Two new diatom species from family *Achnanthidiaceae* in Bulgaria: *Achnanthidium druartii*, an invasive species in Europe and *Achnanthidium straubianum*, new to Bulgarian diatom flora

Plamen N. Ivanov

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, e-mail: plamen_new@abv.bg

Received: November 07, 2017 ▷ Accepted: July 20, 2018

Abstract. *Achnanthidium druartii* and *A. straubianum* (Bacillariophyceae) were observed for the first time in Bulgaria in two reservoirs (A. Stamboliyski and Yovkovtsi) in 2015–2016. *Achnanthidium druartii* was first observed in 2004 in France and subsequently the species was reported from the USA. It is considered as probably invasive in France and Europe in the last decade. Although *Achnanthidium straubianum* is probably widespread in Europe, it has not been reported so far from Bulgaria, presumably because of its small valve size and small numbers. The maximum relative abundance of both species in the studied samples from the two reservoirs exceeded 10 %. A plate with 49 light microscope photographs presents both valves of the two species.

Key words: Bacillariophyceae, diatoms, distribution, invasive species

Introduction

Achnanthidium Kützing is a genus of monoraphid diatoms (Bacillariophyceae) common in fresh water. *Achnanthidium druartii* Rimet & Coute and *A. straubianum* (Lange-Bertalot) Lange-Bertalot were observed for the first time in Bulgaria in two reservoirs during the diatom analyses of a great number of epilithic diatom samples from rivers and stagnant waters across Bulgaria sampled in 2015–2016.

Achnanthidium druartii belongs to the group of taxa leaning to *A. pyrenaicum* (Hustedt) Kobayasi with distal fissures deflected to the same side of the valve (as *A. convergens* (Kobayasi) Kobayasi, *A. deflexum* (Reimer) Kingston, *A. delmontii Peres* & al., *A. japonicum* (Kobayasi) Kobayasi, *A. latecephalum* Kobayasi, and *A. rivulare* Potapova & Ponader). The species was first observed in 2004, in a single locality in France (river Rhone), in low abundance, but in later years appeared in increasing numbers in more rivers and canals across France and in river Ebro in Spain. The taxon was described as a new species invading the rivers in France and Spain (Rimet & al. 2010). Subsequently, the species was reported from rivers in Kentucky and Virginia in the USA (Potapova 2011) and from the Netherlands (Guiry 2017). According to its original description, *A. druartii* is epilithic and alkalophilous, prefers well oxygenated waters, with relatively low trophic and very low saprobic levels, and is typical for rivers with relatively high conductivity and calcium content (Rimet & al. 2010). *A. druartii* is considered as probably invasive in France and Europe in the last decade (Rimet & al. 2010, Guiry 2017).

Achnanthidium straubianum belongs to the group of taxa around *A. minutissimum* (Kütz.) Czarnecki with straight distal endings of the raphe, averagely shorter in the group. It was first published as *Achnanthes minutissima* «Sippe mit breit elliptischen Schalen» (Krammer & Lange-Bertalot 1991), and subsequently, as a new taxon *Achnanthes straubiana* Lange-Bertalot

(Lange-Bertalot & Metzeltin 1996), which was finally transferred as a new combination *Achnanthidium straubianum* (Lange-Bertalot 1999). It has been reported from calcium-rich mesotrophic and eutrophic rivers, springs and lakes, collected from stones, mud and *Vaucheria* sp. filaments (Krammer & Lange-Bertalot 1991; Lange-Bertalot & Metzeltin 1996; Cantonati 1998; Wojtal 2004).

This study is part of a larger-scale investigation of the diatom flora of reservoirs and stagnant waters in Bulgaria. For most reservoirs, it was the first research into periphitic diatoms.

