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Notes on the thermal habitat: Thirteen new records for the freshwater algal flora of Turkey

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Thermal springs are extreme environments for organisms. Cyanobacteria are the group that adapts with success in these environments where there is little diversity of life. In this study, eight different thermal water springs were studied in Kütahya province. Thirteen cyanobacteria taxa were identified as new records for the freshwater algal flora of Turkey. Some morphological and taxonomical characteristics of the taxa were briefly described in this paper, with original photos.

[**Keywords**: Cyanobacteria, Freshwater algae, New record, Turkey]

Introduction

Turkey has many freshwater resources and freshwater algae researches[1-3]. However, algal flora of all freshwater ecosystems is still unknown. Algae are essential for all freshwater ecosystems due to their ecological roles (oxygen, carbon dioxide, and nitrogen cycle, primary production). Moreover, the composition of freshwater algae is varying with changing environmental parameters (pollution, industrialization, urbanization and global warming).

Researches on freshwater algae in Turkey has been mostly interested in lakes, ponds, and rivers. However, apart from all these areas, there are many different environments where algae spread, such as thermal springs. Cyanobacteria show a widespread distribution in natural thermal springs. The thermal tendency in cyanobacteria should be expected because of some basic features of prokaryotic cells[4].

In related literature, there are lots of freshwater algae studies in Turkey[1,5-8]. Checklists were published, based on the freshwater algal flora studies of Turkey[2,3]. After these checklists, many new records have been given for freshwater algal flora of Turkey[6-11]. Recently, there is a digital checklist database of the freshwater algal flora of Turkey[1].

Thermal springs are unique ecosystems for organisms because of their ecological features. Algae, especially cyanobacteria members, are the living group that adapts with success in thermal springs. In terms of thermal springs, there is only some study in Turkey[12-14]. However, there are still many thermal springs with unknown algal flora.

In the present study, thirteen new taxa have been identified with their morphological and ecological features.

Materials and Methods

The material of the study is algal flora of thermal springs. For sampling, eight thermal springs were studied in Kütahya (Fig. 1; Table 1). Within the scope of the study, samples were collected periodically for 12 times between February 2014 and January 2015, and species composition was determined. While sampling, all thermal water outlet areas, and water pools were examined. Two separate samples were taken from the same point, placed in 50 ml sterile falcon tubes, 4 % formaldehyde solution was added to one of them, and the other was used for live diagnosis. Collected samples were labeled and transported to the laboratory immediately. Algae samples were examined in the laboratory under the "Olympus BX 50 (phase-contrast) microscope", taxonomical characteristics determined, and photographed using the "Sony DSC-TX7" camera for morphological identification. The determinations of the taxa were made according to previous studies; Komárek & Anagnostidis[15,16]. The systematic classification was done following Anagnostidis & Komárek[17,18]. The nomenclature was checked from the Algae Base database[19]. New records were checked from Gönülol[1].

Fig. 1 — Study area and location of the sampling sites (Kütahya province, Turkey)

Table 1 — The sampling site details in Kütahya

Thermal Springs	GPS coordinates	pH	T (°C)	EC (ohm)	TDS (mg/L)
Gediz Ilıca	38°56′22′′ N; 29°15′24′′ E	7.7	30–48	-	1530
Tavşanlı Göbel	39°29′51′′ N; 29°26′17′′ E	6.7	30	410	-
Sarpaşan	39°12′10′′ N; 29°16′40′′ E	7.2	32	1010	490
Hisarcık Tarihi Hamam	39°12′07′′ N; 29°16′35′′ E	7.7	35–41	1060	520
Hisarcık Sefaköy	39°10′33′′ N; 29°15′37′′ E	8	32	1020	500
Günlüce Dereli	39°27′45′′ N; 29°15′54′′ E	7	28–32	1460	710
Simav Eynal	39°07′38′′ N; 28°59′33′′ E	8.7	45	-	1340

Results

Collected samples were identified based on morphological and ecological features. As a result, thirteen cyanobacteria taxa were identified for the first time for Turkey freshwater algal flora (Fig. 2A–M).

