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Morphology and taxonomy of the *Aphanizomenon* spp. (Cyanophyceae) and related species in the Nakdong River, South Korea

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Abstract

Background: The purpose of this study is to describe the morphological characteristics of the *Aphanizomenon* spp. and related species from the natural samples collected in the Nakdong River of South Korea.

Results: Morphological characteristics in the four species classified into the genera *Aphanizomenon* Morren ex Bornet et Flahault 1888 and *Cuspidothrix* Rajaniemi et al. 2005 were observed by light microscopy. The following four taxa were identified: *Aphanizomenon flos-aquae* Ralfs ex Bornet et Flahault, *Aphanizomenon klebahnii* Elenkin ex Pechar, *Aphanizomenon skujae* Komárková-Legnerová et Cronberg, and *Cuspidothrix issatschenkoi* (Usačev) Rajaniemi et al. *Aph. flos-aquae* and *Aph. klebahnii* always formed in fascicles; the others only occurred in solitary. *Aph. flos-aquae* was similar to *Aph. klebahnii*, whereas these species differed from each other by the size and shape of fascicles, which was macroscopic in *Aph. flos-aquae* and microscopic in the *Aph. klebahnii*. One of their characteristics was that trichomes are easily disintegrating during microscopic examination. *C. issatschenkoi* could be clearly distinguished from other species by hair-shaped terminal cell. Its terminal cell was almost hyaline and markedly pointed. Young populations of the species without heterocytes run a risk of a misidentification. *Aph. skujae* was characterized by akinete. Morphological variability of akinetes from natural samples collected in the Nakdong River was rather smaller than those reported by previous study.

Conclusions: *C. issatschenkoi* are described for the first time in the Nakdong River. In addition, *Aph. klebahnii* and *Aph. skujae* are new to South Korea.

Keywords: *Aphanizomenon*, *Cuspidothrix*, Cyanobacteria, Nakdong River, Nostocales

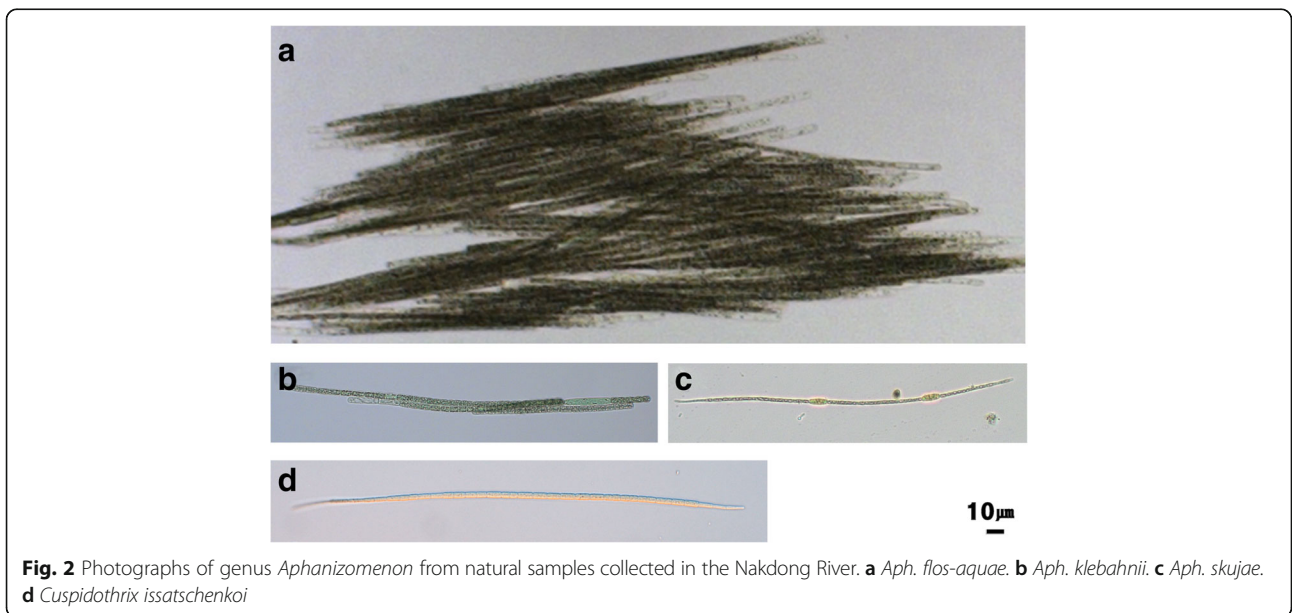
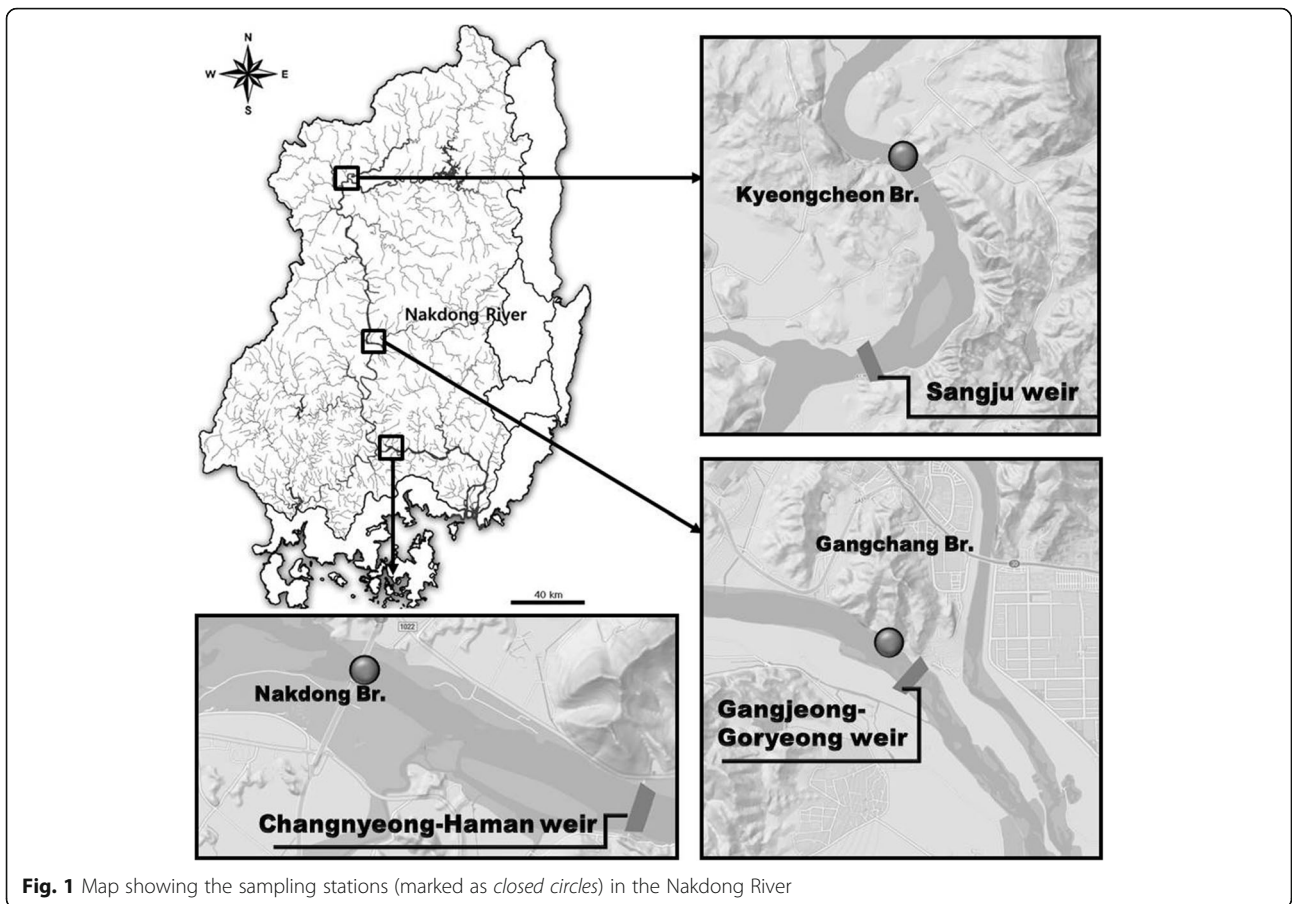
Background