Material and methods

Study area

Diatom samples used in the present study were collected from two artificial reservoirs – Alexander Stamboliyski and Yovkovtsi – within the framework of the investigation *Update of the typology and classification system for surface water evaluation of the categories "river", "lake" and "transitional waters" in the period of the first River Basin Management Plan* during 2015–2016. A. Stamboliyski Reservoir is the first large reservoir in Bulgaria built on river Rositsa. It is 18 km long, covers an area of 10.86 km² and elevation at its dam is 190 m a.s.l. Yovkovtsi Reservoir on river Veselina is one of the largest reservoirs in Bulgaria, with an area of 5.75 km² and elevation of 335 m a.s.l. at the dam. According to the physical and chemical data measured several times during the investigation, water in both reservoirs during 2015–2016 was alkaline, with medium conductivity and varying between low and high content of different nitrogen and phosphorus forms (Table 1). Both reservoirs were sampled twice for diatoms (once per year) at the following sampling points: 43°07'04"N, 25°10'00"E in A. Stamboliyski Reservoir and 42°55'38"N, 25°46'08"E in Yovkovtsi Reservoir.

Table 1. Physical and chemical data of water in A. Stamboliyski and Yovkovtsi Reservoirs during 2015–2016.

	A. Stamboliyski Reservoir			Yovkovtsi Reservoir		
	Min.	Median	Max.	Min.	Median	Max.
pH	7.7	8.3	8.8	7.8	8.3	8.9
conductivity (µS/cm)	200	279	365	169	234	257
N total (mg/L)	0.50	0.50	2.90	0.50	0.62	0.86
$N\text{-}NH_4^+$ (mg/L)	0.002	0.017	0.420	0.010	0.011	0.12
$N\text{-}NO_3^-$ (mg/L)	0.03	0.51	1.10	0.11	0.20	0.75
$N\text{-}NO_2^-$ (mg/L)	0.001	0.006	0.030	0.001	0.003	0.004
P total (mg/L)	0.004	0.069	0.350	0.010	0.022	0.069
$P\text{-}PO_4^{2-}$ (mg/L)	0.001	0.017	0.042	0.001	0.013	0.082

Sampling and diatom analyses

Sampling, laboratory pretreatment, light microscopy, identification and enumeration of diatoms were carried out according to the Bulgarian and European guiding standard EN 13946:2014. Cleaning and mounting for light microscopy followed the method of Hasle & Fryxell (1970). Epilithic diatoms were brushed from at least five boulders, cobbles or other available natural hard substrates situated in the water. Samples were directly fixed with 4 % formaldehyde. In the laboratory, the diatom samples were treated with cold sulphuric acid (H_2S0_4) and potassium permanganate ($KMnO_4$) to get a clean diatom suspension and then mounted on permanent microscope slides using Naphrax®. At least 400 diatom valves were identified to the lowest taxonomic level possible and counted on each slide in random transects, in order to calculate the relative abundance (%) of each taxon. Light microscopy was performed with Olympus BX40 and Olympus BX51 equipped with 100× oil-immersion objectives, and the latter with a digital camera for light micrographs. Diatoms were determined mainly according to Krammer & Lange-Bertalot (1986–1991), Lange-Bertalot (2001), Hofmann & al. (2013). For *Achnanthidium,* the following publications were mainly consulted: Lange-Bertalot & Krammer (1989), Rimet & al. *(*2010), Hlúbiková & al. (2011), Pérès & al. (2012), Novais & al. (2015), Wojtal (2004), Wojtal & al. (2011).

Results

Two species of genus *Achnanthidium* – *A. druartii* and *A. straubianum* – were observed for the first time in Bulgaria in samples collected from stones of the two reservoirs A. Stamboliyski and Yovkovtsi in 2015 and 2016.

