HAPALOSIPHON FONTINALIS (C. AGARDH) BORNET, A NEW RECORD OF STIGONEMATALEAN CYANOPHYTA FOR ALGAL FLORA OF IRAN

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In this study, a new morphospecies, *Hapalosiphon fontinalis* (C. Agardh) Bornet (*Stigonemataceae, Stigonematales*) is reported for Iran. Identification was done in a multidisciplinary way including morphological variations in relation to pH and carbon dioxide concentration fluctuations at limited irradiance (2 µE m^{-2} s^{-1}). Regarding biological versatility of Cyanophyta, it has been tried to emphasize on the most prominent traits for identification and determination. A new description of the species has been presented regarding morphological characterization in Golestan Province.

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Key Words. Cyanophyta, Hapalosiphon, Iran, Paddy-Field, Taxonomy.

گزارش یک گونه جدید از سیانوفیتای راسته Stiginematales برای فلور Hapalosiphon fontinalis (C. Agardh) Bornet جلبکی ایران

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در این مطالعه، یک گونه جدید *Hapalosiphon fontinalis* (C. Agardh) Bornet از خانواده و راسته *Stigonemataceae*، *Stigonematales* برای اولین بار از ایران گزارش شده است. شناسایی با روش چند وجهی شامل تنوع مورفولوژیکی در ارتباط با تغییرات اسیدیته و غلظت دی‌اکسید کربن در شرایط محدود نوری (۲ µE $m^{-2}s^{-1}$) صورت پذیرفت. نظر به تنوع بیولوژیکی سیانوفیتا، سعی بر تاکید بر ویژگی‌های غالب برای شناسایی بوده است. در این مقاله توصیفی جدید از گونه *Hapalosiphon fontinalis* در ارتباط با ویژگی‌های مورفولوژیکی در استان گلستان بیان شده است.

Introduction

The species of stigonematalean cyanophytes are distributed all over the world (Anagnostidis & Komárek, 1990). Many populations of stigonematales show considerable morphological variation (John et al. 2002). However a combination of traditional and modern taxonomy (in addition of physiology and biochemistry) is needed to determine their exact taxonomy.

It seems that in north paddy fields of Iran, especially Golestan province, some strains of stigonematales are common and even dominant (Siahbalaie et al. 2008, Soltani et al. 2010; Shokravi et al. 2002) but there is no clear report about their morphological characterizations and taxonomic situations. Morphological variability, degree of polymorphism and geographical variation in form of the *Fisherella*, *Hapalosiphon* and *Stigonema* make some problems in studying of these organisms. The proposed experiments in the study have shown that

using famous common manuals for determination of the genus *Hapalosiphon* like the other *Stigonematales* have no useful results and seriously need to revise these manuals or even identification keys with regard to special morphological variations of specimens with emphasize on local conditions (Soltani et al. 2010).

In recent years, molecular identification by partial sequence of 16S rRNA gene has regrouping of cyanophyta in some cases, correspond to phylogenetically coherent taxa (Shokravi et al. 2007) whereas in other grouping the traditional classification drastically underestimates extant diversity (Gugger & Hoffman 2004).

In bacteriology, in particular the tolerances to the carbon dioxide concentrations of the environment have been recognized as an important phenotypic properties correlating with phylogeny (Gugger & Hoffman 2004). Light is evidently one of the most important factors for natural distribution of cyanophyta (Valiente & Leganes 1989). Additionally pH is the other factor that clearly affects the distribution of cyanophyta. Most cyanophyta grow in environments that are neutral to alkaline, and in laboratory cultures, the optimal pH ranges from 7.5 to 10. Generally, a wide range of adaptation to pH has been observed not only among different genera but also between different isolates of the same species (Poza-Carrion et al. 2001; Soltani et al. 2006). The aim of this research was to characterize the exact taxonomical position and to determine a comprehensive description of mentioned species. However, a comparative study on the morphology needs more consideration regard to the variations cause by illumination and combined effect of pH and carbon dioxide concentration. We propose the clearly branched trichoms for revised description at species level of the mentioned cyanobacterium in the paddy- field habitat.