Cyanosarcina thermalis (Hindák) Kovácik, nom. illeg. 1988

Phylum: Cyanobacteria

Class: Cyanophyceae

Order: Chroococcales

Family: Chroococcaceae

Genus: *Cyanosarcina* L. Kovácik, 1988

Cyanosarcina thermalis (Hindák) Kovácik, *Algol Stud/ Arch Hydrobiol*, 50–53 (1988), p. 176. *Myxosarcina thermalis* Hindák, *Algol Stud/ Arch Hydrobiol*, 21 (1978), p. 368. *Myxosarcina hindakii* Bourrelly, *Lesalguesd'eaudouce Tome*, III (1985), p. 571 (Fig. 2–A).

Location and Habitat: Hisarcık Tarihi Hamam Thermal Spring, sampled at the bottom of the shallow thermal water pool at 38–41 °C. Date of collection: 16.10.2014.

Description: Small colonies arrange as a packet with four cells. Cells 3–4 µm in diameter, nearly rounded and blue-green color.

Leptolyngbya boryana (Gomont) Anagnostidis & Komárek 1988

Order: Synechococcales

Family: Leptolyngbyaceae

Genus: *Leptolyngbya* Anagnostidis & Komárek, 1988

Leptolyngbya boryana (Gomont) Anagnostidis & Komárek, *Arch Hydrobiol*, 80 (1988), p. 391. *Plectonema boryanum* Gomont, *Bull Soc Bot Fr*, 46 (1899), p. 36. *Lyngbya pseudoramosa* L. Hoffmann, *Bull Jard Bot Natl Belg*, 56 (1986), p. 101 (Fig. 2–B).