The genus *Aphanizomenon* Morren ex Bornet et Flahault 1888 (type species: *Aph. flos-aquae*) belongs to order Nostocales and family Nostocaceae, which has a worldwide distribution (Rajaniemi et al. 2005a). The species of genus *Aphanizomenon* and several of its members have been described as the cause for harmful bloom (McDonald and Lehman 2013; Ma et al. 2015). Some species can produce hepatotoxic and neurotoxic, such as aphantoxin, anatoxin-a, cylindrospermopsin, and saxitoxin, cyanobacterial secondary metabolites which can cause critical

problems (Paerl and Huisman 2009; Ballot et al. 2010; Zhang et al. 2015). Therefore, it is very important for the accurate species identification of *Aphanizomenon* because of water bloom with several toxin-producing species (Guzmán-Guillén et al. 2015).

In the Nakdong River, *Microcystis* and *Anabaena* have been considered as the representative bloom-forming cyanobacteria genera (Yu et al. 2014). After the construction of eight weirs, the number of its bloom has been recently growing in mid-upperstream (Ryu et al. 2016). Nevertheless, two *Aphanizomenon* floras (*Aph. flos-aquae* and *Aph. issatschenkoi*) have been described until a recent date (Park 2004) in South Korea; only one *Aphanizomenon* species has been reported in the ecological study of

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Nakdong River: *Aph. flos-aquae* (Choi et al. 2007; Yu et al. 2014). Recent studies using polyphasic approach, e.g., involving morphology but also ecology and phylogenetics, have revealed that the genus *Aphanizomenon* is in reality very heterogeneous (Cirés and Ballot 2016). According to newly defined approach, 22 taxa identified and described throughout the world have been assigned to the new genera *Aphanizomenon* (e.g., *Aph. flos-aquae* Ralfs ex Bornet et Flahault), *Cuspidothrix* (e.g., former *Aph. issatschenkoi* (Usačev) Proshkina-Lavrenko), *Sphaerospermopsis* (e.g., former *Aphanizomenon aphanizomenoides*

(Forti) Hortobágyi and Komárek), *Chrysochlorum* (e.g., former *Aphanizomenon ovalisporum* Forti), *Anabaena*/*Aphanizomenon* like (e.g., *Aphanizomenon gracile* (Lemmermann) Lemmermann), and *Anabaena*-like group (e.g., *Aphanizomenon volzii* (Lemmermann) Komárek) (Lyra et al. 2001; Gugger et al. 2002; Rajaniemi et al. 2005b; Komárek and Komárková 2006; Zapomělová et al. 2012; Komárek 2013).

