Comparative Study of Cyanobacteria of Desert and Semi-Desert Crusts of Two Different Continents: Africa (Ethiopa) and North America (Usa)

Melaku Mesfin
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COMPARATIVE STUDY OF CYANOBACTERIA OF DESERT AND SEMI-DESERT CRUSTS OF TWO DIFFERENT CONTINENTS: AFRICA (ETHIOPIA) AND NORTH AMERICA (USA)

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To my beloved wife Mitike Chaka, my daughters Absera and Melekte and my son Gihon; for their love, understandings and support throughout my studies.
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COMPARATIVE STUDY OF CYANOBACTERIA OF DESERT AND SEMI-DESERT CRUSTS OF TWO DIFFERENT CONTINENTS: AFRICA (ETHIOPIA) AND NORTH AMERICA (USA)

MELAKU MESFIN

ABSTRACT

Cyanobacterial flora of microbiotic crusts of the Ethiopian Rift Valley System and the Great Basin in Idaho and Oregon were studied. Nine species of cyanobacteria were identified and morphologically characterized from the microbiotic crusts of the Rift Valley in Ethiopia. These included one species of Chroococcales, five species of Oscillatoriales, and three species of Nostocales. Similar studies were made for the microbiotic crusts of the Great Basin of Idaho and Oregon. A total of six morphospecies were morphologically characterized and identified, three species of Oscillatoriales, and three in Nostocales, were recorded in the regions. The cyanobacterial flora of the two continents was compared based on their molecular and morphological differences. Partial sequence data of the 16S rRNA gene (~85%) (Small sub-unit rRNA gene) and the 16S-23S ITS (Internal Transcribed Spacer) genes were obtained for all taxa from the Rift System and for selected morphospecies of cyanobacteria of the Great Basin. Based on the sequence data provided, morphospecies of cyanobacteria of the two continents tend to show variation in their phylogeny. The ITS regions of morphospecies of cyanobacteria of the two continents were also compared in relation to the D1-D1’, V2, and box B helices. Based on morphological, ecophysiological and molecular data; four new species of cyanobacteria were defined from microbiotic crusts of the Rift Valley of Ethiopia. Among these new species, one belongs to Chroococcales, one belongs to Oscillatoriales, and two belong to Nostocales.
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CHAPTER I
INTRODUCTION

1.1 Deserts- Environmentally stressed ecosystems

The maintenance of species diversity, productivity and biogeochemical cycling is integral in the preservation and stability of an ecosystem (Chapin et al. 1996), accomplished through the interaction and regulation of soil sources, major functional groups of organisms, climate and disturbance regime (Chapin et al. 1996; Jenny, 1941). Key ecosystems are greatly suffering at a global level through changes in biogeochemistry, climate and species invasions and extinctions caused by anthropogenic activity (Chapin et al. 1996; Vitousek, 1994; Vitousek et al. 1997). There have been large increases in concentrations of gases such as CO₂, NO₂, CH₄ and CFC’s due to emissions from industry and agriculture (Houghton et al. 1990). Nitrogen is fixed more by anthropogenic activity than by natural processes, and global levels of CO₂ have risen nearly 30% from preindustrial levels (Vitousek, 1994). In turn, this has caused large changes in productivity and biogeochemical cycles within many ecosystems (Aber et al. 1989; Kinzing and Socolow, 1994; Koch and Mooney, 1996; Lubchenco, 1991; Schulze, 1989; Vitousek, 1994). Along with changes in biogeochemical cycling and atmospheric chemistry, species invasions and extinctions are responsible for
large-scale changes in both species diversity and composition. Recent estimates show that 50% of the Earth’s ice-free terrestrial ecosystems have been altered or exploited by anthropogenic activity (Kates et al. 1990) and some of the most dramatic and rapid transformations are occurring in arid and semi-arid regions. A total of 33% to 40% of terrestrial land surfaces are covered by these ecosystems (Kassas, 1995) and 35% of the world’s population inhabit these areas (Brooks and Pokshishevsky, 1986). Climate change and desertification will most likely cause an increase in the spatial coverage of arid ecosystems. It has been predicted that there will be a 20% increase for dry lands in general and a 50% increase in desert, as a direct response to the increase in atmospheric CO$_2$ concentrations (Melillo et al. 1993). There has been a large expansion of arid regions caused by desertification resulting from land use (Monod, 1986; Schlesinger et al. 1990). An estimated 4.5% of the annual net primary productivity is lost to desertification each year (Vitousek et al. 1997) and some estimate that 35% of the world’s land area faces the risk of desertification (Cloudsley-Thompson, 1988). Arid ecosystems have been made especially susceptible to even small changes in critical ecosystem processes that determine species composition and primary productivity due to their inherent low resource availability and species diversity (Kleiner and Harper, 1972; Noy-Meir, 1979; Verstraete and Schwartz, 1991).

1.1.1 Current stresses: USA, Ethiopia

Most arid and semi-arid lands of the United States have been heavily affected by human use since the late 1800s. Historically, most of this impact has been from livestock grazing near areas with forage and water. Most recently, substantial increases in off road
vehicle use and hiking have greatly expanded direct and indirect human impacts both spatially and temporarily. The combination of recreational use and livestock grazing is resulting in unprecedented levels of local and regional disturbances accelerating desertification processes in this region (Belnap and Gardner, 1993; Harper and Marble, 1988; Johansen et al. 2001; Metting, 1991; Rushforth and Brotherson, 1982).

Desertification in Ethiopia is generally a result of human actions in fragile environments, particularly during a sequence of dry years. The main human actions concerned are steppe clearing for cultivation, overstocking and over-grazing, fuel gathering, uprooting of woody species, and intensive villagization (Bishaw, 2001; Mesfin, 1984; Mulugeta et al. 2005; Tewolde, 1989; Zerihun and Mesfin, 1999). The intensities of these actions have increased with human density. Evaluations based on remote sensing studies, coupled with ground observations result in an average figure of several tens of thousands of square kilometers becoming sterile every year in Sub Sahara Africa (George et al. 2008; Kassas, 1995; Le Houerou, 1985).

1.2 Ecological importance of microbiotic crusts

Microbiotic crusts are characteristic of soil surfaces in semi-arid and arid ecosystems in the world (Bhatnagar et al. 2008; Belnap and Lange, 2001; Eldridge and Greene, 1994; Lange et al. 1992, 1993; Zhang et al. 2006). Through microscopic examination, microbiotic crust communities are found to contain microflora of cyanobacteria, other bacteria, eukaryotic algae, fungi, lichens and bryophytes (Evans and Johansen, 1999). In the desert and semi desert regions of the world, soils and their associated microflora have developed under the influence of an arid climate, a short or long geological time period, a rugged topography subject to severe erosive forces, and
parent materials. These materials show relatively moderate or no soil development and formation, except in terms of smaller size particles, which are subject to movement by wind, water or gravity. The primary factors in the development and formation of typical, well-developed terrestrial soils are the availability of water, a favorable temperature regime and abundant vascular plant vegetation. However, these factors play a minor or negligible role in the genesis of most desert soils. In general, semi-arid and arid regions are deficient in available moisture for extended time periods, and annual precipitation is low, infrequent, and irregularly distributed. High evaporation rates, high wind velocities, high solar radiation, temperature extremes, low humidity, and generally rapid runoff are other common features. Severe erosive forces have usually contributed to a rugged topography. The amount of water, which remains in the surface soil, depends upon such factors as rate of evaporation, topography, ground water and nature of soil properties, including infiltration rate, percolation rate, texture, structure, porosity, mineralogy, color and surface reflectivity (albedo) (Broady, 1996; Cameron, 1969; Danin et al. 1998; Davey, 1991; Elster et al. 1998; Karnieli and Tsor, 1995; Kidorn and Yair, 1997; Skujins, 1984). In order to maintain and develop sustainable management of dry and semi-arid regions, knowledge of the roles of microbiotic crusts is vital to the management and balance of these regions. The microbiotic crusts of semi-arid and arid regions of the world may be critical to the environments in which they occur. The following functional attributes of the microbiotic crusts are often cited as important for ecosystem stability.

1.2.1 Soil stabilization

Stability of soil surfaces is promoted through two mechanisms. First, organisms can aggregate soil particles by providing extracellular polysaccharide (Anastasio, 1990; Bell and Sommerfield, 1987; Bailey et al. 1973; Campbell, 1979; Mazor et al. 1996). Second, the filaments of cyanobacteria, rhizoids of mosses and fungal hyphae of lichens further promote consolidation by aggregating soil particles (Belnap and Gardner, 1993; Buttars et al. 1998; Campbell, 1979; Fletcher and Martin, 1948; Schulten, 1985). McKenna-Neuman et al. (1996) compared the surface stability of soils that had been inoculated with a filamentous cyanobacteria (Nostoc commune) or nonfilamentous green algae (Chlamydomonas acidophila). Both taxa excrete extracellular polysaccharides. Filamentous species were much more effective at binding particles together because filaments were present during periods of dormancy, while nonfilamentous species typically form solitary thick-walled cells considered to be resting spores. McKenna-Neuman et al. (1996) concluded that the combination of filament entanglement and cementation by excretions was much more effective than cementing alone. The effect of entanglement was clearly demonstrated by Schulten (1985), who found that the hyphae of lichens were able to consolidate soil particles for up to two years following the death of the organisms. Aggregates from soil surfaces with microbiotic crusts have also been shown to have greater stability than aggregates from bare surfaces (Bond and Harris, 1964; Graetz and Tongway, 1986; Greene et al. 1990; Zhang et al. 2006). Microbiotic crusts are often only 1-2 mm in thickness. Therefore, it is critical when sampling soils with microbiotic crusts, for aggregate stability measurement, to take samples at 1-2mm in depth (Eldridge and Greene, 1994; Johansen, 1993). Several studies have addressed the effects of microbiotic crusts on surface erosion due to wind. Williams et al. (1995)
showed that higher threshold friction velocities (TFV) were needed for the initiation of wind erosion where undisturbed microphytic crusts were present. Threshold friction velocity (TFV) is the wind speed where erosion of the soil surface begins (Williams et al. 1995), and provides a measure of surface stability. Williams et al. (1995) found that removing the microbiotic crust decreases TFV over 50% and increased sediment transport over 500%. Belnap and Gillete (1998) found that only microbiotic crusts that had been undisturbed for at least 10 years had TFVs great enough to protect soil surfaces from wind gusts that were expected at least some of the time. They showed, with wind tunnel experiments, that fragile soil crusts can reduce wind erosion at a wind speed of 48 km h\(^{-1}\) by 10 fold and that there was 5 times the amount of wind erosion from a disturbed crust than one left intact (Leys, 1990). Zhang et al. (2006) found that wind erosion rates for sandy soil with a 0% crust cover was about 46,21,17 times the soil with a 90% crust cover at wind velocities of 18,22,25 m s\(^{-1}\), respectively, in the field experiment conducted in Gurbantunggut Desert of Northwestern China.

1.2.2 Nutrient retention

Microbiotic crusts can have a significant influence on ecosystem nitrogen and carbon dynamics because many of the dominant groups of organisms such as cyanobacteria are capable of nitrogen fixation and photosynthesis. Estimates of the rate of nitrogen input resulting from nitrogen fixation by microbiotic crusts exhibit considerable variation. Rychert et al. (1978) and West and Skujins (1977) report rates of nitrogen fixation from 10 to 100 kg N ha\(^{-1}\) y\(^{-1}\) in cold desert ecosystem, while Jeffries et al. (1992) estimated rates of inputs from 0.7 to 3.6 kg N ha\(^{-1}\) y\(^{-1}\) for an ecosystem on the Colorado plateau. Rychert et al. (1978) also provided estimates for microbiotic crusts in
the Sonoran desert (7 to 18 kg N ha\(^{-1}\) y\(^{-1}\)) and Australia (1.3 kg N ha\(^{-1}\) y\(^{-1}\)). Renaut et al. (1975) estimated the \textit{in situ} nitrogenase activity of continuously wetted cyanobacterial crusts from Morocco at about 300 nmol N\(_2\) g\(^{-1}\) dry crust h\(^{-1}\) with significant inhibition at high light (>60,000 lx) and high temperature (50 °C) regimes in the field. Estimated N\(_2\) fixation rates by \textit{Scytonema}-dominated algal crusts from five semi-arid sites in Nigeria corresponded to approximate annual inputs ranging from 0.3 to 3 g N m\(^{-2}\) across a geographical gradient from dry to relatively more dry savanna (Isichei, 1980; Stewart et al. 1978). Skarpe and Henriksson (1987) compared dark and light colored cyanobacteria crusts from western Kalahari (Botswana) for nitrogenase activity in the laboratory. Within 24 hours the dark crust, which covered only 1.5% of the collection site, showed a maximum activity corresponding to 0.6 mmol m\(^{-2}\) h\(^{-1}\) for the sand–colored crust, which covered nearly 10 times more surface area. Species of \textit{Nostoc} and \textit{Scytonema} were associated with both kinds of crusts (Skarpe and Henriksson, 1987).

1.2.3 Water retention

The effects of microbiotic crusts on soil hydrology have been reported. The rough microtopography of soil crust surfaces promotes infiltration and thus increases the depths of water penetration into the soil while decreasing overland flow (Brotherson and Rushforth, 1983; Eldridge and Greene, 1994). Microbiotic crusts influence infiltration by two mechanisms. First, they maintain the structural integrity of soil pores, and fungal hyphae and cyanobacterial sheaths act as entry points into the soil providing a stable mechanism for water infiltration (Eldridge, 1993b; Falchini et al. 1996). Second, microbiotic crusts protect the soil surface against erosion caused by the impacts of raindrops (Eldridge, 1993a; Eldridge and Greene, 1994). In general, however, the effects
of microbiotic crusts on soil water dynamics depends on the composition of the crust as well as local soil properties and topography. Some crusted areas on sand dunes decreased infiltration and increased runoff (Kidron and Yair, 1997). In areas with fine textured soils, crusts reduce or have no effect on infiltration (Savage et al. 1969; Williams et al. 1995). In many of semi-arid and arid regions of the world, undisturbed crusts increase infiltration, with significantly higher runoff generated in disturbed soil (Evans and Johansen, 1999; Salama and Kobbia, 1982).

1.2.4 Enhancement of vegetation

Microbiotic crusts may have a significant impact on seed germination, seedling establishment, and growth of higher plants by providing nutrients and more favorable microenvironments in cracks and depressions within the crust (Belnap, 2003; Belnap and Lange, 2001; Harper and Pendleton, 1993). Plants growing in association with microbiotic crusts in field and greenhouse studies have been shown to have higher minerals of nutrients such as nitrogen, phosphorous, and potassium (Belnap, 1995; Harper and Pendleton, 1993). Harper and Pendleton (1993) outlined four mechanisms that could cause higher nutrient concentrations in plants growing in association with microbiotic crust. First, microbiotic crusts may concentrate essential elements at the soil surface. Second, soil temperature under microbiotic crusts is often warmer, and this may enhance root growth and mineralization. Third, a sheath of cyanobacteria may contain chelating agents that increases solubility of essential nutrients (Lange, 1974, 1976). Fourth, conditions that favor growth of organisms within the microbiotic crust may also enhance development of mycorrhizal or rhizosheath associations with higher plants (Harper and Pendleton, 1993). Micromorphological examination of thin sections of
surface crusts revealed layers of aggregate, litter and microbiota, which could provide refuge for seeds by providing entrapment niches during wind and water erosion events (Mucher et al. 1988).

1.3 Fragility of microbiotic crusts

Maintaining soil stability and normal water and nutrient cycles in semi-arid and arid ecosystems is critical in avoiding desertification. These particular ecosystem processes are threatened by the trampling of livestock and people, by off-road vehicle use, range fire, overgrazing and oil exploration. From recent studies of the effect of disturbance on microbiotic crusts, range fire is more disruptive to species composition and crust biomass than other factors (Belnap, 1995; Eldridge and Rosentreter, 1999; Johansen et al. 1993; Thomas and Dougill, 2006; Zhang et al. 2006). Soil compaction and disruption of microbiotic crusts can result in decreased water availability to vascular plants through decreased water infiltration and increased albedo with possible decreased precipitation. Surface disturbance may also cause accelerated soil loss through wind and water erosion and decreased diversity and abundance of soil biota. In addition, nutrient cycles can be altered through lowered nitrogen and carbon inputs and slowed decomposition of soil organic matter. This results in lower nutrient levels in associated vascular plants (Belnap, 2002; Zaady et al. 1998). Mature microbiotic crusts take at least 15 years to develop, and lichen and bryophyte species richness and diversity are known to increase over a period of up to 40 years. The nitrogen fixation capability of microbiotic crust requires at least 100 years to fully recovering (Belnap, 1995, Evans and Johansen, 1999). Recovery of microbiotic crusts can be hampered by large amounts of moving sediments and re-establishment can be extremely difficult in some areas. Given
the sensitivity of these resources and extended recovery time, desertification threatens many arid and semi-arid lands in the world (Belnap, 1995; Evans and Johansen, 1999; Eldridge and Greene, 1994; Kassas, 1995; Le Houerou, 1985; West, 1990; Zhang et al. 2006).

1.3.1 Development and post-disturbance recovery

Substantial evidence exists for the important roles that biological crusts have in soil formation, maintenance of soil fertility, and stabilization of soil against erosion by wind and water. Nevertheless, forced development of biological crusts for reclamation of disturbed semi-arid lands and deserts has received scant attention. Natural establishment of mature plant cover in dry ecosystems is low. Processes that initiate or hasten desertification or degradation of useful rangelands are numerous and cumulative, and without human intervention the rate of habitat destruction is greater than the rate of repair. Therefore, desert reclamation efforts that include microbiotic crust species would be of high value (Buttars et al. 1998; Johansen, 1986; Metting, 1991).

Microbiota are commonly thought to be pioneering species in the revegetation of degraded soils (Baily et al. 1973; Booth, 1941; Johansen et al. 1982; Johansen and St.Clair, 1986; Johansen et al. 1993). After an initial landscape disturbance such as fire or severe overgrazing, non-vascular plants are the primary colonizers of a site. In fine textured soils, raindrop action and erosion can lead to the formation of a rain-impact crust, slowing infiltration and interfering with seedling emergence (Moss, 1991). This crust may later be colonized by biological elements to form a microbiotic crust (Fuller, 1974; Johansen and St. Clair, 1986; Scott, 1992).
Several efforts have been made to stimulate the recovery of microbiotic crusts in disturbed areas. St. Clair et al. (1986) used soil crust slurry to enhance recovery rates at a fire disturbed site in the Great Basin in the U.S.A. They found that soils inoculated with surface soil slurry had significantly higher cyanobacterial numbers than soil receiving just water or sub-crust slurry. Belnap (1993) tested the use of inoculation using nearby biotic crust material to increase biotic recovery rates of disturbed crusts. *Microcoleus vaginatus*, *Nostoc muscorum*, and *Phormidium tenue* were all notably higher in crust treated plots. *Microcoleus vaginatus* is the cyanobacterium that makes up the bulk of crustal organisms in semi-arid environments and contributes up to 95% of the crust biomass (Belnap, 1993).