A. druartii (Plate I, Figs 1-27) had a typical linear-lanceolate valve shape with subrostrate ends, valve length of 19–27 µm, valve width of 4.5–5.5 µm and

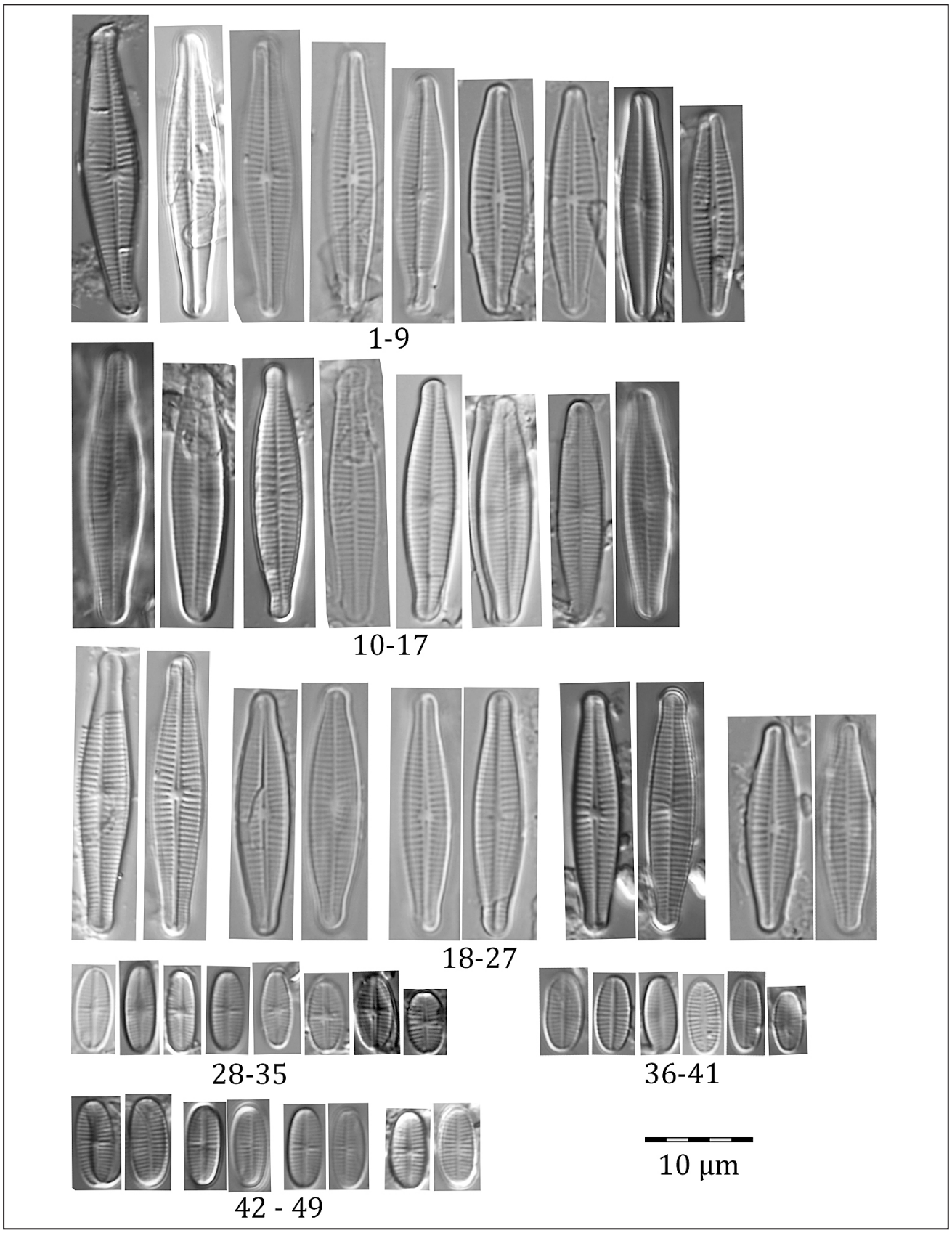

Plate I. *Achnanthidium druartii* (Figs 1-27) and *A. straubianum* (Figs 28-49). Figs 1-9, 28-35: raphe valves; Figs 10-17, 36-41: rapheless valves; Figs 18-27, 42-49: raphe and rapheless valves of a single individual.

slightly radiate stria with density of 17–22 per 10 µm in the central part for both valves.