The classification of genus *Aphanizomenon* which frequently form blooms is in some cases difficult that is due to lack of the study for morphology and taxonomy

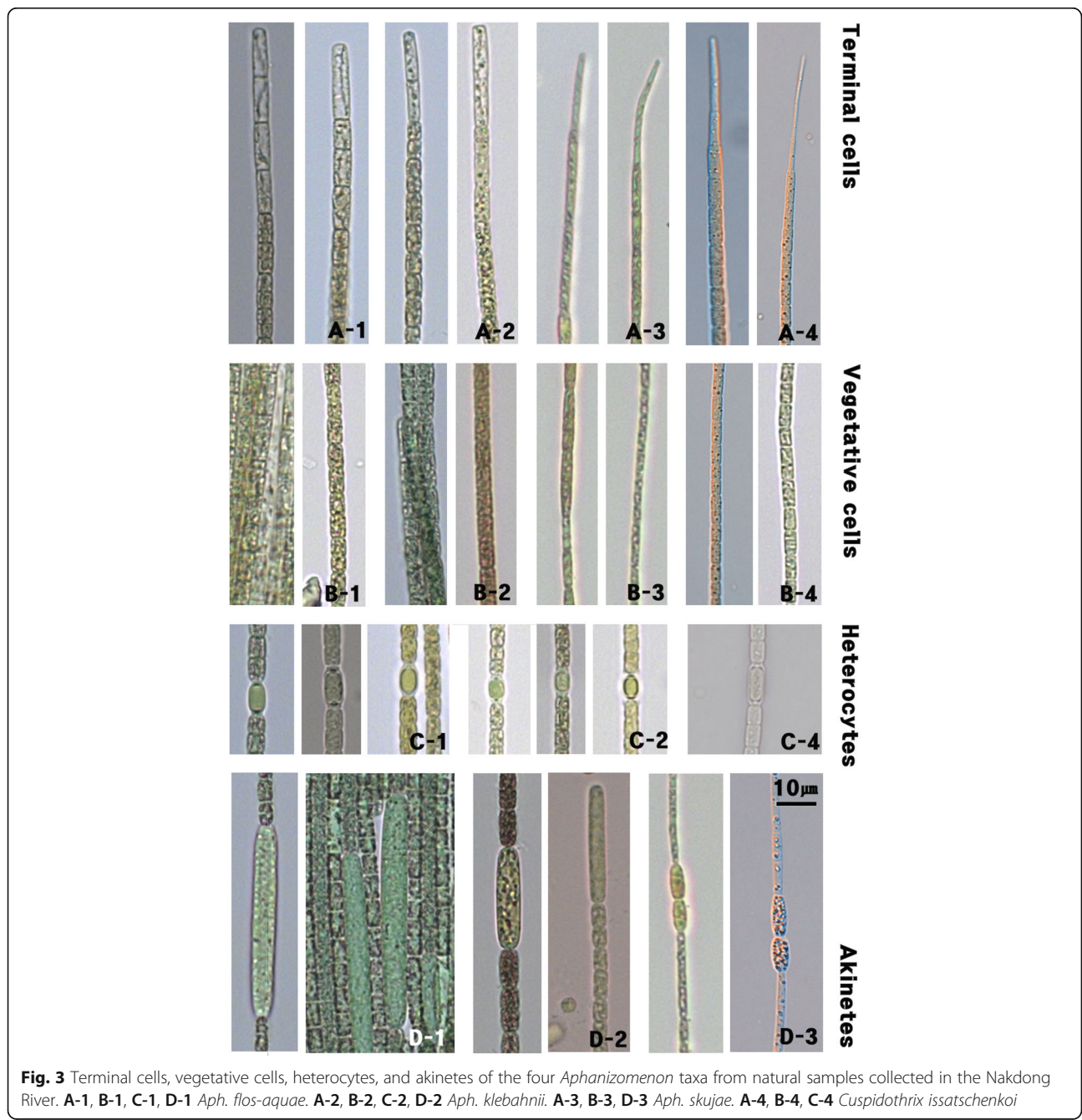


Fig. 3 Terminal cells, vegetative cells, heterocytes, and akinetes of the four *Aphanizomenon* taxa from natural samples collected in the Nakdong River. **A-1, B-1, C-1, D-1** *Aph. flos-aquae*. **A-2, B-2, C-2, D-2** *Aph. klebahnii*. **A-3, B-3, D-3** *Aph. skujae*. **A-4, B-4, C-4** *Cuspidothrix issatschenkoi*

in South Korea. The purpose of this study is to describe the morphological characteristics of the *Aphanizomenon* spp. and related species from the natural samples collected in the Nakdong River, South Korea.