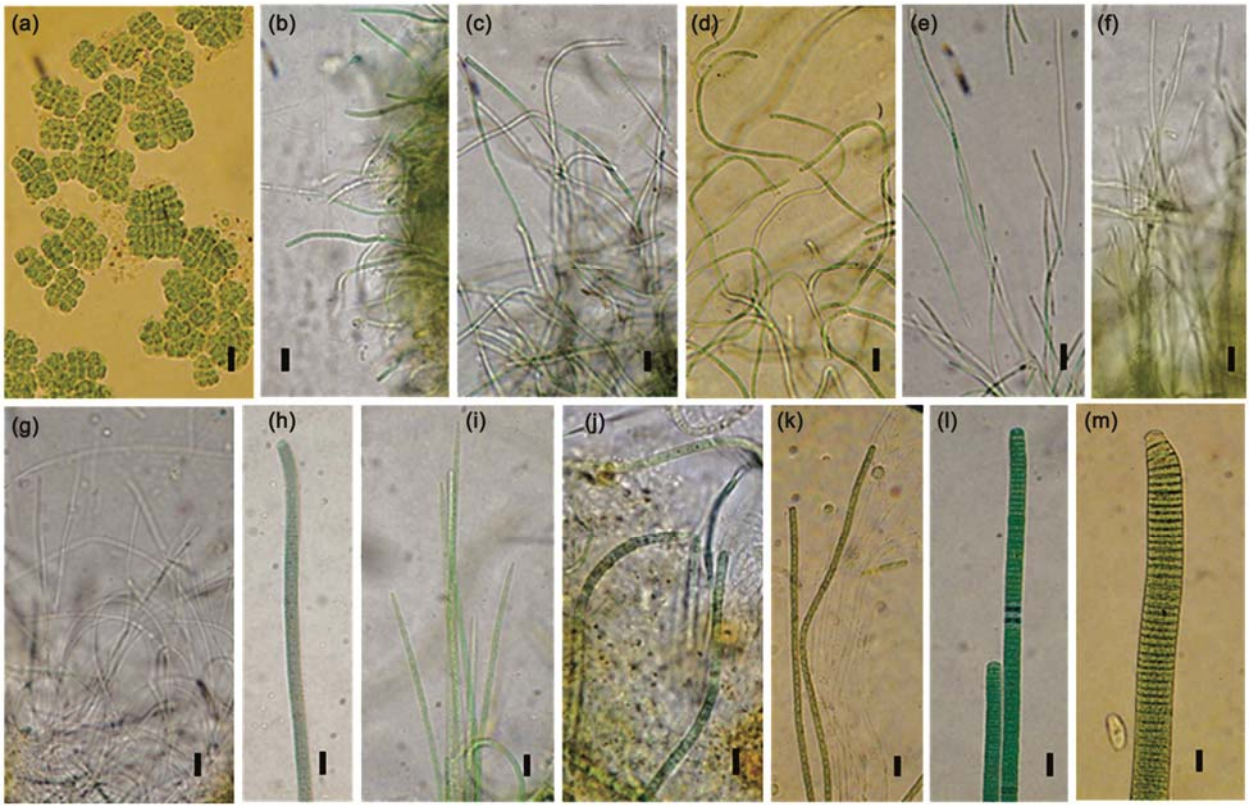

Fig. 2 — General view of the cyanobacteria taxa were identified for the first time for Turkey freshwater algal flora: A – *Cyanosarcina thermalis*, B – *Leptolyngbya boryana*, C – *Leptolyngbya tenerrima*, D – *Leptolyngbya gelatinosa*, E – *Leptolyngbya granulifera*, F – *Limnothrix mirabilis*, G – *Geitlerinema nematodes*, H – *Planktothrix clathrata*, I – *Microcoleus lacustris*, J – *Phormidium incrustatum*, K – *Phormidium thermobium*, L – *Oscillatoria subcapitata*, M – *Oscillatoria proboscidea*. Scales: 10 µm.

Location and Habitat: Tavşanlı Göbel Thermal Spring, sampling at thermal water channel edge, among other algae at 30 °C. Date of collection: 24.04.2014.

Description: Erect filaments densely entangled in the mucilaginous thallus. Filaments 2.5–3 µm wide. Trichomes curved, bright blue-green color, strongly constricted at the distinct cross-walls, 1.8–2 µm wide, rarely with pseudobranched. Sheaths colorless and thin. Cells shorter than wide or isodiametric. Apical cells rounded.

Leptolyngbya tenerrima (Hansgirg) Komárek 2001

Leptolyngbya tenerrima (Hansgirg) Komárek, *Preslia*, 73 (2001), p. 374. *Lyngbya tenerrima* Hansgirg, *Algenflora von Böhmen*, 8 (4) (1892), p. 84. *Oscillatoria thrix* Corda, *Almanach de Carlsbad*, 6 (1836), p. 208. *Oscillaria tenerrima* Kützing, *Phycologia Generalis*, 2 (1843), p. 184. *Lyngbya tenerrima* Hansgirg, *Algenflora von Böhmen*, 8 (4) (1892), p. 84. *Oscillatoria tenerrima* D. Prain, *Rec Bot Surv India*, 3 (1905), p. 333 (Fig. 2–C).

Location and Habitat: Günlüce Dereli Thermal Spring, sampled from the edge of the swimming pool at 32 °C. Date of collection: 24.04.2014.

Description: Slightly coiled filaments irregularly arranged in clusters, densely intricate, rarely solitary. Sheaths colorless, diffluent, and thin. Cells blue-green color, 2–2.5 µm wide, isodiametric, or shorter than wide. Cross-walls indistinctly constricted. Apical cells slightly narrowed at the end.

Leptolyngbya gelatinosa (Woronichin) Anagnostidis & Komárek 1988

Leptolyngbya gelatinosa (Woronichin) Anagnostidis & Komárek, *Arch Hydrobiol*, 80 (1988), p. 391. *Phormidium gelatinosum* Woronichin, *Botanicheskie Materialy Instituta Sporovykh Rastenij Glavnogo Botanicheskogo Sada* RSFSR, 2 (1923), p. 97 (Fig. 2–D).