### 1.4 Floristic studies of microbiotic crusts

Floristic studies of microbiotic crusts have been sporadic and nearly always incomplete. No single researcher has the taxonomic expertise to study all crust components (lichens, bryophytes, eukaryotic algae, cyanobacteria, nonlichenized fungi, heterotrophic bacteria and protozoa) (Evans and Johansen, 1999). The most extensively studied algal crusts are those of semi-arid and arid lands of North America (Alwathnani, 2006; Cameron, 1964; Cameron and Blank, 1966; Durrell, 1962; Flechtner et al. 1998; Hunt and Durell, 1966; Johansen, 1993; Shields and Drouet, 1962). The most frequent taxa in North America deserts include (1) the cyanobacteria: *Microcoleus vaginatus*, *Schizothrix calciola*, *Nostoc commune*, *Nostoc muscorum*, *Nostoc paludosum*, *Nostoc punctiforme*, *Phormidium minnesotense*, *Phormidium tenue*, *Trichormus variabilis*, and *Tolypothrix tenuis*, (2) the xanthophyte: *Xanthonema debilis* (formerly placed in *Heterothrix*) (3) the diatoms: *Hantzschia amphioxys*, *Luticola mutica*, *Luticola cohnii*
(the two latter taxa formerly placed in *Navicula*) and *Pinnularia borealis* (Johansen, 1993). Several sites in arid western North America have shown that a number of coccoid chlorophyte genera are widespread, including *Bracteacoccus, Chlorosarcinopsis, Chlorella, Stichococcus, Desmococcus, Apatococcus, Myrmecia, Chlorococcum*, and *Neochloris* (Flechtner *et al.* 1998; Johansen *et al.* 2001).

An extensive study of dry algal crusts in Senegal, Africa was made over a 10-year period by Reynaud (1987). The floristic make up of the crusts included *Pseudanabaena* species, *Lyngbya* species, *Scytonema* species, *Nostoc* species, *Anabaena* species and *Calothrix* species (Reynaud, 1987). Cyanobacteria were found to be the dominant organisms on rock surfaces collected from inselbergs in the Ivory Coast, Africa (Büdel *et al.* 1997; Büdel, 1999). Twenty-three species of cyanobacteria and 17 cyanobacterial lichen species were reported (Büdel *et al.* 1997). In many previous studies, cyanobacteria have been reported as dominant taxa in the tropics. Fremy (1930) observed the dominance of cyanobacteria over eukaryotic algae on exposed rocks in the central part of Africa. Reynaud and Laloe (1985) also reported species composition and cyanobacterial biomass in the desert crusts of Senegal. Cyanobacteria were reported as the dominant taxa in the desert crust of Egyptian soil (Salama and Kobbia, 1982). *Stigonema mintum, Gloeocapsa minor, Microcoleus testarum, Oscillatoria limosa, Phormidium valderia*, and *Phormidium inundatum* (Salama and Kobbia, 1982) represented species in the desert crusts of Egypt.

Cyanobacteria are the most widely distributed photosynthetic prokaryotes in nature. They are constituted of a cell typical of gram-negative bacteria with a photosynthetic apparatus similar in functional and structural respects to that contained in
eukaryotic chloroplasts (Stanier and Cohen-Bazire, 1977). Their ability to form stable crusts on desert soil surfaces was recognized early in this century (Booth, 1941). Certain soil cyanobacteria have a cosmopolitan geographic distribution. Species of Microcoleus, Schizothrix, Nostoc, Scytonema, Phormidium and several additional genera are repeatedly reported from soils all over the world. These include North America (Cameron, 1964; Flechtner et al. 1998; Johansen, 1993); Brazil (Azevedo, 1991); Equatorial Africa (Fremy, 1930); Israel (Dor and Danin, 1996; Friedmann and Galun, 1974; Vinogradova et al. 2000); Himalayas (Watanabe and Komárek, 1988); China (Reynaud and Lumpkins, 1988; Ying et al. 1992; Zhang et al. 2006); Egypt (Salama and Kobbia, 1982); Botswana (Skrape and Henrikson, 1987); Ivory Coast (Büdel et al. 1997); South Africa (Thomas and Dougill, 2006); Saudi Arabia (Arif, 1992); Argentina (Bouza et al. 1993); Rejasthan (Anantani and Marthe, 1974), Chile (Forest and Weston, 1966); Senegal (Reynaud and Roger, 1981); India (Thar desert) (Bhatnagar et al. 2008). Species composition and thickness of desert crusts may differ considerably from place to place following local environmental conditions such as soil texture and fertility, ionic strength and pH, amount of annual precipitation and degree of local disturbance (Flechtner et al. 1998; Hoffmann, 1989; Lange et al. 1992; Metting 1991; Ying et al. 1992; Zhang et al. 2006).

1.4.1 Traditional systematics of cyanobacteria

Cyanobacteria are a widely distributed group of oxygenic photosynthetic prokaryotes. They possess chlorophyll a and phycobiliproteins and their 16S and 5S rRNA gene sequences are similar to other members of the eubacteria (Boone and Castenholz, 2001; Castenholz and Waterbury, 1989). Despite their widespread occurrence and ecological importance, the alpha-level taxonomy of cyanobacteria is
currently in a state of chaos. The old botanical nomenclature for the group (Geitler, 1932) has been challenged, particularly with reference to sheath characteristics, which vary widely in culture (Rippka et al. 1979, Rippka, 1988). Drouet’s revisions (Drouet and Daily, 1956; Drouet, 1968, 1973, 1978, 1981) are now considered overly drastic and incorrect. Komárek and Anagnostidis have proposed recent revisions in the genera, but their work is botanically based and without consistent use of culture material and bacteriological methods. There are only two monographs that give a detailed modern treatment of the cyanobacterial species (Komárek and Anagnostidis, 1999; 2005).

Bornet and Flahault (1888) began the morphology-based taxonomic classification for the heterocystous blue-green algae and they were soon followed by Gomont (1892), who proposed the initial classification for nonheterocystous, filamentous blue-green algae (Oscillatoriaceae). Geitler (1932) produced a comprehensive review recognizing 1300 species, 145 genera, 20 families and 3 orders. Other classification schemes followed, though most were based on the Geitlerian format. Drouet (Drouet, 1968, 1973, 1978, 1981; Drouet and Daily 1956) produced a major revision of Geitler’s classifications and consolidated a number of the blue-green names, reducing many of the recognized genera and species into 62 species and 24 genera. Drouet, basing his classifications on the microscopic examination of a large number of collected specimens, presumed that relatively few cyanobacterial genotypes existed but that there were many different phenotypes expressed depending on environmental conditions. However, Drouet did not include biochemical, physiological or genetic information in his classification and did not establish any clonal cultures. Stanier et al. (1978) began another classification scheme that sought to correct the same problems Drouet addressed. This system differed from
Drouet's in that it used axenic, clonal cultures and included some of the biochemical and genetic information that Drouet did not use.

The most recent re-classification scheme, proposed by Komárek and Anagnostidis focuses on the presence or absence of specific cell types and different modes of cell division to define families and identify intergeneric relationships (Anagnostidis and Komárek, 1985, 1988, 1990; Komárek, 1994; Komárek and Anagnostidis, 1986, 1989, 1999, 2005; Komárek and Hindak, 1975). Anagnostidis and Komárek (1988) believe that cyanophyte re-classification is problematic for at least eight reasons. First, traditional taxonomic criteria were established more than a hundred years ago and were based on unstable criteria such as sheath characteristics and false branching. Second, environmental factors are known to influence cyanobacterial morphology, and the degree and importance of phenotypic plasticity were not recognized by early workers. Third, typification has been very poor and type materials in herbaria have not been easily available for comparison with field specimens. Fourth, many different species concepts are found among authors of varying backgrounds and working in varying localities. Fifth, a number of large genera with hundreds of species do not correspond to evolutionary trends. Sixth, biogeography has not been taken into account; Northern Hemisphere temperate keys have been used worldwide across climate types and oceanic barriers. Seventh, the authors argue that taxonomists have been very conservative. Many do not like to adopt new taxonomic criteria and continue to use old names that do not correspond with modern taxonomic systems. Eighth, many ecologists force the isolates they observe into old classification schemes based upon European populations without understanding that many species should probably be described as new.
Komárek and Anagnostidís have tried to correct these problems by developing a classification system that utilizes objective criteria, selecting features that occur obligately across genera, which can be used to distinguish species (Anagnostidís and Komárek, 1985, 1990; Komárek and Anagnostidís, 1986, 1989, 1999, 2005). Their approach is more similar to the botanical approach in their use of morphological and life history characteristics. They limit the use of sheath criteria for the definition of genera and emphasize details of cell division, hormogonia formation, tapering, polarity, methods of false branch formation, as well as other features to identify a different set of genera than that proposed by Geitler (1932). They recognize more genera than Drouet and a majority of bacteriologists.

1.4.2 The utility of molecular systematics approaches

During the last decade, microbiologists have begun to employ molecular techniques to answer questions about cyanobacterial taxonomy, evolution, and population diversity. Analysis of 16S rRNA and, more recently, its associated Internal Transcribed Spacer (ITS) has figured heavily in these studies. Giovannoni et al. (1988) used the 16S rRNA gene to determine evolutionary relationships between cyanobacteria and green chloroplasts. The use of the 16S rRNA gene has provided insight into the relationships of cyanobacterial genera. The members of the Nostocales are a monophyletic clade but the Chroococcales and Oscillatoriales are not monophyletic when traditional classification is followed (Turner, 1997). Currently, a new higher order of cyanobacterial taxonomy has been proposed based on molecular and ultra structural methods (Hoffmann et al. 2005; Komárek, 2005). Coccoid and non-heterocytous filamentous cyanobacteria tend to have two phylogenetic lines characterized by similar cytological organization. Heterocytous
cyanobacteria are monophyletic, with subclades having different types of branching, polarity and akinet formation (Hoffmann et al. 2005; Komárek, 2005; Rajaniemi et al. 2005).

The 16S rRNA genes have also been useful for identifying morphological entities that clearly belong to a single clade. For example, Garcia-Pichel et al. (1996) found that the 16S rRNA gene sequences of seven geographically distant field populations of *Micorcoleus chthonoplastes* were identical or nearly identical. They concluded that *M. chthonoplastes* was therefore a single, well-determined, monophyletic taxon. In more recent work, Garcia-Pichel et al. (2001) found that *M. vaginatus* from widely separated habitats is likewise a distinct, monophyletic group based on the 16S rRNA data. Fox et al. (1992) found more than 99.5% sequence identity in the 16S rRNA genes among three phenotypically similar psychrophilic strains of *Bacillus*. Previous DNA-DNA hybridization proved that these strains were different species, which led Fox et al. (1992) to conclude that identity in the 16S rRNA sequence data is not sufficient grounds to conclude identity in species. Particularly, they felt that distinguishing recently diverged species was problematic. Some cyanobacterial workers have used similarity greater than 96-99 % to recommend the combination of a distinct morphospecies into a single species (Palinska et al. 1996; Otsuka et al. 1998). Due to its evolutionary highly conserved regions, 16S rRNA genes are not suitable for separating most closely related species (Boyer et al. 2002; Palys et al. 1997). Near identity in 16S rRNA has proved useful for demonstrating monophyly (Garcia-Pichel et al. 1996), but not for the separation of species within generic clades. Other systematists have also questioned the utility of 16S
rRNA data in delimitation of taxa at the subgeneric level (Komárek, 1994; Mollenhauer et al. 1994; Wilmotte and Golubic, 1991).

The 16S-23S internal transcribed spacer (ITS) region has shown some utility in examining phylogenetic relationships. Flechtner et al. (2002) found that configuration patterns in the ITS were congruent with morphological separation of the families Microchaetaceae, Scytonemataceae, and Rivulariaceae in the Nostocales. The 16S-23S ITS regions are found to be informative, and generally support the conclusions based on the 16S rRNA data (Boyer et al. 2001, 2002; Johansen and Casamatta, 2005; Li, 2000; Payne, 2001; and Scheldeman et al. 1999). ITS regions can be used in estimating relationships below the genus level and separate highly similar strains (Casamatta et al. 2006; Otsuka et al. 1999; Rocap et al. 2002; Taton et al. 2006). There are ITS regions, which have conserved structures that are used as autapomorphic characters in defining species or species clusters. The D1-D1’ helix near the beginning of the 16S-23S ITS region is reliable in characterizing cyanobacterial genera and sometimes species (Alwathnani, 2006; Casamatta et al. 2006; Iteman et al. 2000; Johansen et al. 2008; Řeháková et al. 2007). Even though the 16S-23S ITS regions are reliable in identifying cyanobacterial species, there are some associated problems. Due to the different ribosomal operons expressed in the same strain, the ITS sequence can vary in length and may or may not contain tRNA genes (Alwathnani, 2006; Boyer et al. 2001; 2002; Iteman et al. 2000). However, the use of ITS sequences, which are far more variable among operons than 16S rRNA genes, may be the best way to distinguish species. The homology of operon-specific ITS regions can be determined more easily (Boyer et al. 2001, 2002).
1.5 Research objectives

The current project has several different objectives. The main objective of the research is to investigate and document the cyanobacterial flora of Idaho and Oregon in the U.S.A (North America), and Awassa and Zewai in Ethiopia (Africa). Extensive study and identification of selected cyanobacteria of the semi-arid and arid regions of the two continents are made. Species identification and comparisons of cyanobacterial distributions in the two continents are provided. Two or more morphologically identical species were cultured from the two continents, and sequence analysis the 16S rRNA and the 16S-23S ITS genes were analyzed and compared.

This study is the first of its kind in Sub Sahara regions of Africa in general and in Ethiopian semi-arid regions in particular. Cyanobacteria are the dominant taxa among most microbiotic desert crusts, and their active roles in stabilizing surface soil, nitrogen and carbon dynamics, establishing plant seedlings of vascular plants and in sealing moisture into the desert soils have been established through the studies of microbiotic crusts in different parts of the world. Even though there have been studies conducted on North American desert crusts, nothing has been documented on the prokaryotic algae of Idaho and Oregon. This study will provide an extensive study of soil cyanobacteria of the two continents as well as evaluate the phylogenetic relationship of taxa identified through morphological and molecular characterization.

1.6 Rationale for site selection

I have chosen sites in Ethiopia, Idaho, and Oregon in part because the soils of these regions have never been studied with respect to their cyanobacterial floristic composition. Most of the arid and semi-arid lands within the Ethiopian Rift System have
been heavily affected by human use. The main human impacts resulted from steppe clearing for cultivation, overstocking and over-grazing, fuel gathering, uprooting of woody plant species, intensive villagisation, and oil exploration (Bishaw, 2001; Mulugeta et al. 2005; Mesfin, 1984; Tewolde, 1989; Zerihun and Mesfin, 1999; Zhang et al. 2006). The intensities of these actions have increased with human population density. It is necessary to maintain soil stability and normal water nutrient cycles in the Ethiopian Rift System, which is critical in avoiding desertification in the region. In many ways, most arid and semi-arid lands of the United States have been heavily affected by human use since the late 1800s. Historically, most of this impact has been from livestock grazing near areas with forage and water. Most recently, substantial increases in off-road vehicle use and hiking have greatly expanded direct and indirect human impacts both spatially and temporarily. The combination of recreational use and livestock grazing is resulting in unprecedented levels of local and regional disturbances accelerating desertification processes (Belnap and Gardner, 1993; Harper and Marble, 1988; Johansen, 1986; Metting, 1991).

It is expected that strains of cyanobacteria from different continents will have highly similar morphology and ecophysiology, but will actually be different genospecies based on DNA sequence analysis. It is also possible that some strains of cyanobacteria, such as Microcoleus vaginatus, truly are cosmopolitan and have worldwide distribution. It is expected that the isolation of the two continents on geological time scale will lead to speciation and endemism of cyanobacteria. From the aspects of the above basic and applied sciences, the two semi-arid regions of the continents are studied.
1.7 Literature Cited


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CHAPTER II
MORPHOLOGICAL CHARACTERIZATION OF CYANOBACTERIA IN MICROBIOTIC CRUSTS OF THE ETHIOPIAN RIFT VALLEY SYSTEM

2.1 ABSTRACT

This article deals with the study of the floristic compositions of cyanobacteria in microbiotic crust of the Ethiopian Rift Valley System. Nine species of cyanobacteria were identified and morphologically characterized. One species of Chroococcales, five species of Oscillatoriales, and three species of Nostocales were identified. Among the isolates, four new species were diagnosed, one Chroococcales, Oscillatoriales, and two Nostocales. The new species of the taxa identified are named provisionally as Cyanosarcina abyssinica, Symplocastrum sheleko, Nostoc oromo, and Trichormus sidamae in the text.

Keywords: Cyanobacteria, Microbiotic Crust, Ethiopian Rift Valley System
2.2 Introduction

Microbiotic crusts are integral components of the soil surface in many arid and semi-arid regions of the world. Microbiotic crust communities contain a microflora of cyanobacteria, bacteria, eukaryotic algae, and fungi. They are found in mesic environments, tropical and temperate deserts and in polar regions of the globe (Arif, 1992; Broady, 1996; Büdel et al. 1997; Büdel, 1999; Cameron, 1964; Danin et al. 1998; Johansen, 1993; Issa et al. 1999; Salama and Kobbia, 1982; Thomas and Dougill, 2006). Increasing evidence indicates that microbiotic crusts play several roles in semi-arid lands and in hot and cold deserts around the world. Their ability to form stable crusts on desert soil surfaces was recognized early in the last century (Booth, 1941). They play a significant role in carbon and nitrogen dynamics in the crusts of different parts of the world (Evans and Johansen, 1999; Issa et al. 1999; Isichei, 1990; Renaut et al. 1975; Rychert et al. 1978; Thomas and Dougill, 2006; Zhang et al. 2006).

Microbiotic crusts also play roles as constituents of mature semi-arid and arid ecosystems and as pioneers in primary and secondary plant succession (Metting, 1991; Johansen et al. 2001). Among the component organisms of microbiotic crust, cyanobacteria are the most widely distributed photosynthetic prokaryotes in nature. They are constituted of a cell typical of gram-negative bacteria with a photosynthetic apparatus similar in functional and structural aspects to that contained in eukaryotic chloroplasts (Stanier and Cohen-Bazire, 1977). Certain soil cyanobacteria have a cosmopolitan geographic distribution. Species of Microcoleus, Schizothrix, Nostoc, Scytomena, Phormidium and several additional genera are repeatedly reported from a variety of soils all over the world.
Thus far, no research has been conducted on the microbiotic crusts of the Ethiopia Rift Valley System. The geologically and biologically defined regions of the Ethiopian Rift Valley contain a great variety of habitats, making it an ideal part of Africa in which to commence microbiotic crust studies. The objective of the present study is to characterize cyanobacterial species found in the microbiotic crusts of the Ethiopian Rift Valley System and to compare and contrast their ecology and distribution with similar taxa in different parts of the world.

