

A TAXONOMIC STUDY ON SOIL TAXA OF CYLINDROSPERMUM KÜTZING EX BORNET & FLAHAULT (NOSTOCACEAE) IN IRAN

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In the study of the genus *Cylindrospermum* Kützing ex Bornet & Flahault (*Nostocaceae*) in terrestrial habitats, 18 specimens belonging to eight species were identified. Specimens were collected from 11 paddy field soils located in seven provinces of Iran. Five taxa, including *Cylindrospermum sphaericum* B. N. Prasad, *Cylindrospermum catenatum* (Ralfs) Bornet & Flahault, *Cylindrospermum marchicum* Lemmermann, *Cylindrospermum minutissimum* Collins and *Cylindrospermum muscicola* Kützing ex Bornet & Flahault are considered as new records to Iran. An identification key, description and pictures of these species are presented in this study.

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Key words. *Cylindrospermum*, morphospecies, new record, geographical distribution, Iran.

مطالعه نمونه‌های خاکزی جنس *Cylindrospermum* Kützing ex Bornet & Flahault از تیره *Nostocaceae* در ایران

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جهت بررسی نمونه‌های خاکزی جنس *Cylindrospermum* Kützing ex Bornet & Flahault از تیره *Nostocaceae* تاکسون‌های موجود در خاک ۱۱ ایستگاه از ۷ استان دارای قابلیت کشت برنج کشور مورد مطالعه قرار گرفت. طی این تحقیق در مجموع ۱۸ نمونه متعلق

به هشت گونه از جنس *Cylindrospermum* شناسایی شد. در میان گونه‌های شناسایی شده پنج گونه زیر

Cylindrospermum sphaericum B. N. Prasad, *Cylindrospermum catenatum* (Ralfs) Bornet & Flahault, *Cylindrospermum marchicum* و *Cylindrospermum minutissimum* Collins

muscicola Kützing ex Bornet & Flahault برای نخستین بار از ایران گزارش می‌شوند. همچنین کلید شناسایی، شرح و تصاویر

گونه‌های شناسایی شده ارائه می‌گردد.

INTRODUCTION

The genus *Cylindrospermum* Kützing ex Bornet & Flahault, is one of the filamentous, heterocystous and non-branched cyanobacteria, classified traditionally in *Nostocaceae* family. Taxa of this genus characterized by symmetrical filaments, which their heterocysts develop solitary from the terminal cells and the akinetes adjoining to their terminal heterocysts (Komárek 1989). However, formation of intercalary heterocysts in young cultures of this genus at response to different sources of nitrogen was reported (Kapustka & Rosowski 1976). Up to now around 35 taxonomically accepted species of this genus recorded which can flourish in several habitats such as aquatic and terrestrial ecosystems. Moist soils of paddy fields

are favorable terrestrial ecosystems for the growth of *Cylindrospermum* species. Optimal condition of light, temperature, humidity and nutrient availability, to bring about high cyanobacteria diversity in these ecosystems. Up to now, several species from different genera of *Nostocaceae* were reported from paddy fields of Iran, but reports about this genus and its diversity in paddy soils of Iran is very limited. Report of one species of *Cylindrospermum* from paddy fields of Golestan province was the first record of this genus from paddy soils of Iran (Nowruzi & Ahmadimoghadam 2006). Shariatmadari & Riahi (2010) reported two species of *Cylindrospermum* from paddy fields of Gilan province. In present study, an attempt was made to contribute a new knowledge about *Cylindrospermum* species and

Table 1. Geographical and some ecological details of the sampling locations.

Location	Latitude/Longitude	pH	EC (dS/m)
Tazehabad (Mazandaran)	36°39' N 51°25' E	8.1	1.16
Rahim abad (Gilan)	36°51' N 50°13' E	8	1.47
Saravan (Gilan)	37°05' N 49°24' E	8.1	2.79
Alamut (Qazvin)	36°23' N 50°33' E	8.1	2.47
Visan (Lorestan)	33°49' N 48°07' E	8.4	1.03
Ebrahimabad (Fars)	29°00' N 52°56' E	8.1	9.55
Esmaelabad (Fars)	28°85' N 53°83' E	8.3	2.38
Fathabad (Fars)	29°19' N 52°37' E	8	18.92
Firozabad (Fars)	28°51' N 52°31' E	8.3	1.51
Kalat (Khorasan Razavi)	36°59' N 59°47' E	8.1	5.82
Zarrinshahr (Esfahan)	32°22' N 51°22' E	8.3	3.31

their distribution in seven main rice cultivation provinces situated in north, centre, south, west and east of Iran.

MATERIALS AND METHODS

Soil samples were collected from 11 paddy fields from April 2008 to May 2010 (Table 1) according to Rangaswamy method (1996). The collected soil samples were transferred to sterile petri dishes and sterilized nitrate free BG-11 medium (Stanier & al. 1971) was added. The petri dishes were placed in a culture chamber at 25±5°C and a 12/12h light-dark cycle at artificial illumination (2000-2500 Lux) for two weeks. After colonization, cyanobacteria were transferred to the agar plates for purification. Taxonomic determination was carried out by light microscopy and based on Desikachary (1959), Prescott (1970), Wehr & al. (2002), Whitford & Schumacher (1973), John & al. (2002) and Komárek (1989) by prepared semipermanent slides. The morphological studies were focused on 18 populations of eight *Cylandrospermum* morphospecies isolated from paddy soils of diverse geographic locations in Iran. The vegetative and reproductive characters such as shape, colour and size of the thallus; wide and length of trichomes; shape, size and colour of vegetative cells, heterocysts and akinetes; length/width ratio of akinetes as well as texture, colour and ornamentation of cell walls of the akinetes were used in the taxonomic determination.