Location and Habitat: Hisarcık Sefaköy Thermal Spring (35 °C), Simav Eynal Thermal Spring (45 °C), sampled near to thermal spring side. Date of collection: 16.10.2014.

Description: Gelatinous thallus blue-green color. Trichomes thin, slightly curved or straight, 2 µm wide, with distinct and colorless sheaths. Cells 2 µm long or longer than wide. Some cells contain granules. Cross-walls visible and not constricted. Apical cells rounded. The end of trichomes straight.

Leptolyngbya granulifera (J. J. Copeland) Anagnostidis 1936

Leptolyngbya granulifera (J. J. Copeland) Anagnostidis, Published in Anagnostidis, *Preslia*, 73 (2001), p. 366. *Phormidium tenue* var. *granuliferum* J. J. Copeland, *Ann N Y Acad Sci*, 3 6 (1936), p. 178 (Fig. 2–E).

Location and Habitat: GedizIlıca Thermal Spring, sampled to outflow point of thermal spring at 48 °C. Date of collection: 16.10.2014.

Description: Filaments blue-green color, flexuous and erectly arranged at membranous thallus. Colorless sheaths thin. Trichomes 1.5 µm wide. Cells longer than wide, sometimes with a granule either side of cells. Apical cells rounded.

Limnothrix mirabilis (Bocher 1949) Anagnostidis 2001

Family: Pseudanabaenaceae

Genus: *Limnothrix* M. E. Meffert, 1988

Limnothrix mirabilis (Böcher) Anagnostidis, *Preslia*, 73 (2001), p. 363. *Oscillatoria mirabilis* Böcher, *Kongelige Danske Videnskabernes Selskab Biologiske Meddelelser*, 21 (1949), p. 23. *Jaaginema mirabile* (Böcher) Anagnostidis & Komárek, *Arch Hydrobiol*, 80 (1988), p. 396 (Fig. 2–F).

Location and Habitat: Gediz Ilıca Thermal Spring, sampled from the muddy bottom of the thermal water channel at 36 °C. Date of collection: 28.06.2014.

Description: Forming mats and bright blue-green color. Trichomes motile, 1–1.3 µm wide, straight, bright blue-green color, without sheaths. Cells longer than wide, with granules. Cross-walls hardly visible and not constricted. Apical cells rounded. End of the trichomes straight, not attenuated.

Geitlerinema nematodes (Skuja) Anagnostidis 2001

Order: Oscillatoriales

Family: Coleofasciculaceae

Genus: *Geitlerinema* (Anagnostidis & Komárek) Anagnostidis, 1989

Geitlerinema nematodes (Skuja) Anagnostidis, *Preslia*, 73 (2001), p. 364. *Oscillatoria nematodes* Skuja, *Nova Acta Regiae Soc Sci Ups*, 18 (3) (1964), p. 52. *Pseudanabaena nematodes* (Skuja) Anagnostidis & Komárek, *Arch Hydrobiol*, 80 (1988), p. 383 (Fig. 2–G).

Location and Habitat: Gediz Ilıca Thermal Spring, sampling with other cyanobacteria, especially *Spirulina* spp., at 42 °C. Date of collection: 28.06.2014.

Description: Forming mats with other cyanobacteria, olive-green color. Trichomes 0.7 µm wide, motile, olive-green color, straight, flexuous, or irregularly curved. Cross-walls hardly visible and not constricted. Cells longer than wide.

Planktothrix clathrata (Skuja) Anagnostidis & Komárek 1988

Family: Microcoleaceae

Genus: *Planktothrix* K. Anagnostidis & J. Komárek, 1988

Planktothrix clathrata (Skuja) Anagnostidis & Komárek, *Arch Hydrobiol*, 80 (1998), p. 416. *Oscillatoria mougeotiii var. clathrata* Skuja, *Nova Acta Regiae Soc Sci Ups*, 16 (3) (1956), p. 58. *Oscillatoria mougeotii* f. *clathrata* Skuja ex Starmach, *Flora Slodkowodna Polski*, 1966, p. 325 (Fig. 2–H).

