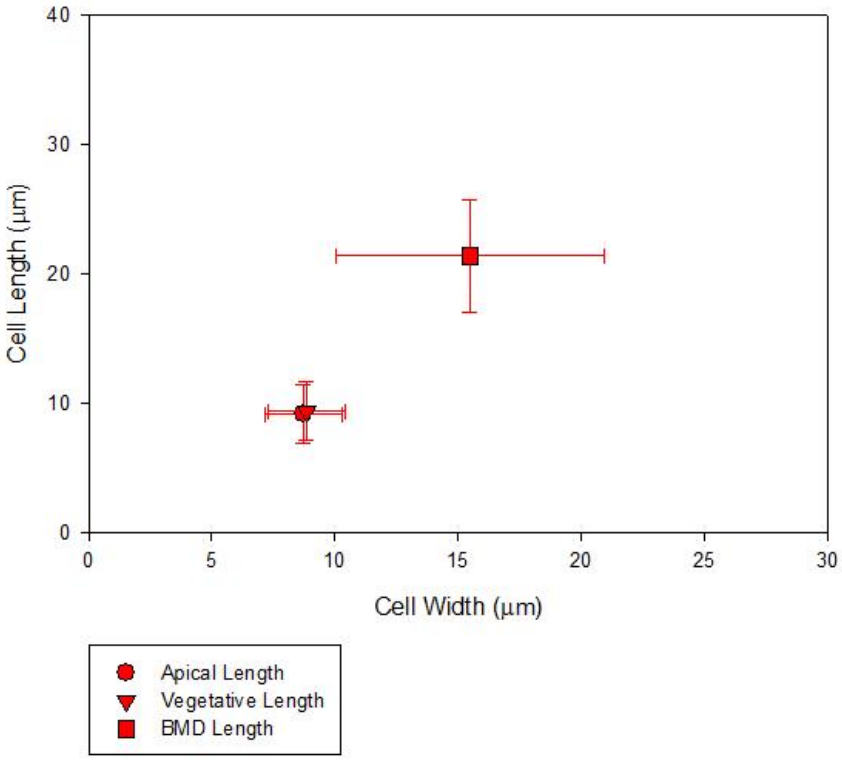


Figure 59 graphical representations of the morphometric measurements for SP 3. apical length=circle, vegetative length=inverted triangle, baeocyte mother cell= square

CHAPTER FIVE

DISCUSSIONS

Until recently some members of the Hyella species group of euendolithic cyanobacteria were thought to be exclusively restricted to the Arabian Gulf. A study by Silva and Pienaar (2000) conducted in South Africa revealed the presence of three out of the six Hyella assemblage described from the Arabian Gulf by Al-Thukair and Golubic (1991) in an earlier study. Two of the species of Hyella (*H. inconstans* and *H. reptans*) are only the second world records, and are the first from Europe; they are known otherwise only from the original locality at Khafji, Arabian Gulf (Al-Thukair & Golubic, 1991b). The other taxon of *Hyella* (*H. caespitosa var. arbuscula*) is reported for the third time in the world and first time from Europe; it was found in the Arabian/Persian Gulf (Al-Thukair & Golubic, 1996) and in carbonate rocks in Papua New Guinea (Taton & Hoffmann, 2003) Three species described from sample of calcareous sediment collected from Costa Rica in this study, share morphometric resemblance to already known euendoliths based on morphometric data and illustrations obtained from publications dealing with the taxonomy of euendoliths. Values of Apical cell length are used to rank the relative sizes of described euendoliths and those obtained from the literature survey. Apical cells are the most important determinants in the development of these organisms, as they are the sites of the primary apical growth and cell differentiation in all compared taxa. Apical

cells are also the sites of carbonate penetration. Filament width and branching pattern are most helpful in relating the organisms to their borings (Golubic *et al.* 1996).

A dichotomous taxonomic key is constructed to identify the species earlier described in this study. The key was initial constructed by Al-Thukair (unpublished) based on published data of known endolithic cyanobacteria. *Hyella* and *Solentia* species described from the Arabian Gulf (Al-Thukair and Golubic 1991) deferred slightly in nature of branching of cells, sizes of apical and vegetative cells, thickness of sheath (Table 6) etc. According to the widths of filaments and apical cells, Hyella species can be grouped in two size-classes. *Hyella immanis*, *H. racemus*, *H. salutans*, *H. stella*, and *H. conferta* have filaments that are considerably wider than 10 μm, whereas filaments of *Hyella reptans*, *H. inconstans* and *H. arbuscula* are much narrower. *H.immanis* has the largest cells of all species compared. *H. racemus* is distinguished by its densely clustered branching mode, and *H. salutans* by long branches and forming thick and layered envelopes *H. conferta* has characteristic bag-like filament morphology, and short and wide apical cells.

Table 6 Published and the result of current study morphometric data for different cell types Euendolithic Cyanobacteria (m ±s.d (n))

Sample	Apical Cells		L/W	Vegetative Cells		L/W
	Width (µm)	Length (µm)		Width (µm)	Length (µm)	
H. conferta **(Al-Thukair and Golubic 1991)**	9.40	6.5	0.7	7.50	10.80	1.4
Hyella balani **(Silva and Pienaar , 2000)**	6.52	6.25	0.99	6.13	9.42	1.59
H. racemus sp. Nova **(Bahamas)**	13.9	14.6	1.0	13.7	14.1	1.1
Hyella immanis **(Silva and Pienaar , 2000)**	19.70	19.95	1.02	14.93	8.33	0.71
H. racemus sp. nov. **(Arabian Gulf)**	14.1	15.3	1.1	18.3	15.1	0.8
H. stella **(Al-Thukair and Golubic 1991)**	10.0	11.4	1.1	11.30	10.10	0.9
SP 3 (Current Study)	11.43	16.55	1.1	9.43	12.02	1.3
SP 2 (Current Study)	8.73	9.16	1.1	8.85	9.39	1.1
H. Inconstans **(II) (Al-Thukair and Golubic 1991)**	6.10	7.5	1.2	6.00	5.50	0.9
H. Inconstans **(III) (Al-Thukair and Golubic 1991)**	5.50	7.3	1.3	4.30	4.60	1.1
H. Inconstans **(IV) (Al-Thukair and Golubic 1991)**	5.50	7.3	1.3	3.10	2.70	0.9
Hyella immanis **(Al-Thukair and Golubic 1991)**	13.2	17.4	1.3	14.9	15.6	1.1
Hyella **sp 2 (Silva and Pienaar , 2000)**	8.79	11.46	1.38	8.87	31.27	3.7
H. salutans **(Al-Thukair and Golubic 1991)**	11.1	16.3	1.5	12.1	11.4	0.9
SP 1 (Current Study)	6.50	10.14	1.6	7.11	9.78	1.4
Solentia sanguinea **sp. nova (Golubic *et al.* 1996)**	14.82	24.3	1.7	-	-	-
H. Inconstans **(I) (Al-Thukair and Golubic 1991)**	5.80	11.3	1.9	5.80	8.00	1.4
H. reptans **(Al-Thukair and Golubic 1991)**	5.20	10.3	2.0	5.50	8.90	1.6
Hyella **sp 1 (Silva and Pienaar , 2000)**	9.02	32.03	3.62	8.65	15.68	1.87
H. arbuscula **(Al-Thukair and Golubic 1991)**	4.20	17.0	4.0	4.1	14.40	3.5
Hyella caespitosa **(Silva and Pienaar , 2000)**	4.95	17.8	4.75	4.80	21.31	3.77
Hyella inconstans **(Silva and Pienaar , 2000)**	6.19	26.34	6.01	5.90	4.5	2.74

Dichotomous keys for euendolithic cyanobacteria identification

1A. Cells in isodiametric colonies, no pseudofilaments ……*Cyanosaccus*

1B. Cells arranged in Pseudofilaments: ……..(2)

2A. Cells displaced at the end of long gelatinous stalks *Solentia*

I. Persistent deeply red pigmentation with apical cells clubbed shaped longer than wide (L/W > 1) *Solentia sanguinea*

II. Cell closely packed

2B. Cells closer together in single or multiple series *Hyella*

3A. Pseudofilaments more than 10 µm wide: Branching predominantly dichotomous:

1. Envelopes thin, simple, uniformly pigmented *H. racemus*

2. Envelopes wide, double, inner pigmented outer colorless *H. stella* and **SP 3 (Current Study)**

3B. Branching predominantly lateral:

1. Apical cells lensoid, shorter than wide (L/W < 1) *H. conferta*

2. Apical cells isodiametric or longer than wide (L/W> 1):

a. Envelopes thin, simple, yellow-brown *H. immanis*

b. Envelopes thick, multilayered, gray-blue *H. salutans*

c. Branching mainly by longitudinal division **SP 1 (Current Study)**

II. Pseudo-filaments less than 10 µm wide:

A. Apical cells much longer than wide (L/W = 3-4) *H. arbuscula*

B. Apical cells shorter (L/W = 1-2):

1. Envelops tight, boxy, colorless *H. inconstans*

2. Envelops tight, , layered, yellow-brown **SP 2 (Current Study)**

3. Envelopes wide, layered, yellow-brown *H. reptans*

SP1 has similar pseudo filament size and cell diameters to *H. salutans* (Al-Thukair and Golubic 1991). The Apical cells are on the average 11.43 ± 1.9 μm (SP1) vs 11.10 ± 1.9 μm (*H.salutans*: Al-Thukair and Golubic 1991) wide and 16.55 ± 2.8 μm (SP1) vs 16.30 ± 3.2 μm (*H.salutans*: Al-Thukair and Golubic 1991) long while the vegetative cells are 9.43 ± 2.8 μm (SP1) vs 12.21 ± 2.8 μm (*H.salutans*: Al-Thukair and Golubic 1991) wide, and 12.02 ± 2.8 μm (SP1) vs 11.40 ± 3.3 μm (*H.salutans*: Al-Thukair and Golubic 1991) long. They differ remarkably in the nature of branching: dichotomous in SP1 prominent while lateral in *H.salutans* (Al-Thukair and Golubic 1991). Natural populations studied appeared brownish yellow when illuminated, probably due to the action of formaldehyde. Adaptation, nutrient availability and other selective environmental factors might be responsible for the observed difference. The result of this comparison is graphically presented in **Figure 60**

There is a pronounced difference in the apical cell length and diameter of tunnels bored by SP 2 when compared to H. *reptans* (Al-Thukair and Golubic 1991). The Apical cells are on the average 6.50 ± 1.3 μm (SP 2) vs 5.20 ± 1.2 μm (*H. reptans* Al-Thukair and Golubic 1991) wide and 10.14 ± 2.3 μm (SP 2) vs 10.30 ± 3.3 μm (*H. reptans*: Al-Thukair and Golubic 1991) long while the vegetative cells are 7.11 ± 1.4 μm (SP 2) vs 5.50 ± 1.4 μm (*H.reptans*: Al-Thukair and Golubic 1991) wide, and 9.78 ± 1.9 μm (SP 2) vs 8.90 ± 2.4 μm (*H.reptans*: Al-Thukair and Golubic 1991) long. The result of this comparison is graphically presented in **Figure 61.** The appearance of cells (apical and vegetative), nature of branching and the behavior of vegetative cells that branches more prominently by slippage remain the same.

SP 3 has comparatively (**Figure 62)** small cell size to *Hyella stella* described from the Arabian Gulf, they are remarkably same in the nature of their cells and dichotomy. The Apical cells

are on the average 8.73 ± 1.6 µm (SP 3) vs 10.0 ± 1.8 µm (*H. stella* Al-Thukair and Golubic 1991) wide and 9.16 ± 2.2 µm SP3 vs 11.40 ± 2.3 µm (*H. stella*: Al-Thukair and Golubic 1991) long while the vegetative cells are 8.85 ± 1.6 µm (SP 3) vs 11.3 ± 2.5 µm, *H.stella*: (Al-Thukair and Golubic 1991) wide, and 9.39± 2.2 µm (*H.sp2*) vs 10.10 ± 2.5 µm (*H.stella*: Al-Thukair and Golubic 1991) long.

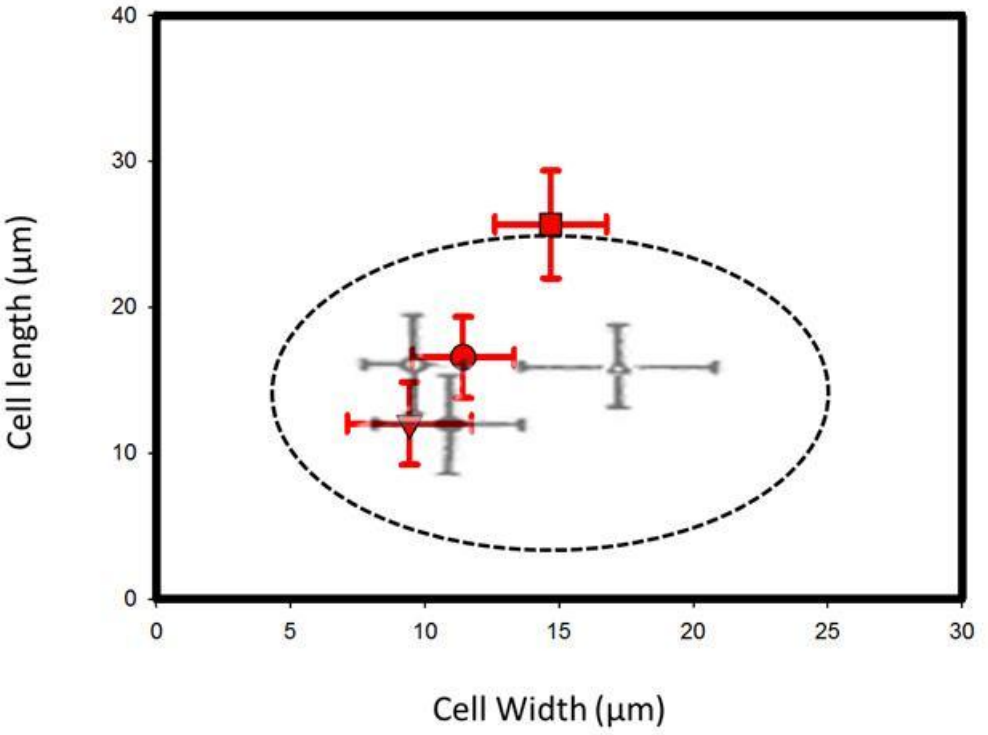


Figure 60 Compares cell sizes of SP1 and H. salutans (Al-Thukair and Golubic 1991) in an effort to taxonomically classified the new species

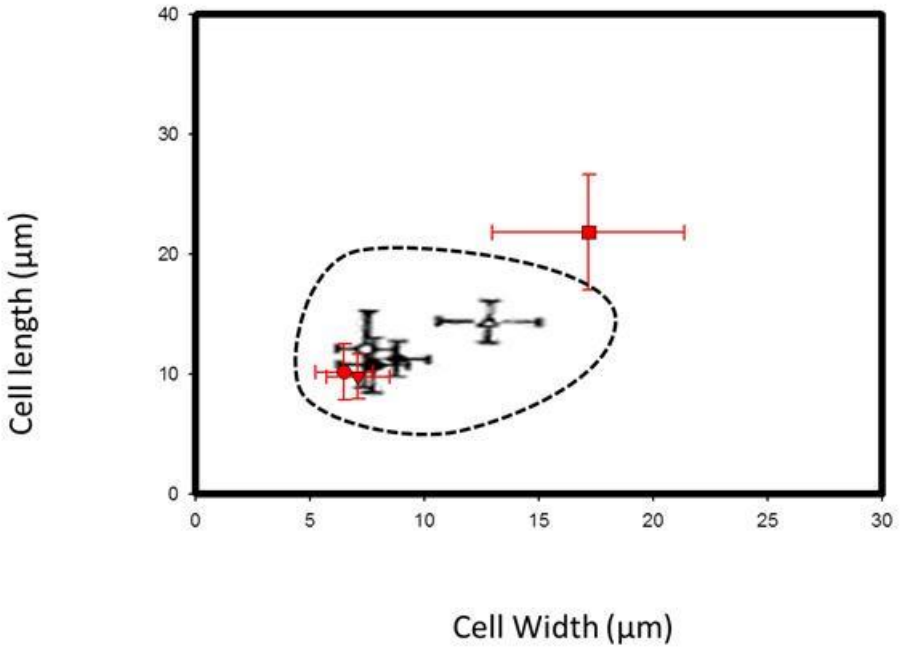


Figure 61 Compares cell sizes of SP 2 and H. reptans (Al-Thukair and Golubic 1991) in an effort to taxonomically classified the new species

