

***Cylindrospermum voukii* and *Wollea saccata*, new records for algal flora of Iran**

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**Zeinab Shariatmadari:** Assistant Prof., Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, G.C. Tehran, Iran**Maedeh Davari:** MSc Student, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, G.C. Tehran, Iran**Hossein Riahi**✉: Prof., Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, G.C. Tehran, Iran (h-riahi@sbu.ac.ir)**Zakieh Modaressi:** MSc Student, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, G.C. Tehran, Iran**Abstract**

Cyanobacteria represent a small taxonomic group of photosynthetic prokaryotes which some of them are capable of N<sub>2</sub> fixation and also possess a tremendous potential for producing a wide range of secondary metabolites. It has been suggested that, some of these microorganisms, especially heterocystous cyanobacteria, assist higher plant growth by supplying growth stimulating substances. During a floristic study of algae in the terrestrial habitats of Iran, cyanobacterial taxa presented in natural habitats of a medicinal plant (*Tanacetum parthenium*), located in Lorestan province (Iran) is isolated and identified. Among identified taxa, two species, *Cylindrospermum voukii*, and *Wollea saccata* are reported as new records from *Nostocaceae* family in Iran. Beside description of the new taxa, general distribution, as well as camera lucida images are included herewith.

**Keywords:** Blue-green algae, cyanobacteria, diversity, *Nostocaceae*, terrestrial habitat**معرفی دو گونه جدید *Cylindrospermum voukii* و *Wollea saccata* برای فلور جلبکی ایران\***

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زینب شریعتمداری: استادیار دانشکده علوم و فناوری زیستی، دانشگاه شهید بهشتی، تهران، ایران

مائده داوری: کارشناسی ارشد، دانشکده علوم و فناوری زیستی، دانشگاه شهید بهشتی، تهران، ایران

حسین ریاحی✉: استاد دانشکده علوم و فناوری زیستی، دانشگاه شهید بهشتی، تهران، ایران (h-riahi@sbu.ac.ir)

زکیده مدرسی: کارشناسی ارشد، دانشکده علوم و فناوری زیستی، دانشگاه شهید بهشتی، تهران، ایران

**خلاصه**

سیانوباکتری‌ها گروهی از موجودات زنده فتوسنتز کننده دارای ساختار سلولی پروکاریوت هستند که برخی از آن‌ها از قابلیت تثبیت نیتروژن و توانایی تولید طیف وسیعی از متابولیت‌های ثانویه تحریک کننده رشد گیاه برخوردارند. تا آنجا که برخی نمونه‌ها، بویژه گونه‌های دارای سلول‌های اختصاصی هتروسیست، قادرند شرایط رشد گیاهان عالی را بهبود بخشند. با توجه به اهمیت کاربردی این گروه از موجودات زنده فتوسنتز کننده و نیز با توجه به اهمیت مطالعه فلورستیک جلبک‌های موجود در اکوسیستم‌های خشکی ایران، سیانوباکتری‌های خاکزی موجود در بستر رویشی برخی از جمعیت‌های گیاه دارویی *Tanacetum parthenium* واقع در استان لرستان جداسازی و شناسایی شدند. از میان گونه‌های شناسایی شده، دو گونه *Cylindrospermum voukii* Pevalek و *Wollea saccata* Bornet & Flahault از تیره نوستوکاسه به عنوان گونه‌هایی جدید برای فلور جلبکی ایران گزارش می‌شوند. همچنین در این مطالعه، موقعیت جغرافیایی دقیق، ترسیم و نیز شرح گونه‌های معرفی شده ارائه می‌گردد.

**واژه‌های کلیدی:** اکوسیستم خشکی، تنوع، جلبک سبز-آبی، سیانوباکتری، نوستوکاسه

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## Introduction

Algae are an important part of the surface soil and play a special role in the soil health and dynamics (Metting 1981). Blue-green algae or cyanobacteria are one of the most important and most efficient groups of algae, because they can affect the physical, chemical and biological properties of the soil. In addition, they play a special role in the growth and productivity of plants due to their ability to stabilize nitrogen and the production of growth-promoting metabolites (Shariatmadari *et al.* 2013). Cyanobacteria are also capable of releasing complex organic carbon compounds in the rhizosphere of plants and improve soil density, texture, permeability, and water holding capacity (Chamizo *et al.* 2018, Kaushik 2007).

