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**Shades of red and green : the colorful diversity and ecology of picocyanobacteria in the Baltic Sea**

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# *Chapter 6*

*General discussion*



Cyanobacteria are photoautotrophic organisms. They use solar energy to sustain their metabolic processes, which enable cyanobacteria to proliferate in a wide range of aquatic and terrestrial environments. In aquatic environments cyanobacteria are an important component of the phytoplankton, together with a plethora of photoautotrophic eukaryotic microorganisms. Phytoplankton species compete for resources such as light and nutrients. Although pelagic ecosystems offer rather unstructured habitats lacking the intricate physical structure of, say, tropical rain forests or coral reefs, phytoplankton is known for its unexplained high species diversity. This conundrum stimulated Hutchinson (1961) to formulate the paradox of the plankton, which has been a source of inspiration for many studies investigating the mechanisms maintaining biodiversity.

This thesis aimed at the identification of the genetic background of one possible solution for the paradox of the plankton, that is, niche differentiation with respect to the color of light. For this purpose, we investigated the distribution of differently pigmented picocyanobacteria as a function of the underwater light spectrum (Chapter 2). Furthermore, we investigated the phylogeny and diversity of two genera belonging to the picocyanobacteria, namely unicellular picocyanobacteria of the *Synechococcus* group (Chapters 3 and 4) and the tiny filamentous *Pseudanabaena* spp. (Chapter 5). Our phylogenetic studies focused on the genes coding for the phycobilin pigments phycoerythrin (PE) and phycocyanin (PC), which are responsible for the characteristic red and green pigmentation of cyanobacteria. This chapter discusses the results obtained in the preceding chapters, and evaluates our hypothesis on the spectral niche differentiation of cyanobacteria.

## Colorful phytoplankton

The color of light could be one important factor allowing the maintenance of the high species diversity observed in the phytoplankton (Stomp *et al.*, 2004). Chemostat experiments showed that differently pigmented *Synechococcus* strains can coexist in white light, because their pigments enable utilization of different parts of the light spectrum. Moreover, further chemostat experiments showed that the filamentous cyanobacterium *Tolypothrix* can coexist with either a red or a green strain of *Synechococcus* by using the part of the light spectrum left unused by its competitors (Stomp *et al.*, 2004). This is possible since *Tolypothrix* is capable of tuning its pigmentation to the prevailing light spectrum through the mechanism of complementary chromatic adaptation (CCA).

In this thesis, the coexistence of differently pigmented picocyanobacteria was verified for natural environments. Seventy aquatic ecosystems characterized by a range of different turbidities were compared with respect to the underwater light spectrum and attenuation in the water column (Chapter 2). This led to the conclusion that red light dominates in turbid waters. This favors the growth of PC-rich picocyanobacteria. Aquatic ecosystems with lower turbidity enable light to penetrate deeper into the water column while the underwater light spectrum shifts towards green and blue light. In transparent oligotrophic waters, such as the open oceans,

PE-rich picocyanobacteria predominate. In water bodies with intermediate turbidity, such as the Baltic Sea, PE-rich and PC-rich picocyanobacteria co-exist with more or less equal cell abundances (Stomp *et al.* 2007a; Chapter 2).

### *Pigmentation of Synechococcus in the Baltic Sea*

In the Baltic Sea, PE-rich and PC-rich picocyanobacteria reveal distinct depth distributions. PE-rich picocyanobacteria dominate the phytoplankton in the deeper part of the euphotic zone, while PE-rich and PC-rich picocyanobacteria coexist in the upper mixed layer (Stomp *et al.*, 2007a; Haverkamp *et al.*, 2008; Chapter 2 and 3). This distribution was the result of the underwater light spectrum, changing from white light at the surface towards green light at depth. Water mainly absorbs red light. Red light is therefore not available in the deeper part of the euphotic zone, which restricts the growth of PC-rich cyanobacteria to the upper mixed layer.