Location and Habitat: Günlüce Dereli Thermal Spring, sampled as planktonic from the swimming pool at 30 °C. Date of collection: 24.04.2014.

Description: Trichomes solitary, straight, 6.5 µm wide, constricted at cross-walls, motile, blackish blue-green color. Cells shorter than wide, 2 µm long, with aerotopes. Apical cells rounded, with aerotopes.

Microcoleus lacustris Farlow ex Gomont 1892

Genus: *Microcoleus* Desmazières ex Gomont, 1892

Microcoleus lacustris Farlow ex Gomont, *Ann Sci Nat*, Bot, 15 (1892), p. 359 (Fig. 2–I).

Location and Habitat: Günlüce Dereli Thermal Spring, sampled from channels the edge of the swimming pool at 30 °C. Date of collection: 24.04.2014.

Description: Thallus thin, and dark green color. Numerous trichomes arranged as parallel in the same sheath. Trichomes straight, blue-green, 3.5 µm wide, constricted at the cross-walls. Cells longer than wide, 6 µm long, with granules. Apical cells acute-conical.

Phormidium incrustatum Gomont ex Gomont 1892

Family: Oscillatoriaceae

Genus: *Phormidium* Kützing ex Gomont, 1892

Phormidium incrustatum Gomont ex Gomont, *Ann Sci Nat*, *Bot*, 16 (1892), p. 170. *Hypheothrix incrustata* Nägeli, *Species Algarum*, 1849, p. 269. *Hypheothrix cataractarum* Nägeli, *Species Algarum*,

1849, p. 269. *Phormidium cataractarum* (Nägeli) Gomont, *Journal de Botanique*, 4 (1890), p. 355. *Lyngbya cataractarum* (Nägeli) Hansgirg, *Sitzungsberichte der Königl Böhmischen Gesellschaft der Wissenschaften Mathematisch-naturwissenschaftliche Classe Jahrgang*, 1891, p. 130. *Lyngbya incrustata* (Nägeli) Hansgirg, *Physiologische Und Phycophytologische Untersuchungen*, 1893, p. 224 (Fig. 2–J).

Location and Habitat: Günlüce Dereli Thermal Spring, sampled from the edge of thermal water channels and pool, petrified, on travertine surface at the edge of the outdoor pool at 28–30 °C. Date of collection: 24.04.2014.

Description: Forming thin and blackish blue-green mats with the other algae. Filaments erect, petrified in the thallus. Sheath thin, lamellate, and transparent. Trichomes blue-green, 5 µm wide. Cells shorter than wide, 3.5–4 µm long, with large granules. Apical cells obtuse-conical.

Phormidium thermobium Anagnostidis 2001

Phormidium thermobium Anagnostidis, *Preslia*, 73 (2001), p. 371 (Fig. 2–K).

Location and Habitat: Günlüce Dereli Thermal Spring (28 °C), Hisarcık Sefaköy Thermal Spring (32 °C), sampled from outflow point of thermal springs. Date of collection: 24.04.2014, 16.10.2014.

Description: Thallus thin, and blue-green color. Long filaments curved and entangled with the others. Sheaths thin and transparent. Trichomes blue-green color, 4.5–5 µm wide, cross-walls constricted. Cells granulated, isodiametric or longer than wide, 4.5–7 µm long. Apical cells longer than other cells, acute-conical.

Oscillatoria subcapitata Ponomarev ex Elenkin 1949

Genus: *Oscillatoria* Vaucherex Gomont, 1892

Oscillatoria subcapitata Ponomarev ex Elenkin, Monographia algarum Cyanophycea rum aquidulcium et terrestrium in finibus URSS inventarum *Pars specialis (Syatematica)*, 1949, (Fig. 2–L).

Location and Habitat: Günlüce Dereli Thermal Spring, sampled from channels the edge of the swimming pool at 28–32 °C. Date of collection: 24.04.2014, 16.10.2014.

Description: Trichomes blue-green color, 9–10 µm wide, straight. Cross-walls distinctly constricted. Cells granulated and 2.5 µm long. Apical cells rounded, with thickened outer cell wall.

