

琉球大学学術リポジトリ

沖縄の黒い気生シアノバクテリアの多様性、分子系統と分類

メタデータ	言語: 出版者: 琉球大学 公開日: 2018-04-12 キーワード (Ja): キーワード (En): 作成者: Nguyen, Xuan Hoa, ゲィン, スァン ホア メールアドレス: 所属:
URL	http://hdl.handle.net/20.500.12000/39225

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Dissertation Title: Diversity, molecular phylogeny and taxonomy of black terrestrial cyanobacteria from Okinawa

SUMMARY

The limestone walls of Shuri Castle represent one of the blackened buildings in Okinawa, and such monuments also include Angkor Wat in Cambodia, the Mayan ruins in Mexico, and many other blackened monuments or buildings all over the world. These black stains have been caused mainly by the growth of terrestrial cyanobacteria. Studies on black terrestrial cyanobacteria are worldwide in scope but many of them are only morphological observations and classification, and available culture strains are limited. In Okinawa and Japan, almost no studies of terrestrial cyanobacteria, especially molecular phylogeny and taxonomy, have been conducted. In this work, I first examined diversity of terrestrial cyanobacteria using molecular phylogenetic and morphological observations of culture strains established from specimens collected within the campus of the University of the Ryukyus. In the next chapter, a very black colored strain from Iriomote Island was studied. Next, the type species of *Porphyrosiphon* was discovered from Kochi and was examined to determine its phylogenetic position. Finally, general conclusions, future studies and possible applications will be discussed.

Current situation of cyanobacterial taxonomy

Recently, a number of new genera and taxa have been proposed for cyanobacteria using a polyphasic approach. Within the past five years, over 40 genera have been proposed. To do so, detailed morphological observations with related taxa and especially type species are now necessary. As well, a 16S rRNA gene sequence should be obtained. If the type and the undescribed taxon are at the same position (same clade) in the phylogenetic tree, they obviously must belong to the same genus. If morphologically related but genetically different undescribed taxon exist, the undescribed specimen becomes a new genus. Hence, culture strains and data of the type species are very important now.

Establishment of terrestrial cyanobacteria strains from campus of University of the Ryukyus (Figs. 1 and 2).

Terrestrial cyanobacterial strains were isolated from the Nishihara campus of the University of the Ryukyus, Okinawa, Japan. The 13 sampling sites were distributed in a 200m radius and appeared as dry blackened stains. From these small areas, 143 cyanobacterial strains were established. The strains were divided into five morphotypes, including unicells, unicells with baeocytes, non-blanching filaments, false blanching filaments, and heterocystous strains (Fig. 2). From the strains, 105 partial 16S rRNA gene sequences were obtained and could be classified into 30 generic types. These data suggest that the terrestrial cyanobacteria are very diverse even within small sampling areas such as within the campus of the University of the Ryukyus. The established strains are not only important for classification of terrestrial cyanobacteria but also for possible application studies in the future.

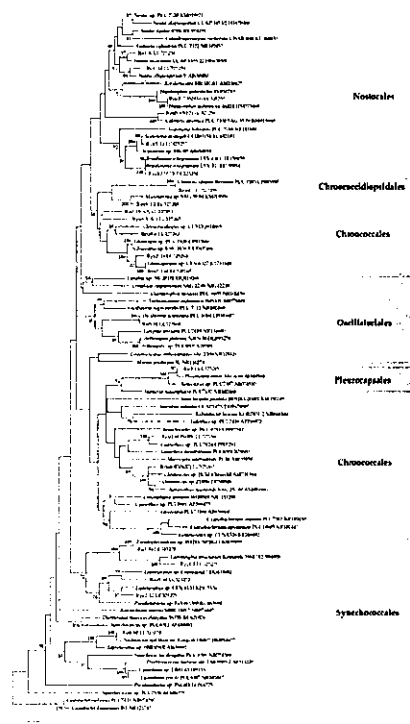


Fig. 1. ML based on 16S rDNA gene sequences of terrestrial cyanobacteria from Nishihara campus of university of the Ryukyus, Okinawa