Material and Methods

Soil samples were obtained from paddy fields of different stations of Golestan province in the north of Iran. A complete description about stations and their geographical and environmental conditions have been reported in Siahbalaei et al. (2010). The collected soils were cultured by usual methods (Andersen 2005). After colonization and isolation, the blue- green algae *Hapalosiphon* sp., was purified and incubated in axenic condition (Andersen 2005). At this phase, it was coded as *Hapalosiphon* sp. FS 56 and preserved in algal culture collection of ACECR of Shahid Beheshti University. Stock cultures were grown in BG110 solid and liquid medium ($NaNO_3$, 17.65 mM; $MgSO_4.7H_2O$, 0.3 mM; $CaCl_2.2H_2O$, 0.25 mM; $K_2HPO_4.3H_2O$, 0.18 mM; $Na_2MgEDTA$, 0.003 mM, Citrate ferric ammonium, 0.02 mM; Acid Citric, 0.029 mM; Na_2CO_3 0.188 mM; microelements 1 ml l^{-1}). The cultivation was done under limited illumination (2μE m^{-2} s^{-1}), a gradient of pH (5, 6, 7, 8, 9) and carbon dioxide concentrations (without aeration, with 200 ml min^{-1} aeration). The temperature was adjusted on 30 ± 1 ºC. Illumination was supplied with 40W cool white fluorescent tubes. Plates were placed at different distances from the light source to obtain a linear gradient of irradiance**.** Light measurements were made with a Licor LI-1000 Datalogger equipped with a quantum sensor. Alternatively, other experiments were carried out in batch cultures, using 300 ml of inoculated medium in 500 ml. Erlenmeyer flasks stoppered with cotton plugs. Culture was maintained without aeration or stirring and buffered and illuminated as above. After 48h of inoculation, when cells were fully adapted to culture condition, light regime, pHs and carbon dioxide concentrations, aliquots were taken and used for determinations. Morphological observations were made in liquid as well as on solid media. Thallus growth, filament structure and biometrical characteristics were recorded (Gugger & Hoffmann 2004). Colony formation and cells shapes were evaluated by binocular and light microscope (in addition to phase contrast and epifluorescence microscopy) each day in two weeks. The growth curves were obtained via measurement of chlorophyll change daily using Jensen method (1978). Statistical analysis was done using software SPSS ver.10. Identification at the species level was done according to John et al. (2002), Anagnostidis and Komarek (1990), Tiffany and Britton (1971), Prescott (1962), Desikachary (1959) and Tiffany (1958).

Result

Aggregations of the strain tend to make a creeping manner of growth especially in solid cultures (Fig. 1). In liquid cultures tendency to make a crustose form may be dominated. In both cases, intensive tendency to produce microbial mats seems noticeable (Stal 1995). This behaviour seems interesting and may be regarded as a constitutive trait. The form of branches, either unilateral or bilateral may be remained at liquid and solid cultures and have not been influenced by pH, carbon dioxide and irradiance fluctuations. Therefore may be regarded as a constant trait for identification (Fig. 2a). Branching is quite different from *Westiella* groups and seems near to *Fischerella*/*Hapalosiphon* kinds of branching (Fig. 3).

For instance, fluorescence microscopy analysis, showed a relatively thin sheath at pH 9 and relatively limited carbon dioxide concentration conditions (Fig. 4) but not at acidic conditions or high level of carbon dioxide concentrations. Heterocysts may be affected by environmental fluctuations. The form and size of

Fig. 1. Aggregations of *Hapalosiphon fontinalis*.