RESULTS

In this study, 18 specimens belonging to eight *Cylandrospermum* morphospecies were identified (Figs 1-6). All species and their distribution are listed in Table 2.

A key to *Cylandrospermum* species distributed in paddy fields soil of Iran

1. Akinetes spherical or sub-spherical *C. sphaericum* (7)
 - Akinetes not spherical 2
2. Akinetes occurring in a series 3
 - Akinetes often solitary 4
3. Akinetes 8-9 µ in diameter, two or three adjoining to heterocyst *C. catenatum* (1)
 - Akinetes 4-6 µ in diameter, more than three in a catenate series adjoining to heterocyst *C. marchicum* (3)
4. Heterocyst and akinete with papillae *C. majus* (2)
 - Heterocyst and akinete often without papillae 5
5. Akinetes cylindrical *C. minutissimum* (5)
 - Akinetes oval or oblong 6
6. Akinetes with colourless cell walls *C. michailovskoense* (4)
 - Akinete with yellowish – brown cell walls 7
7. Heterocyst elongate, akinetes sub-cylindrical with rounded ends *C. stagnale* (8)
 - Heterocyst oval, akinetes ovate to broadly ovate *C. muscicola* (6)

1. *Cylandrospermum catenatum* (Ralfs) Bornet & Flahault, Ann. and Mag. Nat. Hist., 5 (Ser. 2): 321-343. Fig. 1A

Description. Thallus mucilaginous, blue-green. Trichome single, slightly constricted at the cross walls. Vegetative cells sub-quadrangle or sub-cylindrical, 4 µ broad, 5-7 µ long. Heterocysts obvoid or oblong-ovate, 4.5-5 µ broad, 8-11 µ long. Akinetes ovate, formed in a series adjacent to the heterocysts, 8-9 µ broad, 13-25 µ long; episporium smooth, yellowish brown.

Cylandrospermum catenatum was reported from Europe, America and Pacific Islands (Carau 2002, Sherwood 2004).

Distribution in studied sites. Fars: Esmaelabad (28°85' N 53°83' E), Ebrahimabad (29°00' N 52°56' E). – Khorasan Razavi: Kalat (36°59' N 59°47' E).

2. *Cylindrospermum majus* Kützing, Phyc. gene., 212. 1843. Fig. 1B

Description. Thallus mucilaginous, brownish-green. Trichome constricted at the cross walls, light blue-green. Vegetative cells sub-quadrangle, 5 μ broad, 5-6 μ long. Heterocysts sub-spherical or ovoid, 6 μ broad, 5-6 μ long. Akinetes ovate, 10-14 μ broad, 20-33 μ long; epispore brownish with distinct papillae.

Cylindrospermum majus was reported from moist soils and aquatic ecosystems of several regions such as Europe, Asia, Australia and New Zealand (Caraus 2002, Alvarez-Cobelas & Gallardo 1988, Desikachary 1959, Day & al. 1995). Also this species was recorded from paddy field soils of Guilan and Golestan provinces of Iran (Shariatmadari & Riahi 2010; Nowruzi & Ahmadi-Moghadam 2006).

Distribution in studied sites. Gilan: Saravan (37° 05' N 49° 24' E).

3. *Cylindrospermum marchicum* Lemmermann, Algen. I. Leipzig, p. 196, 1910. Fig. 2A

Description. Thallus mucilaginous, blue-green. Trichome single, straight, constricted at the cross walls. Vegetative cells barrel shape or sub-cylindrical, 4-4.5 μ broad, 6-6.5 μ long. Heterocysts ovoid with rounded apex, 5 μ broad, 10 μ long. Akinetes ovate to barrel shape, in a catenate series adjoining the heterocyst, 4-6 μ broad, 6-9 μ long.

Cylindrospermum marchicum was reported from aquatic ecosystems of America and Europe (Prescott 1970, Caraus 2002).

Distribution in studied sites. Fars: Ebrahimabad (29° 00' N 52° 56' E).

4. *Cylindrospermum michailovskoense* Elenkin, Neu selt. Od. Interess. Art. u. Form. d. Alg., in Mitt. Russl., II, Bull. Jardin Imp. Pierre Ie Gr., 11: 162, 1911. Fig. 2B

Description. Trichome pale blue-green, more or less flexuous. Vegetative cells quadrangle or slightly longer than broad, more or less constricted at the cross-walls, 4-4.5 μ broad, 4-5 μ long. Heterocysts subspherical or ovoid, 7 μ broad, 8-9 μ long. Akinetes single, ovate, 10-15 μ broad, 21-27 μ long; epispore colourless.

Cylindrospermum michailovskoense were reported from several regions of the world. Europe, Caribbean Islands and Asia are the main distribution regions of this species (Caraus 2002, Alvarez-Cobelas & Gallardo 1988, Komárek 1989, Desikachary 1959).

Distribution in studied sites. Gilan: Rahimabad (36° 51' N 50° 13' E). –Mazandaran: Tazehabad (36° 39' N 51° 25' E). –Fars: Easmaelabad (28° 85' N 53° 83' E), Fathabad (29° 19' N 52° 37' E).