Oscillatoria proboscidea Gomont 1892

Oscillatoria proboscidea Gomont, *Ann Sci Nat, Bot*, 16 (1892), p. 209 (Fig. 2–M).

Location and Habitat: Gediz Ilıca Thermal Spring (30 °C) and Sarpaşan Thermal Spring (32 °C), sampled as a mat with other cyanobacteria from the bottom of the thermal water channels. Date of collection: 28.06.2014, 16.10.2014.

Description: Forming membranous thallus with other filamentous cyanobacteria. Thallus thin, blackish blue-green color. Trichomes olive-green color, 17.5–20 µm wide, straight, motile. Cells granulated and 5–6 µm long. Cross-walls somewhat constricted. End of trichomes distinctly attenuated and slightly curved. Apical cells hemispherical and with thickened outer cell wall.

Discussion

The taxa listed in the results have not been recorded up to now in Turkey[1-3]. A total of thirteen taxa belonging to eight genera under the Cyanobacteria are new records for freshwater algal flora of Turkey. Cyanobacteria are the most commonly reported microbial groups constituting thermophilic mats and considered as the dominant primary producers in these kinds of habitats[4]. Similarly, eight of the thirteen taxa are commonly found in thermal waters. These are; *Cyanosarcina thermalis* (Hindák) Kovácik 1988, *Leptolyngbya boryana* (Gomont) Anagnostidis & Komárek 1988, *L. gelatinosa* (Woronichin) Anagnostidis & Komárek 1988, *L. granulifera* (J. J. Copeland) Anagnostidis 1936, *Phormidium incrustatum* Gomont ex Gomont 1892, *P. thermobium* Anagnostidis 2001, *Oscillatoria subcapitata* Ponomarev ex Elenkin 1949, *O. proboscidea* Gomont1 892.

Cyanosarcina thermalis is a thermal taxon found in thermal mud at Slovakia[16], thermal springs from Iran[20]. In this study, sampled at the bottom of the shallow thermal water pool and channels at Hisarcık Tarihi Hamam Thermal Spring (38–41 °C). Arman *et al.*[20] recorded this taxon in lower temperature range (34–35 °C). Komárek & Anagnostidis[16] reported that this taxon's cells could max 6 µm in diameter. Besides this, it is measured max 4 µm in diameter in this study. *Leptolyngbya boryana* is freshwater algae widespread in Europe also reported in thermal springs in Georgia[16]. It was sampled at thermal water channel, entangled with other cyanobacteria in Tavşanlı Göbel Thermal Spring. *Leptolyngbya gelatinosa* known as a thermal

and mineral water taxon. Recorded from Georgia thermal spring[16]. It was sampled from Hisarcık Sefaköy (35 °C) and Simav Eynal (45 °C) thermal springs at different degrees. *Leptolyngbya granulifera* is a typical thermal water taxon, described from Yellowstone National Park springs in the USA and Greece thermal springs[16,21,22]. In this study, sampled from Gediz Ilıca Thermal Spring geysers at 48 °C. On the contrary, Kaštovský & Komárek[21] has detected this taxon in a much lower temperature range (24–36 °C and below 24 °C). *Phormidium incrustatum* finds travertine a suitable environment for growth[16,23-25]. Pentecost[23-25] studied ecology and biology of this taxon in detail. Especially in the limestone environment, this taxon is maybe cosmopolite[16,24,25]. Also, Kanellopoulos *et al.*[26] reported that, this taxon is the most common carbonate-incrusted cyanobacteria. Similarly, it formed calcareous tuffs the edge of thermal water channels at Günlüce Dereli Thermal Spring. It has created a small, yellowish layer on travertine surface. According to Komárek & Anagnostidis[16], this taxon is denoted a cosmopolitan distribution and indicated as recorded in Turkey. However, there is no record in the literature to confirm this information.

