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Research Article

Unveiling *Westiellopsis akinetica*: a novel species in the Iraqi habitat, and contrasting its distinctive attributes with *Westiellopsis prolifica*

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Abstract

This is the first record of a new species of cyanobacteria *Westiellopsis akinetica* in the Iraqi environment, Samples were collected on June 2013 and the existence of it was not documented before. We isolated and purified this species ten years ago in Iraq, but we couldn't identify accurately based on all taxonomic handbooks. This is due to the species features being different from the other documented species in the available taxonomic lectures. It resembled many species by morphological characteristics such as *Fischerella muscicola*, *Fischerella thermalis*, *Westiellopsis biateralis* SA16, *Westiellopsis interrupta*, *Westiellopsis persica* SA33, *Westiellopsis prolifica* and *Symphyonema bifilamentata*. Describing a new species of the *Westiellopsis* genus to be named *Westiellopsis akinetica* sp by polyphasic approach. It's a new species in the local environment, based on all local research published within Iraqi Academic Scientific Journals – IASJ and depending on the updated Iraqi checklists of local freshwater algal. It was isolated from the Iraqi Tigris River passing through Tikrit city. There may be confusion between *Westiellopsis akinetica* and *Westiellopsis prolifica* because of the similarity between their phenotypical characteristics, so the most important identify differences between them must be clarified, in terms of cell shape, heterocytes length, and lateral branch shape. further some of the physical and chemical properties of water were measured at sampling time; in addition, some activities of *Westiellopsis akinetica* were determined such as growth, Chlorophyll *a* content, Phycocyanin pigments and protein within 16 days.

Keywords: Cyanobacteria, *Westiellopsis akinetica*, *Westiellopsis prolifica*, Nostocales, Chlorophyll *a*, Protein.

Introduction

The cyanobacteria form one of the largest subgroups of Gram negative prokaryotes. They are a very diverse group of microorganisms (Nienaber and Steinitz-Kannan, 2018). They function an important role in the ecosystems and biogeochemistry of environments. They grow in benthic, periphytic and free-floating mats and assemblages (Komárek et al., 2017). Because of their conventional position to the algae, the classification of cyanobacteria was accomplished by phycologists, depending on Botanical Code (Staffleu et al., 1972). The discriminatory properties, both generic and specific, are either structural or ecological, these being virtually the only

characters determinable in the field (Rippka et al., 1978). Nostocales Order is a monophyletic group. It includes filamentous cyanobacteria, which produce akinetes and heterocytes. This order comprises the most morphologically diverse, existing as single filaments, unstructured clusters, either colonies of diverse arrangements, which may show true or false branching, or without branching (McGregor, 2018). Many earlier researches demonstrated that the order of Nostocales is very analogous in the sequence of 16S rRNA gene and frequently of that genera in this order the similarity between them is more than 98% (Kaštovský et al., 2014; González-Resendiz et al., 2018). Many studies such as Nabot et al., (2013) and Hauer et al., (2013) explained that even with a large number of papers being published about the matter, the

taxonomic knowledge of cyanobacterial diversity in several environments is still poorly known. Many of special morphotypes that represent new cyanobacterial species and genera have lately been described in different environments and extreme habitats (Komárek and Komárkova, 2007; Gama et al., 2014; Komárek et al., 2013) pointed out that there is serious taxonomic confusion because of the fact that the type species for both Fischerellaceae and Hapalosiphonaceae vary similarly species. Therefore, Gugger & Hoffmann (2004) They recommended to redesigning *Westiellopsis*, *Fischerella*, *Mastigocladus* and *Hapalosiphon* into a single genus and family, Whereas Kastovsky and Johansen, (2008) recommended revision Fischerellaceae types to solve this problem. For these reasons the polyphasic approach, which include two systems; morphological characteristics and molecular methods. This is a more accurate method of identifying taxonomic diversity (Miscoe et al., 2016; Alvarenga et al., 2016), as it shows the exact phenotypic characteristics to accurately identify each species.