5. *Cylindrospermum minutissimum* Collins, Erythea, 4: 119-121, 1896. Fig. 3A

Description. Thallus not mucilaginous, blue-green. Trichomes slightly constricted at the cross walls, pale blue-green. Vegetative cells sub-quadrangle or cylindrical, 3-3.5 μ broad, 5-6 μ long. Heterocysts oblong-ovate, 5-8 μ broad, 6-11 μ long. Akinetes solitary, cylindrical, 6-8 μ broad, 11-22 μ long; epispore smooth, colourless.

Cylindrospermum minutissimum was recorded from aquatic ecosystems of several regions such as Europe, North America, Australia and New Zealand. Also this species was reported from paddy fields of Cuba (Komárek 1989, Day & al. 1995).

Distribution in studied sites. Esfahan: Zarrinshahr (32° 22' N 51° 22' E).

6. *Cylindrospermum muscicola* Kützing ex Bornet & Flahault, Revision des Nostocacées heterocystées, 254, 1888. Fig. 3B

Description. Thallus mucilaginous, green to brownish-green. Trichomes constricted at the cross walls, pale blue-green. Vegetative cells sub-quadrangle or slightly longer than broad, 4-6 μ broad, 5-6 μ long. Heterocysts oblong or ovoid, 4-6 μ broad, 6-9 μ long. Akinetes oval or broadly oval, 7-10 (-12) μ broad, (-10) 17-23 (-25) μ long, epispore smooth, brownish or yellowish brown.

Cylindrospermum muscicola was reported from damp soils such paddy soils as well as aquatic ecosystems in several regions of the world. Europe, Asia, Pacific Islands and Caribbean Islands are the main distribution regions of this species. From Asia this species was recorded from India, Pakistan and China (Caraus 2002, Leghari & al. 2005, Hu & Wei 2006, Desikachary 1959).

Distribution in studied sites. Mazandaran: Tazehabad (36° 39' N 51° 25' E). –Qazvin: Alamut (36° 23' N 50° 33' E). –Esfahan: Zarrinshahr (32° 22' N 51° 22' E). – Lorestan: Visan (33° 49' N 48° 07' E). –Khorasan Razavi: Kalat (36° 59' N 59° 47' E).

7. *Cylindrospermum sphaericum* B. N. Prasad, J. Indian bot. Soc., 31: 358, 1952. Fig. 4A

Description. Thallus mucilaginous, blue-green. Trichome single, curved or straight, slightly constricted at the cross-walls. Vegetative cells sub-quadrangle or cylindrical, 4-4.5 μ broad, 5-8 μ long. Heterocysts ovoid, sub-spherical or ellipsoidal, 4-5 μ broad, 6-7 μ long. Akinetes spherical, 7-17 μ diameter, terminal at both ends of the trichome, rarely with intermediate akinet, formed singly, occasionally in pairs, epispore smooth, brownish.

Cylindrospermum sphaericum was reported from submerged soils of India (Desikachary 1959).

Table 2. List of *Cylandrospermum* species recorded from several paddy soils of Iran and their distributions.

Species	1	2	3	4	5	6	7
<i>C. catenatum</i> (Ralfs) Bornet & Flahault	-	-	-	-	-	+	+
<i>C. minutissimum</i> Collins	-	-	-	-	+	-	-
<i>C. marchicum</i> Lemmermann	-	-	-	-	-	-	+
<i>C. michailovskoense</i> Elenkin	+	+	-	-	-	-	+
<i>C. muscicola</i> Kützing ex Bornet & Flahault	-	+	+	+	+	+	-
<i>C. majus</i> Kützing	+	-	-	-	-	-	-
<i>C. sphaericum</i> B. N. Prasad	-	-	-	-	-	-	+
<i>C. stagnale</i> (Kützing) Bornet & Flahault = <i>Anabaena stagnalis</i> Kützing = <i>Cylandrospermum macrospermum</i> Kützing	-	-	-	-	-	-	+

1. Gilan province, 2. Mazandaran province, 3. Qazvin province, 4. Lorestan province, 5. Esfahan province, 6. Khorasan province, 7. Fars province.

Table 3. Main diacritical features of *Cylandrospermum* species.

Species	Diacritical features
<i>C. catenatum</i> (Ralfs) Bornet & Flahault	Oval akinetes in a series (two or three) adjacent to the heterocyst
<i>C. minutissimum</i> Collins	Cylindrical akinete with colourless cell wall
<i>C. marchicum</i> Lemmermann	Oval akinetes in a catenate series adjoining the heterocyst
<i>C. michailovskoense</i> Elenkin	Oval akinete with colourless cell wall
<i>C. muscicola</i> Kützing ex Bornet & Flahault	Oval akinete with brownish cell wall
<i>C. majus</i> Kützing	Oval akinete with distinct papillae on brownish episore
<i>C. sphaericum</i> B. N. Prasad	Spherical akinetes with brownish cell walls
<i>C. stagnale</i> (Kützing) Bornet & Flahault	Oblong or sub-cylindrical akinete with brownish cell wall

Distribution in studied sites. Fars: Ebrahimabad (29°00' N 52°56' E).

8. *Cylandrospermum stagnale* (Kütz.) Born. et Flah., Revision des Nostocacées, 250, 1888. Fig. 4B

Syn.: *Anabaena stagnalis* Kütz., Phyc. gene., 210, 1843; *Cylandrospermum macrospermum* Kützing, Phyc. germ, 173, 1845.