The depth distribution of PE-rich and PC-rich picocyanobacteria in Baltic Sea waters resembles that of spectrally different pigment types (PC versus bacteriochlorophyll) in microbial mats (Ward, 2006). In hot spring microbial mats various ecotypes of a thermophilic PC-rich *Synechococcus* differed in their photosynthetic characteristics because of light quality gradients and were therefore found at different depths (Ramsing *et al.*, 2000; Allewalt *et al.*, 2006; Ward *et al.*, 2006; Kilian *et al.*, 2007). This example is also analogous to the distributions of high- and low-light ecotypes of *Prochlorococcus* species (Moore *et al.*, 1998; West and Scanlan, 1999). In *Prochlorococcus* the chl  $b/a_2$  ratios are different in the high- and low-light ecotypes (Moore and Chisholm, 1999). The low-light ecotypes have the highest chl  $b/a_2$  ratios and are able to grow under low light conditions found at the bottom of the euphotic zone. In the Red Sea and other (sub)-tropical oligotrophic marine environments *Prochlorococcus* is the dominant phototroph when the water column is stratified. In oceanic environments deep mixing causes *Prochlorococcus* to disappear in favor of other phytoplankton species, particularly *Synechococcus* (Olson *et al.*, 1990; Lindell and Post, 1995; Campbell *et al.*, 1998; Fuller *et al.*, 2005; Ahlgren and Rocap, 2006; Al-Najjar *et al.*, 2007).

Studies on marine *Synechococcus* species have identified different ecotypes that show distinct geographic distributions (Fuller *et al.*, 2006; Zwirgmaier *et al.*, 2007; Zwirgmaier *et al.*, 2008). However, the typical vertical distribution of *Synechococcus* ecotypes in the Baltic Sea, characterized by coexistence of PC-rich and PE-rich ecotypes near the water surface and the predominance of PE-rich picocyanobacteria in the deeper water layers underneath (Stomp *et al.*, 2007a; Haverkamp *et al.*, 2008; Chapters 2 and 3), has not been found in open-ocean ecosystems. In the Baltic Sea, the underwater light climate has predominantly a green color, which is effectively absorbed by PE-rich picocyanobacteria. In open ocean waters, water clarity is typically much higher than in the Baltic Sea, which shifts the underwater light color towards the blue part of the spectrum. As a result, the deeper water layers of the open ocean are dominated by low-light *Prochlorococcus* ecotypes, whose spectra are much better tuned to the prevailing blue light than the PE-rich picocyanobacteria of the Baltic Sea (Stomp *et al.*, 2007b).

*Synechococcus* is more abundant than *Prochlorococcus* in coastal oceanic regions, where water transparency is much lower than in the open ocean (Partensky *et al.*, 1999) and the underwater light color is shifted towards the green part of the light spectrum (Stomp *et al.*, 2007b). The *Synechococcus* species found in the different oceanic regions belong to clades I or III of *Synechococcus* cluster 5.1. The cluster 5.1 strains require elevated levels of  $\text{Na}^+$ ,  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  for growth reflecting the marine environment in which they thrive. *Synechococcus* species belonging to other clusters are often just halotolerant (Herdman *et al.*, 2001; Fuller *et al.*, 2003). A unique feature found only in *Synechococcus* strains belonging to clades I and III is the capacity for type IV complementary chromatic adaptation (CCA) (Fuller *et al.*, 2003; Palenik *et al.*, 2006; Zwirgmaier *et al.*, 2007; Zwirgmaier *et al.*, 2008). Many marine *Synechococcus* strains contain phycobilisome complexes with two pigments, phycourobilin (PUB) and phycoerythrobilin (PEB) that are present in the cell in a constant, strain specific ratio (Alberte *et al.*, 1984; Wood *et al.*, 1985; Fuller *et al.*, 2003; Six *et al.*, 2004). *Synechococcus* species capable of CCA are able to change the PUB/PEB ratio in response to changes in the spectral quality of light. Blue light stimulates a high PUB/PEB ratio, while green light induces a low PUB/PEB ratio. CCA leads to subtle but critical changes in the absorbance characteristics of their phycobilisomes (Palenik, 2001; Everroad *et al.*, 2006). During deep mixing marine *Synechococcus* species encounter changes in spectral light quality from white light near the surface to blue or green light at greater depth. It is therefore likely that the CCA capacity found in *Synechococcus* clades I and III is an adaptation to the mixing conditions they experience in marine environments (Fuller *et al.*, 2006; Stomp *et al.*, in press).