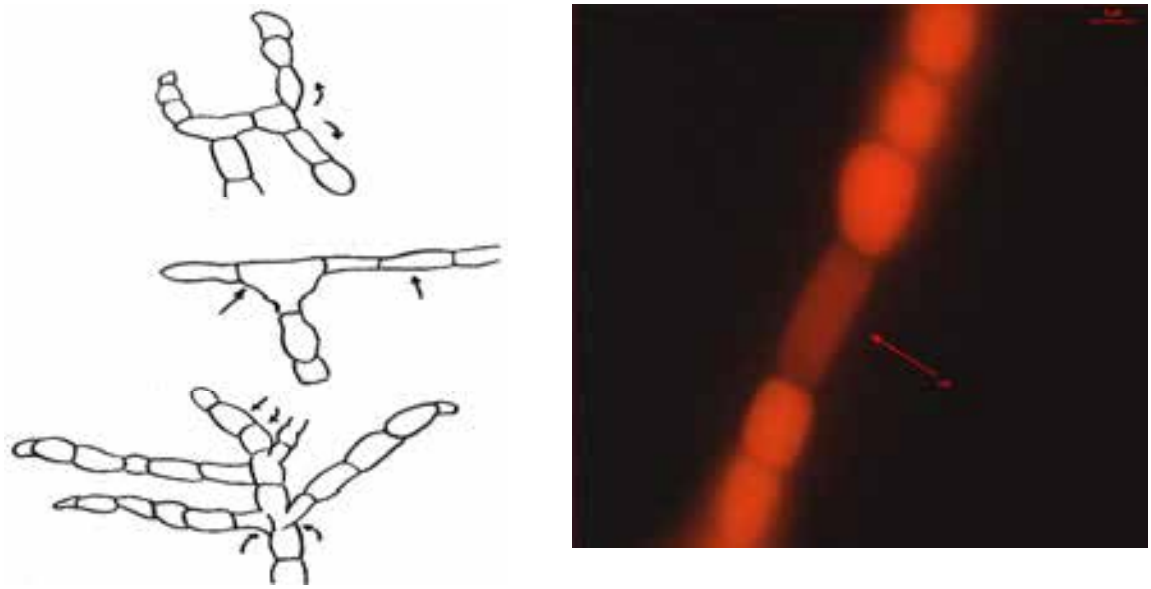

Fig. 2. Branch forms (left) and hetrocyst with fluerosence microscopy (right) of *Hapalosiphon fontinalis*.

heterocyst may be depended on environmental conditions but usually it tends to keep cylindrical or oval-cylindrical form.

Biometrical analysis (Tables 1 & 2), showed the length and the diameter of the vegetative cells and spores are slightly more than that have already been recorded by John, et al. (2002) and lower than Desikachary (1959). At this species tendency to make spherical and sub-spherical spores seems dominant and possibly constitutive trait, but in contrast, vegetative cells showed a wide flexibility and change their form from quadrate to oval and oval-cylindrical (and even spherical).

Discussion

Research on cyanophyta is a new field of study in Golestan province and Iran. So just a few stigonematalean morphotypes have been cultured and

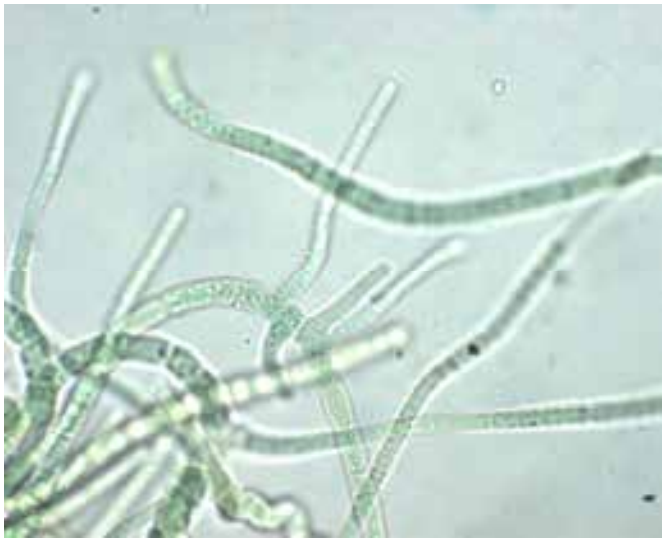

Fig. 3. Branch formation in *Hapalosiphon fontinalis.*

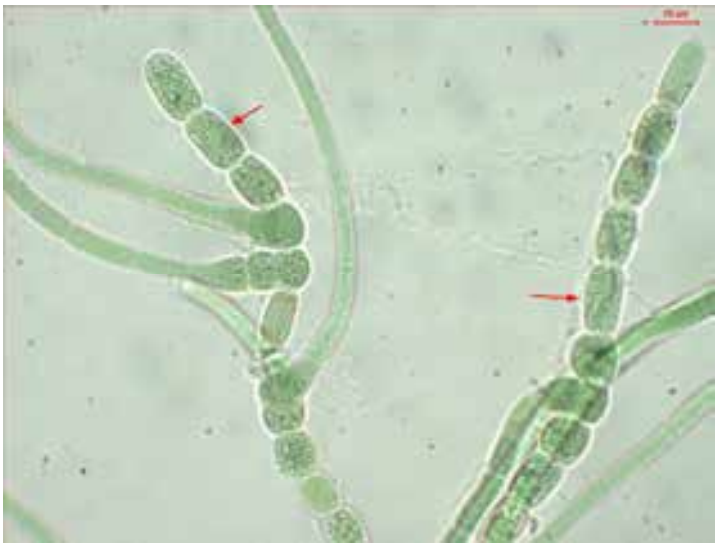

Fig. 4. Sheath of *Hapalosiphon fontinalis* (Fluerosence microscopy).

investigated, therefore the high variability of morphotypes found in nature is not represented in culture (Shokravi et al. 2002). Only some genera of stigonematalean and nostocalean cyanophyta have been characterized from axenic culture strains yet, including some strains of *Fischerella* and *Nostoc* (Soltani et al. 2006, 2007; Ramzannejad-Ghadi 2008; Zarei-darki 2009; Siahbalaei et al. 2010; Shokravi 2003; Sepehri & Nejad- Satari 2003). Results could show a primitive picture of the morphological and taxonomical situation of stigonematalean cyanophyta in paddy-fields of North Iran. These organisms showed relatively variable characters in morphological point of view. It seems pH, irradiances and carbon dioxide fluctuations caused noticeable changes in the morphology of the organism. It seems that pHs above 9 and below 5 affected the variations. On solid medium, all isolates had a creeping growth. This was in agreement with other papers (Perrona et al. 2003).