Methods

The cyanobacteria samples were collected on three stations of the Nakdong River where the stations located in Sangju (N 35° 27' 14.69"/E 128° 15' 27.11"), Daegu (N 35° 50' 35.58"/E 128° 27' 33.92"), and Haman (N 35° 23' 40.89"/E 128° 31' 11.84"), respectively (Fig. 1). The samples were collected from June 2015 to May 2016 with 1-month interval using the plankton net (mesh size 32 µm). It was preserved in 4% Lugol's solution or formaldehyde water and was transferred at the laboratory. The morphology of trichomes, vegetative cells, heterocytes, and akinetes were studied using Nikon ECLIPSE 80i light microscope with a digital camera. NIS-Elements F 3.0 software was used for image analysis. The following parameters were selected to describe the morphology of the studied specimens: length and width of vegetative cell, heterocytes, and akinetes; morphology of terminal cell; distance between heterocytes and distance between a heterocyte and the nearest akinete (counted as the number of cells); presence or absence of terminal heterocytes and gas vesicles; and shape of trichomes and its aggregation in colonies. All measurements were obtained with the preserved materials.

Results and discussion

Within the genus *Aphanizomenon*, three clusters were distinguished by Komárek and Komárková (2006) and

Komárek (2013) for classification. The first cluster (i) included the type species *Aph. flos-aquae* Ralfs ex Bornet et Flahault 1888 and *Aphanizomenon klebahnii* Elenkin ex Pechar 2008, together with *Aph. yezoense*, *Aph. paraflexuosum*, *Aph. flexuosum*, *Aph. solvenicum*, *Aph. platense*, and *Aph. hungaricum*. Trichomes of *Aph. flos-aquae* and *Aph. klebahnii* taxa always formed macroscopic and microscopic fascicles, and those were able to cause intensive water blooms in eutrophic stagnant water (Hindák 2000). *Aph. flos-aquae* was common species with *Microcystis* spp. and *Anabeana* spp. and the major component of the water bloom in the Nakdong River (Park et al. 2015; Yu et al. 2014). Whereas *Aph. klebahnii* was described for the first time in the South Korea. Cluster (ii) included species with slightly curved or flexuous trichomes. The terminal cells were narrowed, elongated, and hyaline with sharply pointed. Akinetes were distant to heterocytes. This cluster included *Cuspidothrix issatschenkoi* (Usačev) Rajaniemi et al. 2005, together with *C. elenkinii*, *Aph. tropicalis*, *Aph. capricorni*, and *Aph. ussatchevii*. *C. issatschenkoi* was described for the first time in the Nakdong River. Cluster (iii) was comprised of species described as morphotype of *Aphanizomenon gracile* with straight, solitary trichomes and with narrowed ends, which belong into the vicinity of *Dolichospermum* according to molecular sequences. *Aph. skujae* Komárková-Legnerová et Cronberg 1992 belonged to this cluster, together with *Aph. gracile*, *Aph. Schindleri*, *Aph. manguinii*, *Aph. chinense*, and *Aph. sphaericum*. Identification of the species is the first report in South Korea.

Table 1 Diacritical morphological characteristics of four *Aphanizomenon* taxa reviewed from natural samples collected in the Nakdong River

Species	Fascicles trichomes	Terminal cells	Vegetative cells	Heterocytes	Akinetes
<i>Aphanizomenon flos-aquae</i>	Band-like, up to 2 cm long, straight or bent, often grouped in fascicles	Elongated cylindrical, not narrowed, without aerotopes, almost hyaline	Cylindrical to slightly barrel-shaped, 4.0–12.1 × 3.6–5.6 µm ^a (n = 45)	Intercalary, solitary, cylindrical, 6.6–8.5 × 3.3–3.9 µm ^a (n = 19)	Intercalary, long cylindrical, distant from heterocytes, 30–62 × 5.2–7.5 µm ^a (n = 22)
<i>Aphanizomenon klebahnii</i>	Spindle-like, up to 3 mm long, straight or slightly arcuated, often grouped in fascicles	Elongated cylindrical, without aerotopes, and with remaining cytoplasm in the form of fine granulation	Cylindrical or slightly barrel-shaped, 3.9–8.3 × 3.6–4.9 µm ^a (n = 31)	Solitary, intercalary, oval to cylindrical, 5.5–6.7 × 3.2–4.0 µm ^a (n = 6)	Intercalary, solitary, elongated cylindrical, 26–39 × 4.5–5.9 µm ^a (n = 17)
<i>Aphanizomenon skujae</i>	Solitary, straight, bent or irregularly curved	Narrowed and elongated, bluntly pointed, containing a smaller amount of pigment and sporadic aerotopes	4.8–8.4 × 1.2–2.5 µm ^a (n = 19)	Solitary, intercalary, oval to cylindrical, 6–15 × 2–3 µm ^b	Solitary or up to 3 in a row, cylindrical with rounded ends, wider than trichomes, 7.6–11.8 × 3.8–4.6 µm ^a (n = 13)
<i>Cuspidothrix issatschenkoi</i>	Solitary, straight, bent, or slightly coiled	Tapered like hair-shaped, almost hyaline, continually pointed	Cylindrical to long-cylindrical, usually with scarce aerotopes, 4.4–7.0 × 2.5–3.3 µm ^a (n = 36)	Solitary, intercalary, 6.6–8.7 × 3.4–3.7 µm ^a (n = 5)	Solitary or 2–3 in a row, distant from heterocytes, long cylindrical with rounded ends, 8.5–12.5 × 4–4.6 µm ^c

n number of identified samples

^aThis study

^bKomárek 2013

^cRajaniemi et al. 2005b

The four investigated species in the Nakdong River were classified in the genus *Aphanizomenon* (*Aph. flos-aquae*, *Aph. klebahnii*, *Aph. skujae*) and in the genus *Cuspidothrix* (*C. issatschenkoii*). Morphological characteristic of trichomes, heterocytes, and akinetes from natural samples collected in the Nakdong River is shown in Figs. 2 and 3 and Table 1 and that investigated from studies cited is shown in Fig. 4.