During this study, picocyanobacteria related to the *Synechococcus* cluster 5.1 were not encountered in the Baltic Sea. Instead, analysis of the 16S rRNA and ITS-1 region of clone libraries and isolated strains confirmed the presence of *Synechococcus* cluster 5.2 or *Cyanobium*-like species (Crosbie *et al.*, 2003; Ernst *et al.*, 2003; Chen *et al.*, 2006). Cluster 5.2 and *Cyanobium* are closely related to the *Synechococcus* cluster 5.1 (Herdman *et al.*, 2001). Isolates of *Synechococcus* cluster 5.2 and *Cyanobium* have been obtained from brackish as well as freshwater environments and it is therefore not surprising that species related to these groups were identified in the brackish Baltic Sea (Crosbie *et al.*, 2003; Ernst *et al.*, 2003).

So far, CCA type IV has not been detected among picocyanobacteria other than *Synechococcus* cluster 5.1. Hence, it did not come as a surprise that CCA was absent among the Baltic Sea *Synechococcus* strains that were isolated in the course of this study. Variation in pigmentation was observed within several PE-rich or PC-rich isolates from the Baltic Sea, but this variation was attributed to differences in nitrogen source (i.e., ammonium versus nitrate) and not to chromatic adaptation (Chapter 4). The absence of CCA type IV is further supported by the light regime in the Baltic Sea. Green light dominates throughout the photic zone and this is not absorbed by the PUB pigments used in CCA type IV.

### *Chromatic adaptation in Pseudanabaena.*

While the Baltic Sea *Synechococcus* isolates were negative with respect to complementary chromatic adaptation, many Baltic Sea isolates of the filamentous *Pseudanabaena* were able to

perform CCA type III. The *Pseudanabaena* isolates were divided into two groups with respect to pigment and CCA: a PC-rich group and the CCA+ group (Chapter 5). The CCA+ group comprises strains that are able to regulate the amounts of both the PE and PC pigments. In this respect the CCA+ *Pseudanabaena* species are similar to the *Tolypothrix* sp. used by Stomp *et al.* (2004) in their study of the competition and coexistence of differently pigmented cyanobacteria. Remarkably, every PE-rich *Pseudanabaena* isolate known and tested so far is capable to perform CCA type III, except for strain PCC 7367 (Castenholz *et al.*, 2001). The latter strain is also unique among *Pseudanabaena* because it was isolated from a marine intertidal sediment and it grows in full strength seawater medium ASNIII (Castenholz *et al.*, 2001). As far as known, all other *Pseudanabaena* isolates have been obtained from brackish and freshwater environments (Postius *et al.*, 2001; Stal *et al.*, 2003; Zwart *et al.*, 2005). It is not clear why no other PE-rich *Pseudanabaena* strains incapable of CCA type III have been isolated from the environment. If such strains do not exist it would suggest that CCA provides *Pseudanabaena* with a selective advantage over PE-rich species that are unable of chromatic adaptation.

Despite extensive research, the ecological role of CCA has not yet fully been elucidated (Kehoe and Gutu, 2006; but see Stomp *et al.*, in press). Detecting CCA in the environment is difficult, because cyanobacteria capable of CCA may be mixed with other phytoplankton with similar pigmentation. However, one study assigned the capability of type III CCA to isolates from the periphyton of macrophytes in the deep littoral zone but only to a few isolates from the pelagic zone (Postius *et al.*, 2001). In their study, the capability of type III CCA seemed to be connected to the presence of the *nifH* gene and anaerobic N<sub>2</sub>-fixation. In the deep littoral zone, biofilms are formed on macrophytes creating microhabitats characterized by low oxygen concentrations, poor nitrogen availability and strong light attenuation (Thomas *et al.*, 2006; Vis *et al.*, 2006; Uku *et al.*, 2007). Postius *et al.* (2001) concluded that the ability to perform anaerobic N<sub>2</sub> fixation in combination with CCA type III might give an advantage to organisms colonizing macrophytes because it would enable them to adapt to changing light and nutrient conditions that affect the growth of the organisms.