Description. Thallus blue-green. Trichomes straight, constricted at the cross walls, pale blue-green. Vegetative cells sub-cylindrical, 3.5-4 µ broad, 6 µ long. Heterocysts elongate or ovoid, 5-6 µ broad, 8-10 µ long. Akinetes oblong or sub-cylindrical with rounded ends, 13-18 µ broad, 20-29 µ long, with smooth brown outer layer.

Cylandrospermum stagnale was reported from several regions of the world. This species was recorded from Asia, Europe, Pacific Islands, Australia and New Zealand (Day & al. 1995).

Distribution in studied sites. Fars: Ebrahimabad (29°00' N 52°56' E), Firozabad (28°51' N 52°31' E).

DISCUSSION

The genus *Cylandrospermum* comprises diazotrophic filamentous cyanobacteria, which can occur as planktic

or terrestrial species. The *Cylandrospermum* species have been reported from several regions of the world, from Asia to America and from other regions such as Australia and Pacific Islands. Among distribution regions of this genus, Iran as an Asian country with diverse ecological situations can comprise several species of this genus. Before this study, *Cylandrospermum majus* and *Cylandrospermum michailovskoense* had been reported from paddy fields of Guilan and Golestan provinces (Nowruzi & Ahmadimoghadam 2006, Shariatmadari & Riahi 2010). In present study, eight species from *Cylandrospermum* was reported. Five new taxa, *Cylandrospermum sphaericum* B. N. Prasad, *Cylandrospermum catenatum* (Ralfs) Bornet & Flahault, *Cylandrospermum marchicum* Lemmermann, *Cylandrospermum minutissimum* Collins and *Cylandrospermum muscicola* Kützing ex Bornet & Flahault are presented among these taxa. Morphological study on these species showed that among several morphological characters, akinete shape, episore colour and akinete number are the main diacritical features of these species (Table 3). However, recognition of the some species boundaries in this genus is not being easy. For instance, *Cylandrospermum michailovskoense* is similar to *Cylandrospermum muscicola* and recognition of