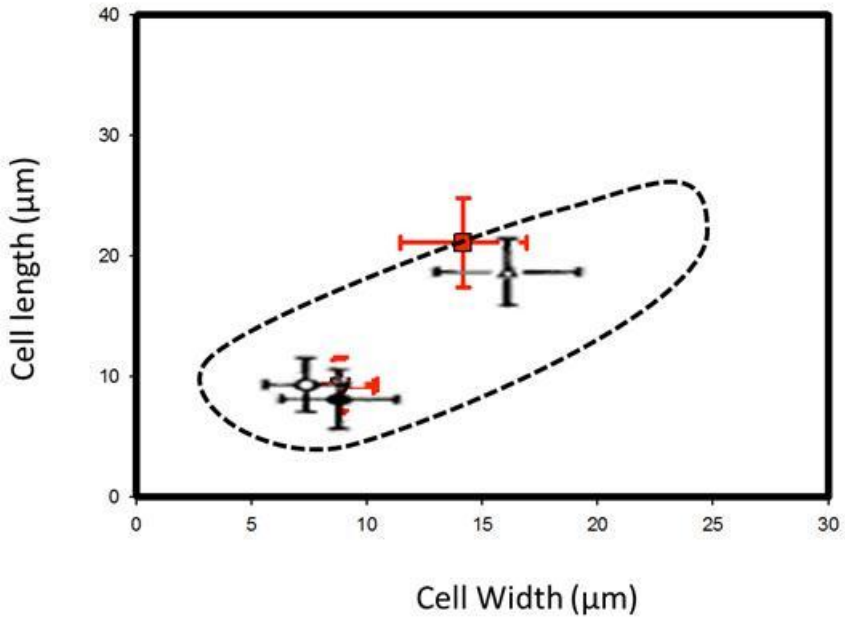


Figure 62 Compares cell sizes of SP 2 and H. stella (Al-Thukair and Golubic 1991) in an effort to taxonomically classified the new species

Culturing

Release of baeocytes in culture was intermittent resulting in difficulties faced in sustaining the emergence of endolithic growth in both solid and liquid media. Species of euendolithic cyanobacteria isolated from calcareous sediments collected from the Arabian Gulf and Costa Rica in this study have shown. After 3 week of initial culture, the baeocyte released from Tarut samples were streaked and used to inoculate calcite crystal, to test if they retain their boring capabilities. The re-boring test yielded a promising result, boring was initiated as evidenced by the SEM resin cast images of calcite grain. However, unialgal species could not be determined since the baeocytes where undifferentiated and bare similar morphology in culture, the cells produced thick shealth, which do not bear resemblance to known species. Golubic (1970) demonstrated that the planes of crystal cleavage and twinning determine the tunnel direction. In such highly crystalline substrates, the tunnel then resembles a sequence of negative microcrystals. This is consistent with the presence of a focused dissolution front around the apical cell. Culturing of the Arabian Gulf euendolithic cyanobacteria was reported to have been successfully carried out by Al-Thukair and Golubic (1991, 1996) using both liquid and solid enrich and natural seawater and they successfully maintained unialgal cultures of 6 out of ten assemblages they identified. The studies reveal that successful culturing is possible if carried out in the laboratory and uttermost care is taken. Also in a study conducted in South Africa, Silva and Pienaar (2000) cultured euendolithic using Provasoli Enrichment Seawater Medium and identified 5 species of euendolithic cyanobacteria, 3 of which were identified to species taxonomic unit.

CONCLUSION

The initial objectives of this study was to culture and genetically identified euendolithic cyanobacteria of the Arabian Gulf, Sadly, the objectives were never satisfied due to a failure to maintain a viable culture of the micro borers in the laboratory. Genetic and phylogenic analyses are both largely dependent on the success of the culturing process and were not carried out because the experiment did not proceed as expected. Notwithstanding, other objectives were sort after to satisfy the requirements of a Master's Thesis. The new objectives include the morphometric identification of euendolithic cyanobacteria found in calcareous sediments collected from the pacific coast of Costa Rica (Playa Concha).

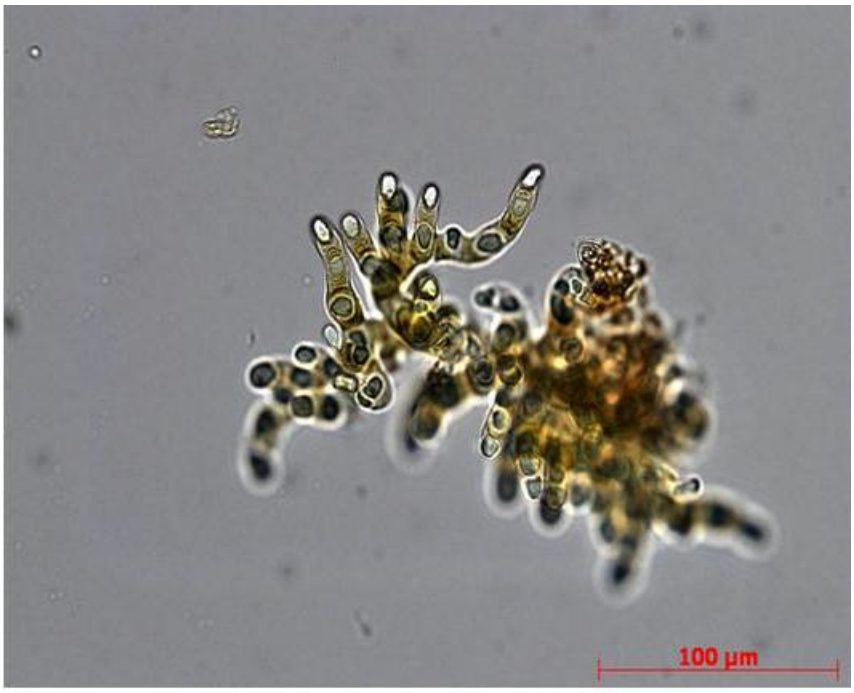
Six species of euendolithic cyanobacteria (*Hyella reptans, H.inconsistant, H.arbiscular, H.salutans, H.immanis,* and *H.stella*) were identified from the Arabian Gulf samples (Tarut, Abu Ali and Mussalima) using culture independent techniques (SEM and LM). The Six species observed in this study from the Arabian Gulf have been described in an earlier study by Al-Thukair and Golubic (1991). As such, this study validates the existence of the endolithic in the Arabian Gulf. Five new species of euendolithic cyanobacteria studied from natural populations resident in calcareous sediments were found in the Costa Rican samples, using culture independent techniques. Three of this species were described up to the species taxon and compared with their Arabian Gulf counterparts. They bear resemblance to their Arabian counterparts and shows adaptive variations required for their unique tropical environment. Two other species were encountered from the Costa Rican sample but not formally described due to their rare occurrences and difficulties to obtain sufficient numbers for a conclusive morphometric measurement.

The diversity of euendolithic cyanobacteria found in the Arabian Gulf and Costa Rica in this study validates previous claims of euendolithic cyanobacteria distribution in lower latitudes and marine extreme environment (high salinity and temperature). Comparative study of the calcareous sediments was conducted with the hope of finding new species still unknown to science. Morphometric methods previously described Al-Thukair and Golubic 1991; Brent et al. 1970; Lukas and Golubic 1981; Lukas and Golubic 1983 and species illustrations were extensively depended upon to described this species.

RECOMMENDATIONS

There is a need to successfully establish endolithic cyanobacteria in culture so as to compare the behavior of the newly identified Costa Rican euendoliths with their natural population, this will help to minimize taxonomic errors and in line with previous works. Areas of suggested future studies include optimal growth factors regulating euendolithic cyanobacteria growth and morphometric identification of euendolithic cyanobacteria from Costa Rica.