Changing light and nutrient conditions also occur in the Baltic Sea where stratification and mixing of the water column are important factors for the development of large cyanobacterial blooms (Janssen *et al.*, 2004). *Pseudanabaena* can become dominant in environments with high nutrient concentrations, irradiance levels and strong mixing (Chomerat *et al.*, 2007). In the Baltic Sea *Pseudanabaena* is part of the phytoplankton (Suikkanen *et al.*, 2005; Kangro *et al.*, 2007; Riemann *et al.*, 2008; this thesis) Under certain conditions *Pseudanabaena* can become dominant at the surface following a bloom of *Aphanizomenon* (Riemann *et al.*, 2008). Similar observations have been made in the German Melangsee (Mischke and Nixdorf, 2003). The exact reasons causing the succession from *Aphanizomenon* to *Pseudanabaena* are not clear. For the German Melangsee it was concluded that the collapse of the *Aphanizomenon* bloom released nitrogen into the lake waters which could be used by the smaller filamentous species *Limnothrix* and *Pseudanabaena* (Mischke and Nixdorf, 2003). A similar event might be possible in the Baltic Sea. Another reason for the succession from *Aphanizomenon* to *Pseudanabaena* could be the difference in buoyancy of the cells of the two species. Buoyancy

is regulated by the volume of the gas vesicles and the density of the cell, the latter depends mainly on the carbohydrate content (Visser *et al.*, 1995; Walsby, 2005). For *Limnothrix* spp. it was estimated that their sinking speed was less than 1 m per month (Walsby, 2005). This suggests that *Pseudanabaena*, which has a size comparable to *Limnothrix*, has a very slow sinking velocity. Moreover, *Pseudanabaena* possesses only 2 small gas vesicles at the polar ends of the cell and these are built of only one protein (gvpA), whereas other cyanobacteria require three proteins to make gas vesicles (Damerval *et al.*, 1991). The gas vesicles of *Pseudanabaena* may therefore serve another function than providing buoyancy. Larger PC-rich filamentous, heterocystous cyanobacteria like *Aphanizomenon* will float faster upwards and form surface blooms that change the spectral quality of light at greater depth. Under such conditions it might be beneficial for *Pseudanabaena* to use PE for light absorption. In contrast, when thriving at the surface, *Pseudanabaena* encounters a rather white light spectrum, and will thus benefit from a combination of PE and PC pigments. Accordingly, the capability of CCA might be beneficial because of the fluctuations in spectral quality encountered during vertical mixing in the water column.

In addition, *Pseudanabaena* might survive during periods of low nitrogen availability by means of N<sub>2</sub>-fixation. In *Pseudanabaena* N<sub>2</sub>-fixation has only been found under anaerobic conditions (Kallas *et al.*, 1985; Bergman *et al.*, 1997; Postius *et al.*, 2001). However, in the dark, microaerobic patches may be formed within aggregates of cyanobacteria creating a niche for anaerobic N<sub>2</sub>-fixation (Ploug *et al.*, 1997; Postius *et al.*, 2001; Tuomainen *et al.*, 2003). This might alleviate N-limitation in *Pseudanabaena* in surface waters. Together with their ability for CCA, this would enhance the competitive potential of *Pseudanabaena*. This suggests that *Pseudanabaena* spp. are opportunistic species that are capable to cope with a wide variety of environmental conditions.

In summary, the capacity of CCA type III or IV might be a prerequisite for cyanobacteria that migrate or are exposed to vertical mixing through the water column because they will experience considerable spectral changes in the underwater light. Obviously, vertical migration or mixing is not sufficient to explain the presence of CCA in cyanobacteria, since not all cyanobacteria show this ability. The Baltic Sea *Synechococcus* isolates were not able to perform CCA of any kind, while many of the *Pseudanabaena* isolates were capable of CCA. Factors such as the generation time, the speed of vertical mixing and/or vertical migration and the depth of the photic zone all influence the success of organisms and the benefits they may obtain from the ability of CCA.