By statistical analysis, it is difficult to reach a unique pattern in morphological variation in vegetative cells of this strain. However, with this exception (cross expanding of the main axis), possibly high alkalinity (pH 9), has been caused noticeable morphological variations especially in carbon dioxide limited conditions. In this condition, cyanophyta tends to get a different topological configuration. In minimum light intensity (2 $\mu E\ m^{-2}\ s^{-1}$) and pH 9 cross enlargement of the trichome was seen. However, results showed that these organisms could be considered an alkalophilic

Table 1. Vegetative cell dimensions after the 6th day of inoculations (*um*) at different pH and carbon dioxide concentrations in *Hapalosiphon fontinalis*.

CO2	pH 5	pH 6	pH 7	pH 8	pH 9
NA	17 x 8	13.2 x 7.2	12.5 x 6.5	12.5 x 8	9 x 8
AE	13.5 x 8.75	12 x 8	13.5 x 9	11.5 x 8	11 x 5.25
CDE	14.5 x 9	14 x 6	9.5 x 7.5	13.5 x 6.5	10.5 x 7.75

NA; no aeration, AE: Aeration, CDE: Carbon dioxide enrichment

Table 2. Spores cell dimensions at the main axes after the 6th day of inoculations (*um*) at different pH and carbon dioxide concentrations **in** *Hapalosiphon fontinalis*.

CO2	pH 5	pH 6	pH 7	pH 8	pH 9
NA	17 x 8	13.2 x 7.2	12.5 x 6.5	12.5 x 8	9 x 8
AE	13.5 x 8.75	12 x 8	13.5 x 9	11.5 x 8	11 x 5.25
CDE	14.5 x 9	14 x 6	9.5 x 7.5	13.5 x 6.5	10.5 x 7.75

NA: no aeration, AE: Aeration, CDE: Carbon dioxide enrichment

organism. Optimal growth rates were observed at pH 9 which is nearly equal to pH that usually found in the rice fields from which the cyanophyta was isolated (pH 8.8) (Soltani et al. 2007).

Regarding the above, *Hapalosiphon fontinalis* and *Stigonema minutum* are two candidates that may be considered using identification keys for determination. In *Stigonema minutum*, main filaments is uniseriate, lateral branches show turf like appearance and filaments seems near biometrically (30-40 µ wide). Multiseriates main axis was not seen. In addition, turf like appearance may be affected strongly by environmental conditions especially acidity and limited carbon dioxide concentrations. In contrast, completely uniseriate main axis, long lateral branches, and thin colorless sheath, and a *Hapalosiphon hibernicus* like biometry and spherical spores, are strong evidence to choice *Hapalosiphon fontinalis* for determination though there is small difference in length and diameter of the vegetative cells both at the main axis and branches. It seems that sheath production in this species may be affected completely by environmental fluctuations. However, sheath breath is not as large as have been reported by John et al. (2002) that may be related to environmental fluctuations. Accordingly, more realistic polyphasic description of the species may be as the following.

Hapalosiphon fontinalis (C. Agardh) Bornet (Bornet; 1849, P, 894; emend. Borzi, 1917, p.90).

Thallus nonmucilaginous, aggregations mostly brown, rarely green specially at the first days after inoculation, creeping, branched, uniseriate trichomes; sheath not permanent, if present close and firm but irregular and present at different acidic and alkaline conditions depend on carbon dioxide concentration, unlamellated, or a close, firm sheath; The uniseriate trichome, bearing true branches which rarely branches, branching usually at right angles, branch vegetative cell broad 2.2- 5.3µ; branches unilateral and bilateral independent on the pH and carbon dioxide condition; cells cylindrical, subcylindrical and obovoid, 2.2-5.3 x 5.5 µ; sometimes elliptical and constricted at the cross walls; heterocysts quadrate–cylindrical, the contents homogeneous, 4.9 x 10 µ; the highest growth rate in alkaline conditions, pH 9 and relatively limited carbon dioxide concentration, be able to grow at a wide range of pHs from extremely acidic to alkaline; having a powerful bilateral CO_2 concentrating mechanisms; spores spherical and subspherical with bilayer envelopes, numerous when pH and carbon dioxide and irradiance flactuate, 16-40 µ in diameter and 12-44 µ in length; spores spherical and with a bilayer envelope.

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