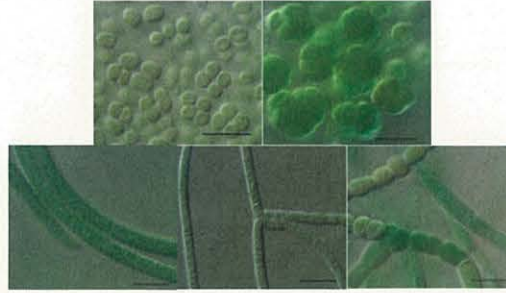


Fig. 2. Example of terrestrial cyanobacteria from Nishihara campus of university of the Ryukyus

Very black strain IRH12 from Iriomote Island (Figs. 3 and 4)

Terrestrial black filamentous cyanobacterium IRH12 was isolated from Iriomote Island. The sampling site was a fish statue along the roadside in the northern part of the island. A strain was established and kept by agar slant culture, and notably the old slant of strain IRH12 was very blackened, and appeared very different from other strains. Strain IRH12 was a filamentous cyanobacterium with a thin hyaline sheath with false branching, the apical cells rounded, and filaments were slightly narrowing. Morphologically, the strain was classified into *Leptolyngbya* or *Pseudophormidium*. Phylogenetic analyses revealed that the closest relatives were *Leptolyngbya boryana* (LC215287) and *Plectolyngbya* sp. (KT899565), but both sequence identities were only 95%. These data suggest that strain IRH12 is an undescribed genus and species of filamentous cyanobacterium.

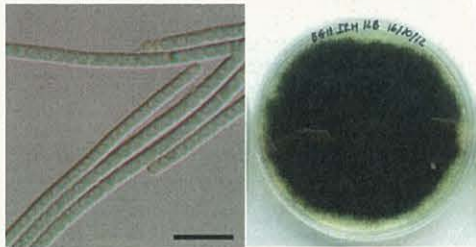


Fig. 3. Black terrestrial cyanobacterium IRH12 from Iriomote Island:

A: Morphology, Scale bar = 10 μ m,
B: Cultivation in petri dish agar

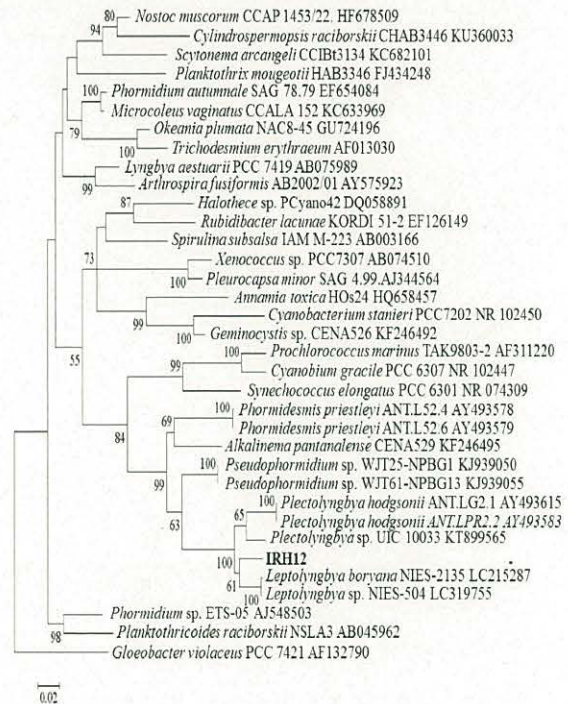


Fig. 4. ML tree based on 16S rRNA gene sequences of IRH12 from Iriomote Island.