APPENDIXES



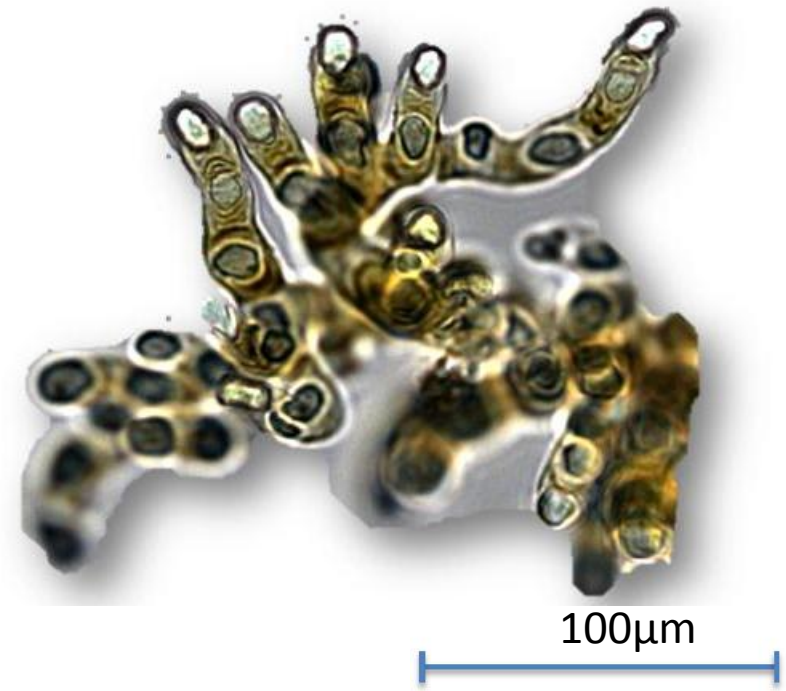


Figure 63 species 4 from Costa Rica

Figure 64 Species 5 from Costa Rica

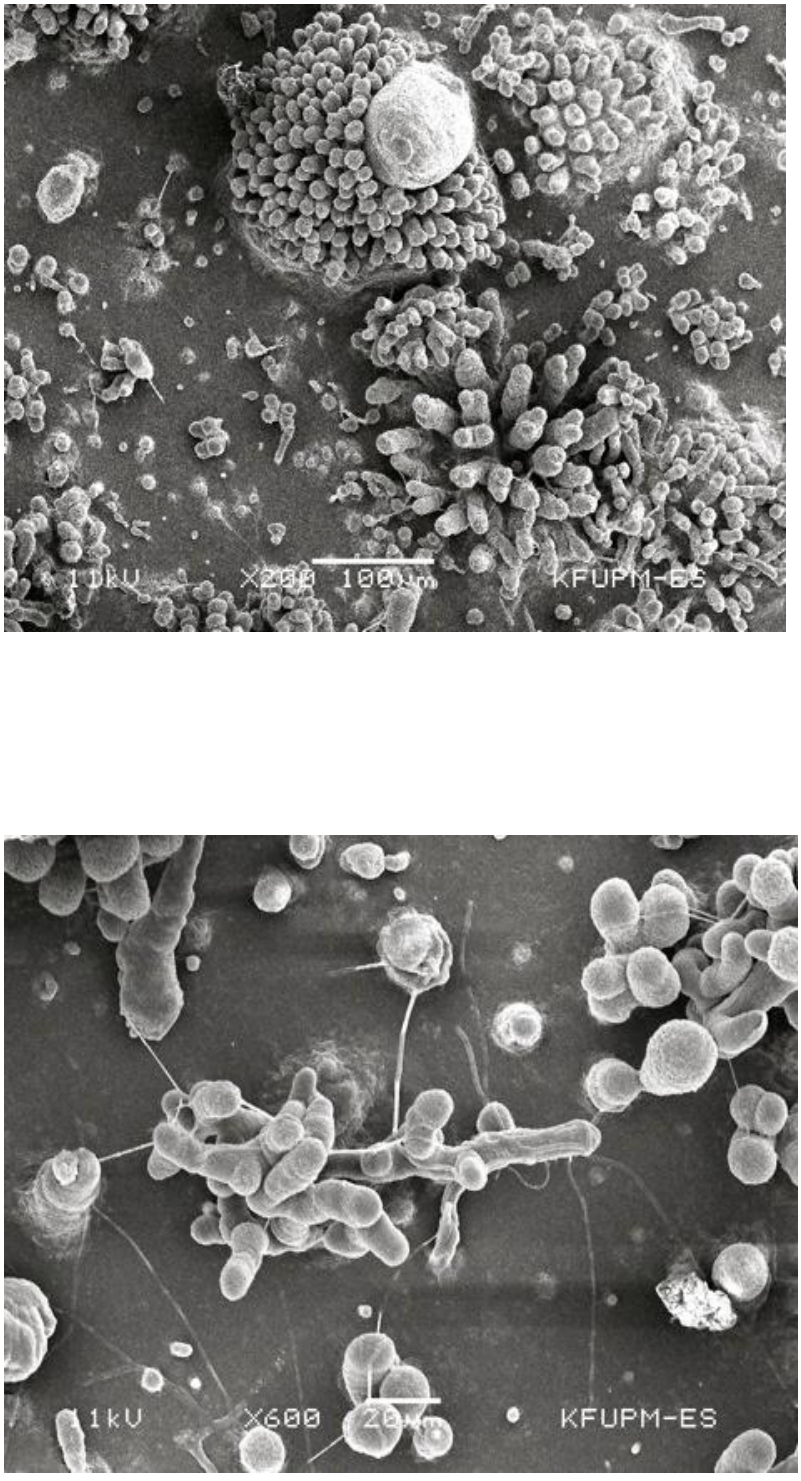
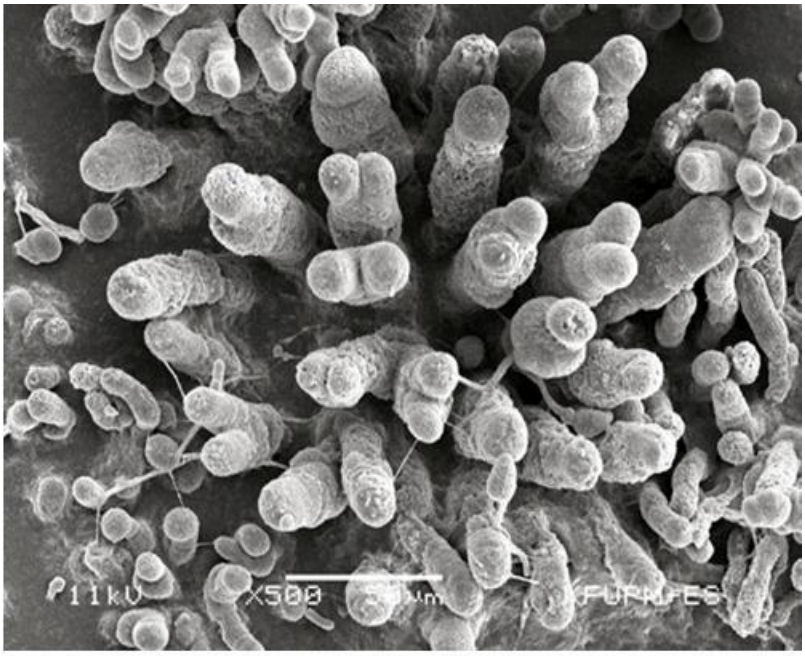