Systematics of genus *Aphanizomenon* and genus *Cuspidothrix*

Class Cyanophyceae Sachs 1874

Order Nostocales Borzi 1914

Family Nostocaceae C.A. Agardh 1824 ex Korchner 1898

Genus *Aphanizomenon* Morren ex Bornet et Flahault 1888

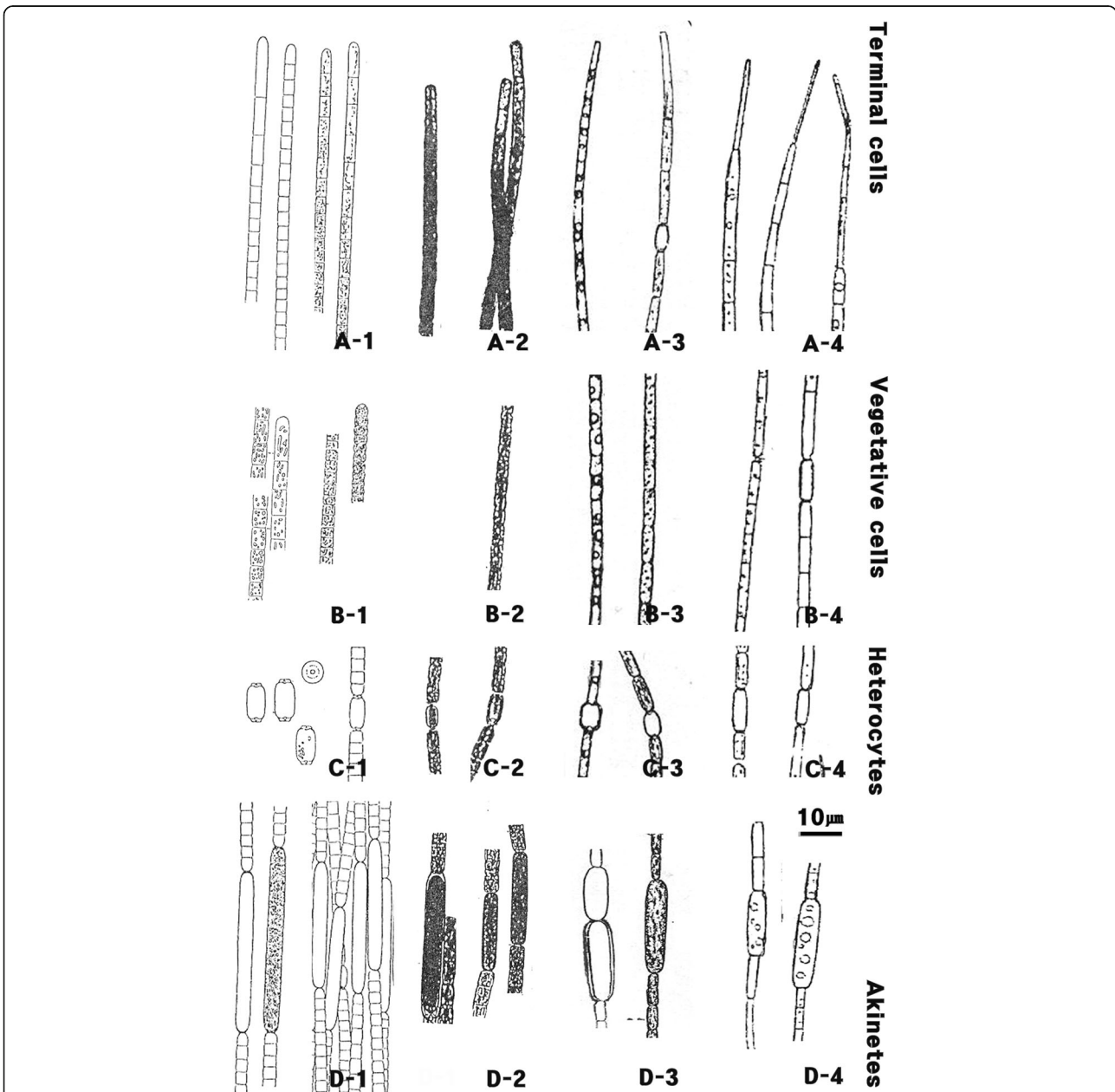


Fig. 4 Terminal cells, vegetative cells, heterocytes, and akinetes of the four *Aphanizomenon* taxa investigated from studies cited. **A-1, B-1, C-1, D-1** *Aph. flos-aquae*—after Komárek (1958). **A-2, B-2, C-2, D-2** *Aph. klebahnii*—after Komárek et Kováčik (1989). **A-3, B-3, D-3** *Aph. skujae*—after Skuja (1956) and Komárková-Legnerová et Cronberg (1992). **A-4, B-4, C-4** *Cuspidothrix issatschenkoii*—after Usačev from Kondraeva 1968 and after Hindák et Moustaka (1988)