Phylogenetic position of type species of *Porphyrosiphon* (Fig. 5)

One sample was collected from Kochi Prefecture and we established a culture strain. Morphologically, the sample was identified as the type species of *Porphyrosiphon*, *P. notarisii* (LC331913), and our phylogenetic results showed the strain has no closely related cyanobacteria relatives.

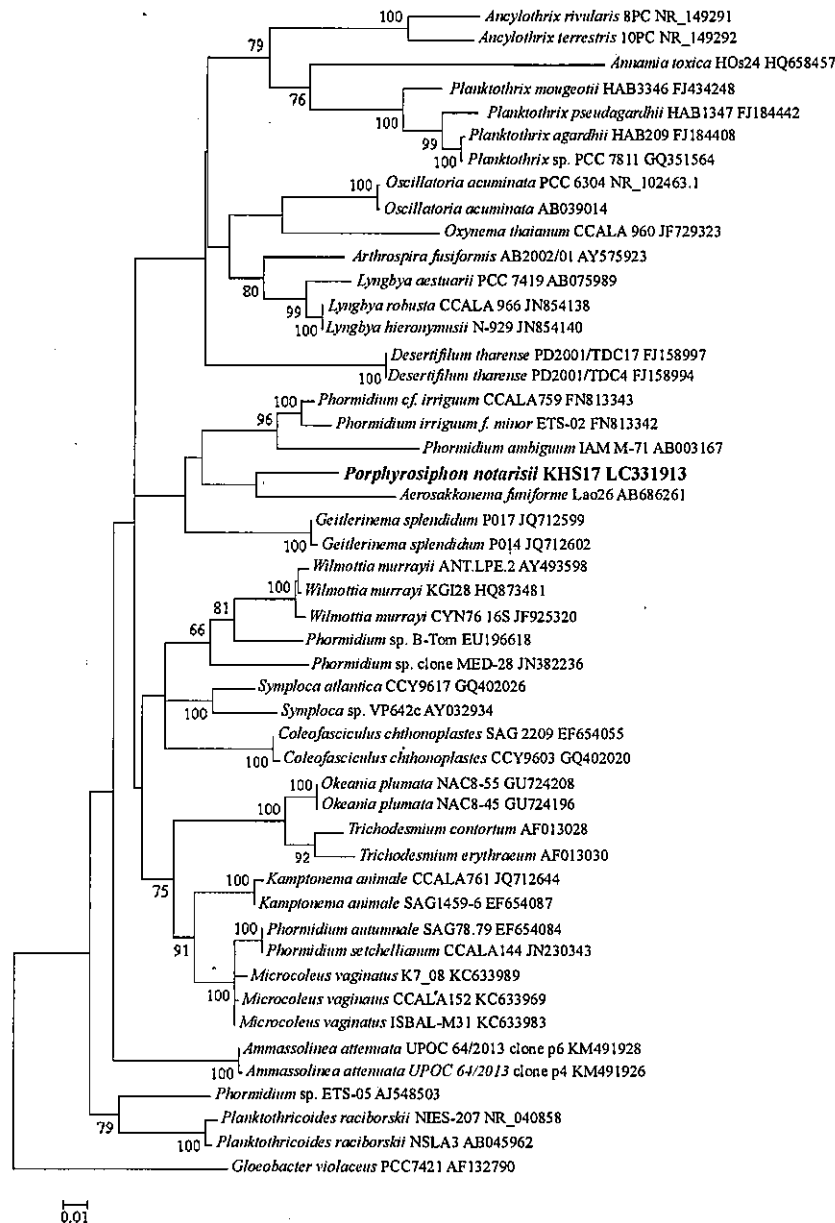


Fig. 5. ML tree based on 16S rRNA gene sequences of *Porphyrosiphon notarisii* from Kochi

General discussion

This study has provided novel and substantial contribution to our knowledge in understanding the diversity, molecular phylogeny and taxonomy of terrestrial cyanobacteria. The newly established strains may contribute to some applied research in the future.