Materials and Methods

Sites of sample collection

Samples were collected from water in the Tigris River, east of Tikrit as Fig. 1 shows. Sterile plastic bottles were prepared to store the samples collected for the purpose of the study.

Physical and Chemical Parameters

Air & Water Temperature

The temperature of air and water was measured using a mercury thermometer with a range of -10 °C to 50.

Turbidity

Turbidity was measured using the HANA-LP 2000 Turbidity Meter, and it is expressed in NTU.

Conductivity

The ability of water to conduct an electric current was recorded using an Oyster Conductivity Meter µg/ml.

pH

An Oyster pH meter was used after calibration with 3 standard solutions, which are 9-7-4.

Dissolved Oxygen

The method Winkler followed, as Helm et al., (2009), mentioned it.

Nitrite (NO₂), Nitrate (NO₃) and phosphate PO₄³⁻

Nitrite, nitrate and phosphate were measured according to the

APHA (1998) method.

Salinity

Salinity values were calculated based on electrical conductivity values according to the method of Golterman et al., (1978).

Preparation of the culture medium

BG11 medium is adopted, it was suitable for *Westiellopsis* sp. pH was adjusted at 7.1 using Dilute NaHCO₃ and HCl (Parks, 2004). Distribute 100 mL of the medium into 250 mL flasks. The mouths of the flasks are closed by medical cotton. Then the media was sterilized by autoclave Sanshe modl yx280B. at 121°C for a 20 minutes under a pressure of 15 pounds/inch².

Cyanobacteria isolation and purification

After diluting the samples, they were cultured on petri dishes continuing BG11 medium with 1% agar. Then incubated in a cooled incubator, made in England by Gallen Kamp, at 28°C with 2500 lux intensity light for a period of 10 hours/day. The distinct colonies were transferred to a new Petri dish containing the same culture medium under the same conditions. This process will be repeated until axenic cultures are obtained. Then transformed to liquid media under standard conditions with refrigerated shaking incubator at 100 cycles/min.

Identifications and Taxonomic classification

Several species were identified using an Olympus microscope according to several references and descriptions available in the literature that specialized in Cyanobacteria (Rippka et al., 1979; Desikachary, 1959; Wehr et al., 2015; Komárek et al., 2013; Casamatta et al., 2020; Mishra et al., 2020). Phenotypic characteristics were observed. The cells' sizes and dimensions were measured using micrometry 0.01 mm. and documented as microphotographs.

Physiological Parameters

Growth measurement

The species were grown on liquid BG11 for 16 days, under standard conditions referred to previously, the optical density was measured with 5 ml of culture at wavelength 436 nm by a spectrophotometer type Lapo Med. inc (Gibson and Fay, 1983).

Chlorophyll a Measurement

A 5 ml aliquot of cyanobacteria culture was harvested by centrifuge tube, and centrifuged at 3000 rpm, in a type Hettich centrifuge, for 10 min. Acetone 90%v/v is added to the precipitate (Kurtis, 2007). Chl a concentration was calculated using the Jeffrey equation (Jeffrey et al., 1997). Optical density was measured by a spectrophotometer with 1.0 q. cm cell at several wavelengths: A630 nm, A645 nm, A663 nm. Using blank by same acetone 90%. The Chl a concentration was calculated in µg/ml through the application

of the following equation:

$$\text{Chl a } \mu\text{g/ml} = (11.64A663 - 2.16 A645 + 0.10A630) v / IV$$

v = acetone volume (ml)

l = cell length of the spectrophotometric (cuvette) cm

V = volume of the sample (ml)

Protein Estimation

The protein amount was determined according to Lowry's method (1951) Modified by Waterborg (1984). The standard stock solution by 200 $\mu\text{g/mL}$ of bovine serum albumin Fig. 2.