Aphanizomenon flos-aquae Ralfs ex Bornet et Flahault 1888

Aphanizomenon klebahnii Elenkin ex Pechar 2008

Aphanizomenon skujae Komárková-Legnerová et Cronberg 1992

Genus *Cuspidothrix* Rajaniemi et al. 2005

Cuspidothrix issatschenkoi (Usačev) Rajaniemi et al. 2005

Morphology and taxonomy of individual species

Aphanizomenon flos-aquae Ralf ex Bornet et Flahault (Fig. 2a; Fig. 3A-1~D-1)

(Smith 1950, p. 585, fig. 503; Hirose et al. 1977, p. 85, pl. 36 3a-3d; Komárek and Kováčik 1989, fig. 8; John et al. 2002, p. 96, pl. 18g-j; Rajaniemi et al. 2005b, Fig. 7. a; Komárek and Komárková 2006, Fig. 6; Komárek 2013, p. 688, Fig. 853)

Synonyms: *Aphanizomenon incurvum* Morren 1835; *Aphanizomenon cyaneum* Ralfs 1850; *Aphanizomenon holsaticum* Richter 1896; *Aphanizomenon americanum* Reinhard 1941

This species was common in Asian freshwater not only in South Korea (Park et al. 2015; Ryu et al. 2016) but also in China (Wu et al. 2010; Ma et al. 2015) and in Japan (Takano and Hino 2009; Yamamoto 2009). Studied samples were collected from all stations. The species was characterized by a tendency to aggregate trichomes in parallel fascicles which can reach a macroscopic size of up to 2 cm. Trichomes of the species were straight or bent, cylindrical, and slightly constricted at the cross-walls. Other morphological features include an isopolar and at the ends cylindrical-rounded (Fig. 2a). Trichomes were easily disintegrating by shaking or fixing solution during microscopic examination (Fig. 5). Cells were cylindrical to slightly barrel-shaped, isodiametric with olive-green protoplast and numerous aerotopes, $4\text{--}12.1 \times 3.6\text{--}5.6 \mu\text{m}$; terminal cells elongated and up to 19.1 long, without aerotope, almost hyaline, usually with characteristic remains of cytoplasm in form of an irregular (Fig. 3A-1). Heterocytes were intercalary, solitary (up to 3) in a trichome, cylindrical, and $6.6\text{--}8.5 \times 5\text{--}8.5 \mu\text{m}$. Morphological variability of akinetes from natural samples collected in the Nakdong River was rather smaller. Akinetes were reported by Komárek (2013) as $40\text{--}220 \times 6\text{--}10.8 \mu\text{m}$, however mostly $30\text{--}62 \times 5.2\text{--}7.5 \mu\text{m}$, intercalary, long cylindrical, and distant from heterocyte (Table 1).

Ecology: This species is planktonic in eutrophic reservoir (Komárek 2013). It has shown positive growth within a wide range of temperatures ($16\text{--}25 \text{ }^\circ\text{C}$) (Preussel et al. 2009) and can grow below $10 \text{ }^\circ\text{C}$ (Üveges et al. 2012). It has a competitive advantage under situations of low light intensities (Mehnert et al. 2010). We collected this specimen in waterbodies of mesotrophic or eutrophic status (range of total phosphorus $0.017\text{--}0.040 \text{ mg L}^{-1}$).