Due to the importance of cyanobacteria in soil texture optimization and the improvement of plant growth conditions, accurate recognition of cyanobacterial taxa presented in the soil is very important. So, there is an attempt to identify and introduce efficient cyanobacterial taxa, especially strains with practical and economic value (Shariatmadari *et al.* 2013, Seyed Hashtroudi *et al.* 2013a, b, Heidari *et al.* 2018).

In the present study, therefore, cyanobacterial taxa presented in rhizosphere of some medicinal plant populations (*Tanacetum parthenium*), were identified. In this paper, two new species of *Nostocaceae* family from Lorestan province (Iran) are reported and described.

## Materials and Methods

### - Studied sites

Soil samples were collected in the summer 2012–13 from natural habitats of *Tanacetum parthenium* located in Lorestan province (Darr-e Durak village, 33° 11' N 49° 56' E) of Iran, according to Rangaswamy method (1966).

### - Identification of cyanobacterial species

The sieved soils were transferred to sterile Petri dishes containing sterile liquid nitrate free BG-11 medium (Stanier *et al.* 1971). The Petri dishes were incubated in a culture chamber at 25±2 °C for two weeks under artificial light illumination (74 μmol photons m<sup>-2</sup> s<sup>-1</sup>) with a 12/12 hr light-dark cycle. After colonization, isolates were

transferred to agar plates for purification. For taxonomic determination, the semi permanent slides of colonies were prepared and morphometric study was performed by light microscopy (Olympus, Model BH-2), based on some valid references such as Desikachary (1959) and Komárek (2013). The microscopic images were drawn by means of camera lucida (Olympus, Japan).

## Results and Discussion

A survey on cyanobacterial flora of the natural habitats of *Tanacetum parthenium* in Lorestan province revealed that, despite the rocky habitat of this plant in the study area, several taxa of cyanobacteria can be seen in these environments. Selection of efficient characteristics for identification of these taxa was an important step in identification process. In general, characteristics such as thallus structure, akinete number, position, size, and shape of akineses were among the most important determinant characteristics. Comparison of taxonomic characteristics of two isolates with previously identified taxa revealed that two nostocacean morphospecies in the present study can be considered as new records for Iran. The descriptions of these taxa are as follows:

### 1. *Cylindrospermum voukii* Pevalek 1916 (Fig. 1a)

Thallus mucilaginous, dark green. Trichomes constricted at the cross walls, with pale blue-green in color. Vegetative cells barrel-shaped or slightly longer than broad, 3–4 μm broad, 4–5 μm long. Heterocysts ovoid or obovoid, 4 μm broad, 7–8 μm long. Akinetes oblong, 5–8 μm broad, 20–27 μm long, with colorless exospores.

General distribution: Bulgaria (Stoyneva-Gärtner *et al.* 2015), and Macedonia (Krstić & Aleksovski 2016).

The genus *Cylindrospermum* consisting at least 52 accepted taxonomically species, which are characterized by filaments with special cells or heterocysts and akinetes. Heterocysts always terminal, developing at both ends of trichomes. Akinetes develop only close to heterocysts at both trichome ends, solitary or in rows, sometimes with sculptured outer cell wall layers (Guiry & Guiry 2019).

The species of this genus can be seen in several habitats, including aquatic or terrestrial ecosystems. Some species of this genus grow in soils with a low or high humidity such as agricultural soils. Previously, various species of this genus have been reported from paddy fields of Iran (Shariatmadari & Riahi 2012). One of the distinguishing characteristic of this isolate is its long and narrow akinetes. *Cylindrospermum zonatum* Komárek, and *C. minutissimum* Collins are other taxa of this genus with cylindrical akinetes (Komárek 1989). But length/width ratio of akinetes in our taxon is far more than these taxa and is consistent with the *Cylindrospermum voukii* (3.7–4 µm, respectively). In general, characteristics such as the shape and size of akinetes as well as epispore color can be considered as the most important characteristics separating several taxa in this genus.