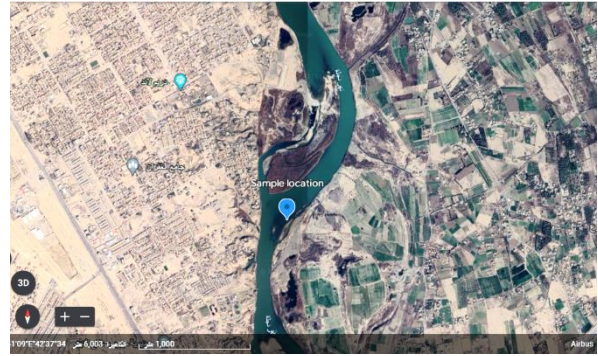


Fig. 1 sample location by Google earth

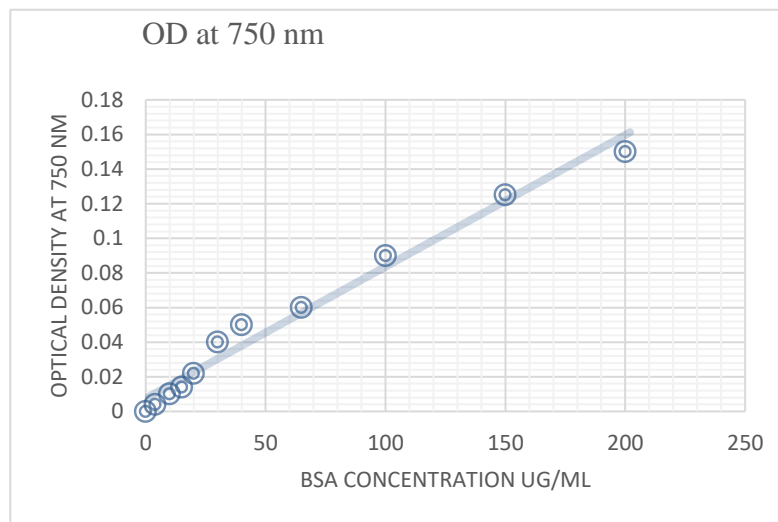


Fig. 2 Standard curve of bovine serum albumin

Results and discussion

In this study, seven cyanobacteria species were isolated and purified from the local habitat of the Tigris River; *Anabaena oryzae*, *Anabaena laxa*, *Oscillatoria chlorine*, *Oscillatoria splendida*, *Gloeotheca sp.*, and *Westiellopsis akinetica* were identified in correspondence to Morphological characteristics using the previously mentioned references in Materials and Methods. Only one species, was unable to be identified accurately due to it exhibiting multiple distinct morphological characteristics. It displayed similarities with many species such as *Fischerella muscicola*, *Fischerella thermalis*, *Westiellopsis interrupta*, *Westiellopsis prolifica*, *Westiellopsis persica* SA33 and *Westiellopsis bilateralis* SA16, *Symphyonema bifilamentata*. It was identified as such according to the exact description it, from Mishra et al., (2020). They documented a new member of the *Westiellopsis* with the name proposed being *Westiellopsis akinetica* by the polyphasic approach that pointed out new strain based on the International Code of Nomenclature for Algae, Fungi and Plants. As demonstrated in Fig. 3, the vegetative cells are irregular in shape, with sizes ranging

between 3.7 – 7 μm in length to 3.6 - 6 μm in width. The trichome uniseriate and prostrate, sometimes divide to result in a biseriata trichome. The emerging lateral true uniseriate form T-type branches on both sides of the main filaments; which is different from the main filaments. The initial cell of the lateral branches was 3.8-5.6 μm in length and 3.5-4.8 μm in width. The branches were thin and tapering, Akinetes was from 6.5–7.5 μm in length to 3.4–4.4 μm in width, sometimes formed a chain of akinetes. heterocysts had various shapes, from enlarged, spherical, to oblong, elongated and cylindrical are intercalate or sometimes lateral positions come with two shapes; rectangular 6.2-7.4 μm in length and 2.3-4.1 μm in width and sometimes spherical with size 3.6–7.1 μm in length to 3.6– 6.1 μm in width. The sheath of trichomes was colorless.