Figure 65 SEM micrograph of Costa Rican Sample



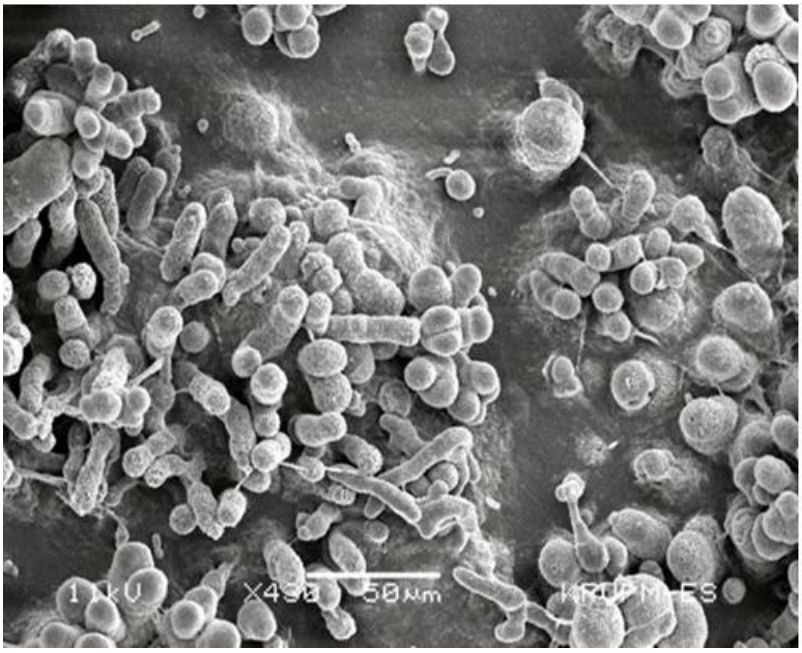


Figure 66 SEM micrograph of Costa Rican Sample

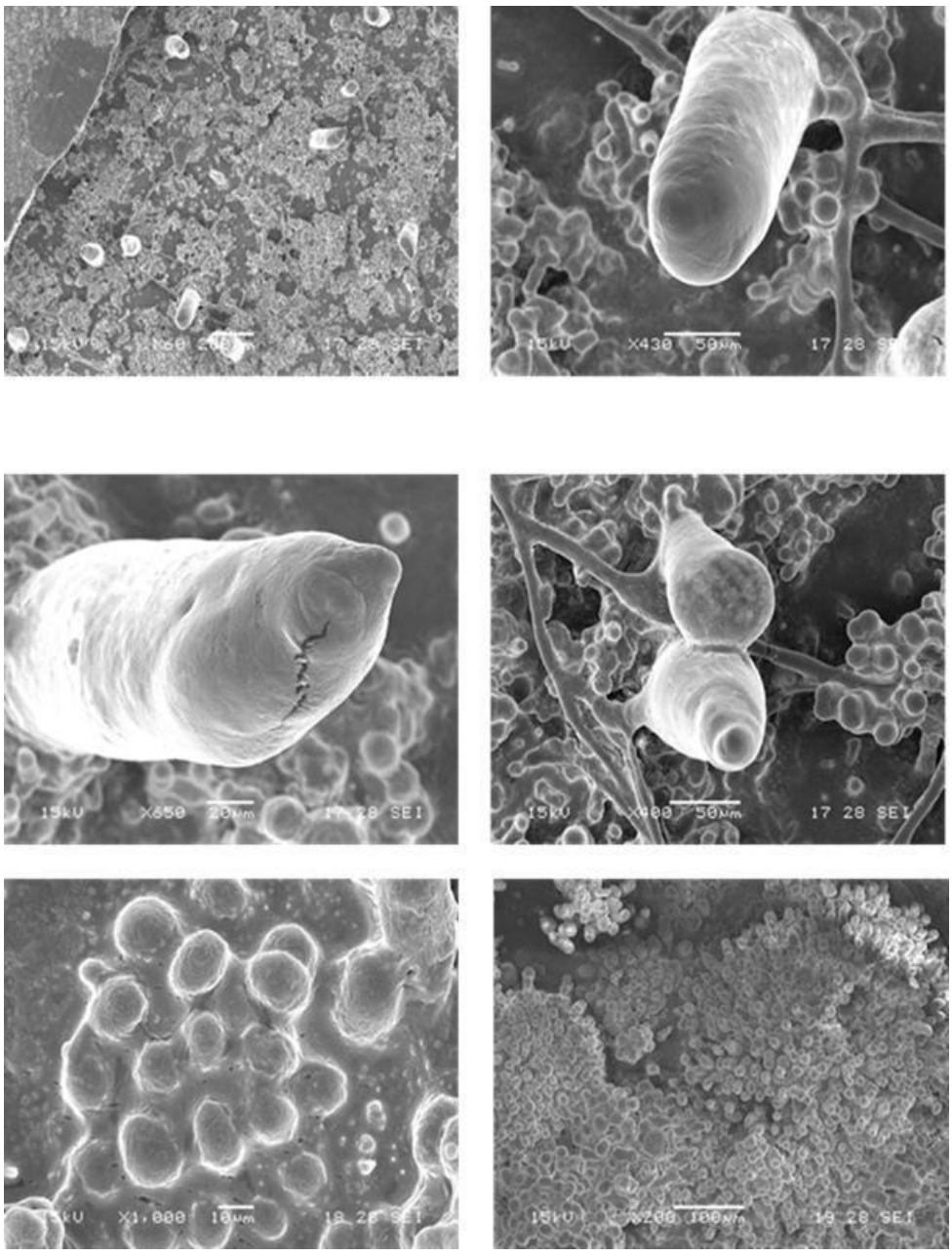


Figure 67 SEM micrograph of Costa Rican Sample

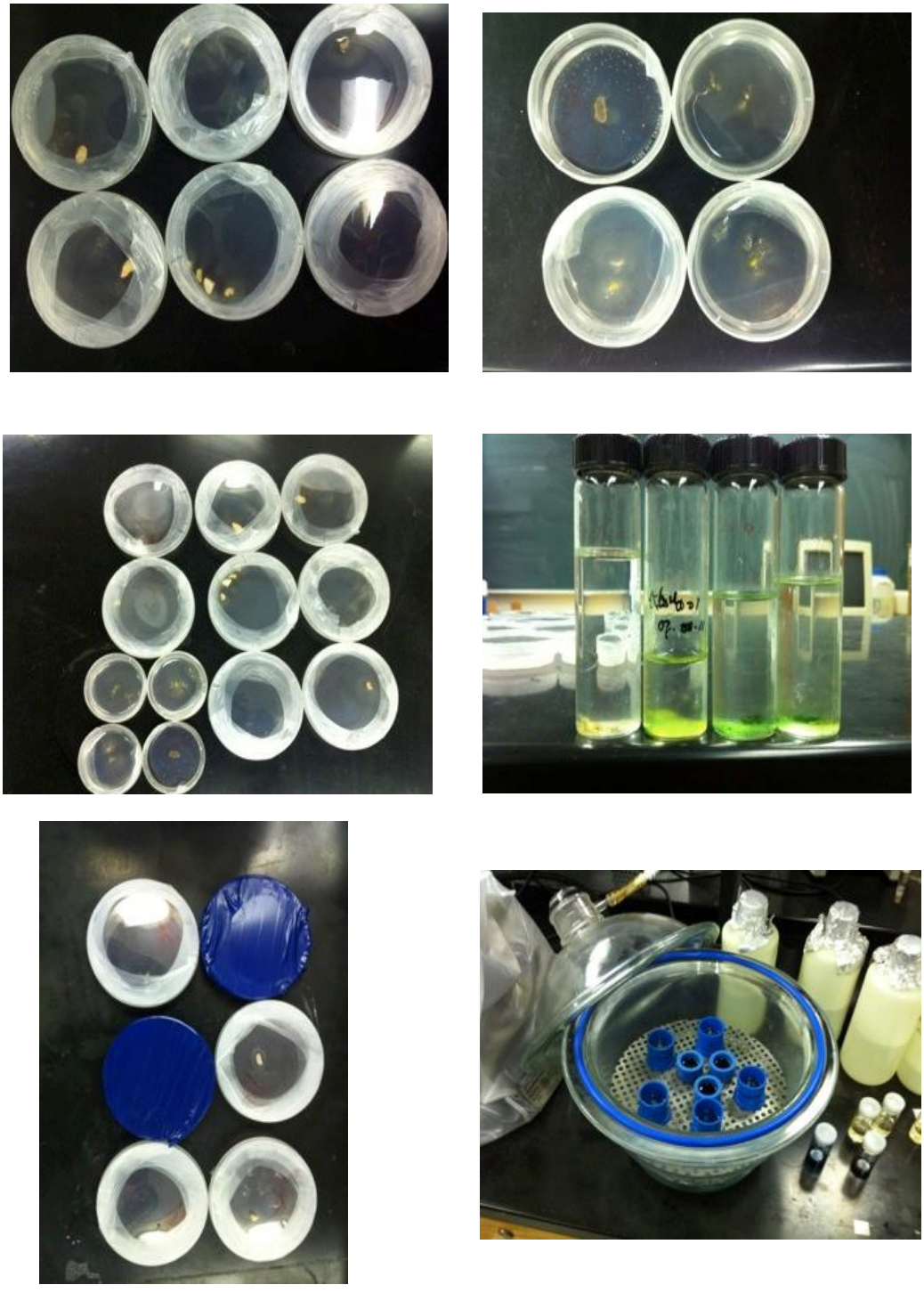


Figure 68 Culture plates, bottles and desiccator used in the experimentation.