2.3 Description of Study Areas

The Ethiopian Rift System comprises the Afar Depression in the northeast, the centrally placed Main Ethiopian Rift, and the 300-km-wide rifted zone located in the southwest of the country (between 4° 25’ and 15° 30’ Lat. N) (Fig. 1). This Rift System covers 310981 km², which is estimated to be 28% of the total area of the country (WoldeGabriel et al. 2000; http://www.fao.org/docrep/W4347E/w4347e01.htm).

The study areas are located in the southern part of the Ethiopian Rift Valley System (7°.09’ N, 38°. 29’ E, and 7°. 9’ N, 38°. 7’ E) in the vicinity of Awassa and Zewai towns. Both places are semi-arid regions and have annual temperature and annual precipitation ranging from 13-27 °C and 760-1000 mm, respectively (FAO, 1984).

Depending upon the climate, the Ethiopian Rift System supports different forms of vegetation, which are subjected to ecological stresses and anthropogenic pressures. These have led to poor farming and range conditions in the region (Feoli and Woldu, 2000; Girma, 2001; Mulugeta et al. 2005; Oba et al. 2000; Zerihun and Mesfin, 1999). The regions have been heavily affected by human activities through steppe clearing for cultivation, overstocking and over-grazing, fuel gathering, uprooting of woody plant
species, and intensive villagisation (Tewolde, 1989; Mulugeta et al. 2005; Zerihun and Mesfin, 1990). The dominant vegetation cover in the region has been described and includes *Acacia albida*, *Olea europaea*, *Acacia tortilis*, *Acacia seyal*, *Balanites aegyptiaca*, *Euphorbia candelabrum*, *Croton dychogamus*, and *Solanum schimperianum* (Zerihun and Mesfin, 1990). The Rift System has the right climate and suitable physical and chemical properties of soil (Appendix B) that are capable of supporting microbiotic crusts when protected from mechanical disturbances (trampling by livestock and humans). Surface soil structures and the types of vegetation cover during sampling were photographed (Fig. 2, 3, 4).
Figure 1. Sampling locations in Ethiopia.
Figure 2. Sampling locations in Ethiopia, satellite photograph.
Figure 3. Soil surface structures and vegetation cover at Awassa location in Ethiopia.
Figure 4. Soil surface structures and vegetation cover at Zewai location in Ethiopia.

2.4 Materials and Methods

2.4.1 Field sampling

Ten sites were sampled from the Awassa and Zewai study locations (five in each area). The sampling sites were chosen using the following criteria. 1. Each site had to be at least 5 km away from highways and urban areas. 2. Each site had to be in untilled range. 3. Each site was 2-5 km away from the nearest site. Sites were all on communal land with minimal disturbances. The five sampling sites in Awassa are located southeast of the town (site-1), near lake Awassa, east of the town (site-2), near mount Tabor, south of the town (site-3), north of the town (site –4), and west of Awassa at the road side of
lake Awassa (site-5). The five sampling sites in Zewai are located southeast of the town (site-1), south of the town (site-2), west of the town (site-3), northwest of the town (site-4) and north of the town (site-5). A total of 10 samples of microbiotic crusts were collected from both locations. A 1.0 m$^2$ quadrat was placed within each sample site, and the top 2 mm of microbiotic crust was sampled within the quadrat by taking five teaspoons of the crust and sealing the soil plastic whirlbag bags. Soil samples consisted of composite samples of the surface 5 cm of the profile was taken at random in each site for soil chemical and physical analyses. Soil chemical and physical analyses were conducted by Soil Testing Laboratory at Brigham Young University, Provo, Utah, using standard soil methods (Soil Conservation Service, 1972; Soil Survey Staff 1962). The isolation, characterization and identification of cyanobacteria were done in the phycology laboratory at John Carroll University in Ohio.

2.4.2 Isolation and characterization of strains

Half a gram (0.5 g) of microbiotic crust from each sampling site was put into a flask containing 50 ml of Z-8 medium, which is designated for the growth of cyanobacteria (Carmichael, 1986). Samples were cultured in Z-8 medium solidified with 4% (w/w) agar plates. Triplicate plates were prepared for each site and incubated at 23 °C in 16:8 hr light dark cycle at 200 uE.s$^{-1}$.cm$^{-2}$. The cyanobacterial isolates made from the plates were studied and identified using a Ziess Axioskop Photomicroscope with Nomarski DIC optics at 1000X magnification. The cyanobacterial isolates were measured with a graduated eyepiece and photographs were taken of all isolates. A number of individuals from each isolate were identified using standard botanical references (Albertano and Kovacik, 1994; Desikachary, 1959; Geitler, 1930-1932;
Komárek and Anagnostidis, 1999, 2005). New taxa were identified based on various species concepts, and contemporary theories about speciation in the recognition of cyanobacterial diversity (Johansen and Casamatta, 2005; Řehákova et al. 2007).

Type materials were prepared in several ways. The holotype was prepared by filtering young, healthy cultures of the new taxa described onto Whatman filter paper. The paper was allowed to air dry, attached to lichen herbarium cardstock, and placed in herbarium envelopes. Two isotypes were additionally prepared in this way. Two vials containing each strain preserved in 2% glutaraldehyde were also prepared, and these are considered isotypes as well. Finally, a feral soil sample from the each site was sealed in the plastic whirlbag bags, and temporarily deposited together with the above prepared types at the Phycological and Molecular Systematics Laboratory at John Carroll University. Upon completion of the current work, the types for all the taxa will be deposited at the Herbarium of Nonvascular Cryptogams, Brigham Young University, Provo, Utah (USA). The Ethiopian taxa will be sent to Ethiopia upon the establishment of a Herbarium of cryptogams of Ethiopian flora. This is expected to be an extension of the work undertaken after these initial stages of investigation. In this dissertation, provisional species names for new taxa were given. These names are not correctly described and represent only place-holder names (*nomen nuda*) until they are properly described in the peer-reviewed literature.

2.5 Results

Nine species of cyanobacteria were identified and morphologically characterized. One species of Chroococcales, five species of Oscillatoriales, and three species of Nostocales were identified. Among the isolates, four new species were recognized. In the
new species, one belongs to Chroococcales, one belongs to Oscillatoriales, and two belongs to Nostocales (Table I). Description and comments on species studied are provided below.
Table I. Cyanobacteria species distribution in the study areas (X=present). Awassa (A) & Zewai (Z) sites.

<table>
<thead>
<tr>
<th>Species</th>
<th>1A</th>
<th>2A</th>
<th>3A</th>
<th>4A</th>
<th>5A</th>
<th>1Z</th>
<th>2Z</th>
<th>3Z</th>
<th>4Z</th>
<th>5Z</th>
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</thead>
<tbody>
<tr>
<td><em>Cyanosarcina abyssinica</em> provisional sp. nov.</td>
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<td>X</td>
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<td><em>Leptolyngbya cf. africanum</em> (Men. ex Gom.) Anag. &amp; Kom.</td>
<td>X</td>
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<tr>
<td><em>Leptolyngbya cf. foveolarum</em> (Zopf ex Hansg.) Anag. &amp; Kom.</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td><em>Leptolyngbya cf. tenue</em> (Men. ex Gom.) Anag. &amp; Kom.</td>
<td>X</td>
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<tr>
<td><em>Microcoleus vaginatus</em> (Vauch.) Gom.</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td><em>Symplocastrum sheleko</em> provisional sp. nov.</td>
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<tr>
<td><em>Nostoc oromo</em> provisional sp. nov.</td>
<td>X</td>
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<tr>
<td><em>Trichormus sidamae</em> provisional sp. nov.</td>
<td>X</td>
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<tr>
<td><em>Scytonema javanicum</em> Bornet</td>
<td>X</td>
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</table>
2.5.1 Morphological characterization

*Cyanosarcina abyssinica* Mesfin et Johansen provisional sp. nov. (Fig.5, 6).

Thallus flat, blue green, somewhat shiny. Sheaths thin, diffuent, not very evident. Cells bright blue-green in fresh cultures, becoming yellowish in older cultures, persistently nongranular, dividing in three planes, forming cubical packets of 8-16 oval to spherical cells (1.5 μm wide by 2 μm long), later separating to produce larger spherical cells (up to 3 μm in diameter), with peripheral thylakoids apparent in LM, with division by binary fission into two morphologically equal daughter cells, which reach the original globular shape before next division (never forming baecocytes).

**Diagnostic features:** This strain is clearly *Cyanosarcina* based on the formation of small cubical aggregations of cells in rapidly growing cultures. In older cultures, when cell packets disintegrate to form solitary or paired cells loosely arranged in mucilage, the strain has some similarity to *Synechocystis*. Most *Cyanosarcina* strains have been described from thermal or marine habitats, and all have larger cell sizes than our taxon. The oval cells in *C. abyssinica* are also quite distinct. Given the range of ecological and morphological differences, it is unclear which of the species in the genus are sister taxa to this species (see Komárek and Anagnostidis, 1999 for a treatment of all taxa described to date). It is the only representative of Chroococcales isolated from the microbiotic crusts of the Ethiopian Rift Valley.

*Type locality:* Microbiotic crust of the Ethiopian Rift Valley System, Zewai location.

Reference strain ETH 2. 4. M4

*Etymology:* named after the ancient name of Ethiopia, which was Abyssinia.
Leptolyngbya cf. africanum (Lemm.) Anagnos. & Kom. (Fig. 7-9).

Thallus flat, creeping when first transferred, eventually forming a slightly raised globular colony, blue-green when young, becoming dark olivaceous in old cultures. Filaments unbranched with a single trichome per sheath, up to 4-5 μm wide. Sheath colorless, firm to diffusive, unlamellated. Trichomes distinctly constricted at cross walls, which are easily visible in LM, rarely forming meristematic zones, untapered to distinctly tapered, 1.5-1.7 μm wide. Cells with peripheral thylakoids along the outside walls, occasionally at cross walls as well, non-granulated or containing a single granule, with pseudanabaenaceous type of cell division, occasionally giving rise to oblique cross walls, isodiametric, or shorter or longer than broad, 1-2 μm long. Apical cells distinctly tapered, up to 3-4 μm long. Fragmentation occurs at oblique walls. The tapering trichomes with elongated conical end cells make this strain distinct from all morphospecies in both this genus and in the easily confused genus Schizothrix. It is likely a new species, but should not be described as such without molecular sequence data, since a growing number of soil strains have been sequenced.

The strain is in the subgenus Leptolyngbya due to cells being shorter than wide. Cell length is a critical feature in this genus, although probably not sufficient in and of itself for defining monophyletic clades (see Komárek and Anagnostidis, 2005, figure 245A).

This species was observed in multiple samples from the soils of our study sites.

Reference strain ETH 2.3. M8
Leptolyngbya cf. foveolarum (Zopf ex Hansgirg) Anagnos. & Kom. (Fig.10-12).
Thallus flat spreading, dark green. Filaments with only one trichome per sheath, unbranched or very rarely false branched, thickly entangled, often twisted, under 400 μm long, up to 4.0 μm wide. Individual sheaths thin, firm, rigid, unlamellated, open, colorless, persistent and evident, absent only immediately following transfer to fresh media when the majority of trichomes are sheath-less. Trichomes constricted at cross walls, without necridia, 1.8-2.0 μm wide. Cells roughly isodiametric, having cell division as in the Pseudanabaenaceae, nongranular or with 1-2 minute granules in the centroplasm, with thylakoids peripheral along the outer walls and crosswalls, 1.3-2.0-(3.0) μm long. End cells sharply to bluntly conical, longer than wide. It is difficult to decide whether this strain belongs in subgenus Leptolyngbya (cells isodiametric, with necridia) or in subgenus Protolyngbya (cells longer than wide, without necridia). If in Leptolyngbya, it keys to L. foveolarum, but differs distinctly from that taxon in the possession of distinctly conical end cells (Komárek and Anagnostidis, 2005). If in Protolyngbya, it keys to L. tenuis, but differs from that taxon in many regards (no motility, no polar granules, distinct constrictions at crosswalls, distinct and evident sheath material in most stages). It is likely a new species, but we prefer to wait for molecular data before proceeding with a description.
Reference strain ETH1.1. M5
*Leptolyngbya* cf. *tenue* (Menegh. ex. Gom.) Anagnos. & Kom. (Fig.13-15).

Thallus flat and spreading from the center, dark blue green. Filaments unbranched, mostly running parallel to each other, mostly straight, with distinct sheath in older cultures, but without sheath in freshly transferred material, 2.0-4.0 μm wide. Sheath firm, colorless, open, not lamellated, 0.5-1.0 μm wide. Trichomes slightly constricted to distinctly constricted at the cross walls, non-motile, lacking meristematic regions, lacking necridia, 1.8-2.2 μm wide. Cells isodiametric, with Pseudanabaenaceae type of cell division, non-granular or with 1-2 minute granules in the centroplasm, with thick thylakoid layer peripheral at cross walls and outside walls, (1.0)-1.5-3.5-(4.0) μm long. End cells bluntly rounded to bluntly conical, longer than wide, or not set off from vegetative cells after recent fragmentation. The strain has close morphological similarities with *L. tenue* described in & Komárek & Anagnostidis (2005).

Reference strain: ETH 2. 3. M1

*Microcoleus vaginatus* (Vauch.) Gom. (Fig.16-18).

Thallus flat and blue green. Filaments with many trichomes in a common sheath, up to 40 μm wide. Sheath colorless, not lamellated, becoming diffluent, open. Trichomes not constricted at cross walls, with gliding motility, with necridia, 6.5-8.0 μm wide in main part of trichome, tapering to 4.5 μm at the apices. Cells light blue-green, sometimes granulated at cross walls, with successive cell division, with fasciculated thylakoids, 1.5-5 μm long between complete septa. End cells bluntly conical, calyptrate, sometimes capitate, always shorter than wide.
M. vaginatus has probably a cosmopolitan distribution and is found worldwide in microbiotic crusts of arid and semi arid regions. The strain is morphologically similar to the M. vaginatus description in Geitler (1932, pp. 1136). The Ethiopian strain is actually in the M. vaginatus clade, but is distinguished by its cellular swellings, its absence of granules along the crosswalls, and its large size. It may be a cryptic species within this taxon.

Reference strain: ETH 2. 2. M7

Symplocastrum sheleko Mesfin et Johansen provisional sp. nov. (Fig. 19, 20).

Thallus forming a flat blue-green mat with little mucilage when first transferred to fresh media, eventually forming upright acute, pointed, and closed fascicles when growing on agar, also forming creeping flat colonies, yellow to blackish in older cultures. Filaments containing firstly a few and later numerous widely dispersed trichomes in a common sheath. Sheath colorless, not lamellated, becoming diffluent. Trichomes highly motile with gliding motility, slightly constricted at the cross walls, often tapering, straight or hooked at the apices, 4-5.5 (6) μm wide, as narrow as 3 μm wide at the apices. Cells nongranular when first transferred, becoming highly granulated in old culture, with fasciculated thylakoids as evidenced by peripheral irregular chromatoplasm in LM, with cell division as in the Phormidiaceae, 2-4-(6) μm long. Apical cells obtuse conical, longer than wide, sometimes distinctly and capitately calyptrate.

Diagnostic features: This species has close morphological similarities with the species described as Symplocastrum mascarenica Gomont in Jadin (Geitler, 1932, pp. 1074). Both have the same range of trichome width and cells isodiametric to shorter than broad.
However, *S. mascarenica* has lamellated sheaths, unconstricted trichomes, and is found aerophytically on walls. The soil species *Symphocastrum friesii* (Agardh) Forti is also very similar, particular in the production of the upright fascicles and the blackish coloration. However, *S. friesii* has cells isodiametric to longer than wide and lamellated sheath. This species was collected from microbiotic crusts of several sites in the southern part of the Ethiopian Rift Valley System.

*Type locality:* Microbiotic crust of the Ethiopian Rift Valley System

*Reference strain:* ETH1.1. M1

*Etymology:* Named for the Amharic (Ethiopian National Language) word sheleko, which means valley.

*Nostoc oromo* Mesfin et Johansen provisional sp. nov. (Fig. 21-25).

Colony bright blue green to olive, spherical to oblong or irregular, up to 50 μm in diameter, fragmenting to release one-, two- or four-celled colonies, filamentous nature never apparent in colonies, which are always densely packed with cells. After transfer, some colonies will fragment to release few-celled, immotile trichomes. Sheath firm, colorless, in some old colonies becoming compartmentalized and yellowish. Trichomes curved or bent, never straight, never motile, few-celled (up to 16 cells long at most), 3-4 μm wide. Vegetative cells often with a single large central granule, 4-6 μm long. Akinetes thin walled, minutely granular, distinguished primarily by their larger size, oval to spherical, 4-7 μm wide, 6-7.5 μm long. Heterocytes very rare, colorless to yellowish, compressed hemispherical, with a single polar nodule, 4-6 μm wide, 3-4 μm long.
Diagnostic features: This strain resembles *Chroococcidiopsis kashayi*, and at first, it was considered to be a *Chroococcidiopsis* species. Heterocytes are very rare, and were only seen in 6 month-old cultures. The filamentous nature is likewise consistently absent and only seen in very young cultures. The short trichomes released were not motile, and did not resemble typical *Nostoc* hormogonia, although they likely fulfill that role. The release of many few-celled, nonfilamentous propagules from aging colonies was very distinctive, and similar release was not seen in other *Nostoc* kept in culture. Thus, this species, while currently fitting *Nostoc* due to its amorphous colony structure, may actually be in a different clade than the type species, *Nostoc commune*. Molecular sequence analysis of *Nostoc* has shown it to be a polyphyletic genus. Until this species is sequenced and found to be something else, it is described as a new species within the genus *Nostoc*.

*Type locality:* Microbiotic crust of the Ethiopian Rift Valley System, Zewai location

Reference strain ETH 2.4. M5

*Etymology:* Named after the nationality living in the region, the Oromo tribe.

*Trichormus sidamae* Mesfin et Johansen provisional sp. nov. (Fig. 26-29).

Thallus hemispherical, or flattened and spreading dark blue green. Mucilage colorless, soft, diffusent, without firm outer layer. Trichomes short and straight to longer and coiled, 3-4 μm wide. Vegetative cells minutely granular or nongranular, bright blue-green, 2.5-4 μm long. End cells bluntly conical, longer than broad. Heterocytes terminal, 3-4 μm wide, 4-5 μm long. Akinetes apoheterocytic, nongranular at first, becoming granulated with age, spherical to oblong, in series, yellowish green to light
brown, 6-7 μm wide, 6-8 μm long in trichomes, becoming up to 8 μm wide by 12 μm long when solitary, germinating to produce a cluster of 2-4 closely appressed cells, often outside of firm akinete wall which is empty and persistent after germination, subsequently forming short, compact trichomes in a firm, tight sheath.

**Diagnostic features:** The species is morphologically similar to *Nostoc calcicola* (Geitler, 1932, pp. 843) because of distinct empty cell walls after post-reproduction. However, it differs by lacking heterocytes and large sized akinete formation up to 8 by 12 μm in size, typical for this strain. It is one of the dominant taxa in the soil of the region. It lacks mucilaginous colonies of *Nostoc*, and therefore belongs in the genus *Trichormus*. However, it differs from other *Trichormus* strains by the presence of empty cell walls after post-reproduction in culture.