## Bacterial speciation and ecotypes

In taxonomic studies, different species, ecotypes or strains are compared in order to assess their relatedness and evolutionary divergence. In microbial systematics and taxonomy this is achieved by analyzing gene sequences because of the lack of sufficient distinguishable phenotypic characteristics. The analysis of the 16S rRNA gene sequence allows quick identification of



newly isolated strains. However, molecular methods also revealed that closely related strains may display considerable phenotypical variation within what would be regarded as a single species based on their 16S rRNA sequence (Feldgarden *et al.*, 2003; Polz *et al.*, 2006).

In this thesis, we studied the genus *Synechococcus* and its closely related sister-genus *Cyanobium*. We observed extensive phenotypic differences between different isolates. In fact, the within-genus variation was at least as large as the variation between the genera *Synechococcus* and *Cyanobium*. This raises the question whether *Synechococcus* and *Cyanobium* should be pooled into the same genus. The same question can be posed for *Pseudanabaena* and *Limnothrix*. The close relation of *Synechococcus* with *Cyanobium*, and also of *Pseudanabaena* with *Limnothrix* emphasizes that the taxonomical descriptions for these genera are difficult to apply, and that assignment of strains to either genus is not straightforward.

### *Morphology, phenotypes and molecular data*

At the 16S rRNA level the genus *Synechococcus* is closely related to *Cyanobium*, while *Pseudanabaena* is closely related to *Limnothrix*. The use of the 16S rRNA gene to distinguish between the different genera is cumbersome because the phylogenies do not clearly separate the species (Crosbie *et al.*, 2003; Ernst *et al.*, 2003; Zwart *et al.*, 2005; this thesis).

Historically, taxonomists have described genera by using morphological, physiological and other phenotypic characteristics to discriminate species (Herdman *et al.*, 2001). For instance, pigmentation and cell elongation have been used as important descriptive characters to separate *Synechococcus* and *Cyanobium* (Komárek *et al.*, 1999; Rippka *et al.*, 2001). According to the description in Bergey's Manual of Systematic Bacteriology *Cyanobium* spp. do not produce phycoerythrin (PE), while *Synechococcus* species may (Boone and Castenholz, 2001). Within *Synechococcus*, strains are found that produce PE while other strains do not (Herdman *et al.*, 2001). However, in the newest taxonomic descriptions it is mentioned that PE can also be found among *Cyanobium* (Komárek *et al.*, 1999). These authors concluded that the PE/PC ratio among *Cyanobium* spp. is strain or species specific. Moreover, the phylogeny based on 16S rRNA gene and ITS sequences is not congruent with the possession of the genes for PE synthesis. Hence, pigmentation is a characteristic with little taxonomic value (Komárek *et al.*, 1999).

Using the *cpcBA* operon it was possible to phylogenetically separate PC-rich from PE-rich strains. In addition, a further separation of the latter into those producing PUB/PEB and those producing only PEB was also possible (Six *et al.*, 2007; Haverkamp *et al.*, 2008; this thesis). The phylogenies based on the *cpcBA* / *cpeBA* genes form clades of closely related sequences obtained from isolates from marine, brackish and freshwater environments with similar pigmentations (Chapters 3 and 4). This phylogenetic pattern is strikingly different from the pattern observed using the 16S rRNA gene which does not separate different pigmentation types (Chapter 3).

Recently, analysis of a number of cyanobacteria for which the whole genome has been sequenced indicated the existence of a core genome consisting of a set of highly conserved genes giving highly identical phylogenetic topologies (Shi and Falkowski, 2008). The PE and PC

genes are not part of the core genome, but belong to the variable part that exchanges among different closely related lineages, which is in line with our results (Six *et al.*, 2007; this thesis Chapter 4).

Cell elongation has also been used to differentiate between *Cyanobium* and *Synechococcus* (Komárek *et al.*, 1999; Jezberova and Komarkova, 2007). Cell elongation occurs in *Synechococcus* when grown under suboptimal conditions but not in *Cyanobium* (Jezberova and Komarkova, 2007). Furthermore, cell elongation is accompanied by asymmetrical cell division, while under optimal conditions division occurs symmetrically in *Synechococcus* (Komárek *et al.*, 1999). This indicates a close relationship between cell elongation and cell division.