Figure 69 Microscopes, Autoclave, storage bottle and weighing balance

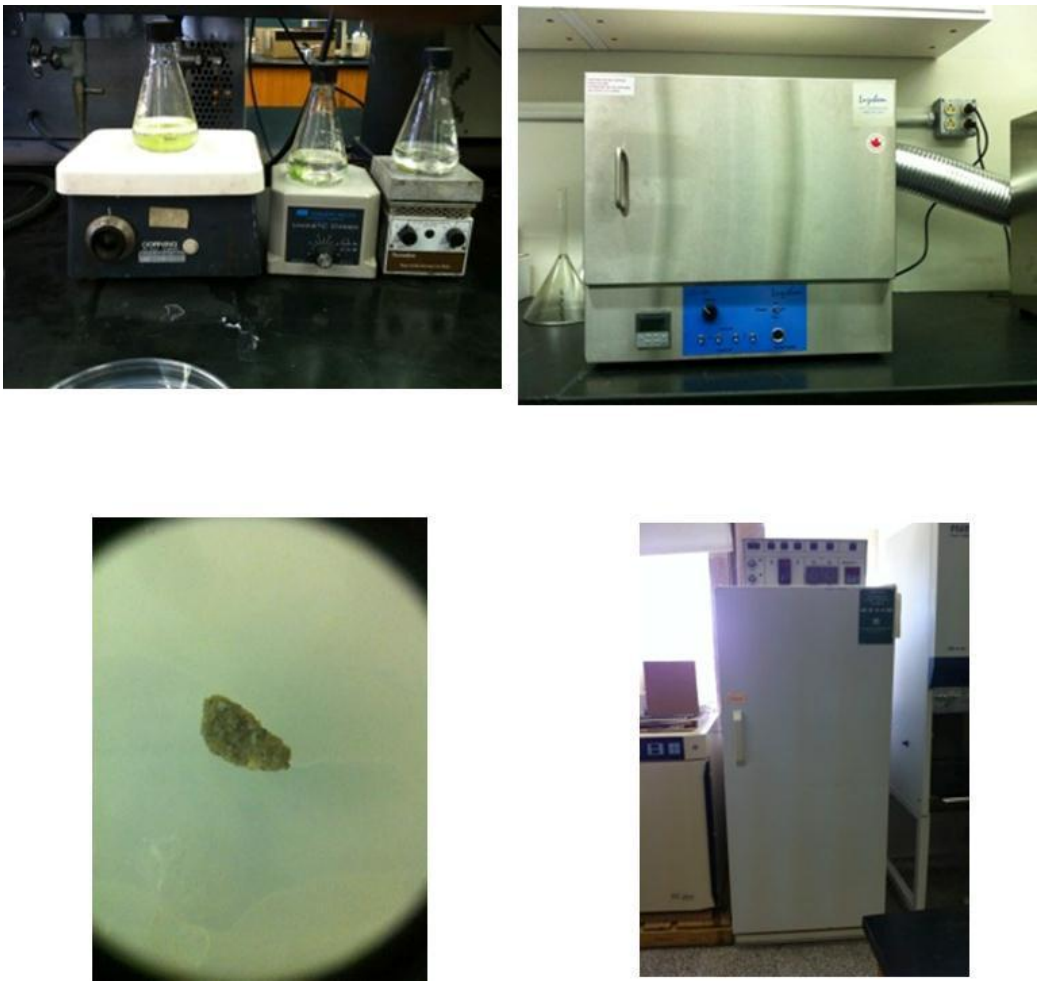


Figure 70 Culture plates, bottles and desiccator used in the experimentation

Morphometric measurements for species 1 (Costa Rica)

APICAL CELL LENGTH	APICAL CELL WIDTH	VEGETATIVE CELL LENGTH	VEGETATIVE CELL WIDTH	BMCE LENGTH	BMC WIDTH
8.8	10.52	10.24	10.49	32.13	32.13
5.51	9.62	6.82	8.91	18.78	18.78
7.68	9.16	10.14	8.6	14.77	15.06
9.75	12.31	9.22	6.19	17.15	18.57
9.41	10.14	13.05	6.61	23.73	12.29
6.25	8.19	8.81	8.75	22.01	13.93
10.65	7.38	7.11	7.6	21.38	14.43
8.99	8.54	10.04	6.48	16.89	11.19
12.02	9.07	5.51	8.47	20.54	10.86
7.8	11.01	9.59	9.39	19.81	12.05
6.48	6.99	11.11	8.7	20.27	17.75
8.8	8.7	7.42	6.33	24.04	18.28
8.34	9.8	8.94	6.99	27.44	13.97
8.4	7.74	14.43	6.76	19.3	11.33
8.66	11.27	5.48	9.08	22.62	11.56
7.68	12.17	9.73	8.72		
8.23	11.78	8.7	9.48		
11.67	9.77	11.11	8.87		
7.44	7.44	9.96	8.69		
7.8	7.38	9.32	7.25		
9.21	8.74	11.81	9.62		
7.46	10.45	7.91	9.66		
8.6	9.06	9.83	9.23		
11.11	5.79	8.7	10.01		
10.8	8.96	8.91	9.96		
8.69	10.07	17.69	8.84		
7.94	8.27	6.91	9.44		
9.15	7.53	13.6	9.05		
6.07	6.42	10.91	10.06		
13.51	11.27	8.55	10.52		
12.26	9.47	14.48	10.86		
9.63	9.08	7.68	9.16		
10.14	8.6	9.75	12.31		
9.22	6.19	9.41	10.14		
13.05	6.61	6.25	8.19		
8.81	8.75	10.65	7.38		
7.11	7.6	8.99	8.54		

10.04	6.48	12.02	9.07
5.51	8.47	7.8	11.01
9.59	9.39	6.48	6.99
11.11	8.7	8.8	8.7
7.42	6.33	8.34	9.8
8.94	6.99	8.4	7.74
14.43	6.76	8.66	11.27
5.48	9.08	7.68	12.17
9.73	8.72	8.23	11.78
8.7	9.48	11.67	9.77
11.11	8.87	7.44	7.44
9.96	8.69	7.8	7.38
9.32	7.25	9.21	8.74
11.81	9.62	7.46	10.45
7.91	9.66	8.6	9.06
9.83	9.23	11.11	5.79
8.7	10.01	10.8	8.96
8.91	9.96	8.69	10.07
17.69	8.84	7.94	8.27
6.91	9.44	8.19	10.42
12.23	8.88	5.12	9.11
10.3	11.59	10.24	11.77
9.97	10.5	12.14	8.87
10.28	9.08	9.96	7.37
9.08	8.95	7.93	5.72
9.51	9.27	8.19	8.74
6.65	7.75	5.62	6.89
7.17	7.18	11.24	5.94
8.24	5.84	9.27	9.61
8.34	6.53	11.22	6.72
4.35	7.77	10.75	8.31
5.59	8.38	10.42	6.41
8.13	8.02	7.97	7.68
6.48	5.98	10.61	6.4
7.03	8.19	11.8	10.75
10.14	10.01	8.96	8.99
9.42	7.96	9.37	6.76
7.68	9.98	10	10.24
7.03	11.34	7.83	9.44
9.83	13.16	11.47	9.59
10.65	7.38	7.11	7.6
8.99	8.54	10.04	6.48

12.02	9.07	5.51	8.47
7.8	11.01	9.59	9.39
6.48	6.99	11.11	8.7
8.8	8.7	7.42	6.33
8.34	9.8	8.94	6.99
8.4	7.74	14.43	6.76
8.66	11.27	5.48	9.08
7.68	12.17	9.73	8.72
8.23	11.78	8.7	9.48
11.67	9.77	11.11	8.87
7.44	7.44	9.96	8.69
7.8	7.38	9.32	7.25
9.21	8.74	11.81	9.62
7.46	10.45	7.91	9.66
8.6	9.06	9.83	9.23
11.11	5.79	8.7	10.01
10.8	8.96	8.91	9.96
8.69	10.07	17.69	8.84
7.94	8.27	6.91	9.44
9.15	7.53	13.6	9.05
6.07	6.42	10.91	10.06
13.51	11.27	8.55	10.52
12.26	9.47	14.48	10.86
9.63	9.08	7.68	9.16
10.14	8.6	9.75	12.31
9.22	6.19	9.41	10.14
13.05	6.61	6.25	8.19
8.81	8.75	10.65	7.38
6.07	6.42	10.91	10.06
13.51	11.27	8.55	10.52
12.26	9.47	14.48	10.86
9.63	9.08	7.68	9.16
10.14	8.6	9.75	12.31
9.22	6.19	9.41	10.14
13.05	6.61	6.25	8.19
8.81	8.75	10.65	7.38
7.11	7.6	8.99	8.54
10.04	6.48	12.02	9.07
5.51	8.47	7.8	11.01
9.59	9.39	6.48	6.99
11.11	8.7	8.8	8.7
7.42	6.33	8.34	9.8