The main morphological characteristics which separate *Cylindrospermum voukii* from another species previously reported from terrestrial habitats of Iran are presented below (Shariatmadari & Riahi 2012):

*Cylindrospermum voukii* (oblong akinete with colorless epispore), *C. catenatum* Ralfs ex Bornet & Flahault (ovate akinetes with yellowish brown epispore), *C. minutissimum* Collins (cylindrical akinete with colorless epispore), *C. marchicum* (Lemmermann) Lemmermann (ovate to barrel-shaped akinetes with colorless epispore), *C. michailovskoense* Elenkin (ovate akinete with colorless epispore), *C. muscicola* Kützing ex Bornet & Flahault (oval or broadly oval akinete with brownish or yellowish brown epispore), *C. majus* Kützing ex Bornet & Flahault (ovate akinete with brownish epispore and distinct papillae), *C. sphaericum* B.N.Prasad (spherical akinete with brownish epispore), and *C. stagnale* Bornet & Flahault (oblong or sub-cylindrical akinete with brownish epispore).

## 2. *Wollea saccata* Bornet et Flahault 1886 (Fig. 1b)

Trichome enclosed in a mucilaginous envelope, straight, clearly constricted at cross walls. Vegetative cells

pale blue-green, sometimes barrel-shaped, shorter than wide or discoid, 4–6 µm broad, 3–5 µm long. Heterocysts intercalary, barrel-shaped, discoid or slightly longer than wide, 4–5 µm broad, 4–6 µm long. Akinetes solitary or several in both sides of heterocysts, ellipsoidal or ovate, 6–8 µm broad, 12–17 µm long, yellowish brown in color in maturity stage.

General distribution: Northern of eastern Siberia, and North America (Kozhevnikov & Kozhevnikova 2011).

The genus *Wollea* consisting at least nine species, which are characterized by unbranched filaments with special cells or akinetes and heterocysts (Guiry & Guiry 2019). Heterocysts intercalary and akinetes arise paraheterocytic at both sides of heterocysts in short series. It should be noted that, *Wollea* is a poorly known genus which is most morphologically similar to genera *Anabaena* and *Nostoc* (Komárek 2010, Kozhevnikov & Kozhevnikova 2011). Several species of this genus was reported from aquatic and terrestrial habitats of Iran (Shariatmadari *et al.* 2014). The difference in the shape and size of akinetes as well as akinete color in maturity stage are important differences of *Wollea saccata* with the other species which previously reported from Iran. It should be noted that, *W. ambigua* (C.B.Rao) R.Y.Singh, and *W. vaginicola* (Fritsch & Rich) Singh, as two species previously reported from terrestrial habitats of Iran, have yellowish green akinetes in maturity stage (Shariatmadari *et al.* 2011), while the akinetes of *W. saccata* is brown in similar stage. The form and size of heterocysts are other characteristics that differed between these taxa. The heterocysts are barrel-shaped or slightly longer than wide in *W. saccata*, but in two other taxa they are spherical or sub-spherical (Shariatmadari *et al.* 2011).

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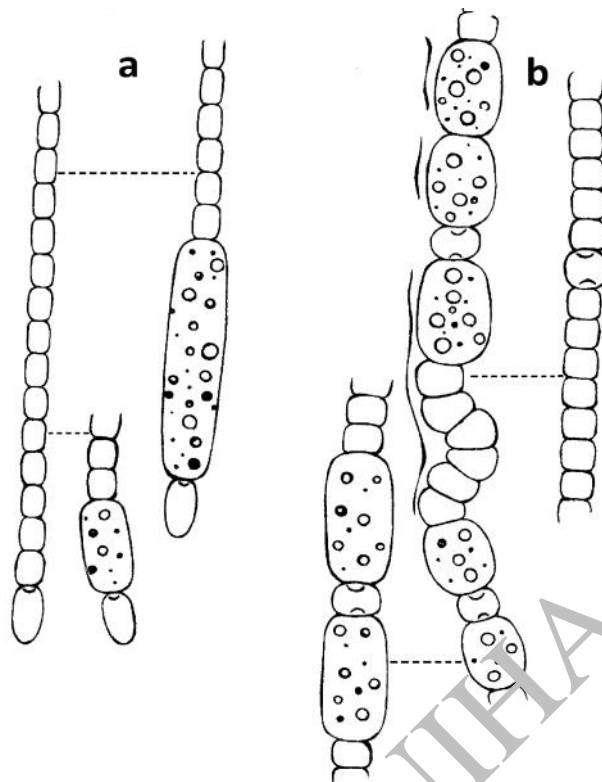


Fig. 1. Camera lucida images of taxa: a. *Cylindrospermum voukii*, b. *Wolleea saccata* (Bar = 10  $\mu$ m).

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