**Type locality:** Microbiotic crust of the Ethiopian Rift Valley System, Zewai location

**Reference strain:** ETH 2.1.M2

**Etymology:** Named after the nationality living in the region, the Sidama tribe.

*Scytonema javanicum* Bornet (Fig. 30-34).

Thallus in upright position in culture, blue green to yellowish green. Sheath colorless to yellowish, soft, diffluent, lamellated. Filaments pseudobranched, mostly geminate branching, 8-13 μm wide. Trichomes unconstricted at the crosswalls to distinctly constricted due to swelling of cells, with necridia, not tapering, 7-11 μm wide.

Vegetative cells blue green and granulated, 4-16 μm long. End cells sometimes set off by light brownish coloration, bluntly conical. Heterocytes distant from branching, oval shaped 6.5-7.5 μm long. The species has wide distribution and is one of the dominant
taxa in the microbiotic crust of the southern part of the Ethiopian Rift Valley System. It is one of important taxa observed. It has close morphological similarity with *S. javanicum* in Geitler (1932, pp. 765).

Reference strain: ETH2. 2. M10
Figure 5-18. *Cyanosarcina abyssinica* 5, 6 (scale= 1 µm), cells forming cubical packets of 8-16 (5) and solitary cells in culture (6). *Leptolyngbya* cf. *africanum* 7-9 (scale=1 µm), tapering trichomes with elongated conical ends in culture. *Leptolyngbya* cf. *foveolarum* 10-12 (scale=1 µm), distinct conical end cells in culture. *Leptolyngbya* cf. *tenue* 13-15 (scale= 1µm), filaments without a sheath in freshly transferred culture (13, 14), filaments with distinct sheath in old culture (15). *Microcoleus vaginatus* 16-18 (scale= 1µm), trichomes not constricted, end cells conical or calyptrated in culture.
Figure 19-31. *Symplocastrum sheleko* (19, 20) (scale=1 µm), filaments with widely dispersed trichomes in a common sheath (19), colorless sheath in a filament (20). *Nostoc oromo* 21-25 (scale=1 µm), colony yellowish in old culture (21, 23, 24), blue green in fresh culture (22). *Trichormus sidamae* 26-29 (scale=1 µm), trichomes short and straight (26), distinct empty cells after post-reproduction (27), large sized akinetes (28), end cells sometimes conical (29). *Scytonema javanicum* (30, 31) (scale=1 µm).
Figure 32-34. *Scytonema javanicum* (scale=1 μm), filament pseudobranched.
2.6 Discussion and conclusion

All studies in biology, whether at the level of molecular, cell, individual or populations are typically referenced to the level of species. In the field of conservation biology, assessments of biodiversity are made at the level of species: typical criteria include species richness, number of endemic species and the number or presence of endangered species in given areas (Johansen and Casamatta, 2005; Myers et al. 2000; Řeháková et al. 2007). The accurate identification of species is crucial both to research in all areas of biology and to biodiversity conservation. Characterization and recognition of cyanobacterial species should be based on species concepts, which are practical and universally applicable within the phylum. Many of the monographs available for cyanobacterial taxonomic work are mainly based on morphological characteristics alone. Individual members of a species are based on a suite of shared morphological characters that were diagnostic and differentiated them from other such morphologically defined groups (Desikachary, 1959; Geitler, 1930). Many of the morphological features that have been applied are differences in cell/trichome size, degree of constrictions at the cross-walls, the degree of sheath production, and thylakoid structures. Even though morphological characterization is informative in characterizing cyanobacterial species, it lacks the information to distinguish among cryptic species. Cryptic species are morphologically similar yet genetically different, exercising the process does not allow this genetic biodiversity to be recognized (Boyer et al. 2002). The Biological Species Concept does not accommodate organisms such as cyanobacteria, which are all asexual. The Evolutionary Species Concept could be practical in the recognition of cyanobacterial
diversity. Based on the Evolutionary species concept, Wiley and Mayden (2000) defined species as a single lineage of ancestor-descendant population which maintains its identity from other lineages, and which has its own evolutionary tendencies and historical fate. The Evolutionary Species Concept has been formalized in various species concepts that focus on phylogenetic lineage, including the Phylogenetic Species Concept in which individual members of a species are considered to be part of a monophyletic group, having descended from a single ancestral taxon (Mishler and Theriot, 2000). The synopsis of the Phylogenetic Species Concept sensu Mishler and Theriot (2000) recognizes species as the smallest monophyletic groups worthy of taxonomic recognition. Monophylly within species is recognized by the presence of autapomorphies. The Monophyletic Species Concept is found to be operational when molecular sequence data for phylogenetic analysis are available (Johansen and Casamatta, 2005; Řehákova et al. 2007).

The Ecotypic Species Concept is also practical in species recognition when only ecological and morphological characteristics are known. The Ecotypic species concept attempts to categorize organisms by capturing the essence of phenotype as an expression of genomic information and environmental influences (Komárek and Anagnostidis, 1999; 2005). The process of allocating individuals to a given species obviously depends on the criteria by which species are defined and delimited, which are in turn determined by concepts of what a species is. The species concept continues to be the subject of much debate (Cracraft, 2000; Řehákova et al. 2007) and, until they obtain a consensus, there can be no well-defined universal criterion by which species may be delimited or identified. Due to inadequacies of evolutionary evidence from morphology, habitat
specificity, and biochemical and molecular characters, it has been difficult to treat cyanobacterial systematics (Casamatta et al. 2005; Komárek, 1985; Řeháková et al. 2007). Currently many taxonomists are proposing a total evidence approach to cyanobacterial systematics. Any one character set such as morphological, biochemical or molecular could be sufficient in separating species if the grouping criterion of monophyly is met (Johansen and Casamatta, 2005; Řeháková et al. 2007). In the identification of cryptic species, molecular or ecological data could reveal the strains as distinct lineages worthy of recognition as separate species. Alternatively, if there are distinct and persistent morphological differences in culture between two strains, the morphological characters are sufficient evidences to separate the species even if the molecular or ecological evidences are weak (Johansen and Casamatta, 2005). Considering geographical isolation, in conjunction with the morphological, ecophysiological, and molecular evidences, the Phylogenetic Species Concept (Mishler and Theriot, 2000) and the Evolutionary Species Concept (Wiley and Mayden, 2000) are effective in recognition of cyanobacterial diversity in both continents. The Evolutionary Species Concept, which stresses the evolutionary fate of species over a period of time and space, could describe the evolutionary divergence of cyanobacteria in both continents. The Phylogenetic Species Concept, which stresses the unique derived characters, could be effective in the identification of species as a separate lineage. The character sets of morphology, ecophysiology and molecular sequence data (16 rRNA and ITS regions) obtained in the study were employed in the recognition and identification of new species in the Ethiopian microbiotic crusts.
This study demonstrates the potential and adaptive features of cyanobacteria in extreme and remote locations around the globe. The types and distributions of cyanobacteria are interesting from ecological and biological points of view. They tend to show cosmopolitan distribution and endemism to a given location (Desikachary, 1959; Johansen, 1993; Komárek and Anagnostidis, 1999, 2005). Most of the species identified in the Ethiopian Rift Valley System show morphology similar to taxa in different parts of the world, but differing in some respects. The cyanobacterial strains identified in the microbiotic crusts of Ethiopia were *Cyanosarcina abyssinica* (Chroococcales), *Leptolyngbya* cf. *africanum*, *Leptolyngbya* cf. *foveolarum*, *Leptolyngbya* cf. *tenue*, *Microcoleus vaginatus*, *Sympleastra sheleko*, (Oscillatoriales), *Nostoc oromo*, *Trichormus sidamae*, and *Scytonema javanicum* (Nostocales). With the exception of *C. abyssinica* species in these genera have been reported in semi-arid and arid regions in other parts of the world. The presences of coccoid cyanophytes, even though they are rare in microbiotic crusts, have been reported in soil flora from different parts of the world (Komárek and Anagnostidis, 2005). The strain identified as *C. abyssinica* is clearly in the genus *Cyanosarcina* based on the formation of small cubical aggregations of cells in rapidly growing cultures (Fig. 5, 6). Based on the morphology and ecology obtained for this taxon, it is identified as a new cyanobacterial species from the region.

The five species of Oscillatoriales identified in the region belong to Pseudanabaenaceae and Phormidiaceae. The strains, *Leptolyngbya* cf. *africanum*, *Leptolyngbya* cf. *foveolarum*, and *Leptolyngbya* cf. *tenue*, which belong to Pseudanabaenaceae are characterized mostly for their unbranched filaments with cell
length up to 3 (4) μm (Alwathnani, 2006; Johansen and Casamatta, 2005; Komárek and Anagnostidis, 2005).

In the current monographs compiled by Komárek and Anagnostidis (1999; 2005), there are evidences that support the presence of endemic cyanobacterial species in different parts of the world based on morphological features and range of ecological distributions of the species. Among the Ethiopian cyanobacterial strains isolated and characterized, four new species were identified and named as *Cyanosarcina abyssinica*, *Symplocastrum sheleko*, *Nostoc oromo*, and *Trichormus sidamae* in the text. Molecular characterization was performed using 16Sr RNA and 16S-23S ITS genes to further support the designation of the four new cyanobacterial strains isolated from the microbiotic crusts of the Rift Valley (Chapter IV of the thesis). The congruency among morphological characteristics, 16S rRNA and 16S-23S ITS contribute to better understanding and describing of new species in cyanobacteria (Alwathnani, 2006; Casamatta *et al.* 2006; Johansen and Casamatta, 2005; Laamanen *et al.* 2001; Řehákova *et al.* 2007; Rocap *et al.* 2002). The endemism seen in Ethiopia could be due to the isolation of the region from other parts of the globe, resulting in establishment of a local flora in the region.

Desertification is the process which turns productive land into non-productive deserts as a result of poor land management. Overgrazing is the major cause of desertification worldwide. Desertification reduces the ability of land to support life, affecting wild species, domestic animals, agricultural crops and people. The reduction in plant cover that accompanies desertification leads to accelerated soil erosion by wind and water. It has been reported that in the Sahel (the semi-arid area south of the Sahara
Desert) the desert expanded 100 km southwards between 1950 and 1975. (Gelletly, 2006). Most of the arid and semi-arid lands in the Ethiopian Rift Valley System have been heavily affected by human activities, which have resulted in environmental degradation, with respect to the soil and vegetation in the region (Tewolde, 1989; Zerihun and Mesfin, 1990).

Characterizations of cyanobacterial populations are important towards conservation of natural resources in the region. Introduction of new cyanobacterial species is also a direct measure of biodiversity, an aspect often used to measure the health of biological systems with respect to ecosystem services such as recycling nutrients and providing fertile soil. Very few studies have been conducted on cyanobacterial taxonomy in desert and semi-desert of Africa. Reynaud (1987) reported species of Pseudanabaena, Lyngbya, Scytonema, Nostoc, Anabaena and Calothrix in the crusts in Senegal. Cyanobacteria were reported as the dominant taxa in the desert crust of Egyptian soil and Molopo Basin in South Africa. The species were identified as Stigonema mintum, Scytonema sp., Gloeocapsa minor, Microcoleus testarum, Oscillatoria limosa, Phormidium valderia, and Phormidium inundatum (Salama and Kobbia, 1982; Thomas and Dougill, 2005) from these regions.

The inherent low resource availability and species diversity of arid ecosystems (Noy-Meir, 1979; Verstraete and Schwartz, 1991) make them especially susceptible to even small changes in critical ecosystem processes that determine species composition and primary productivity. This preliminary study and characterization of cyanobacterial flora is essential towards the conservation of microbiotic crusts in the region. This could help and promote the local and regional environmental protection agencies to incorporate
management and conservation of microbiotic crusts as one of the strategies in conservation of natural resources in the region.

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CHAPTER III

FLORISTIC COMPOSITION OF CYANOBACTERIA IN MICROBIOTIC CRUSTS OF THE GREAT BASIN OF IDAHO AND OREGON

3.1 ABSTRACT

Cyanobacterial flora of microbiotic crusts of the Great Basin in Idaho and Oregon were studied. A total of six morphospecies were characterized and identified. Three species of Oscillatoriales *Leptolyngbya nostocorum*, *Microcoleus steenstrupii*, *Microcoleus vaginatus*, and three species of Nostocales, *Nostoc cf. edaphicum*, *Nostoc indistinguendum*, *Tolypothrix distorta* were recorded in the regions.

The documentation of cyanobacterial species in the Great Basin is vital towards understanding the biodiversity and ecological roles of this population in the North American crusts. The study addresses the impacts of recreational activities on microbiotic crusts and recommends that the regional planners and range managers to develop strategies that conserve this biota in the region.

**Keywords:** Cyanobacteria, Microbiotic Crust, the Great Basin
3.2 Introduction

Dense growth of cyanobacteria, lichens, eukaryotic algae, mosses, and fungi are a common feature of soil surfaces in semi-arid and arid temperate regions worldwide (Evans and Johansen, 1999; Friedmann and Galun, 1974; Harper and Marble, 1988; West, 1990). These soil surfaces known as microbiotic crusts benefit the soil on which they grow (Johansen, 1993).

Microbiotic crusts consolidate soil surfaces against erosion (Belnap, 1995; Booth, 1941; Fletcher and Martin, 1948; Johansen et al. 2001; Loope and Gifford, 1972), improve water infiltration and retention (Eldridge, 1993a, 1993b), enhance seedling establishment (Harper and Pendleton, 1993; St. Clair and Johansen, 1993) and provide nutrients through weathering and biological N\textsubscript{2} fixation (Harper and Marble, 1988) and biomass production (Graetz and Tongway, 1986; Isichei, 1990). Microbiotic crusts also play roles as constituents of mature semi-arid and arid ecosystems and as pioneers in primary and secondary plant succession (Metting, 1991; Johansen and St. Clair, 1984).

Microbiotic crusts in North America are most prevalent in the semi-arid steppe regions in the Great Basin, Colorado Plateau, and Columbia Basin. They also extend into hotter, more arid deserts in the southwestern regions of the United States (Cameron, 1964; Johansen, 1993; Mack and Thompson, 1982). Knowledge of the component organisms of microbiotic crusts and their usefulness in the soil stabilization, fertility, and reclamation processes are important in any region. Among the component organisms of microbiotic crust, cyanobacteria are the most widely distributed photosynthetic prokaryotes in nature. They are constituted of a cell typical of gram-negative bacteria with a photosynthetic apparatus similar in functional and structural aspects to that
contained in eukaryotic chloroplasts (Stanier and Cohen-Bazire, 1977). Their ability to form stable crusts on desert soil surfaces was recognized early in past century (Booth, 1941). Certain soil cyanobacteria have a cosmopolitan geographic distribution. Species of *Microcoleus, Schizothrix, Nostoc, Scytonema, Phormidium* and several additional genera are repeatedly reported from a variety of soil all over the world. These include Antarctic (Broady, 1996), North America (Alwathnani, 2006; Cameron, 1964; Flechtner *et al.* 1998; Johansen, 1993); Brazil (Azevedo, 1991); Equatorial Africa (Fremy, 1930) Morocco (Renaut *et al.* 1975); Israel (Dor and Danin, 1996; Danin *et al.* 1998); Himalayas (Watanabe and Komárek, 1988); China (Ying *et al.* 1992; Zhang *et al.* 2006); India (Bhatnagar *et al.* 2008); Egypt (Salama and Kobbia, 1982); Botswana (Skarpe and Henrikson, 1987); Ethiopia (Mesfin *et al.* 2009), Ivory Coast (Büdel *et al.* 1997); Saudi Arabia (Arif, 1992); Argentina (Bouza *et al.* 1993); Chile (Forest and Weston, 1966); Senegal (Reynaud and Roger, 1976). Few comprehensive floristic studies of microbiotic crusts of the Great Basin exist (Alwathnani, 2006; Flechtner, 1998; Johansen, 1993). The purpose of the present paper is to identify and characterize the cyanobacterial flora in microbiotic crusts of the Great Basin of Idaho and Oregon.

### 3.3 Description of study areas

The Great Basin of North America stretches from southern Idaho in the north to Nevada in the south and through parts of Utah, Colorado, Arizona, and New Mexico. It is the only U.S. desert covering 492,100 km² (the Chihuahuan Desert is larger, but only a small part of it lies in the U.S.A. (Morrison, 1991). The study areas are located in the Great Basin and samples were collected from the cold deserts of Idaho (43° 20’ N, 115° 55’ W) and Oregon (44° 48’ N, 117° 44’ W) (Fig. 35). The Great Basin is considered a
cold desert because precipitation often occurs as snow, and the northern latitudes keep
even summer temperatures down. Elevations range from 914–1981 meters and
precipitation averages 18–30 mm/year (Morrison, 1991). The physical and chemical
characteristics of the soil could support the growth of microbiotic crusts in the region
(Appendix C). Surface soil structures and associated vegetation cover were photographed
during sampling (Fig. 36).

Figure 35. Sampling locations in the USA (** Oregon, * Idaho).
Figure 36. Soil surface structures and vegetation cover at the sampling sites in Idaho.
3.4 Materials and Methods

3.4.1 Field sampling

Six sample sites were systematically chosen based on minimal disturbance and accessibility of the sites in Idaho and Oregon. These include sites at Bogus Basin Road: Ada Co. (site-1), West Road, Elmore Co. (site-2), Boise Road, Elmore Co. (site-3), Leadore, west of Leadore Ranger station, Lemi Co. (site-4), and Kuna Butte, Swan Falls Road, Ada Co. (site-5). Only one site was sampled in Oregon at the Oregon Trail National Historic Site, Interpretive Center, Baker Co., Oregon. A 1.0 m² quadrat was placed, and the top 2 mm of microbiotic crust was sampled within the quadrat. From each sampling site, five teaspoons of the crust was collected and sealed in the plastic whirlbag bags. Soil samples consisted of composite samples of the surface 5 cm of the profile were taken at random in each site for soil chemical and physical analyses. Soil chemical and physical analyses were conducted by Soil Testing Laboratory at Brigham Young University, Provo, Utah, using standard soil methods (Soil Conservation Service 1972, Soil Survey Staff, 1962). The isolation, characterization and identification of cyanobacteria were done in the phycology laboratory at John Carroll University in Ohio.

3.4.2 Isolation and characterization of strains

Half a gram (0.5 g) of microbiotic crust from each sampling site was put into a flask containing 50 ml of Z-8 medium, which is designated for the growth of cyanobacteria (Carmichael, 1986). Samples were cultured in Z-8 medium solidified with 4% (w/w) agar plates. Triplicate plates were prepared for each site and incubated at 23 °C in 16:8 hr light dark cycle at 200 uE.s⁻¹.cm⁻².
The cyanobacterial isolates made from the plates were studied and identified using a Zeiss transmitted light microscopy with an attached camera at 1000X magnification. The cyanobacterial isolates were measured with a graduated eyepiece and photographs were taken of the isolates. Each isolate was identified using standard botanical references (Albertano and Kovacik, 1994; Desikachary, 1959; Geitler, 1930-1932; and Komárek and Anagnostidis, 2005). Dried herbarium materials as well as glutaraldehyde-preserved liquid materials for all isolates were deposited in the Herbarium of Nonvascular Cryptogams, Brigham Young University, Provo, Utah (USA).