Despite the similarities characteristics between *Westiellopsis akinetica* and *Westiellopsis prolifica* (It was the most similar of the previously mentioned species), but there are real differences between them; such as the branched filaments of *Westiellopsis prolifica* are devoid of a sheath and are not enveloped in mucilage, the lateral branches are erect. The length of the heterocysts is up to 22 μm . The erect branches at apical portions widen and become

swollen and pinched along their length and sometimes aggregate in fascicles (JANET, 1941; Desikachary, 1959; Komárek et al., 2013). While the differences between *Westiellopsis akinetica* and *Fischerella muscicola* are, the color

of the *F. muscicola* filaments is dark green or blackish-brown, about 10-12 µm in wide, with rectangular vegetative cells, filaments enveloped with sheath and the lateral branches are erect (Mishra et al., 2019).

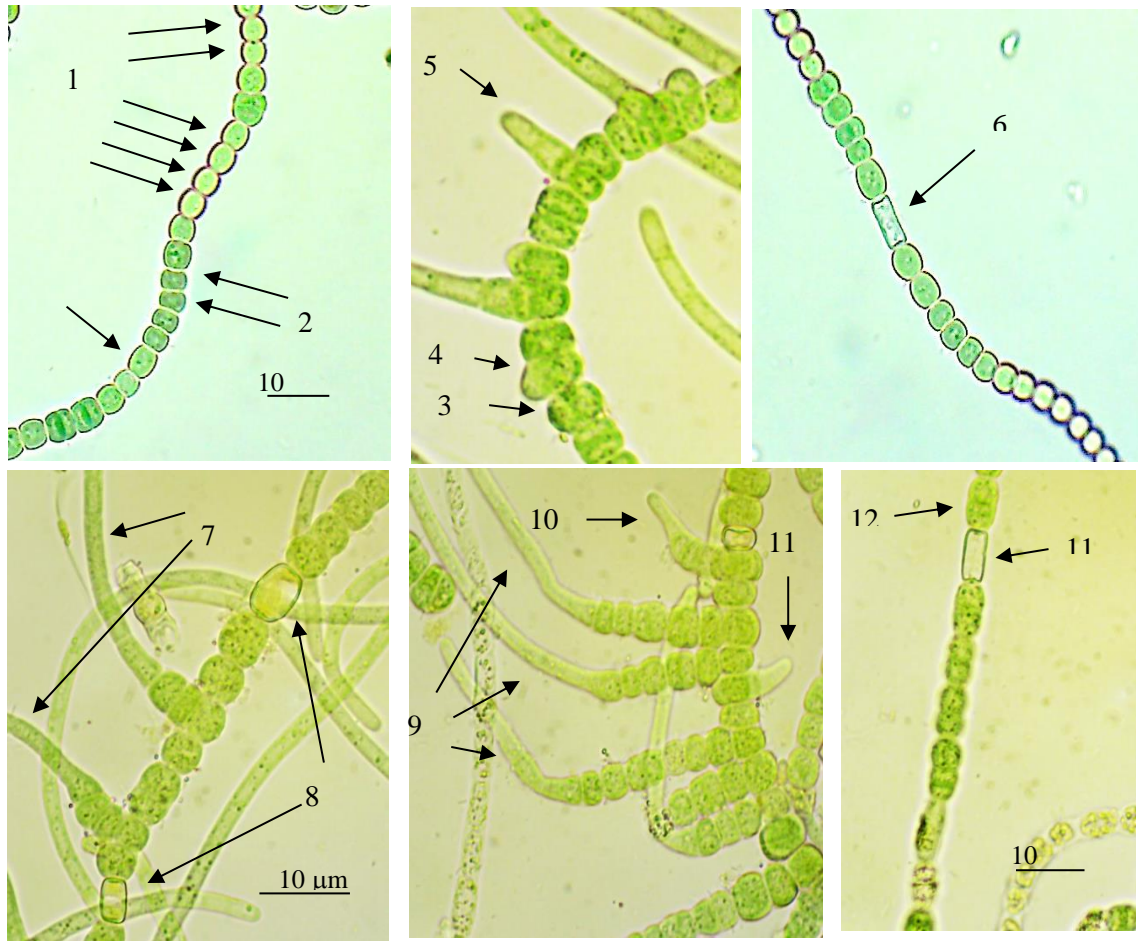


Fig. 3 Shown *Westiellopsis akinetica*; morphological characteristics, (1) akinetes chain; (2) vegetative cells (longitudinal divisions); (3), (4) emerging lateral true branches; (5) thin and tapering branch; (6), (8), (11) Various shaped of heterocytes; rectangular or cylindrical, spherical to oblong; (7), (9) prostrate true-branch; (10), (11) branches on both of sides; (12) vegetative cell adjacent to heterocytes.

Table 1. Physical and chemical parameters of water

Factors	Air Temp °C	Water Temp °C	pH	Dissolved Oxygen mg/L	Turbidity NTU	Conductivity µS/cm	NO ₂ µg/L	NO ₃ µg/L	PO ₄ ⁻³ µg/L	Salinity mg/L
results	39	23.3	7.9	7.3	158	523	0.630	6.3	2.1	0.305

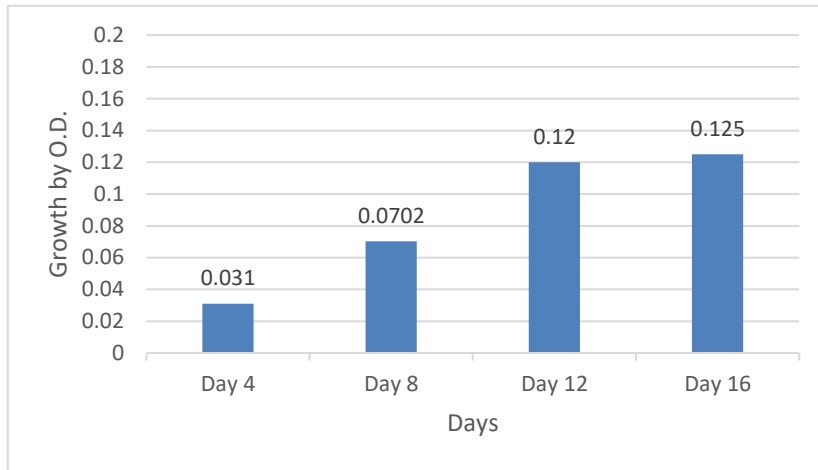


Fig. 4 Growth of *Westiellopsis akinetica* by OD (436 nm)