Phormidium thermobium recorded from thermal springs in Phthiotis, Greece, by Bravakos *et al.*[27]. It was sampled from the outflow point of Günlüce Dereli and Hisarcık Sefaköy Thermal Springs in this study. *Oscillatoria subcapitata* prefers sulfur springs[16] and thermal springs[19]. It was sampled from channels on the edge of the swimming pool from Günlüce Dereli Thermal Spring. In this study, the temperature of the channels was 28–32 °C, but the SO_4^{-2} (ppm) value below the measuring limits. *Oscillatoria proboscidea* is freshwater algae, probably cosmopolite in temperature zone[16]. Also, found in thermal springs[28-30]. It was sampled from Gediz Ilıca Thermal Spring and Sarpaşan Thermal Spring at a temperature gradient between 30–32 °C. *Limnothrix mirabilis* recorded from freshwater springs in Romania[31] and some thermal springs (temperature range 34–36 °C) in Iran[20]. It was sampled from the muddy bottom of the thermal water channel at Gediz Ilıca Thermal Spring at 36 °C. *Leptolyngbya tenerrima* recorded in Canada[32], common in central Europe[16], Romania[31], and Czechia[19]. It was sampled from the edge of the swimming pool in Günlüce Dereli Thermal Spring. *Geitlerinema nematodes* live in shallow, small freshwater lakes[16]. It was sampled as a mat with the other cyanobacteria, especially *Spirulina* spp. In Gediz Ilıca Thermal Spring. *Planktothrix clathrata*, which frequently found in the species composition of freshwater, was also recorded from Großer Vätersee, Templin, Germany[33], Himachal Pradesh, India[34] and Iławskie Lake, Poland[35]. Heidari *et al.*[36] stated that *Plankthothrix iranica* Heidari et Hauer sp. nov. found in thermal water from Iran is very similar to *Planktothrix clathrata* but it differs from it with its characteristics such as movement and ecology. This taxon was detected at Günlüce Dereli Thermal Spring, as a planktonic in the swimming pool. *Microcoleus lacustris* is a benthic freshwater taxon common at the European[19] and Asian countries (India[37], Iran[38], and Nepal[39]). Unlike this, Dominic & Madhusoodanan[40] collected it from acidic soils (pH range 4–2.5). It was sampled from channels the edge of the swimming pool in Günlüce Dereli Thermal Spring (pH 7). Komárek and Anagnostidis[16] reported that trichomes could be 3.5–5.7 µm wide, also Barman *et al.*[37] indicated that 4–5 µm wide. Besides both, the trichomes measured 3–3.5 µm wide in this study.

There are no significant differences in pH, temperature, and TDS between the thermal springs. Even so, Günlüce Dereli (5 taxa) and Gediz Ilıca (4 taxa) thermal spring have the highest number of the taxon, among the others. According to Bhakta *et al.*[41], Cyanobacteria was dominance at a range of temperatures 35–60 °C. Kaštovský & Komárek[21] described 14 Cyanobacteria in a range of temperatures 24–55 °C. Sompong *et al*.[42] mapped the distribution of mat-forming cyanobacteria at thermal gradient 30–80 °C. Arman *et al*.[20] indicated that one of the most important parameters for cyanobacteria diversity in thermal springs is temperature. Whereas, Cyanobacteria was sampled at 28–48 °C in this study.

Conclusion

The diversity of algae are the basis for determining the ecological importance of environment and monitoring changes. Therefore, the determination of the freshwater algal flora in Turkey and monitoring studies should continue. Freshwater algal flora data of Turkey is compiled in recent years at the online checklist[1].

Algae distributed at thermal waters/ springs are least known among other freshwater ecosystems. Therefore, there are several new records in the study

of thermal water algae. From this point, thirteen cyanobacteria were identified, which of 8 were common in thermal waters, in this study. Cyanobacteria are the most adapted organisms for thermal springs. Cyanobacteria were once again shown to be the best living organisms that can adapt to the thermal waters with this study. The result also showed that further detailed studies about the algal flora of thermal springs should be carried out.

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Conflict of Intrest

No conflict of intrests.

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