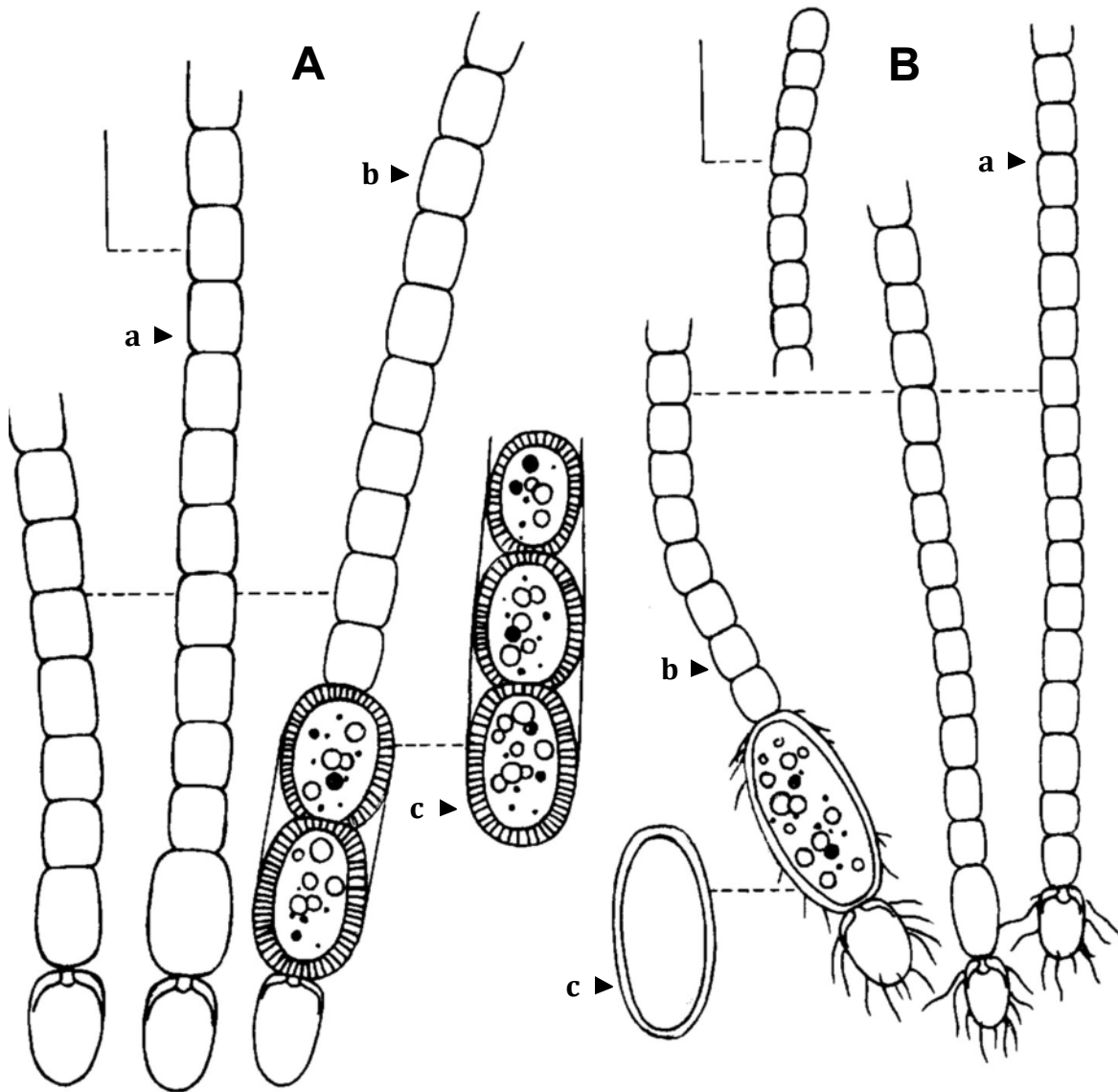


Fig. 1. A. *Cylandrospermum catenatum*: a- part of immature trichome, b- part of trichome with heterocyst and akinetes, c- chain of akinetes., B. *Cylandrospermum majus*: a- immature trichome , b- part of mature trichome with heterocyst and akinete, c- akinete. (Scale: 10 μ m).

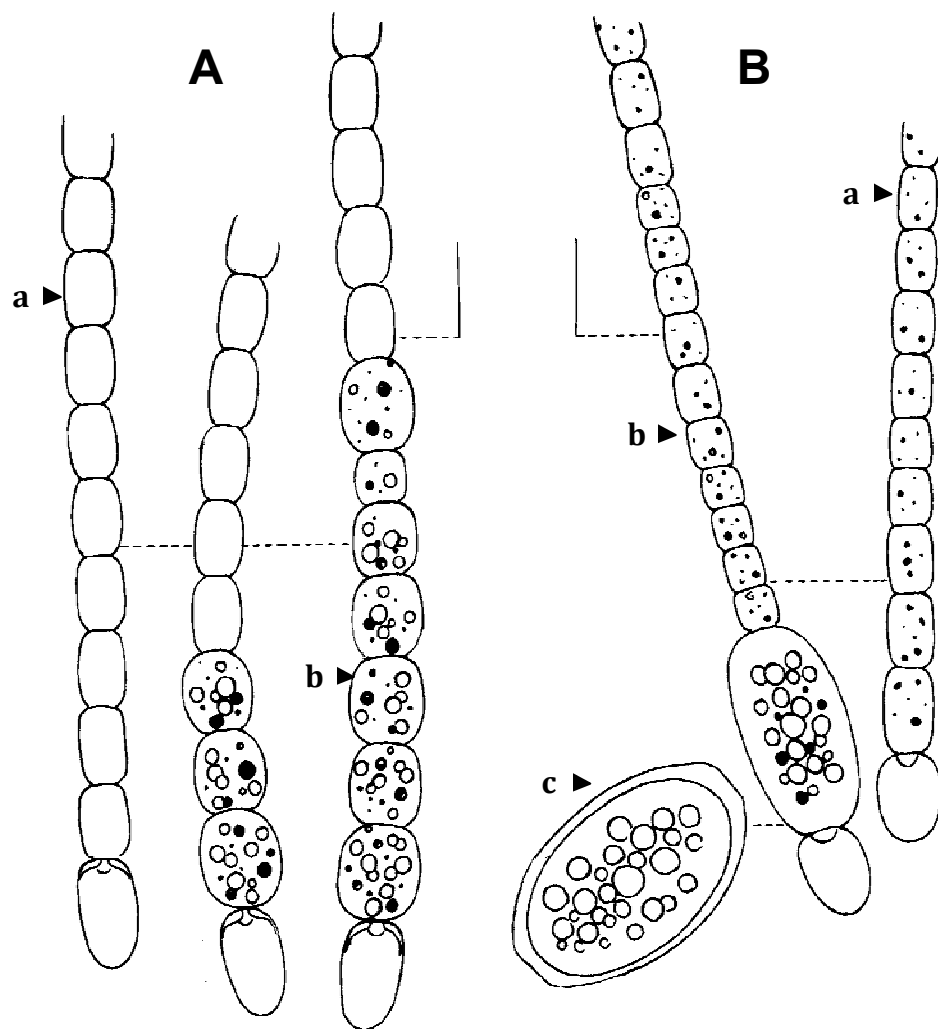


Fig. 2. A. *Cylindrospermum marchicum*: a- part of immature trichome, b- part of trichome with heterocyst and akinetes., B. *Cylindrospermum michailovskoense*: a- part of immature trichome, b- part of trichome with heterocyst and akinete, c- akinete (Scale: 10 μ m).