3.5 Results

A total of six morphospecies of cyanobacteria were isolated and morphologically characterized from the Great Basin of Idaho and Oregon (Table II). The cyanobacteria strains characterized are given in the following sections.
Table II. Cyanobacteria species distribution in the study areas (X= present)
Sites: Idaho (ID. 1-5) Oregon (OR. 1)

<table>
<thead>
<tr>
<th>Species</th>
<th>ID. (1-5)</th>
<th>OR. (1)</th>
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</thead>
<tbody>
<tr>
<td><em>Leptolyngbya nostocorum</em> (Bornet ex Gom.) Anagn. &amp; Kom.</td>
<td>X X X X X</td>
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<tr>
<td><em>Microcoleus steenstrupii</em> Boye-Petersen</td>
<td>X X X X X X</td>
<td></td>
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<tr>
<td><em>Microcoleus vaginatus</em> Boye-Petersen</td>
<td>X X X X X X</td>
<td></td>
</tr>
<tr>
<td><em>Nostoc cf. edaphicum</em> Kondratyeva</td>
<td>X X X X X</td>
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</tr>
<tr>
<td><em>Nostoc indistinguendum</em> Vaucher ex Bornet et Flahault</td>
<td>X X X</td>
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<tr>
<td><em>Tolypothrix distorta</em> Kütz</td>
<td>X X X X X</td>
<td></td>
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</tbody>
</table>

3.5.1 Morphological characterization

*Leptolyngbya nostocorum* (Bornet ex Gom.) Anagnos. & Kom. (Fig. 37-40).

Thallus spreading, flat, blue green to green in culture. Filament with one trichome per sheath, without false branching, 2-3 μm wide. Sheath tight, colorless, unlamellated, extending beyond the trichome apices. Trichomes slightly constricted at the crosswalls, without necridia, without meristematic zones, 2 μm wide. Cells blue-green, nongranular, with thylakoids peripheral along the outside walls, 1.5-3.7 μm long. End cells bluntly rounded. False branching was not observed, yet this is a feature listed for *L. nostocorum* (Komárek and Anagnostidis, 2005). We also did not see necridia after such a search, but these structures are reported as absent in *L. nostocorum*

Reference strain: OR1. M2
Microcoleus steenstrupii Boye-Petersen (Fig. 41-45).

Thallus flat, spreading, and blue green to dirty yellow green. Filaments unbranched, with many trichomes in a common sheath, up to 45 \( \mu m \) wide. Sheath thin, soft, colorless, and becoming diffluent, individual sheaths persistent in culture. Trichomes unconstricted to very slightly constricted at the cross walls, with necridia, motile with gliding motility, subtly tapered at the apices, 4-5 \( \mu m \) wide. Cells blue-green in actively growing cultures, yellowish in senescent cultures, granular throughout, with more granules near the crosswalls, sometimes distinctly granulated at the crosswalls, with cell division as in the Phormidiaceae (cells grow to full size before new cell walls begin to form), with thylakoid structure typical not discernable in LM, but appearing fasciculated when visible in LM, 4.5-8-(11) \( \mu m \) long. It is similar to M. paludosus which is reported from aquatic biotopes and wet soils. M. steenstrupii was originally reported from thermal springs. The name was used for a clade of forms with similar morphology but different ecology (i.e. desert soils) by Boyer et al. (2002). The taxon likely needs revision.

Reference strain: ID2. M2

Microcoleus vaginatus (Vauch.) Gom. (Fig. 46-48).

Thallus flat and blue green to yellowish green. Filaments unbranched with many trichomes running parallel to each other in a common sheath. Sheath colorless, not lamellated, and open at one end. Trichomes motile, not constricted at cross walls, with cell division as Oscillatoriaceae (new wall formation begins before previous wall formation is complete). having successive cell division, 6-7 \( \mu m \) wide in the main part of the trichome, tapering to 4.0 \( \mu m \) wide at the apices. Cells blue green often granulated at
cross walls, with fasciculated thylakoids, 2-9 μm long. End cells shorter than wide to isodiametric, or in very narrow end cells even longer than wide, calyptrate. Calyptra capitate or conical.

Reference strain: ID4. M2

*Nostoc cf. edaphicum* Kondratyeva (Fig. 49-57).

Thallus hemispherical mass, or flattened and spreading, consisting of numerous microcolonies, dark blue green. Microcolonies up to 20 μm in diameter. Sheath firm, tight around the colonies, colorless, with individual sheaths not apparent, absent from hormogonial stages. Trichomes entangled in microcolonies, long and straight following hormogonia production, 3.5-5 μm wide. Cells sometimes granulated, as long as broad to longer than broad, 2.5-6 μm long. Involution cells common in post-hormogonial trichomes. End cells of trichomes conical. Heterocytes intercalary or terminal, oval shaped 4-6-7.5 μm wide, 7.5-8.5 μm long. Akinetes oval, 6-8 μm wide.

Reference strain: ID 2. M2

*Nostoc indistinguendum* Vaucher ex Bornet et Flahault (Fig. 58-63).

Thallus bright blue-green. Colonies microscopic, starting as small spherical colonies or as elongated hormogonia, becoming spherical to subspherical, to elongate colonies, up to 65 μm in diameter. Sheath with a distinct outer layer, tight around the colonies, colorless. Trichomes coiled, densely arranged in mucilage but consistently with filamentous nature evident, 4 μm wide. Vegetative cells mostly spherical, 3.5-4.5 μm long. Terminal heterocyte, oval, 5-5.5 μm wide, 4-5 μm long. Akinetes in series, spherical to oval, 5-5.5 μm.
μm wide, 5.5-7 μm long. Hormogonia with cell dimensions as in the colonies, short, curved and coiled.

Reference strain: OR1b. M1

*Tolypothrix distorta* Kütz. (Fig. 64-71).

Thallus blue-green, becoming yellowish green with age, with upright filaments.

Filaments heteropolar, both singly and doubly false branched, with branches distant from heterocyte, typically not associated with heterocytes, 10-11-(14.5) μm wide. Sheath colorless to yellowish, soft, diffluent, lamellated. Trichomes distinctly to indistinctly constricted at the crosswalls, with necridia, 9-12 μm wide. Cells blue green, granulated, 3.5-9 μm long. End cells slightly yellowish, bluntly conical. Heterocyte oval, mostly terminal, rarely intercalary, when intercalary sometimes forming in pairs, 9-10 μm wide, and 11-12 μm long.

Reference strain: ID 4. M1
Figure 37-48. *Leptolyngbya nostocorum* 37-40 (scale = 2.5 μm), trichomes slightly constricted (37-38), tight and colorless sheath in a filament (39-40). *Microcoleus steenstrupii* 41-45 (scale = 2 μm), filaments with many trichomes in a common sheath, fresh culture (41), old culture (43-45). *Microcoleus vaginatus* 46-48 (scale = 1.6 μm), filaments with many trichomes (46, 47), end cells calyptrated (47, 48).
Figure 49-63. _Nostoc cf. edaphicum_ 49-57 (scale = 2.6 μm), colonies in hemispherical mass (49, 50, 56, 57), sheath firm, tight around colonies (50, 51, 52), sheath absent in hormogonial stages (53, 54). _Nostoc indistinguendum_ 58-63 (scale = 1.6 μm), colonies in spherical mass fresh culture (58, 59), akinetes in series (60), heterocyte terminal (61), sheath distinct outer layer, old culture (62, 63).
Figure 64-71. *Tolypothrix distorta* (scale = 1 μm), single and double false branched (64, 67, 70, 71), end cells bluntly conical (68), necridic cells observed (69, 71), heterocyte oval and terminal (65, 66, 67), rarely intercalary forming in pairs (69).
3.6 Discussion and conclusion

Throughout wide geographical regions, microbiotic crust flora tends to show a strong resemblance. Most of the cyanobacterial species recorded in this paper show a wide geographical distribution (Table II). Their records from any locations are interesting from ecological and biogeographical considerations. Each taxa identified in this study belong to two orders, Oscillatoriales, and Nostocales, common in many of the studies conducted in North American crusts. The most frequent cyanobacterial taxa in North America deserts include Microcoleus vaginatus, Schizothrix calciola, Nostoc commune, Nostoc muscorum, Nostoc paludosum, Nostoc punctiforme, Phormidium minnesotense, Phormidium tenue, Trichormus variabilis, and Tolypothrix distorta (Alwathnani, 2006; Flechtner, 1998; Johansen et al. 2001).

The three species of Oscillatoriales characterized were Leptolyngbya nostocorum, Microcoleus steenstrupii, and Microcoleus vaginatus. The strain characterized as Leptolyngbya nostocorum has common morphological features in culture with the genus Leptolyngbya characterized from the Mojave Desert (Alwathnani, 2006). The filaments have one trichome per sheath, and the trichome width was 2-3 μm wide, which was the range for most of the species recognized in the genus Leptolyngbya (Alwathnani, 2006; Komárek and Anagnostidis, 2005). The strain has close morphological similarities with L. nostocorum described by Komárek and Anagnostidis, (2005) but lacks the features such as false branching and necridic cells (Fig. 37-40). Even though it has certain morphological differences from the strain described by Komárek and Anagnostidis (2005), most of the features fit the description. The strains characterized as Microcoleus steenstrupii, and Microcoleus vaginatus are the most common in microbiotic crusts of
semi arid and arid regions in the world (Alwathnani, 2006; Boyer et al. 2002; Johansen et al. 2001; Mesfin et al. 2008; Metting, 1991; Zhang et al. 2006). Microcoleus steenstrupii is characterized for having unbranched filaments with many trichomes in a common sheath (Fig. 41-45). Trichomes are unconstricted with a cell width ranging from 4-5 μm. The strain has a Phormidiacean type of cell division (cells grow to full size before new cell walls begin to form), which was also reported for the similar strains isolated from North American deserts (Alwathnani, 2006; Boyer et al. 2002). Microcoleus vaginatus has similar morphological features with M. steenstrupii. Filaments are unbranched with many trichomes running parallel to each other in a common sheath (Fig. 46-48). Unlike M. steenstrupii, Microcoleus vaginatus show Oscillatoriae type of cell division in culture (new wall formation begins before previous wall formation is complete). Similar observations were reported for many similar species collected from different microbiotic crusts (Alwathnani, 2006; Boyer et al. 2002; Casamatta et al. 2005).

The three species of Nostocales characterized in the study were Nostoc cf. edaphicum, Nostoc indistinguendum, and Tolypothrix distorta. Both Nostoc species are characterized by the formation of coiled or entangled trichomes in the microcolonies in culture, which is typical of Nostoc strains collected from different biotypes (Alwathnani, 2006; Geitler, 1930; Kondratyeva and Kislova, 1992; Li, 2000). Nostoc cf. edaphicum has intercalary or terminal positioned heterocytes and has oval akinetes with width size ranging from 6-8 μm (Fig. 49-57). Most of the characters described for Nostoc edaphicum by Kondratyeva and Kislova (1992, 2002) are observed in this strain. However, this strain is from North American microbiotic crusts and is designated as Nostoc cf. edaphicum to show its morphological similarities with similar strains of
different biotypes. *Nostoc indistinguendum* has colonies, which are spherical in culture, and tend to grow up to 65 μm in culture. It has terminal heterocyte with oval akinetes with width size ranging from 5-5.5 μm. Most of the morphological characteristics described for this strain as *Nostoc indistinguendum* (Geitler, 1930) are observed in the culture of this isolate (Fig. 58-63). *Tolypothrix distorta* is also one of the important taxon characterized from the North American microbiotic crust (Alwathnani, 2006; Johansen et al. 2001). The filaments are heteropolar and show single or double false branching with branches distant from the heterocyte (Fig. 64-71). The end cells are rounded to bluntly conical, which characterize this taxon. Alwathnani (2006) reported similar characteristic features for similar species isolated from the Mojave Desert.

Most of cyanobacterial strains identified in this study were previously reported from the North American microbiotic crusts (Alwathnani, 2006; Flechtner et al. 1998; Johansen, 1993). All of the taxa identified in this study belong to two orders, Oscillatoriales, and Nostocales, which is common in many of the studies conducted in North American (Alwathnani, 2006; Johansen, 1993; Johansen et al. 2001). In many of the microbiotic communities dominated by filamentous algae, *Microcoleus vaginatus* is the most ubiquitous and abundant algal taxon in the deserts of North America and possibly in the world (Alwathnani, 2006; Johansen, 1993; Mesfin et al. 2009; Metting, 1991; Zhang et al. 2006).

The cyanobacterial strains characterized in the Great Basin of Idaho and Oregon could play significant ecological roles in the region. There are evidences that most of the species identified contribute to soil fertility through nitrogen fixation, organic carbon
contribution and accumulation of soil fines (Harper and Marble, 1988). The most important of their roles is the stabilization of soil surfaces, which reduces erosion caused by wind and water actions in arid and semi-arid regions of the North American crusts (Bailey et al. 1973; Fletcher and Martin, 1948; Johansen, 1993).

Maintaining soil stability and normal water and nutrient cycles in semi-arid and arid ecosystems is critical in avoiding desertification. These particular ecosystem processes are threatened by trampling of livestock and people, and by off-road vehicle use and range fire (Belnap, 1995; 2002). From recent studies of the effects of disturbances on microbiotic crusts, the range fire is more disruptive to species composition and crust biomass than other factors (Johansen et al. 1993). Mature microbiotic crusts take at least 15 years to develop. Lichen and bryophyte species richness and diversity are known to increase over a period of up to 40 years. The nitrogen fixation capability of microbiotic crusts requires at least 100 years recovery if destroyed by human activity (Belnap, 1995; St. Clair and Johansen, 1993). Recovery of microbiotic crusts can be hampered by large amounts of moving sediments. Re-establishment can be extremely difficult in some areas. It is possible that if proper conservation measures are not taken, the microbiotic crust of Idaho and Oregon will be destroyed, which leads to the loss of biodiversity with respect to the cyanobacterial population in the region. Studying the biodiversity of cyanobacteria in microbiotic crusts in the Great Basin is critical in understanding the ecological roles played by these organisms. This distribution record is the first documentation of cyanobacteria for the Great Basin of Idaho and Oregon. Given the sensitivity of these resources and recovery
time, understanding the microbiotic communities, particularly the cyanobacterial population, is essential in conserving the natural resources in the region.

3.7 Literature Cited


Li, X. 2000. Phenotypic and genotypic characterization of *Nostoc* species from six different sites in the Mojave Desert. Master’s Thesis, John Carroll University, Cleveland, OH.


CHAPTER IV

MOLECULAR COMPARISONS OF SELECTED TAXA OF CYANOBACTERIA
OF TWO CONTINENTS: AFRICA (THE ETHIOPIAN RIFT SYSTEM), AND
NORTH AMERICA (THE GREAT BASIN)

4.1 ABSTRACT

Selected cyanobacterial taxa from the soils of Africa and North America were characterized based on the molecular sequence of the 16S rRNA and 16S-23S internal transcribed spacer (ITS) genes. About 85% of the gene from 16S rRNA was sequenced for selected taxa of cyanobacteria of the Rift System and the Great Basin. In the process of the investigation, the phylogenetic positions of the respective morphospecies were analyzed using parsimony, distance and maximum likelihood methods. The ITS region of the taxa was compared based upon the presence/absence of tRNA genes and the secondary structure of conserved within the 16S-23S ITS, including: D1-D1’helix, V2 helix, and box B domain. During the process of molecular and morphological characterization of the taxa, four new species of cyanobacteria were defined from the microbiotic crusts of the Rift Valley System. Of the putative new species, one belongs to Chroococcales, one belongs to Oscillatoriales, and two belong to Nostocales.

Key words: Cyanobacteria, 16S-23S ITS, D1-D1’, phylogeny; the Rift Valley System, the Great Basin
4.2 Introduction

The classification of cyanobacteria has become very problematic since researchers have learned that taxonomy based on morphological features is not always congruent with ultrastructure, physiology, and molecular sequence data. The use of the sequence of the small subunit rRNA (SSU rRNA) gene to determine the evolutionary relationships among cyanobacteria has become fairly common in recent years (Gray et al. 1984; Garcia-Pichel et al. 1998; Ishida et al. 2001; Nelissen et al. 1995; Turner, 1997; Turner et al. 1999; Wilmotte et al. 1993; Wilmotte et al. 1994). In addition to phylogenies based on the sequence of the SSU rRNA, recent workers have additionally used secondary structure of the 16S-23S internal transcribed spacer (ITS) to understand relationships among species and genera of cyanobacteria (Boyer et al. 2001; Flechtner et al. 2002; Johansen and Casamatta, 2005; Řehakova et al. 2007). The rRNA operon, consisting of three RNA molecules (16S, 23S, 5S) separated by internal transcribed spacers (ITS), has been a target for sequence analysis. The 16S-23S ITS region has also been found to be effective in understanding population structure in cyanobacteria (Rocap et al. 2002). Currently, the primary utility of the ITS region seems to be the study of sequence configuration (tRNA gene presence, large sequence gaps, stem and loop structures, and intergenic spacer region length). Some ITS regions contain the structural genes for tRNA\textsuperscript{ile} and tRNA\textsuperscript{ala}; other ITS regions lack these genes, and some have only one tRNA gene, which is tRNA\textsuperscript{ile} (Boyer et al. 2001; Flechtner et al. 2002; Řehakova et al. 2007).

At present, the molecular approach of species identification of cyanobacteria focuses on the combination of the 16S rRNA gene and the 16S-23S internal transcribed
spacer (ITS) regions. The sequence of SSU rRNA is still critical for determining phylogeny. Knowing the phylogeny of terminal taxa (i.e. strains) helps define monophyletic clades. Both species and genera should be monophyletic in cyanobacteria, which are reproductively isolated asexual organisms. Once phylogeny is determined, the search for morphological, ultrastructural, and even molecular synapomorphies that define clades can be conducted. The 16S-23S ITS sequences encoding structural products of tRNA genes (tRNA\textsubscript{ala} and tRNA\textsubscript{ile}) and intervening sequence (IS) regions that do not encode structural products vary at the species level in cyanobacteria. Specific areas of the ITS region have been identified as critical to proper folding of the primary transcript for the release of structural ribosomal and transfer RNAs during processing. One of these areas, the D1-D1’ helix that occurs at the very 5’ end of the 16S rRNA gene, is found to be a highly conserved region assumed to be important in forming the secondary structure essential for rRNA processing (Alwathnani, 2006; Casamatta \textit{et al.} 2006; Iteman \textit{et al.} 2000). The variable region V2 and box B appear to give additional information in the designation and characterization of species in cyanobacteria. These regions have been vital in resolving taxonomic questions in cyanobacteria when used in conjunction with the 16S rRNA gene (Alwathnani, 2006; Casamatta \textit{et al.} 2006; Flechtner \textit{et al.} 2002; Iteman \textit{et al.} 2000; Johansen and Casamatta, 2005; Řehakova \textit{et al.} 2007). The V3 helix and D5-D5’ helix are defined by interactions between the 16S-23S ITS and 23S-5S ITS, and consequently the structure of these regions cannot be reliably determined with 16S-23S ITS sequence alone (Iteman \textit{et al.} 2000).