Cell division is a highly coordinated process. The characteristics of cell elongation and asymmetrical cell division typical for *Synechococcus* but absent in *Cyanobium* could be the result of differences in the regulation mechanisms in these species when dealing with suboptimal conditions. Furthermore, it is known that different culture conditions can have profound effects on cell size and growth efficiency in *Synechococcus* (Burns *et al.*, 2005; Ernst *et al.*, 2005; Schwarz and Forchhammer, 2005; Burns *et al.*, 2006; Jezberova and Komarkova, 2007). In *Synechococcus* PCC7942 a large number of genes have been identified that are involved in the regulation of cell division (Koksharova and Wolk, 2002; Miyagishima *et al.*, 2005; Koksharova *et al.*, 2007). Knock-out mutants of these genes show different length phenotypes in PCC7492 under logarithmic growth (Koksharova and Wolk, 2002; Miyagishima *et al.*, 2005). The expression of many genes involved in processes such as protein synthesis, cell division, cell morphology, chromosome segregation, photosynthesis and carbon fixation, are either up- or down-regulated when genes involved in cell division are knocked-out in PCC7492 (Koksharova *et al.*, 2007). It should be noted that when regulatory genes become mutated this may have important consequences for the phenotype. Hence, mutations in regulatory genes and culture conditions affecting the phenotype and morphology make the use of such characteristics for taxonomic purposes cumbersome. Nonetheless, observations on phenotypes under laboratory conditions can be meaningful when the different phenotypes can be related to specific changes in culture conditions. For instance, *Synechococcus*-like cells grown on BG11 exhibited larger cell size compared to those grown on WC medium which contains much lower nutrient concentrations (Guillard and Lorenzen, 1972; Jezberova and Komarkova, 2007). In contrast with *Synechococcus*, one strain of *Cyanobium* did not show differences in cell size when grown on BG11 or the WC-medium (Jezberova and Komarkova, 2007).

These observations imply that, possibly, the environmental conditions separating *Cyanobium* and *Synechococcus* could involve the nutrient status of the ecological niches that they inhabit. *Cyanobium* might be better capable to cope with suboptimal conditions than *Synechococcus*. As to date, however, the ecological preferences for either species remain unclear. If *Cyanobium* prefers eutrophic or mesotrophic conditions and *Synechococcus* rather occurs in mesotrophic to oligotrophic environments, one might consider *Cyanobium* and *Synechococcus* as different nutrient ecotypes of the same genus. If this were the case, using the appropriate genes, one might be able to identify these ecotypes with higher confidence than when using the 16S rRNA gene.

Obviously, speciation among picocyanobacteria is affected by a range of environmental factors including spectral light quality, salinity and nutrient levels.

Another example is the *Pseudanabaena* / *Limnothrix* group that was investigated during this study. A polyphasic approach was applied in this study, combining morphology and molecular data, in order to determine the phylogenetic relationships of our isolates with known strains. *Pseudanabaena* / *Limnothrix* show overlap in their phenotypic characters (e.g. cell size, pigmentation, etc.) (Komárek, 2003). At the molecular level, the 16S rRNA gene sequences indicate that *Pseudanabaena* and *Limnothrix* are polyphyletic and strains with different taxonomical assignments appear in monophyletic clusters (Herdman *et al.*, 2001; Zwart *et al.*, 2005; Chapter 5). Using highly divergent sequences such as the ITS and the *cpcBA*-IGS region, several clades of the *Pseudanabaena* / *Limnothrix* cluster were found with different cell morphologies and geographical distributions (Chapter 5). Some of these clusters contain only strains with *Limnothrix*-like morphologies, while others contained isolates with morphologies characteristic of both *Pseudanabaena* and *Limnothrix*. These results indicate that, despite our detailed molecular analysis, it was still impossible to give a proper taxonomical assignment of our isolates. The small morphological differences between *Pseudanabaena* and *Limnothrix* could be the result of genetic or environmental differences that affect morphology. Unfortunately, experimental data describing the effect of environmental conditions on the morphology of *Pseudanabaena* and *Limnothrix* is lacking.