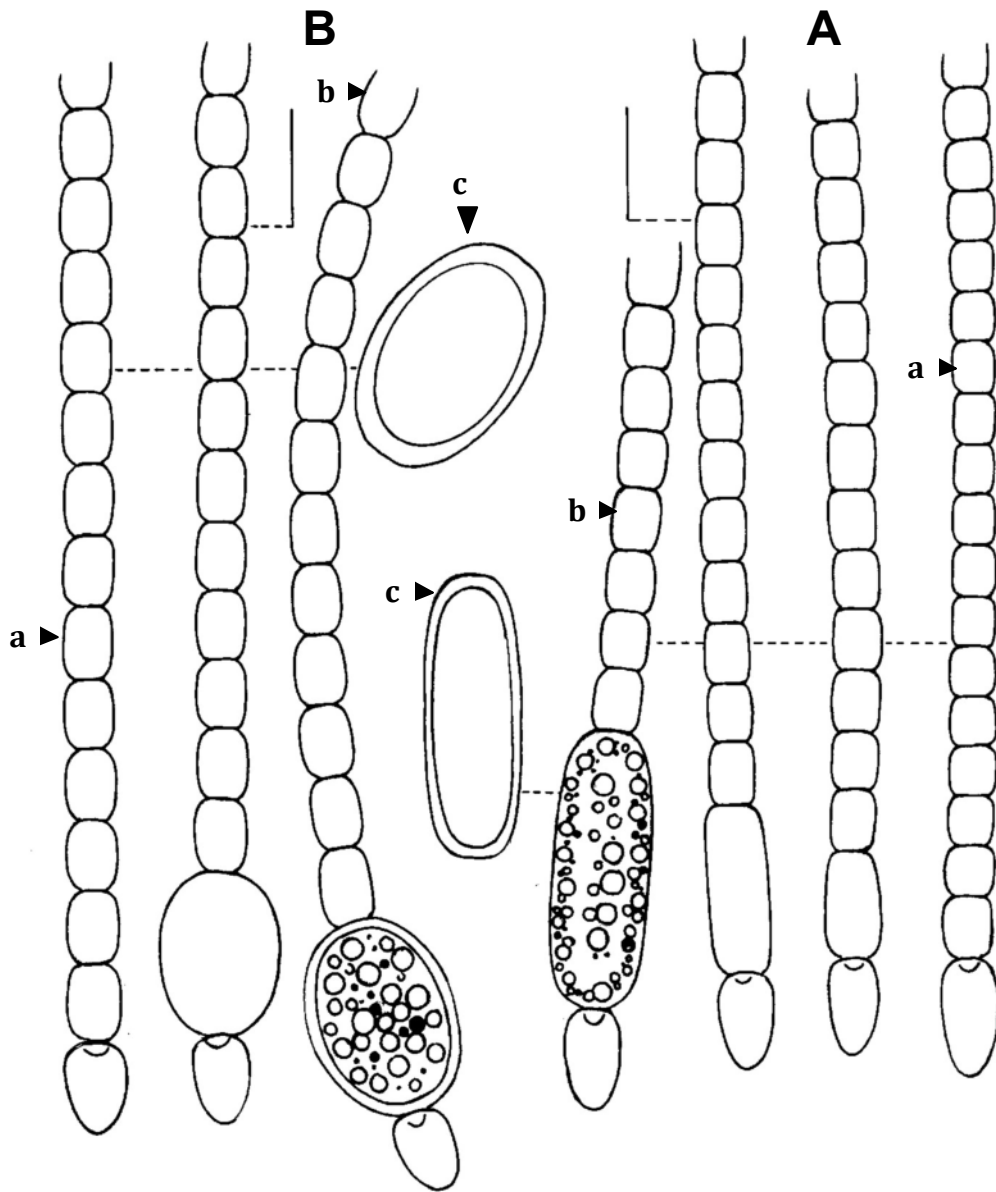


Fig. 3. A. *Cylindrospermum minutissimum*: a- immature trichome, b- part of trichome with heterocyst and akinete, c- akinete., B. *Cylindrospermum muscicola*: a- immature trichome, b- part of trichome with heterocyst and akinete, c- akinete (Scale: 10 μ m).