8.94	6.99	8.4	7.74
14.43	6.76	8.66	11.27
5.48	9.08	7.68	12.17
9.73	8.72	8.23	11.78
8.7	9.48	11.67	9.77
11.11	8.87	7.44	7.44
9.96	8.69	7.8	7.38
9.32	7.25	9.21	8.74
11.81	9.62	7.46	10.45
7.91	9.66	8.6	9.06
9.83	9.23	11.11	5.79
8.7	10.01	10.8	8.96
8.91	9.96	8.69	10.07
17.69	8.84	7.94	8.27
6.91	9.44	8.19	10.42
12.23	8.88	5.12	9.11
10.3	11.59	10.24	11.77
9.97	10.5	12.14	8.87
10.28	9.08	9.96	7.37
9.08	8.95	7.93	5.72
9.51	9.27	8.19	8.74
6.65	7.75	5.62	6.89
7.17	7.18	11.24	5.94
8.24	5.84	9.27	9.61
8.34	6.53	11.22	6.72
4.35	7.77	10.75	8.31
5.59	8.38	10.42	6.41

Morphometric measurements for species 2 (Costa Rica)

APICAL CELL LENGTH	APICAL CELL WIDTH	VEGETATIVE CELL LENGTH	VEGETATIVE CELL WIDTH
15.24	5.18	12.86	6.11
8.88	5.34	7.8	5.77
14.73	8.02	8.58	9.22
12.38	6.91	9.8	8.13
8.48	7.68	12.03	5.38
7.17	6.91	9.47	6.15
11.05	5.43	9.15	6.58
12.8	6.23	10.99	7.7
12.16	6.7	11.31	5.38
8.38	4.47	8.27	5.99
14.57	5.18	13.95	4.61
14.06	7.32	10.51	5.42
8.13	6.46	5.61	6.76
11.08	6.61	10.09	6.72
11.59	9.8	14.44	11.82
14.7	7.56	10.19	5.98
14.71	5.64	10.42	6.19
12.35	7.12	11.78	5.95
13.51	5.95	9.73	7.13
15.24	5.18	12.16	5.4
15.75	8.69	13.12	8.96
6.19	7.21	7.7	5.59
9.47	7.43	11.82	7.64
9.94	10	11.5	8.88
12.29	7.13	11.34	9.5
7.61	5.98	7.06	6.34
12.26	7.37	12.39	8.52
11.5	6.23	8	7.49
6.76	5.89	7.48	5.64
13.55	5.99	11.17	6.25
7.25	5.84	9.08	7.03
11.91	6.72	14.1	7.32
9.08	5.62	11.11	7.78
11.61	7.78	9.32	6
12.44	7.56	13.61	6.27
10.28	6.23	13.31	5.28
9.44	6	11.44	6.72
7.7	6.44	7.78	5.28

9.81	7.88	9.3	8.58
12.35	5.89	9.59	7.06
5.64	5.14	8.23	5.91
8.9	6.16	6.7	3.62
9.02	4.89	11.01	5.63
8.51	6.58	13.05	8.96
8.76	5.68	7.74	6.19
11.57	8.83	10.99	8.01
11.78	6.34	6.72	6.04
13.3	6.7	7.85	6.83
9.97	8.24	14.84	8.38
10.28	9.05	14.34	8.51
9.56	5.68	10	9.19
5.89	4.1	7.44	6.92
13.05	5.12	11.88	8.48
11.33	7.8	13.39	8.31
6.91	5.38	8.71	6.23
11.26	5.63	9.25	8.02
12.86	5.68	9.8	7.75
15.58	8.72	8	6.7
7.68	6.11	12.43	6.41
16.3	9.5	9.47	8.63
12.05	8.13	11.12	9
7.75	7.44	10.52	7.97
9	4.78	8.6	6.99
14.2	5.91	12.55	6.34
13.08	7.08	11.91	7.13
13.97	6.19	8.45	6.64
7.33	5.91	10.44	6.66
9.68	5.75	8.81	5.33
11.91	5.97	10	7.28
8.88	5.03	9.47	6.99
10.8	6.48	8.34	6.4
10.1	7.46	11.31	11.05
10.49	11.8	6.93	9.98

Morphometric measurements for species3 (Costa Rica)

APICAL LENGTH	APICAL WIDTH	VEGETATIVE LENGTH	VEGETATIVE WIDTH	BMC Length	BMc Width
17.26	11.18	15.1	12.03	27.29	15.16
16.45	9.96	8.81	8.36	22.09	16.73
14.2	10.37	9.89	11.24	30.62	19.25
15.34	9.39	17.73	10.7	24.45	11.74
16.95	9.32	10.58	7.46	35.61	17.48
17.24	8.25	10.24	7.88	28.48	13.83
13.82	8.7	13.51	7.72	28.74	17.26
18.18	9.42	7.7	9.96	26.43	16.64
13.82	9.73	12.31	8.38	24.69	17.56
18.73	9.34	10.93	7.03	25.78	14.12
14.98	10.37	11.05	8.74	27.76	13.63
18.67	10.76	10.77	12.03	34.74	12.05
14.66	13.13	11.56	9.14	22.85	12.87
13.85	11.02	8.91	6.7	27.82	15.92
15.57	8.81	15.49	11.5	26.69	12.85
16.93	8.27	8.96	14.08	23.75	15.05
18.57	9.56	11.86	8.75		
12.54	10	10.6	7.28		
15.95	10	13.57	11.43		
17.98	11.68	10.91	9.27		
19.3	9.05	11.33	10.77		
14.11	8.19	9.75	12.86		
19.31	9.53	10.32	11.08		
16.84	9.39	11.91	8.17		
13.88	12.07	18.69	15.88		
18.94	10.01	18.68	15.52		
18.44	10.27	15.87	13.36		
16.03	7.56	17.67	11.56		
14.36	14.22	11.09	8.81		
17.03	10.5	9.25	7.94		
16.71	10.86	10.06	12.33		
11.08	11.08	10.06	9.44		
16.74	8.95	15.46	7.21		
14.33	11.04	13.82	9.25		
14.66	10.99	11.47	11.61		
14.74	10.42	9.85	11.34		
18.79	11.02	11.53	8.19		
17.96	9.23	12.8	9.73		

14.09	9.32	16.03	11.23
13.51	11.59	8.48	10.01
10.67	9.88	14.53	8.38
18.01	11.78	17.2	8.51
11.16	9.44	13.43	5.05
20.02	13.75	13.75	4.97
17.47	11.01	10.03	8.24
18.19	9.76	15.9	7.17
14.44	13.68	9.23	11.56
18.66	9.32	13.13	11.88
19.03	11.59	17.71	5.13
18.18	13.57	12.47	4.71
20.22	11.01	13.84	10.33
15.78	15.02	9.06	6.52
20.8	14.79	16.03	6.04
16.48	11.59	13.6	8.74
16.16	8.69	15.53	7.24
19.24	10.45	11.56	7.33
17.98	12.98	10.66	8.54
15.23	9.56	13.41	7.08
15.64	8.83	8.03	8.47
15.61	11.67	6.76	12.82
19.49	11.91	12.07	13.43
21.13	11.17	9.32	5.95
17.12	10.65	10.22	6.56
15.59	10.65	14.14	9.23
13.33	12.07	14.85	7.46
19.35	10.55	8.97	5.89
18.46	10.77	10.86	7.95
12.55	7.95	10.69	8
9.21	12.05	11.07	7.1
19.2	8.19	12.91	6.64
10.65	10.55	5.12	10.52
21.51	10.54	13.21	10.01
17.41	8.71	12.49	8.96
16.07	9.78	9.47	7.7
16.54	10.52	10.69	8.51
18.79	9.85	14.85	7.93
15.51	12.88	14.53	7.24
11.07	12.95	12.13	11.8
18.61	12.03	9.62	10.32
18.75	13	14.69	11.08