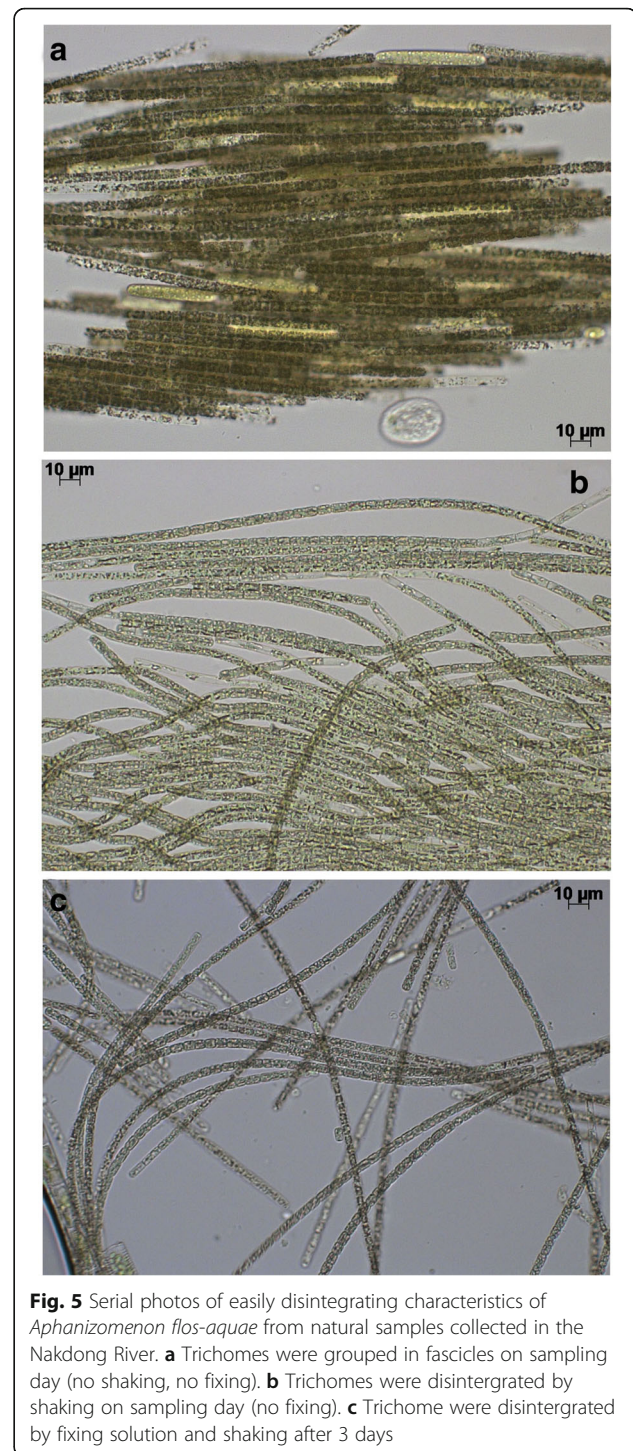


Fig. 5 Serial photos of easily disintegrating characteristics of *Aphanizomenon flos-aquae* from natural samples collected in the Nakdong River. **a** Trichomes were grouped in fascicles on sampling day (no shaking, no fixing). **b** Trichomes were disintegrated by shaking on sampling day (no fixing). **c** Trichome were disintegrated by fixing solution and shaking after 3 days

Material examined: Sangju (Jun. 2015, Oct. 2015, Dec. 2015, Nov. 2015, Jan. 2016, May. 2016), Daegu (Jun. 2015, Oct. 2015, Dec. 2015, Nov. 2015, Jan. 2016, Feb. 2016, Mar. 2016, Apr. 2016, May 2016), Haman (Jun. 2015, Oct. 2015, Dec. 2015, Nov. 2015, Jan. 2016, Feb. 2016, Mar. 2016, Apr. 2016, May 2016)

Aphanizomenon klebahnii Elenkin ex Pechar (Fig. 2b; Fig. 3A-2~D-2)

(Hirose et al. 1977, p. 85, pl. 36 4a-4b; Komárek and Kováčik 1989, fig. 9; Komárek 2013, p. 690, Fig. 855)

Synonyms: *Aphanizomenon flos-aquae* var. *klebahnii* Elenkin 1909; *Aphanizomenon klebahnii* Elenkin 1909 (Nomen alternat.)

Blooms of this species have been frequently observed in the Japanese lakes (Yamamoto 2009); however, the species has not been recorded in South Korea. Single free-floating trichomes of the species were aggregated in parallel in spindle-like fascicles and up to 2 mm long. Trichomes were straight or slightly arcuated cylindrical; almost not or only very slightly constricted at the cross-walls; isopolar, on both ends with elongated cylindrical; and not narrowed cells (Fig. 2b). Cells were cylindrical or slightly barrel-shaped, isodiametric, with olive-green protoplast with numerous aerotopes, and $3.9\text{--}8.3 \times 3.6\text{--}4.9 \mu\text{m}$; terminal cells were elongated, up to $12 \mu\text{m}$ long, without aerotopes, and with remaining cytoplasm in the form of fine granulation (Fig. 3A-2). Heterocytes were solitary, intercalary, 1–2 in the trichome, oval to cylindrical, and $5.5\text{--}6.7 \times 3.2\text{--}4.0 \mu\text{m}$. Akinetes were developed asymmetrically on the trichome, intercalary, solitary or rarely in pairs, elongated cylindrical, and $26\text{--}39 \times 4.5\text{--}5.9 \mu\text{m}$ (Table 1). This species was very similar to the species, *Aph. flos-aquae* Ralfs ex Bornet et Flahault, namely in shape, size, and color of cells, terminal cell, and heterocytes. However, this species differed from *Aph. flos-aquae* only by the size and shape of fascicles, which is macroscopic in *Aph. flos-aquae* and microscopic in *Aph. klebahnii* (Fig. 6, Table 1).

Ecology: This species is planktonic in eutrophic up to hypertrophic reservoir (Komárek 2013). It is adapted to high water temperatures (Yamamoto and Nakahara 2006). We collected this specimen in waterbodies of mesotrophic or eutrophic status (range of total phosphorus $0.028\text{--}0.036 \text{ mg L}^{-1}$).

Material examined: Daegu (Oct. 2015, Nov. 2015), Haman (Oct. 2015, Feb. 2016)

Aphanizomenon skujae Komárková-Legnerová et Cronberg (Fig. 2c; Fig. 3A-3, B-3, D-3)

(Komárek and Komárková 2006, Fig. 17)

Synonyms: *Aphanizomenon* cf. *flos-aquae* var. *klebahnii* sensu Skuja 1956

Identification of this species is the first report in South Korea. Trichomes of the species were solitary, fine, straight, bent or irregularly curved, and without mucilage (Fig. 2c). Cells usually were with aerotopes, particularly in the central part, towards the ends sometimes more hyaline, $4.8\text{--}8.4 \times 1.2\text{--}2.5 \mu\text{m}$; terminal cells were narrow and elongated, containing a smaller amount of pigment and sporadic aerotopes. Terminal cells were bluntly pointed (Fig. 3A-3). Heterocytes were not found from natural samples collected in the Nakdong River. Akinetes were oval to cylindrical with rounded ends, conspicuously wider than vegetative cells, and with an akinete/trichome width ratio often greater than twofold. Morphological variability of akinetes from natural samples collected in the Nakdong River was rather small. It was reported by Komárek (2013) as $20\text{--}34 \times 2.7\text{--}4.7 \mu\text{m}$ but mostly $7.6\text{--}11.8 \times 3.8\text{--}4.6 \mu\text{m}$. Akinetes range from 1 to 2 in number (rarely up to 3) in a row, with smooth and colorless exospore; the content was greenish and granular (Table 1).