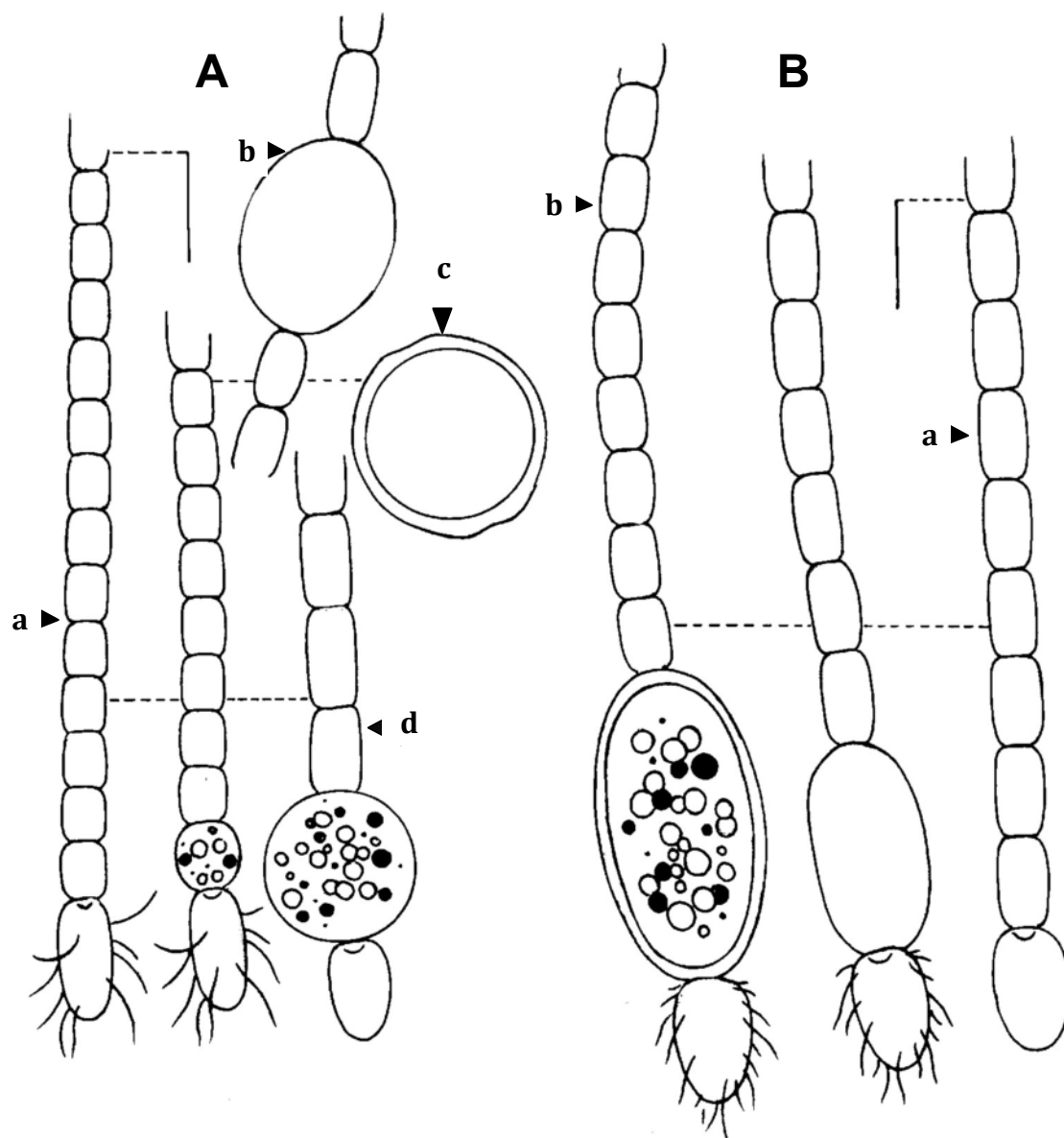


Fig. 4. A. *Cylindrospermum sphaericum*: a- immature trichome, b- Part of trichome with intermediate heterocyst, c- akinete, d. part of trichome with heterocyst and akinete., B. *Cylindrospermum stagnale*: a- immature trichome, b- part of trichome with heterocyst and akinete (Scale: 10 μ m).

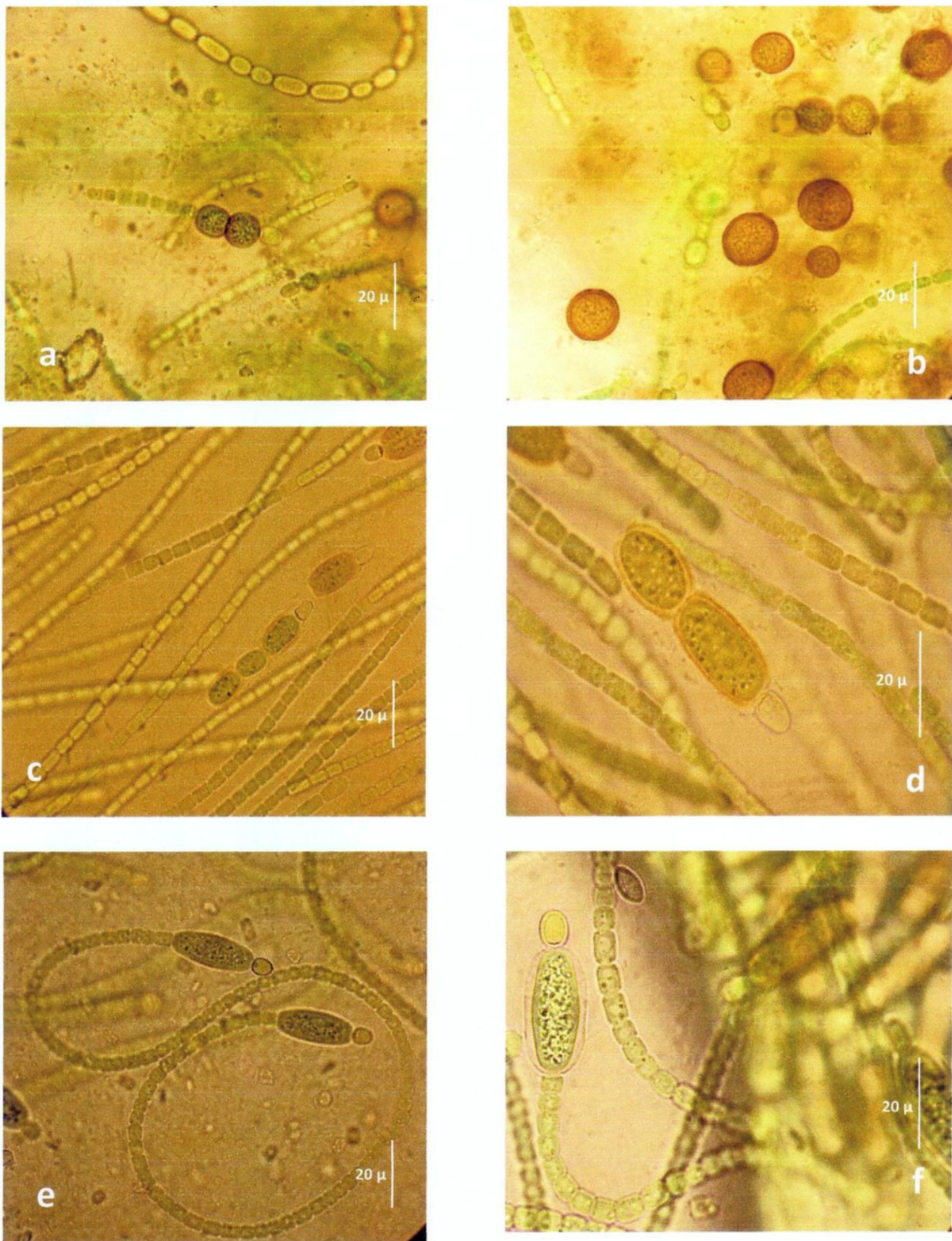


Fig. 5. a, b. *Cylindrospermum sphaericum*., c, d. *Cylindrospermum catenatum*., e, f. *Cylindrospermum michailovskoense* (Scale: 20 µm).