Evidence of multiple non identical operons have been found in cyanobacterial strains, which vary in sequence, presence/absence of tRNA genes (Boyer \textit{et al.} 2001;

Flechtner et al. (2002), Boyer et al. (2001) observed rRNA operons with nonidentical ITS regions in Scytonema hyalinum and Calothrix parietina. Řehakova et al. (2007) observed non identical ITS regions in Nostoc lichenoides and Nostoc indistinguendum where both had no tRNA genes or both genes for tRNA\textsubscript{ala} and tRNA\textsubscript{lle} products. Johansen et al. (2008) observed three distinct operons in the same strain of Leptolyngbya badia. This makes it difficult to know if ITS regions among taxa are homologous. An ITS region with 2 tRNA genes in one species is not homologous to an ITS region in another species with no tRNA genes. Thus, it is best to use those operons with at least one tRNA gene as they are more likely homologous. The presence of different operons means that to get clean sequence, the PCR products must be cloned.

Many cyanobacteria have the ability to exist in harsh environmental conditions, and have the capacity to ecologically modify these ecosystems in which they occur. They are among the most widespread, morphologically distinct, and abundant prokaryotes. With regards to comparisons of species of different localities, similar morphospecies of cyanobacteria have been observed within a single continent and among different continents based on ecological and morphological considerations. Presently, many ecologists consider cyanobacteria species to be cosmopolitan, because morphologically similar forms can be found in many geographical regions. Morphologically similar forms from habitats of North America and Africa have been reported (Mesfin, 2009, chapter 2, 3). However, some evolutionary biologists are very doubtful that “morphospecies” from different climates and continents represent monophyletic species (Johansen and Casamatta, 2005).
The objective of this study is to characterize selected morphospecies of cyanobacterial taxa of the two continents based on molecular markers of the 16S rRNA and 16S-23S internal transcribed spacer (ITS) genes. The ITS domains mentioned as D1-D1’ helix, V2 helix and Box B domain are thoroughly examined as potential genetic markers to identify cyanobacterial species. In the process, new species of cyanobacteria are designated by combining morphological, ecological and molecular approaches as recommended by Komárek and Anagniostidis (1999, 2005), Johansen and Casamatta (2005), and Řehakova et al. (2007).

4.3 Materials and Methods

4.3.1 Selection of morphospecies of cyanobacteria of Africa and North America.

Cyanobacteria strains were obtained from the culture collection in the phycology laboratory at John Carroll University. The cyanobacteria taxa with reference strain numbers are provided below.

4.3.1.1 Cyanobacterial taxa of Africa (The Rift Valley System in Ethiopia)

1. *Cyanosarcina abyssinica* Mesfin et Johansen provisional sp. nov. Code: ETH2.4.M4
6. *Symplocastrum sheleko* Mesfin et Johansen provisional sp. nov. Code: ETH1.1M1
4.3.1.2 Cyanobacterial taxa of North America (The Great Basin of Idaho and Oregon)

1. Leptolyngbya nostocorum (Bornet ex. Gom.) Anagnos. & Kom. Code: OR1.M2
3. Nostoc indistinguendum Vaucher ex Bornet et Flahault. Code: OR1b. M1

4.3.2 DNA extraction

DNA was extracted from unialgal tissue cultures of the above strains using The PowerSoil DNA Isolation Kit, which is recommended for isolation of genomic DNA from environmental samples (www.mobio.com). Cells were mechanically broken using a cell homogenizer (Minibeadbeater, Biospec.). The DNA extractions were stored at -20 °C.

4.3.3 Polymerase chain reaction

Primers were based on those designated by Wilmotte (1993), and Nübel et al. (1997). They were designated as follows:

Primer 1. 5’ CTCTGTGTGCTAGGTATCC 3’ (after Wilmotte et al. 1993).
Primer 2. 5’ GGGAATTT TCGCAATGGG 3’ (after Nübel et al. 1997).
Primer 5. 5’ TGTACACACCGGCCCGTC 3’ (after Wilmotte et al. 1993).
Primer 7. 5’ AATGGGATTAGATACCCAGTAGT C 3’ (after Nübel et al. 1997).
Primer 8. 5’ AAGGGAGGTGATCCAGCCACA 3’ (after Wilmotte et al. 1993).

These primers attached to conserved stretches of the cyanobacterial 16S rRNA and 23S rRNA gene (Boyer et al. 2002). The organization of the ribosomal RNA (rRNA) operon showing the locations of the above primers used for PCR amplification of the
16S rRNA and ITS regions are provided below (Figure 72). Primers were obtained from Mildland Certified Reagent Company in concentrations that were brought to 100 µM for use in PCR, out of which 10µM stock solutions of each primer were made.

Initially, each DNA sample was amplified using primers 1 and 2. This resulted in a product approximately 1600 bp long. Each reaction contained 10 µl 10x buffer (Promega), 0.5 µl of each dNTP (G, A, T, C) at 10 mM, 0.5 µl primer 1 and 0.5 µl primer 2, 0.5 µl Taq polymerase (Promega), and 1µl of genomic DNA, and sufficient sterile water to bring reaction to 100 µl.

The most commonly used profile for the initial long PCR reaction using primers 1 and 2 was 94°C for 1 minute, 57°C for 1 minute, 72°C for 4 minutes (35 cycles), followed by a 10 minute extension at 72°C and then 4°C dwell. Reactions were carried out using Thermolyne’s amplitron thermal cycler (Barnstead International, Dubuque, IA, USA). The presence of PCR products was detected by standard agarose gel electrophoreses with ethidium bromide staining.

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Figure 72. Organization of the ribosomal RNA (rRNA) operon showing the locations of the primers used for PCR amplification of the 16S rRNA and 16S-23S ITS.
4.3.4 Cloning and sequencing.

The PCR product was cloned into plasmids containing the sites for universal primers M13 forward and M13 reverse on either side of the insert site using Invitrogen’s TOPO TA Cloning Kit for Sequencing, Version A (Invitrogen Corp. Carlsbad, CA, USA). Plasmid DNA was purified and obtained from the resultant clones using Qiagen’s QiaPrep Spin Kit. In the case of clones containing PCR products generated with primers 1 and 2; nine minipreps were digested with EcoRI enzyme and run on a long gel to visualize the size of the inserts. The three clones chosen for sequencing included as many different insert lengths as were distinguishable. Automatic sequencing with the universal primers M13 forward and M13 reverse and internal primers 5, 7, and 8 was performed by Cleveland Genomics.

4.4 Data analysis

Forward and reverse primer sequences were checked against each other by generating the reverse compliment of the “reverse” sequence with the Sequence Utilities Service in Baylor College of Medicine’s Search launcher (Smith et al. 1996) at http://searchlauncher.bcm.tmc.edu/seq-util.html. Contigs were constructed by aligning the sequences from 5’ sequencing reactions in ClustalW. Contigs constructed in previous studies were used to assist these alignments (i.e. they were reference strains). This resulted in the longest possible read of sequences, in addition to acting as a check on sequencing. The resulting sequences were carefully checked by eye for ambiguities and sequencing errors. This was accomplished by the examination of sequence chromatograms, and corrections were made when appropriate. All consensus sequences were BLAST searched (Altschul et al. 1997) to ensure they were cyanobacterial in origin.
Phylogenetic trees based on the 16S rRNA sequence data were constructed by a variety of methods. All aligned sequences were analyzed using the program PAUP*4.0b (Swofford, 1998). Heuristic searches (1000 replicates) using distance, maximum parsimony, and maximum likelihood as optimality criteria were conducted (Swofford, 1998). For distance and maximum parsimony analysis, branch swapping was set to TBR, substitution rates were equal, and the MULTREES option was on. For maximum likelihood, tree branch swapping was set to NNI, substitution rate was assumed to follow gamma distribution, and the MULTREES option was off. The logdet distance metric was utilized for the distance analysis. All other options were set at default settings. Bootstrap analysis was performed as part of each analysis, with 1000 replicates for distance and maximum parsimony, and 100 replicates for maximum likelihood.

Two sets of sequence data were constructed for strains of Oscillatoriales and Nostocales. One set was comprised of samples of Oscillatoriales (83 OTU’s) from different habitats, and included *Leptolyngbya* cf. *africanum*, *Leptolyngbya* cf. *foveolarum*, *Leptolyngbya* cf. *tenue*, *Microcoleus vaginatus* (Ethiopia), *Microcoleus vaginatus* (Idaho), and *Symphocapsa strum sheleko* from this study. Similarly, another set comprised of a sample of Nostocales (104 OTU’s) from different habitats, and included *Nostoc oromo*, *Nostoc indistinguendum*, *Scytonema javanicum*, *Tolypothrix distorta*, and *Trichormus sidamae* from this study. Sequences not obtained from this study were obtained from GenBank for both sets of analysis. The following taxa from the GenBank were used as outgroups in the various analyses (accession numbers in parentheses): *Gloeobacter* sp. PCC8105 (BA000045) was used in the analyses of Oscillatoriales.
Microcoleus steenstrupii USPC-MC1 (AF355392) was used in the analyses of Nostocales.

The predicted secondary structure model of the 16S-23S ITS sequence was constructed using the Mfold web server version 2.3 (Zuker, 2003) with the temperature set at 20°C and all other options set at default. Structures were determined by folding and identifying each conserved helix separately first. Analysis of secondary structures also informed sequence proofreading. These models enabled us to validate the divergence and the variation of the alignments among different strains. Terminology of the domains in the ITS regions are based on Iteman et al. (2000). For the comparisons of the ITS domains of the cyanobacterial species isolated, different ITS sequences of closely related taxa were obtained from GenBank and from unpublished sequence from the Phycology Laboratory at John Carroll University.

4.5 Results and discussion

4.5.1 Analysis of 16S rRNA sequence data

Parsimony, distance and maximum likelihood analyses on the 16S rRNA sequence data for 83 OTU’s within the Oscillatoriales and pseudanabaenales were performed. All three optimality criteria gave very similar results, and clustered the strains of interest in this study (ETH. and ID. strains) identically (Fig. 73). Several clades known to be supported in other studies (Boyer et al. 2002; Casamatta et al. 2005; Johansen et al. 2008; Řeháková et al. 2007) were also supported in this work. These include: a) Microcoleus vaginatus, b) Leptolyngbya sensu stricto (the clade containing L. boryana, the type species), and c) LPP Group I. While not fully supported, most strains attributed to Microcoleus steenstrupii in Boyer et al. (2002) cluster together, with internal
support for a subset of 15 of these OTU’s (Fig. 73 top of phylogeny). Both Microcoleus vaginatus strains of the two different regions (ETH 2.2. M7 and ID4. M2) clustered with other M. vaginatus strains forming a clade for this species, with 100% bootstrap support. Besides morphological similarities between these strains (Fig. 17, 18, 46, 47), the secondary structures of the ITS domains of D-D1’, V2 and box B were also identical in their internal and terminal loops (see next section). The strains of Microcoleus vaginatus from Ethiopia and Idaho were both in the M. vaginatus clade and there is no evidence of cryptic species in this group. They both fell into the same subclade in the genus, which supported the idea that M. vaginatus has a worldwide distribution in soils (Garcia-Pichel et al. 2001). The very close similarities of the strains from Ethiopia and various desert regions with the USA, with regard to both morphological and molecular sequence data support the hypothesis that this species is cosmopolitan in arid soils.

This study provides further evidence that Leptolyngbya is polyphyletic, with at least four supported clades, similar to what was found in Johansen et al. (2008). The Leptolyngbya group, LPP Group I (clade I, bootstrap value 51%-63%), contained desert strains mostly collected from North American microbiotic crusts. The Leptolyngbya sensu stricto clade contained the type species Leptolyngbya boryana along with several aerophytic species (L. angustata, L. corticola, L. foveolarum, L. tenerrima), and a group of arid soil taxa from microbiotic crusts collected from New Mexico (bootstrap value 99%-100%). A sister clade to Leptolyngbya sensu stricto (bootstrap value 63%-100%) contained four strains collected from Californian desert soils. Leptolyngbya could be defined as any clade from the node with 55%-57% bootstrap support (clade II) to node with 99%-100% (Fig. 73). Even though there was no support in the analyses, the clade
which contained *Leptolyngbya* CNP1-B3-C9 AY239600 and *Leptolyngbya* Kovacik 1990-54 (clade III) was genetically related to clade II. The two strains in clade III were related with 100% boot strap support irrespective of their geographical differences. *Leptolyngbya* Kovacik 1990-54 was from European soil and *Leptolyngbya* CNP1-B3-C9 was from North American desert soil.

The three *Leptolyngbya* species from Ethiopia were not within the *Leptolyngbya sensu stricto* clade. Consequently, *L. foveolarum* ETH1.1.M5 does not belong in that species or genus, despite the strong morphological similarity. *L. tenue* ETH2.3.M1 and *L. africanum* ETH2.3.M8 are in the same clade (clade IV, Fig. 73), and correspond to the current subgenus within *Leptolyngbya* known as *Protolyngbya* (with cells longer than wide). It appears that this subgenus likely needs to be recognized on the genus level, and is not a subgenus of *Leptolyngbya*. *L. tenue* and *L. africanum* were very similar in their 16S rRNA sequence and in all analyses were clustered together (boot strap value 96%-100%). The molecular characterization using 16S-23S ITS and the secondary structure of

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Figure 73. Parsimony analyses of Oscillatoriales taxa, including six strains from the present study (in red font, two within *Microcoleus vaginatus* clade). Topologies of trees using maximum parsimony, maximum likelihood, and distance were similar. Bootstrap values based are indicated for parsimony analysis and maximum likelihood analysis above the node, while bootstrap values using distance as an optimality criterion are in smaller font and are below the nodes.
the domains did not reveal significant differences among the strains characterized as *L. foveolarum* ETH1.1M5, *L. tenue* ETH2.3. M1, and *L. africanum* ETH2. 3. M8. The secondary structures of the D-D1’, V2, and box B helices showed close similarities to one another and further support that the above strains likely belong to the same genus (see next section). *Leptolyngbya* needs revision; others have recommended that it be split into at least 5 genera (Johansen et al. 2008).

The position of *Symlocastrum sheleko* was not resolved. In all three trees it was within the Pseudanabaenales (*Leptolyngbya sensu lato*), but it had varying positions within the clade depending on optimality criterion. In no case did it have bootstrap support above 60% for the clade containing it. In the maximum likelihood and distance trees, it was associated with *Leptolyngbya* CNP-B3-C9 and *Leptolyngbya* Kovacik 1990-54. The morphological characterization of this strain in the cultures had shown that the cells had Phormidiacean type of cell division (Fig. 19, 20).

Parsimony, distance and maximum likelihood analyses of 16s rRNA sequence data for 104 heterocytous OTU’s was constructed. The topology of the trees was nearly identical and the parsimony tree is shown to represent the analyses (Fig. 74).

**Fig. 74.** Parsimony analyses of heterocytous taxa, including three strains from the present study (in red font). Topologies of trees using maximum parsimony, maximum likelihood, and distance were similar. Bootstrap values are indicated for parsimony analysis and maximum likelihood analysis above the node, while bootstrap values using distance as an optimality criterion are in smaller font and are below the nodes. Clade A is *Nostoc indistinguendum*, Clade B is *Nostoc lichenoides* and *Nostoc oromo*, Clade C contains the Microchaetaceae, and Clade D contains *Trichormus* and *Anabaena* group. Next page has figure.
According to all three of these analyses of heterocytous taxa, three distinct clades contained the strains characterized in this study. Clade ‘A’ contained taxa related to *Nostoc indistinguendum*, clade ‘B’ contained taxa related to *Nostoc lichenoides*, and clade ‘C’ contained taxa related to *Tolypothix distorta* (i.e. the Microchaetaceae).

The reference strain characterized as *Nostoc indistinguendum* was isolated, characterized, and described from soil samples from a Mojave Desert region in California (Řehákova *et al*. 2007). The strain was morphologically similar to the strain studied and characterized as *Nostoc indistinguendum* from Oregon. In culture, both taxa had small spherical microcolonies with comparable vegetative cell sizes up to 5 μm wide and 6 μm long (Fig. 58, 59). Heterocytes were very similar in shape (spherical or oval) in both taxa. Akinete sizes were comparable in both strains, being oval and growing up to 5 μm wide and 7 μm long. In the analysis of the ITS region (see next section) both strains had closely similar D-D1’ and box B helices, which could be taken as taxonomic evidence to consider both strains as identical species. Ecologically, both strains were collected from similar habitats of North American microbiotic crusts, and similar physiology is an additional taxonomically informative character, supporting species identity of the strains.

In the phylogenetic trees constructed based on 16S rRNA, clade ‘A’ contained clusters of several closely related strains (61% bootstrap value) characterized as *Nostoc indistinguendum* including the strain studied in the microbiotic crust of Oregon (Fig. 74). The strains identified by others as *N. commune* and *Nostoc “Peltigera pruinosa” cyanobiont* are likely identified incorrectly, and should be considered *N. indistinguendum* in future studies. In the topology of the trees generated, clade ‘B’ contained the new taxon characterized as *Nostoc oromo* (ETH2.4.M4) from the Ethiopian microbiotic
crusts. *Nostoc oromo* was morphologically similar to the phycobiont strain isolated from *Collema tenax*, which was identified and characterized as *Nostoc lichenoides* (Řehákova *et al.* 2007). Both strains had spherical colonies with vegetative cells growing up to 6 μm long in culture. Akinete sizes in both strains were comparable and measured up to 7 μm wide and 4-10 μm long (Fig. 25). However, there were distinct morphological differences between the two strains. *Nostoc oromo* from Ethiopian microbiotic crusts resembled *Chroococcidiopsis kashayi* in culture. The colony fragmented to release one, two or four celled colonies. The release of many few-celled nonfilamantous propagules from aging colonies was distinctive, and similar release was not seen in other *Nostoc sensu stricto* kept in culture. *Nostc oromo* was geographically different from other sister taxa clustered in clade ‘B’. It is a free-living strain unlike those phycobiont strains isolated and characterized as *Nostoc lichenoides* (Řehákova *et al.* 2007). Even though there were distinct morphological, ecophysiological and molecular (ITS) differences among the taxa of clade ‘B’, the molecular evidence of 16S rRNA reflected the close relatedness within the group (52% bootstrap value). In the topology of the trees generated, the *Nostoc sensu stricto* clade contained *Nostoc commune* species, the type species as well as other terrestrial species, including those in the present study and having bootstrap values ranging from 63%-88% (Fig. 74). Besides distinct reproductive strategies (cell division and life cycle) of *Nosto oromo*, the 16S-23S ITS region of box B helix was distinctive for this strain (see next section).