Ecology: This species is planktonic in lakes. It is distributed in northern and colder parts of temperate zone in Eurasia (Komárek 2013). We collected this specimen in waterbodies of oligotrophic status (total phosphorus 0.009 mg L^{-1}).

Material examined: Sangju (Jun. 2015)

Cuspidothrix issatschenkoii (Usačev) Rajaniemi et al. 2005 (Fig. 2d; Fig. 3A-4~C-4)

(John et al. 2002, p. 96, pl. 18k; Rajaniemi et al. 2005b, Fig. 7. c; Komárek and Komárková 2006, Fig. 31; Figueiredo et al. 2011, Fig. 1. a-c; Ballot et al. 2010. Fig. 1; Komárek 2013, p. 668, Figs. 822-823)

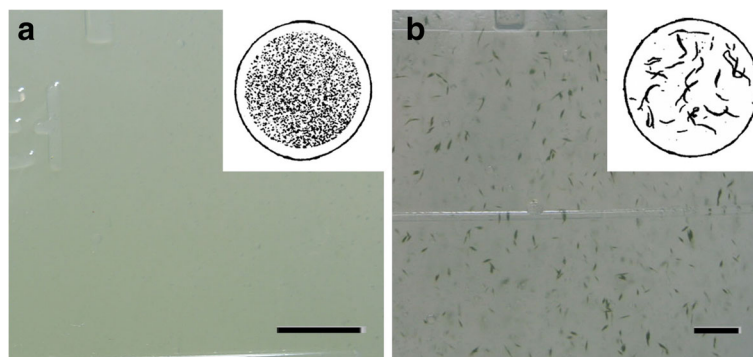


Fig. 6 Comparison of size and shape of fascicles from natural samples collected in the Nakdong River. **a** *Aphanizomenon klebahnii* (microscopic size) and **b** *Aphanizomenon flos-aquae* (macroscopic size). Scale bar is 10 mm. Shape of fascicles in circles was depicted by Pechar and Kalina from Komárek and Komárková (2006)

Synonyms: *Aphanizomenon issatschenkoi* Usačev 1938

The presence of this species has been reported in freshwaters from many European countries (Kastovsky et al. 2010) and in Asia including China (Wu et al. 2010; Ballot et al. 2010), Japan (Watanabe 1985), and Singapore (Pham et al. 2011). In South Korea, this species has been described only one time until a recent date in the Han River (Park 2004) and it is described for the first time in the Nakdong River. The species was characterized by solitary, bent, or slightly coiled trichomes. The trichomes were isopolar, cylindrical in central part, and continually narrowed or pointed towards ends (developed trichomes). Other morphological features include a not or slightly constricted at the cross-walls and subsymmetric (Fig. 2d). Cells were cylindrical to long cylindrical, usually with scarce aerotopes, $4.4\text{--}7.0 \times 2.5\text{--}3.3 \mu\text{m}$; terminal cell was almost hyaline and markedly pointed. The hair-shaped terminal cell was in general narrower than the vegetative cells and continually elongated (Fig. 3A-4). Heterocytes were solitary, intercalary, 1–2 (rarely 3) on a trichome, cylindrical, and $6.6\text{--}8.7 \times 3.4\text{--}3.7 \mu\text{m}$. Akinetes were not found from natural samples collected in the Nakdong River. This species was easily recognized by trichomes with hair-shaped terminal cells. However, young field populations of this species without heterocytes can be easily misidentified as the very similar *Rapidiopsis mediterranea* Skuja or as the non-heterocystous life stages of *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya and Subba Raju (Moustaka-Gouni et al. 2010).

Ecology: This species is sporadically planktonic in mesotrophic and eutrophic reservoirs (Komárek 2013). It has shown positive growth within a moderate range of temperatures ($22\text{--}28 \text{ }^\circ\text{C}$) (Dias et al. 2002). It has also been observed thriving in freshwater, as well as in oligohaline and brackish waters (Marshall et al. 2005). We collected this specimen in waterbodies of eutrophic status (range of total phosphorus $0.032\text{--}0.043 \text{ mg L}^{-1}$).

Material examined: Haman (Oct. 2015, Nov. 2015, Apr. 2016)

Conclusions

The four investigated species in the Nakdong River were classified in the genus *Aphanizomenon* (*Aph. flos-aquae*, *Aph. klebahnii*, *Aph. skujae*) and in the genus *Cuspidothrix* (*C. issatschenkoi*). *C. issatschenkoi* are described for the first time in the Nakdong River. In addition, *Aph. klebahnii* and *Aph. skujae* are new to South Korea.

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Availability of data and materials

Please contact author for data requests.

Authors' contributions

RHS carried out the design of the study, performed the fieldwork, and drafted the manuscript. SRY participated in the microscopic analysis. LJH participated in the design and coordination of manuscript and helped draft the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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