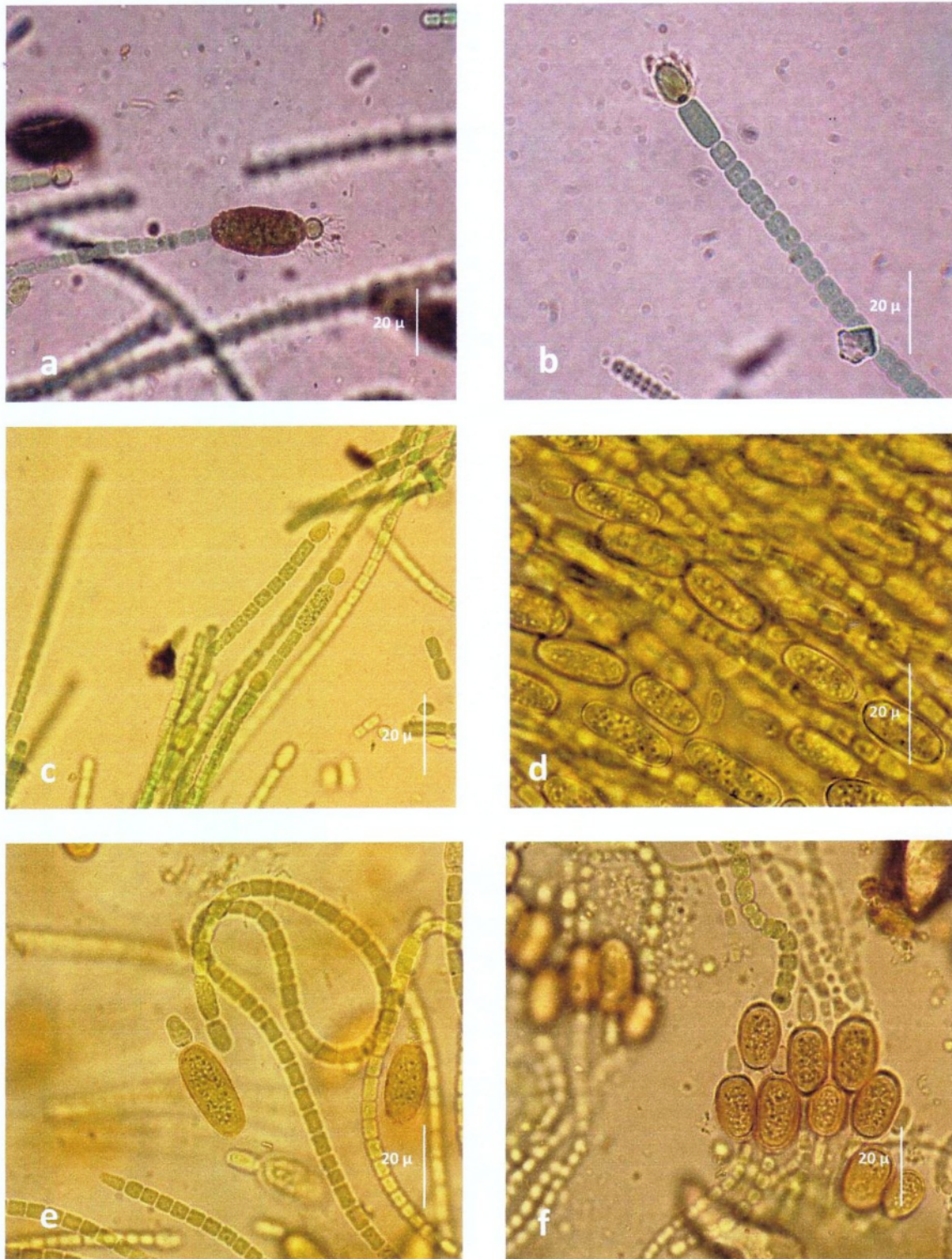


Fig. 6. a, b. *Cylindrospermum majus*., c, d. *Cylindrospermum minutissimum*., e. *Cylindrospermum stagnale*., f. *Cylindrospermum muscicola* (Scale: 20 μm).

populations of these two species is not easy. Geitler (1932) does not separate these species from each other but Elenkin (1938) considers that these species are quite distinct. Akinete cell wall colour is the main diacritical feature of these two species (Komárek 1989), and inattention to this character may be causes to incorrect identification. *Cylindrospermum marchicum* is another example which it differs from *C. catenatum* only by cell size and originally described as a variety of that species (Prescott 1970).

Despite the differences observed between different species of this genus, morphological studies did not show much difference between several populations of each species in different regions. Relative stability of environmental conditions such as light intensity, soil temperature, humidity and pH in several studied sites, may be that reason. Also comparison of species diversity of this genus in several provinces showed that Fars province with five *Cylindrospermum* species has the most species diversity. Wide range of salinity from 1.51 to 18.92 dS/m in several sites of this province may be one of the most important reasons of this diversity.

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