In the phylogenetic analysis, clade ‘C’ contained one of the taxa characterized as *Toylhypothrix distorta* from the microbiotic crust of Idaho (ID4. M1). Most of the taxa observed in clade ‘C’ contained members of the Microchaetaceae such as *Spirirestis*
raphaelensis. The members of this clade are best characterized for having false branching with one or two basal heterocytes in culture (Flechtner et al. 2002; Řeháková et al. 2007). Many of the strains characterized as *Toylypotrhix distorta* from different localities have trichome size ranging from 7-12 μm wide. Necridic cells are usually evident in the thallus, which is also observed in the strain isolated from Idaho (Fig. 71). Even though there are many morphological features shared among members of the Michrochaetaceae, detailed molecular evidence using 16S rRNA and secondary structures of 16-23S rRNA regions have been used to separate very closely related species (Flechtner et al. 2002) of the same clade (see next section).

In addition to clades ‘A’, ‘B’, and ‘C’, other clades were also observed in the phylogenetic analysis (Fig. 74). A clade (D) containing *Trichormus*, *Anabaena cylindrica*, *Cylindrospermum*, *Mojavia*, and some misidentified *Nostoc* and *Tolypothrix* lack bootstrap support. These are cultures that lack aerotopes found in *Anabaena/Aphanizomenon*. They could be included in *Nostoc* to make that a large group unwieldy variable monophyletic taxon, or they could be split into separate genera. Given the absence of bootstrap support, these may represent long-branch attraction in a group of unrelated taxa.

### 4.5.2 Analysis of 16S-23S ITS sequence data

The results from the study of the 16S-23S ITS were analyzed for nine of the Ethiopian and four of the USA cyanobacterial strains (Tables III, IV, V). These were *Cyanosarcina abyssinica* (ETH.), *Leptolyngbya* cf. *africanum*, (ETH), *Leptolyngbya* cf. *foveolarum* (ETH), *Leptolyngbya* cf. *tenue* (ETH), *Microcoleus vaginatus* (ETH), *Symplocastrum sheleko* (ETH), *Nostoc oromo* (ETH), *Trichormus sidamae* (ETH), and
Scytonema javanicum (ETH) from Ethiopia, and Leptolyngbya nostocorum (Oregon), Microcoleus vaginatus (Idaho), Nostoc indistinguendum (Oregon), and Tolypothrix distorta (Idaho) from the USA.

The D1-D1’helix obtained for Cyanosarcina abyssinica (ETH.) was compared with other taxa in Chroococcales (Fig. 75). It was the only coccoid alga identified in this study. The internal and terminal loops observed in the D1-D1’helix of C. abyssinica were distinctly different from other coccoid algae. However, all secondary structures of the D1-D1’helices had the same beginning and ending five nucleotides. These conserved features of the D1-D1’helices are characteristic for all cyanobacterial species. The V2 helix and box B domain of C. abyssinica were also compared with other Chroococcales (Fig. 76, 77). The V2 helix is only present when tRNA\textsuperscript{Ile} and tRNA\textsuperscript{Ala} genes are present in the ITS region (Iteman \textit{et al.} 2000), at times is absent even when both tRNA genes are present.

The V2 of C. abyssinica is longer than all other V2 regions from coccoid taxa. The various Microcystis taxa were remarkably consistent in structure (Fig. 76). The Box B of C. abyssinica was unique among all cyanobacteria compared in this study (Fig. 77).

The secondary structures of the V2 and box B domain have been important in species characterization (Alwathnani, 2006; Casamatta \textit{et al.} 2006; Siegesmund \textit{et al.} 2007), and support the recognition of lineages among cyanobacterial species. C. abyssinica forms clear and small cubical aggregations of cells in rapidly growing cultures (Fig. 5, 6), which are typical of the genus isolated from different locations (Komárek and Anagnostidis, 1999). Given the range of ecological, morphological and molecular
evidence, this taxon was defined as a new species in the region. The species name was given after the former name of Ethiopia, which was Abyssinia.

Figs. 75-77 follow this page

Figure 75. ITS domain (D1-D1’ Helix): *Cyanosarcina abyssinica* ETH2.4.M4 (A*), *Microcystis aeruginosa* UAM 253 (B), *Microcystis novacekii* UAM 241 (C), *Microcystis wesenbergii* (AB015397) (D), *Synechococcus sp.* (AF397726) (E) (*: strain identified in the study).
Figure 76. ITS domain (V2 Helix): 
* Cyanosarcina abyssinica ETH2.4.M4 (A*),
  Microcystis aeruginosa UAM 253 (B), Microcystis flosaquae UAM 242 (C),
  Microcystis novacekii UAM 241(D), Microcystis viridis UAM 260 (E),
  Microcystis wesenbergii (AB015397) (F), Anacystis nidulans (X00346) (G),
  Microcystis ichthyoblabe (AB015373) (H) (*: strain identified in the study).
Figure 77. ITS domain (box B domain): *Cyanosarcina abyssinica* ETH2.4.M4 (A*),

*M. aeruginosa* UAM 253 (B), *M. flosaquae* UAM 242 (C),

*M. novacekii* UAM 241 (D), *M. viridis* UAM 260 (E), *M. wesenbergii* (AB015397) (F), *Prochlorococcus sp.* AF397702 (G), *Synechococcus sp.* (AF397726) (H) (*: strain identified in the study).

The members of Pseudanabaenaceae identified in this study are *Leptolyngbya cf. africanum*, (ETH), *Leptolyngbya cf. foveolarum* (ETH), *Leptolyngbya cf. tenue* (ETH), and *Leptolyngbya nostocorum* (Oregon). It appeared that the D1-D1’helices tend to have
the same structures in phylogenetically related taxa characterized in this study (Fig. 78 A, B, C, D). Komárek and Anagnostidis (2005) concluded that the genus *Leptolyngbya* contains two large subgenera, the subgenus *Leptolyngbya* which consists of the type species (*L. boryana*) as well as other taxa with cells shorter than wide to isodiametric, and the subgenus *Protolyngbya* with cells longer than wide. Within the genus *Leptolyngbya* there are strains which are morphologically similar to *Leptolyngbya sensu stricto*, but belong to different clades. Some strains belong by morphological definition to the subgenus *Protolyngbya*, and this is where the strains sequenced in this study appear to belong. There were distinct differences in the D1-D1’ helices with regard to internal and terminal loops between the *Leptolyngbya* strains such as *L. boryana* and the Ethiopian strains (Fig. 78). The strains characterized from Ethiopian microbiotic crust could best be included in the subgenus *Protolyngbya*, because of the distinct phylogenetic placement based on 16S rRNA analysis. The D1-D1’ helices were different than those found in subgenus *Leptolyngbya*, but did resemble some *Leptolyngbya* outside of the *sensu stricto* group (Fig. 78G, H, compare with clade III in Fig. 62). However, there were still differences of two or more base pairs among the isolates, mainly in their terminal loops that characterized individual species.

The V2 helices for the *Leptolyngbya*, subgenus *Protolyngbya*, strains isolated in this study were similar to each other, but distinct from other *Leptolyngbya* taxa (Fig. 79). They were similar in length and had identical primary sequence in the terminus of the helix (Fig. 79). The Box B for these *Leptolyngbya* strains was similar to each other (Fig. 80). Most had similar basal motif- a bilateral bulge with two cytosine across from a
single adenine or cytosine (Fig. 80). This differs from all other cyanobacteria examined in this study. Figs. 78-80 follow this page.

Figure 78. ITS domain (D1-D1’ Helix): *Leptolyngbya cf. africanum* ETH2.3.M8 (A*), *Leptolyngbya cf. foveolarum* ETH1.1.M5 (B*), *Leptolyngbya cf. tenue* ETH2.3.M1(C*), *Leptolyngbya nostocorum* OR1.M2(D*), *Leptolyngbya corticola* CCALA-085 (E), *Leptolyngbya boryana* UTEX485 (F), *Leptolyngbya sp.* Kovacik 1990/54(G), *Leptolyngbya sp.* Hindak 1982/12(H) (*: strains identified in the study).
Figure 79. ITS domain (V2 Helix): *Leptolyngbya cf. africanum* ETH2.3.M8 (A*),
*Leptolyngbya cf. foveolarum* ETH1.1.M5 (B*), *Leptolyngbya cf. tenue* ETH2.3.M1 (C*),
*Leptolyngbya nostocorum* OR1.M2 (D*), *Leptolyngbya sp.* SEV4-3-c-1(E),
*Leptolyngbya sp.* SEV5-3-c-19(F), *Leptolyngbya sp.* Kovacik 1990/54 (G) *Leptolyngbya sp.* SEV4-3-6c (H) (*: strains identified in the study).

The members of Oscillatoriales identified in this study were *Microcoleus vaginatus* (ETH), *Microcoleus vaginatus* (Idaho), and *Symplocastrum sheleko* (ETH). The D1-D1’ helices constructed for the above three taxa were highly similar in basal structure (Fig. 81) and the two *Microcoleus vaginatus* isolated from two widely
separated regions were identical (Fig. 81A, B). They also had identical V2 helices and box B domains (Fig. 82 A, B; 83 A, B). Besides the morphological similarities between the two *Microcoleus vaginatus* collected from the two continents (Fig. 16-18, 46-48), the molecular evidence of the 16S rRNA and 16S-23S ITS obtained in this study supported the hypothesis that this particular cyanobacterial taxon shows a world-wide distribution.

The strain isolated as *Symplocastrum sheleko* (ETH) had a distinct D1-D1’helix when as compared to other related taxa although the basal portion and most of the helix had structure similar to *M. vaginatus* (Fig. 81A, B, D). The V2 helix was very similar to *M. vaginatus* (Fig. 82A, B, D). It also had a distinct box B domain (Fig. 83). In the phylogenetic trees generated, the strain was positioned between the *Leptolyngbya* clade I and *Leptolyngbya* clade II or it was associated with *Leptolyngbya* strains collected from European soil (*Leptolyngbya* Kovacik 1990-54, clade III) and North American desert soils (*Leptolyngbya* CNP-B3-C9, clade IV). This strain had a comparable cell size with *Microcoleus* species (4-5.5 (6) μm wide) which was a distinct morphological feature that differentiated the strain from *Leptolyngbya* strains. The strain was defined as a new species from the Rift Valley System of Ethiopia. The name of the species is after the word “sheleko”, meaning valley in Amharic (Ethiopian national language).

Figs. 81-83 follow this page.
Figure 81. ITS domain (D1-D1’ Helix): Microcoleus vaginatus ETH2.2M7 (A*), Microcoleus vaginatus ID4.M2 (B*), Microcoleus vaginatus MOA1MC1-10 (C), Symplocastrum sheleko ETH1.1M1 (D*), Microcoleus vaginatus SEV1-KK3 (E), Microcoleus steenstrupii (AF363947) (F), Microcoleus chthonoplastes ASK5 (G), Microcoleus chthonoplastes GNP5 (H) (*: strains identified in the study).
Figure 82. ITS domain (V2 Helix): *Microcoleus vaginatus* ETH2.2M7 (A*), *Microcoleus vaginatus* ID4.M2 (B*), *Microcoleus vaginatus* MI-18 (C), *Symplastrum sheleko* ETH1.1.M1 (D*), *Microcoleus sp.* NDN (E), *Microcoleus sp.* CHI (F), *Microcoleus sp.* ASK1 (G) (*: strains identified in the study).
The ITS domains for the members of Nostocales identified in this study were analyzed and compared with other related taxa collected from different localities (Figs. 84-89). Most of the Nostocales in the genus *Nostoc* have similar D1-D1’ helices, both in basal portions and terminal loops of the helices (Fig. 84). The *Nostoc indistinguendum*
from Oregon and *Nostoc oromo* from the Rift Valley System in Ethiopia were almost identical in primary sequence, and were identical in structure (Fig. 84 A, B). *Nostoc oromo* (ETH) was one of the cyanobacterial strains identified as a new taxon in the microbiotic crusts of the Rift Valley in Ethiopia. This strain showed distinct morphological features in culture, resembling members of the genus *Chroococcidiopsis* (Fig. 21-25). After close observation, the heterocyst in a 6-month-old culture was revealed, which was atypical for most species of the genus *Nostoc* (Kondratyeva and Kislova, 1992; Li, 2001). The V2 for Nostoc oromo was larger than all other Nostoc (Fig. 85), and was very distinct from the V2 in *N. indistinguendum*. The secondary structure of Box B domain was distinct in *N. oromo* compared to other *Nostoc* (Fig. 86). Considering the ecology, morphological features and the molecular evidence for this strain (16S rRNA and 16S-23S ITS), this taxon was recognized and identified as a new species in the microbiotic crusts of the Rift Valley of Ethiopia. The name of the species was given after the nationality of the Oromo people living in the region.

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Figs. 84-86 follow this page.
Figure 84. ITS domain (D1-D1’ Helix): *Nostoc oromo* ETH2.4.M5 (A*),
*Nostoc indistinguendum* OR1b.M1 (B*), *Nostoc commune* EV1-KK 1(C),
*Nostoc indistinguendum* F15-VF1 (D), *Nostoc desertorum* CM1-VF14 (E),
*Nostoc indistinguendum* F15-VF4 (F), *Nostoc lichenoides* CNP-AK1 (G),
*Nostoc indistinguendum* F15-VF12 (H) (*: strains identified in the study).
Figure 85. ITS domain (V2 Helix): *Nostoc oromo* ETH2.4.M5 (A*), *Nostoc linkia* (AF105138) (B), *Nostoc punctiforme* (ATCC29133) (C), *Nostoc commune* EV1-KK1 (D), *Nostoc desertorum* CM1-VF14 (E), *Nostoc lichenoides* CNP-AK1 (F), *Nostoc indistinguendum* F15-VF1 (G) (*: strain identified in the study).
The other Nostocales identified in the study were *Trichormus sidamae* (ETH), *Scytonema javanicum* (ETH) from Ethiopia, and *Tolypothrix distorta* (Idaho) from the USA. The heterocyte differentiating cyanobacteria had a wide range of differences in their morphological characteristics, which were also reflected in the secondary structures.
of the ITS regions (Fig. 87-89). The D1-D1’ helices of *Tolypothrix distorta* (Idaho) identified in the study, and one of similar species recognized as *Tolypothrix distorta* from the North American crusts had similar secondary structures (Fig. 87 A, B). Both taxa also were clustered in the phylogenetic trees generated using the data set for Nostocales (Fig. 74). The secondary structures of V2 helices were similar in both strains (Fig. 88 A, C). On the other hand, the D-D1’ helix of *Spirirestis rafaelensis* had different terminal loops when compared with the secondary structure D1-D1’ of *Tolypothrix distorta* studied (Fig. 87 C). In the topology of the trees generated through 16S rRNA sequence data, however, clade ‘C’ contained the Microchaetaceae members including the strains isolated and characterized as *Tolypothrix distorta* and *Spirirestis rafaelensis*. This evidence reflected the verification of common ancestry with the support of 98%-100% boot strap value. *Trichormus sidamae* (ETH) isolated from the Rift Valley microbiotic crust was defined as a new species in the region. Morphologically it is characterized by its large sized akinetes (up to 8 by 12 μm) and mode of reproduction. It differs from other *Trichormus* strains by the presence of empty cell walls after post-reproduction in culture. (Figs. 28-31). The D1-D1’ helices of *T. sidamae* and other *Trichormus sp*. (PCC7120) were different in their internal and terminal loops (Fig. 87 E, F). The box B domains were also different as compared to PCC7120 (*Trichormus variabilis*) (Fig. 89 C, D). The V2 helix was not expressed in *Trichormus sidamae*. The range of the ecology, morphological characteristics and molecular evidence were used to define this taxon as a new species from Ethiopia. The species was named after the nationality of the Sidama people living in the region. Figs. 87-89 follow this page.
Figure 87. ITS domain (D1-D1’ Helix): *Tolypothrix distorta* ID4.M1 (A)*, *Tolypothrix distorta* SEV-2-5-Ca (B), *Spirirestis rafaelensis* SR56 (C), *Coleodesmium wrangelii* MC-JRJ1 (D), *Trichormus sidamae* ETH2.1.M2 (E*), Trichormus sp. (PCC7120) (F), *Scytonema javanicum* ETH2.2.M10 (G*), *Mojavia pulchra* JT2-VF2 (H) (*: strains identified in the study).
Figure 88. ITS domain (V2 Helix): *Tolypothrix distorta* ID4.M1 (A)*, *
*Scytonema javanicum* ETH2.2.M10 (B*), *Tolypothrix distorta* SEV-2-5-Ca (C), *Trichormus sp.* (PCC7120) (D), *Spirirestis sp.* (AF236659) (E), *Scytonema hyalinum* FI- 8A (F), *Gloeotrichia sp.* (AF1105135) (G) (*: strains identified in the study).
Figure 89. ITS domain (box B domain): *Tolypothrix distorta* ID4.M1 (A)*, *Tolypothrix distorta* SEV-2-5-Ca (B), *Trichormus sidamae* ETH2.1.M2 (C*), *Trichormus sp.* (PCC7120) (D), *Spirirestis sp.* (AF236659) (E), *Scytonema javanicum* ETH2.2M10 (F*), *Scytonema hyalinum* FI-8A (G), *Mojavia pulchra* JT2-VF2 (H) (*: strains identified in the study).
Table III. D1-D1’ sequence alignment of nine strains of Ethiopia and three strains of US in this study. Bold-faced regions at the beginning and end of the sequence indicate complementary binding sites at the base of D1-D1’ helix and letters in boldface types in the center represent those bases in the terminal loop.

<table>
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<tr>
<th>Strain</th>
<th>D1</th>
<th>D1’</th>
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<tr>
<td>Cyanosarcina abyssinica ETH2.4.M4</td>
<td>GACCTTACTTATGAATAC------CGAACCATACTGAGTACGTAAGTACGAAATAGGTCATCTAA------GGTC</td>
<td></td>
</tr>
<tr>
<td>Leptolyngbya cf.africanum ETH2.3.M8</td>
<td>GACCTGACCCCTATTTGACACGCTAGTACGAAATAGGTCATCTAA------GGTC</td>
<td></td>
</tr>
<tr>
<td>Leptolyngbya cf.foveolarum ETH1.1.M5</td>
<td>GACCTGACCCCTATTTGACACGCTAGTACGAAATAGGTCATCTAA------GGTC</td>
<td></td>
</tr>
<tr>
<td>Leptolyngbya cf.teneae ETH2.3.M1</td>
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<td></td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>Symplocastrum sheleko ETH1.1.M1</td>
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<td></td>
</tr>
<tr>
<td>Nostoc oromo ETH2.4.M5</td>
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<td></td>
</tr>
<tr>
<td>Trichormus sidamae ETH2.1.M2</td>
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<td></td>
</tr>
<tr>
<td>Leptolyngbya nostocoram ETH1.1.M2</td>
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</tr>
<tr>
<td>Microcoleus vaginatus ID4.M2</td>
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<td></td>
</tr>
<tr>
<td>Nostoc indistinguendum OR1b.M1</td>
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<td></td>
</tr>
<tr>
<td>Tolypothrix distorta ID4.M1</td>
<td>GACCTGACCCCTATTTGACACGCTAGTACGAAATAGGTCATCTAA------GGTC</td>
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</tr>
</tbody>
</table>
Table IV. V2 sequence alignment of nine of Ethiopian and three of US strains in this study. Bold-faced regions at the beginning and end of the sequence indicate complementary binding sites at the base of V2 helix, and the letters in boldface types in the center represent those bases in the terminal loop.