20.09	11.16	9.47	10.27
16.9	13.05	8.72	8.71
18.29	14.35	8.19	6.65
12.86	12.86	8.99	11.03
18.07	9.88	12.04	6.27
17.41	10.24	11.3	10
11.64	9.58	11.52	8.84
18.46	12.8	11.66	9.94
11.8	13.04	7.17	11.15
13.55	10.87	14.33	8.52
12.72	12	10.86	8.75
15.27	8.69	9.85	10.65
11.93	8.55	17.48	11.59
19.81	11.22	15.4	9.02
13.41	11.23	13.05	7.18
18.43	12.8	10.44	11.72
15.05	12.67	14.88	7.88
13.87	11.22	9.31	11.11
12.08	9.44	12.84	9.96
17.41	11.01	6.15	13.06
17.77	12.5	9.8	9.23
19.06	13.28	12.72	11.47
10.8	10.65	13.72	10.52
12.92	8.54	9.39	8.02
13.98	11.92	9.81	9.02
15.02	10.69	11.01	8.45
11.57	9.44	9.23	7.74
13.05	10.76	10.51	8.45
12.29	14.85	9.96	10.72
13.85	11.37	7.78	10.04
14.27	13.87	11.11	10
19.07	14.88	16.43	10.78
16.25	14.49	8.31	9.42
19.03	9.19	10.76	10.03
15	12.73	12.13	7.95
17.08	12.26	11.08	6.36
16.97	12.8	11.8	6.34
21.72	10.96	5.51	9.32
12.72	10.19	10.8	6.89
17.91	12.35	11.61	7.8
17.15	11.52	12.07	8.97
12.38	11.91	15.96	10.87

19.26	10.24	10.49	8.19
17.24	9.08	12.43	9.39
20.61	8.52	9.25	8.51
14.03	12.08	15.57	7.21
12.31	12.8	16.16	12.54
14.11	9.96	17.56	14.77
18.18	8.75	17.32	12.31
16.67	12.29	15.97	11.82
19.88	12.86	16.33	7.44
19.59	12.57	15.27	7.14
17.89	14.59	13.3	6.65
21.46	12.29	13.78	6.2
19	12.04	11.72	8.96
22.64	13.8	6.14	13.57
15.12	13.18	12.39	10.87
20.53	11.78	14.88	7.24
20.79	11.86	13.68	6.07
18.84	13.22	14.23	11.67
18.03	13.22	14.34	8.97
12.84	11.56	12.54	6.66
19.26	11.23	12.71	10.24
19.33	9.02	11.95	10.3
20.84	10.01	14.66	9.59
14.7	13.06	15.14	10.76
19.58	13.41	17.21	12.03
17.26	12.31	15.56	13.6
14.49	13.16	13.47	8.36
21.38	15.21	13.97	10.27
13.88	12.16	8.4	8.33
13.21	11.95	13	9.78
19.11	13.68	12.23	8.09
16.75	11.98	14.16	16.43
17.18	14.22	16.12	17.09
17.03	11.79	15.06	13.08
16.49	15.24	7.21	5.42
18.47	12.35	8.48	7.46
13.05	15.14	12.54	13.82
17.92	9.73	13.97	11.22
17.17	10.3	14.16	9.56
17.21	16.64	12.87	8.83
18.43	9.74	11.5	8.95
16.17	9.52	12.19	8.19

18.26	16.25	11.01	7.6
18.32	13.8	13.29	7.85
18.44	11.74	9.16	9.97
17.18	12.59	8.58	7.78
14.77	11.97	9.78	8.7
18.49	14.14	10.61	9.75
16.16	11.95	13.58	9.42
14.61	16.13	7.25	7.06
17.69	10.57	15.61	11.52
16.23	14	14.75	15.38
13.41	11.78	11.24	8.27
19.38	15.51	7.6	10.14
22.02	12.92	10.46	9.62
19.31	13.35	10	10
21.65	11.56	9.09	9.09
13.28	11.22	10.39	10.39
15.24	13.58	8.97	8.97
18.61	11.05	9.62	9.62

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CURRICULUM VITAE

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OBJECTIVES:

To be a renowned environmental scientist

PROFILE:

I am adaptable, dependable and enthusiastic to learn new ideas.

BROAD RESEARCH INTEREST

- Bioremediation
- Environmental Impact Assessment (EIA) and Strategic Environmental Assessment (SEA).
- Waste management

EDUCATIONAL QUALIFICATION:

2009 - 2011:	M.Sc. Environmental Sciences, King Fahd University of Petroleum and Minerals (KFUPM), **GPA 3.84 /4**
2003 - 2008:	B.Sc. Biology (2.1) Ahmadu Bello University, Zaria. Nigeria. **GPA 3.98/5**

WORK EXPERIENCE

2009 - 2012.	Research Assistant. Earth Sciences Department, KFUPM.
2008 - 2009.	Field Officer for the Global Fund Roll Back Malaria Project under Planned Parenthood Federation of Nigeria (PPFN). Coordinated the distribution of anti-malaria drugs and permanent treated mosquito nets around eight local Government areas of Kaduna State.

RESEARCH EXPERIENCE

2009 – 2012	Research Institute (Center for Environment and Water), King Fahd University of Petroleum and Minerals, Dhahran

2009 – Till Date Earth Sciences Department, King Fahd University of Petroleum and Minerals

LANGUAGES:

English, Yoruba and Hausa

EXPERIENCE WITH COMPUTER AND SOFTWARE PACKAGES

- Effective use of ArcGIS
- Windows XP, Vista, 7 and Mac OSX Operating ssystems
- Variety of statistical packages, spreadsheet, database, business graphics, word processing, network applications).

SKILLS AND INTERESTS:

- Photo Editing and Graphical Designing enhances my creative personality.
- Reading enhances my thinking process and creates a platform for me to be perceptive to concepts and ideas.
- Taking long walks and jogging are acts I do to maintain a healthy lifestyle.
- Hobbies such as Volleyball, Basketball, Scrabble and Chess improve my precision and analytical abilities.
- Surfing the Internet, is a way of keeping myself updated and learning new ideas.

WORKSHOPS AND SEMINARS ATTENDED

- Joint KFUPM-EAGE: Geological History of CO_2: Atmospheric Change and Natural Sequestration by Alain-Yves Huc Institut Français du Pétrole. Dhahran, Saudi Arabia
- EARTHDAY Arabia: Yearly Specialty Conference on Environmental Progress in Saudi Arabia. Jubail, Saudi Arabia.
- ENVIROARABIA: 10^{th} Specialty Conference on Environmental Progress in the Middle East, Manama, Bahrain

- Special Workshop on the Impact of Groundwater Quality on the Removal of MTBE Using Advanced Oxidation Technology. KFUPM, Saudi Arabia

SHORT COURSES, TRAINING AND VOLUNTARY SERVICES:

- Short course on Air Quality and Pollution Control, Organised by Environmental Technology Management Association (ETMA). Movenpick Corniche, AlKhobar, Saudi Arabia.
- Training course on Marine Taxonomy, Organised by Inter-Islamic Network of Oceanography. Istanbul-Izmir Turkey.
- Summer Training at Microbiology and Chemistry Department EXOVA Environmental Laboratory, Dammam, Saudi Arabia.
- Voluntary Teacher: Developed and Administered Adult Literacy Training. Teaching 50+ mothers how to read and write.

SCHOLARSHIP AND AWARDS:

- 2009-2011 King Fahd University of Petroleum and Minerals Merit Scholarship Awards.
- 2004-2008 Elf Petroleum Nigeria Limited (NNPC/EPNL) Merit Scholarship Awards.

VOLUNTEERING AND PROFESSIONAL ASSOCIATION MEMBERSHIP

- Student Representative and Editorial team Member. Environmental Technology Management Association (ETMA).
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- Society for Exploration Geophysicists (SEG)
- European Association of Geoscientists & Engineers (EAGE)
- Dhahran Geosciences Society (DGS)
- Environmental Technology and Management Association (ETMA)