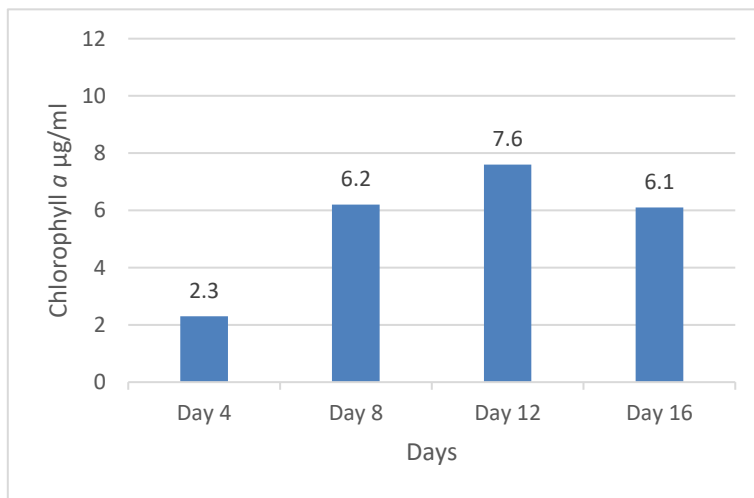


Fig. 5 Chl a of *Westiellopsis akinetica*

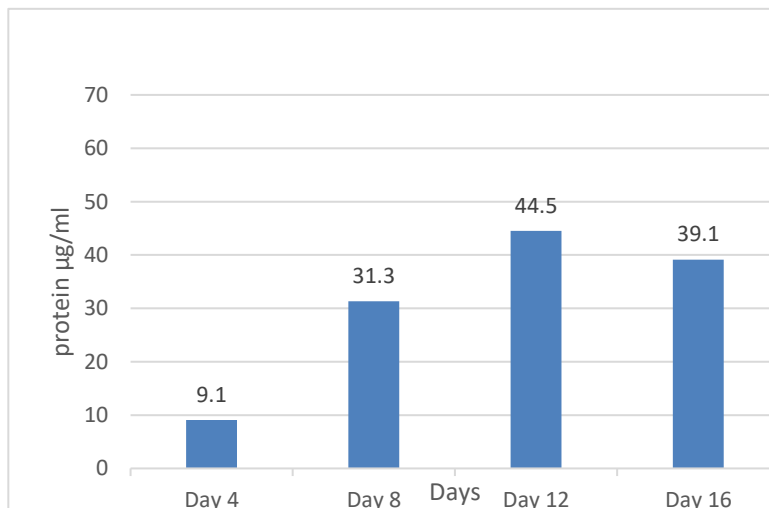


Fig. 6 Protein of *Westiellopsis akinetica*

The current study is the first to document the presence of *Westiellopsis akinetica* in the Iraqi environment. The existence of this species was not mentioned before; based on local research published and research of Iraqi Academic Scientific Journals – IASJ. as well as depending on the updated Iraqi checklist of freshwater algal flora by Aziz and Ahmed (2022) and Checklist of algal flora in Iraq by Maulood et al., (2013). Perhaps this is the first recording after Mishra's (2020). However, Aziz and Ahmed refereed there are 400 taxa of Cyanophyta in Iraqi Kurdistan, which represent 23.5% of the algae.

Physical and chemical parameters of water were measured at the time of sampling, The results are shown in Table 1. Further, Growth and amount of Chlorophylla a, protein and phycocyanin were estimated The *Westiellopsis akinetica* cultured under standard condition for 24 days. The results showed The biomass growth at 16 days was 0.125 O.D. (Fig. 4), concentration of chlorophyll a and protein were 7.6 µg/ml and 44.5 µg/ml at 12 days respectively as shown in (Fig. 5 and 6). While No significant concentrations of phycocyanin pigment were recorded, It may be produced in greater concentrations under different lighting conditions.

Classification

Empire: Prokaryota
Kingdom: Eubacteria
Subkingdom: Negibacteria
Phylum: Cyanobacteria
Class: Cyanophyceae
Subclass: Nostocophycidae
Order: Nostocales
Family: Hapalosiphonaceae
Genus: *Westiellopsis akinetica* (Mishra et al., 2020)

Conclusion

In conclusion, the discovery of a novel cyanobacteria species, *Westiellopsis akinetica*, in the Iraqi environment marks a significant contribution to local algal biodiversity. The polyphasic approach employed for its accurate identification distinguishes it from closely resembling species. Morphological differences, including cell shape, heterocytes length, and lateral branch shape, provide key markers for differentiation, particularly from *Westiellopsis prolifica*. Through rigorous analysis of physical and chemical properties of its habitat, as well as its growth and pigment content, the distinct characteristics of *Westiellopsis akinetica* have been elucidated. This study enriches the understanding of indigenous freshwater algal species and underscores the importance of updated taxonomic considerations.

Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication.

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