<table>
<thead>
<tr>
<th>Strain</th>
<th>V-2 REGION</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cyanosarcina abyssinica</em> ETH2.4.M4</td>
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</tr>
<tr>
<td><em>Leptolyngbya cf.africanum</em> ETH2.3.M8</td>
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</tr>
<tr>
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</tr>
<tr>
<td><em>Leptolyngbya cf.teneue</em> ETH2.3.M1</td>
<td>ATTCAAATTTAGAATTCAAAGTTCAA--AATTTTGAAT</td>
</tr>
<tr>
<td><em>Microcoleus vaginatus</em> ETH2.2.M7</td>
<td>ATTCAAATTTAGAATTCAAAGTTCAA--AATTTTGAAT</td>
</tr>
<tr>
<td><em>Symplocastrum sheleko</em> ETH1.1.M1</td>
<td>ATTCAAATTTAGAATTCAAAGTTCAA--AATTTTGAAT</td>
</tr>
<tr>
<td><em>Nostoc oromo</em> ETH2.4.M5</td>
<td>ATTCAAATTTAGAATTCAAAGTTCAA--AATTTTGAAT</td>
</tr>
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</tr>
<tr>
<td><em>Microcoleus vaginatus</em> ID4.M2</td>
<td>ATTCAAATTTAGAATTCAAAGTTCAA--AATTTTGAAT</td>
</tr>
<tr>
<td><em>Tolypothrix distorta</em> ID4.M1</td>
<td>ATTCAAATTTAGAATTCAAAGTTCAA--AATTTTGAAT</td>
</tr>
</tbody>
</table>
Table V. Box B sequence alignment of nine of Ethiopian and three of US strains in this study. Bold-faced regions at the beginning and end of the sequence indicate complementary binding sites at the base of box B helix, and letters in boldface types in the center represent those bases in the terminal loop.

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<th>Box B Region</th>
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</tr>
<tr>
<td>Leptolyngbya cf.foveolarum ETH1.1.M5</td>
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</tr>
<tr>
<td>Leptolyngbya cf.tenue ETH2.3.M1</td>
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<td>Microcoleus vaginatus ETH2.2.M7</td>
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<td>Symplocastrum sheleko ETH1.1.M1</td>
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<td>Nostoc oromo ETH2.4.M5</td>
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<td>Scytonema javanicum ETH2.2.M10</td>
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</tr>
<tr>
<td>Leptolyngbya nostocorum OR1.M2</td>
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<tr>
<td>Microcoleus vaginatus ID4.M2</td>
<td>AGCAACTGTTCTAGTGATTAGTCTTGATTAACTAGAGAGCC-----TGCT</td>
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<td>Nostoc indistinguendum OR1b.M1</td>
<td>AGCAACTGTTCTAGTGATTAGTCTTGATTAACTAGAGAGCC-----TGCT</td>
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<td>Tolypothrix distorta ID4.M1</td>
<td>AGCAACTGTTCTAGTGATTAGTCTTGATTAACTAGAGAGCC-----TGCT</td>
</tr>
</tbody>
</table>

4.6 Implications of molecular characterization

Considering both approaches of molecular characterization using the 16S rRNA and the 16S-23S ITS, cyanobacterial species tend to reveal their phylogenetic relationships, and at the same time exhibit unique features that enable us to differentiate to the level of species. The 16S-23S ITS regions have been reported to differentiate species of the genus *Microcystis* (Otsuka *et al.*1999), and also used to differentiate toxic and non-toxic strains of the genus *Nodularia* in mats forming in the Baltic Sea.
(Laamanen et al. 2001). Based on morphological and molecular characteristics, 15 strains of cyanobacteria were identified from the two continents of Africa and North America. Among the isolates, four new species were defined from the microbiotic crust of the Rift Valley of Ethiopia. The congruency among morphological characteristics, 16S rRNA and 16S-23S ITS contribute to better understanding and describing of new species in cyanobacteria (Alwathnani, 2006; Casamatta et al. 2006; Johansen and Casamatta, 2005; Laamanen et al. 2001; Řehákova et al. 2007; Rocap et al. 2002). The only coccoid alga identified in this study was *Cyanosarcina abyssinica*. This strain forms clear and small cubical aggregations of cells in a rapidly growing culture. Colonies were microscopic with packet-like or subspherical with densely aggregated cells enveloped in thin colorless sheath (Fig. 5, 6). Similar patterns of reproduction strategy (cell division and cell cycle) were reported for all species in the same genus (Komárek and Anagnostidis, 1999). The molecular evidences revealed that the secondary structures of the D1-D1’helix, V2 helix and box B domain for *C. abyssinica* were distinct and different from other cyanobacteria tested (Fig. 75 A, 76 A, 77 A). Rocap et al. (2002) were able to identify *Prochlorococcus* and *Synechococcus* species based on the 16S-23S ITS where the sequence analysis of only the 16S rRNA could not resolve the differences. The designation of *Cyanosarcina abyssinica* as a new species in the region was based on the recommendations and considerations of the ecophysiology, morphology and molecular evidences currently applied in naming and exploring the biodiversity of cyanobacteria in different regions (Johansen and Casamatta, 2005; Komárek, 2005; Komárek and Anagnostidis, 2005; Řehákova et al. 2007; Taton et al. 2006).
The analysis of the 16S rRNA appeared to be useful for higher-level systematic relationships and was sufficient to assign strains in this study to Pseudanabaenaceae and Phormidiaceae in the Oscillatoriales. Among the nonheterocytous, filamentous members of cyanobacteria characterized using the molecular markers of the 16S rRNA, three belonged to the genus *Leptolyngbya*, two belonged to the genus *Microcoleus*, and one belonged to the genus *Symplocastrum*. Molecular evidences using the 16S rRNA showed significant similarities among related taxa collected from different locations. The phylogenetic trees generated from aligned sequences supported that different genera tend to form clusters with related genera collected from different locations. The three species of *Leptolyngbya* cf. *africanum* (ETH), *Leptolyngbya* cf. *foveolarum* (ETH), *Leptolyngbya* cf. *tenue* (ETH) formed clusters with bootstrap values ranging from 96% - 100% among each other or with other similar taxa (Fig. 73). It is possible that they belong to the sub genus of *Protolyngbya* (with cells longer than wide) which is currently in revision in cyanobacterial systematics (Johansen et al. 2008). The D1-D1’helices of the *Leptolyngbya* species reported or obtained from different localities had similar structures (Fig. 78). Most of the D1-D1’helices of *Leptolyngbya* observed in this study are similar to the secondary F-type structures defined by Alwathnani (2006) from the Mojave Desert.

The two strains of *Microcoleus vaginatus* from Ethiopia and Idaho also formed clusters with other *M. vaginatus* strains with 100% bootstrap value (Fig. 73). Both strains had identical D1-D1’helices, V2 helices and box B domains (Fig. 81 A, B; 82 A, B; 83 A, B). The distinctive synapomorphic features of an 11-bp insert in variable loop six (V6) on the 16S rRNA gene (bp 423-433) was reported by Boyer et al. (2002) was
observed in both strains. These two strains also had similar morphological characteristics in culture (Fig. 16, 46). The other member of Phormidiaceae characterized and defined as a new species was *Symplocastrum sheleko*. The cell size was comparable to the cell size of the *Microcoleus* species (4-5.5 (6) μm) (Fig. 19, 20). A very simple morphological difference in the Oscillatorian strains could hide genotypic diversity (Ward *et al.* 1998). The genetic differences were best revealed in the secondary structures of the D1-D1’ helix, V2 helix, and box B domain, which were distinct for this strain (Fig. 81 D, 82 D, 83 D). The designation of this taxon as a new species in the region was based on the recommendations and considerations of the ecophysiology, morphology and molecular evidence obtained in this study (Johansen, and Casamatta, 2005; Komárek and Anagnostidis, 2005; Řehákova *et al.* 2007; Taton *et al.* 2006).

The conserved 16S-23S ITS regions were analyzed for four of the Nostocales strains in both regions. Among Nostocales, the genus *Nostoc* was well known and a widespread taxa. There was a considerable overlap of characters among the many morphospecies described thus far. Due to the absence of derived characters in naming species, the taxonomy of the genus remains problematic (Drouet, 1978; Kondratyeva, and Kislova, 2002). The analyses of 16S rRNA and 16S-23S ITS secondary structures were found to be important in resolving taxonomic problems in many of the cyanobacterial species (Boyer *et al.* 2002; Casamatta *et al.* 2006; Iteman *et al.* 2000; Otsuka *et al.* 1999; Řehákova *et al.* 2007; Rocap *et al.* 2002). The D1-D1’ helices in the most of the genus *Nostoc* had similar structures in their terminal loops (Fig. 84). *Nostoc indistinguendum* (Oregon) showed similar morphological features in culture with similar to other strains isolated from North American desert soils (Řehákova *et al.* 2007). The
colonies were microscopic, starting as small spherical colonies, growing up to 65 μm in diameter (Fig. 58, 59) with distinct sheath in the outer layers. In the analyses of 16S rRNA it formed clusters with the previously isolated strains of *Nostoc indistinguendum* (Fig. 75). In most cases the terminal loops of the D-D1’ helices of *Nostoc* strains have identical structures (Fig. 84 A-L) and this region was consequently not very informative. The V2 helix was not expressed in *Nostoc indistinguendum* from Oregon. The box B helix of this strain was closely similar to other edaphic *Nostoc* strains (Fig. 86). *Nostoc oromo* (ETH) is one of the cyanobacterial strains identified as a new taxon in the microbiotic crusts of the Rift Valley in Ethiopia. This strain showed distinct morphological features and reproductive strategies (cell division and life cycle) in culture, resembling members of the genus Chroococcidiopsis because heterocytes failed to form in young cultures. (Fig. 21-25). After close observation, the heterocyst was revealed only in a 6-month-old culture. This delayed appearance of heterocytes was not the characteristic for the *Nostoc* cultures examined from soil cultured from different regions (Kondratyeva and Kislova, 1992; Li, 2001). In the 16S rRNA analyses *Nostoc oromo* was positioned with the *Nostoc lichenoides* clade showing relatedness with phycobiont strains isolated from *Collema* (Fig. 74). The D-D1’ helix was similar to other *Nostoc* strains compared in this study (Fig. 84). However, the box B helix was distinct for this strain (Fig. 86). Considering the ecology, morphological features and the molecular evidences of this strain (16S rRNA and 16S-23S ITS), this taxon was recognized and identified as a new species in the microbiotic crusts of the Rift Valley of Ethiopia. The other members of Nostocales characterized were *Trichormus sidamae* (ETH) *Tolypothrix distorta* (Idaho) and *Scytonema javanicum* (ETH) from both
continents. The secondary structures of the D1-D1’, V2 and box B helices were different in all strains (Fig. 87-89). They also had distinct morphological characteristics.

*Trichormus sidamae* (ETH) isolated from the Rift Valley microbiotic crust was defined as a new species in the region. Morphologically it is characterized by its large sized apoheterocytic (up to 8 by 12 μm). It lacked prominent sheath around the colonies like the genus *Trichormus* in culture (Fig. 26-29). But, it produced distinct empty cell walls after post reproduction like certain Nostoc strains in culture (Fig. 27, 28). The D1-D1’ helix and the box B domain are also distinct for this species (Fig. 87 B, 89 B). The V2 helix was not expressed in this strain. The range of the ecology, morphological characteristics and molecular evidences were employed to define this taxon as a new species from the microbiotic crusts of the Rift Valley in Ethiopia.

*Tolypothrix distorta* (Idaho) identified in this study showed similar D1-D1’ secondary structures with other similar strains isolated in the North American microbiotic crusts. (Fig. 87 A, D), and were clustered in the phylogenetic trees generated (Fig. 75, 76) with boot strap support of 98%-100%, suggesting that both are identical species. *Scytonema javanicum* (ETH) isolated from the Rift Valley microbiotic crusts had a characteristic false branched thallus in culture like similar species identified elsewhere (Alwathnani, 2006; Geitler, 1930). It had a distinct D1-D1’ helix, V2 helix and box B domain (Fig. 87 C, 90 B, 89 C) as compared to other Nostocales strains. Applications of the morphological features in culture, molecular evidences (16S rRNA, 16S-23S ITS) and habitat specificity were important in characterizing and defining species in the two regions.
4.7 Conclusions and future directions

The isolation and characterization of cyanobacterial strains from diverse biotypes remain extremely important in the biodiversity study, and in the preservation of species. Cyanobacterial population in the microbiotic crusts of deserts and semi-deserts of the world are essential for many of the ecological roles they play in the respective regions. They are significant in the process of formation and in the stability and fertility of soil. They are important in preventing soil erosion by water or wind, increasing the possibilities of vascular plant colonization and sand dunes in deserts (Belnap, 2003; Belnap and Lange, 2001; Eldridge and Greene, 1994; Evans and Johansen, 1999; Friedmann and Galun, 1974). Through the study of morphological and molecular characterizations of cyanobacterial species, phycologists were able to identify different patterns in terms of species richness and in the recognition of endemic and regional species (Alwathnani, 2006; Johansen and Casamatta, 2005; Komárek and Anagnostidis, 2005; Řehákova et al. 2007; Scheldeman et al. 1999; Taton et al. 2006).

The sampling regions of the Great Basin and the Rift Valley of Ethiopia have many ecological similarities. Both are desert or semi-desert habitats with moisture limitation, which prevent vegetation growth in the respective regions. The physical and chemical properties of the soil in both regions have the minimum concentrations of cations and anions (Fitter and Hay, 2002) that could support vegetation growth. The types of vegetation that prevail in the regions are the desert adapted *Acacia* species in Ethiopia (Fig. 3, 4) and desert adapted shrubs in the Great Basin (Fig. 36). The profiles of the soil in both regions are dominated and covered by microbiotic crust communities. These communities are ones, which have the ability to survive desiccation and extreme
temperatures (up to 70 °C) and high pH and salinity, usually characterized in arid and semi-arid climates. (Evans and Johansen, 1999; Friedman and Galun, 1974; West, 1990; Zhang et al. 2006).

The microbiotic crusts of the two regions are also heavily affected by human activities such as overgrazing, over-increasing recreational and commercial activities and deforestation. These result in accelerating desertification in the region (Bishaw, 2001; Belnap, 1995; Evans and Johansen, 1999; Mulugeta et al. 2005; Thomas and Dougill, 2006; Zerihun and Mesfin, 1990). Studying the biodiversity of cyanobacteria in microbiotic crusts is critical to understanding the ecological roles played by these organisms. Currently, cyanobacterial taxonomists apply a polyphasic approach where the ecophysiology, phenotypes, and genotypes are used to characterize and define new species from different regions (Komárek and Anagnostidis. 2005; Johansen and Casamatta, 2005). The congruency among morphological characteristics, 16S rRNA and 16S-23S ITS contribute to better understanding and describing of new species in cyanobacteria (Alwathnani, 2006; Casamatta et al. 2006; Johansen and Casamatta, 2005; Laamanen et al. 2001; Řehákova et al. 2007; Rocap et al. 2002). In this study, the significance of the 16S-23S ITS secondary structures are emphasized in the recognition and identification of cryptic species in these regions. Although the genetic features (16S rRNA, 16S-23S ITS) are important in cyanobacterial taxonomy, there are still limitations in the application of molecular methods used in cyanobacterial systematics. For example, the 16S rRNA sequences are sometimes very similar to species and genera of Nostocales (Casamatta et al. 2006), or the 16S-23S ITS can vary significantly in different ribosomal operons in the same strain (Boyer et al. 2001, 2002; Coleman and Mai, 1997; Iteman et
The cyanobacterial strains characterized as *Leptolyngbya* cf. *africanum*, *Leptolyngbya* cf. *foveolarum*, and *Leptolyngbya* cf. *tenue* did not cluster with true *Leptolyngbya* clade in the phylogenetic analyses (Fig. 73). These cryptic species show similar morphological characterization, but tend to be different with regard to molecular characterization. More molecular study is required, such as sequence analyses of the whole 16S rRNA gene which could resolve the taxonomic position of these strains. Future studies should also focus on sequencing multiple strains, which could lead to more diversity of the cyanobacterial taxa in the region. Through a broader range of sampling sites, with greater variety plant association and soil types, observed cyanobacterial diversity will increase. It is highly recommended that additional related studies be undertaken to comprehend the systematics of cyanobacteria in different parts of the world.

A better understanding of the roles cyanobacteria play in arid and semi-arid ecosystems is imperative so that proper conservation and preservation strategies can be implemented to prevent the disturbance of microbiotic crusts. Once the microbiotic crusts are disturbed and destroyed, it takes 45-250 years to recover the original state (Belnap, 1993). It is also the intention and recommendation of this study to initiate the establishment of Herbarium of Non-Vascular Plants in Ethiopia with the intention that regional species could be preserved and maintained in the country. The cyanobacterial strains characterized from microbiotic crusts of the Rift Valley in Ethiopia are temporarily kept in the Phycology Laboratory at John Carroll University in Ohio. The establishment of a Herbarium of cryptogams of Ethiopian flora is critical during this
period when human activities and the global climatic changes are eroding the genetic resources of the region.

4.8 Literature Cited


APPENDICES
Appendix A. Soil Chemical and Physical Analyses: Laboratory Methods

Soil chemical and physical analyses for the two regions were conducted by Soil Testing Laboratory at Brigham Young University, Provo, Utah, using standard methods (Soil Conservation Service, 1972; Soil Survey Staff, 1962). Analyses included percent gravel, soil texture (gravimetric method), and sand fractionation by sieving, pH (saturated paste), electrical conductivity, and percent organic matter (Walkely-Black Method). Nitrate-N, calcium, magnesium, and sodium levels were determined from soluble extracts. Phosphorus and potassium were extracted using sodium bicarbonate via Olsen method (standard for alkaline soils). Sodium absorption ratio (SAR) was calculated using levels of calcium, magnesium and sodium.

Next pages: Tables of soil chemical and physical analyses of Ethiopian and US soils.
**Appendix B.** Soil chemistry of 10 sites in Ethiopian Rift Valley. OM= organic matter, EC= electrical conductivity (dS/m), SAR= sodium absorption ratio. Percent sand, silt, and clay were calculated after removal of gravel. Mineral nutrients in ppm (ETH. 1.1-1.5 Awassa location, ETH. 2.1-2.5 Zewai location) (date of sampling: 4/11/1999)

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<tr>
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<th>Site1.3</th>
<th>Site1.4</th>
<th>Site1.5</th>
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Appendix C. Soil chemistry of sites in Idaho (1-5) and Oregon (site 1) (USA). OM= organic matter, EC= electrical conductivity (dS/m), SAR= sodium absorption ratio.

Percent sand, silt, and clay were calculated after removal of gravel. Mineral nutrients in ppm (date of sampling: 7/19/1998).

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