### *Species and ecotypes*

The use of combined molecular or morphological and physiological characteristics may be confusing when attempting to understand the taxonomy and speciation of closely related microorganisms. This is especially true when only a few genes are used to delineate species. Genomics and multi-locus sequence approaches could improve the resolution for discriminating species.

Ideally, a polyphasic taxonomy of microorganisms using molecular as well as morphological and physiological phenotypic characteristics as complementary tools should produce similar phylogenies displaying the same bifurcations in the phylogenetic tree. For the cyanobacteria investigated in this thesis it was found that the species relationship at one locus differed from that at another shared locus. One explanation for this observation is that different genes may be under different selective forces causing different evolutionary patterns between closely related species. Other processes such as lateral gene transfer, recombination and gene exchange through conjugation or viral intermediates could also disturb clonal propagation of species.

Analysis of the available microbial genomes indicates that, within the cyanobacteria and especially between the closely related genera *Synechococcus* and *Prochlorococcus*, extensive lateral gene transfer must have taken place (Beiko *et al.*, 2005; Zhaxybayeva *et al.*, 2006; Shi and Falkowski, 2008). Analysis of the *Prochlorococcus* and *Synechococcus* genomes indicates that extensive reshuffling of genes has taken place (Hess, 2004). Since *Cyanobium* is sister to *Synechococcus* and *Prochlorococcus*, it can be expected that also between these genera extensive lateral gene transfer might have occurred. This could have obscured the possible boundaries between these species. The identification of core genes in cyanobacterial isolates and using them

for MLSA typing may help to solve taxonomical questions as has been done for pathogenic prokaryotes (Zhaxybayeva *et al.*, 2006; Shi and Falkowski, 2008).

Cluster 5 *Synechococcus* and *Cyanobium* spp. are closely related, they have only small and often overlapping morphological and other phenotypic differences, and equally important, they have ecologically similar or overlapping roles in the environment (Komárek *et al.*, 1999; Ernst *et al.*, 2003; this thesis). Therefore, we suggest that they should be classified as different ecotypes within the same genus (Konstantinidis and Tiedje, 2005; Cohan and Perry, 2007). The differences between *Pseudanabaena* and *Limnothrix* are equally small as within the picocyanobacteria. Therefore *Pseudanabaena* and *Limnothrix* might also be classified as one genus comprising closely related ecotypes. These ecotypes could be assigned based on their response to light, salinity and nutrients (Ahlgren and Rocap, 2006; Zwirgmaier *et al.*, 2008; this thesis). Obviously, much more ecological, physiological, and genetic data is required from many more isolates of the *Synechococcus/Cyanobium* and *Pseudanabaena/Limnothrix* groups in order to solve this taxonomical conundrum.

## Conclusions

Cyanobacteria are a monophyletic group of phototrophic organisms with considerable variation in genetic, physiological and morphological characteristics. Their extensive diversity allows cyanobacteria to thrive in many different habitats occupying a large variety of ecological niches. One important selective factor creating different niches in pelagic habitats is the color of light in the euphotic zone. Cyanobacteria with different photosynthetic pigments are capable of occupying niches characterized by different light colors. Here it was shown how the relative abundance of red (PE-rich) and green (PC-rich) picocyanobacteria varies with the underwater light spectrum. Although these differently pigmented picocyanobacteria cannot be distinguished on the basis of their 16S rRNA gene sequences, they can be distinguished using the genes encoding PC and PE proteins. For *Pseudanabaena* species it was found that the non-coding regions of these genes separated groups according to their geographic distributions. However, much more knowledge on the ecology of the different *Pseudanabaena* geotypes is required in order to determine their ecological distinctiveness and dispersal capabilities and to understand their success in many freshwater environments.

Taken together, the results presented in this thesis show that highly divergent gene sequences can be used to identify distinct ecotypes. This is only possible when DNA sequences can be linked to well-defined phenotypes, which requires the availability of cultured strains.