A. straubianum (Plate I, Figs 28-49) had a typical elliptical to linear-elliptical valve shape, with valve length of 5.8–8.7 µm, valve width of 2.7–3.5 µm and slightly radiate stria with density of 27–30 per 10 µm for both valves.

Relative abundance of both species in A. Stamboliyiski Reservoir for 2015 and 2016, and in Yovkovtsi Reservoir for 2015 was near 10 % (Table 2). In 2016, in Yovkovtsi Reservoir, *A. druartii* was not found and *A. straubianum* was found in very small numbers, near 1 %.

Dominant species and those with relative abundance above 5 % in the samples were (in alphabetical order): *Achnanthidium eutrophilum* (Lange-Bertalot) Lange-Bertalot, *A. minutissimum, A. saprophilum* (H.Kobayashi & Mayama) Round & Bukhtiyarova, *Amphora pediculus* (Kützing) Grunow, *Caloneis lancettula* (Schulz) Lange-Bertalot & Witkowski, *Cyclotella ocellata* Pantocsek, *Eolimna minima* (Grunow) Lange-Bertalot, *Navicula cryptotenelloides* Lange-Bertalot, *N. reichardtiana* Lange-Bertalot, *Nitzschia tabellaria* (Grunow) Grunow, and *Pseudostaurosira brevistriata* (Grunow) D.M.Williams & Round (Table 2).

Table 2. Relative abundance (%) of *Achnanthidium druartii, A. straubianum* and species with relative abundance above 5 % in the samples from A. Stamboliyski (S) and Yovkovtsi (Y) Reservoirs in 2015 and 2016.

	S 2015	S 2016	Y 2015	Y 2016
Achnanthidium druartii	10	12	9	
Achnanthidium straubianum	11	10	12	<1
Achnanthidium eutrophilum	15			11
Achnanthidium minutissimum	12	25		10
Achnanthidium saprophilum	21			13
Amphora pediculus		22	52	
Caloneis lancettula				10
Cyclotella ocellata	13			
Eolimna minima			9	19
Navicula cryptotenelloides		9		
Navicula reichardtiana			5	
Nitzschia tabellaria				8
Pseudostaurosira brevistriata				11

Discussion

Characteristics of *Achnanthidium druartii* found in both reservoirs in Bulgaria are very close to its original diagnosis: linear-lanceolate valve shape, valve length 12–29 µm, average 21 µm, and valve width 3.9–5.8 µm, average 5 µm (Rimet & al. 2010). In light microscopy, *A. druartii* is easily distinguishable, being longer and wider on the average as compared to *A. convergens, A. deflexum, A. delmontii*, *A. japonicum, A. latecephalum,* and *A. rivulare*.

A. druartii, which is considered an epilithic river diatom, has never been reported before for Bulgaria, although the rivers in Bulgaria have been regularly object to routine diatom monitoring for almost a decade. The species certainly was not overlooked or misidentified because of its relatively large valve size within the genus *Achnanthidium*. As far as we know, these are the first records of the species in reservoirs although it has been found in large rivers and navigation canals in France. Whether the species *A. druartii* is invasive in Bulgaria, as it is considered to be in France and Europe in the last decade (Rimet & al. 2010, Guiry 2017), cannot be assessed during this study because benthic diatoms were studied for the first time in A. Stamboliyski and Yovkovtsi Reservoirs.

Achnanthidium straubianum found for the first time in Bulgaria has a typical elliptical to linear-elliptical valve shape, with broadly rounded apices with valve length and width and stria density typical of the species (originally length of 5.3–11.5 µm, width of 2.6–4.5 µm, and stria density 25–29 in 10 µm).

Although *A. straubianum* is probably widespread in Europe, it could be easily overlooked when it appears in small numbers because of its small valve size. This could explain why it was not reported so far in Bulgaria. Its identification in the present study was made possible by virtue of the fact that it was in relatively large amounts (10–12 %) in three of the studied samples. Another reason for not being reported so far could be its misidentification as another *Achnanthidium,* especially when found with only few valves per sample as it was in the fourth of the studied samples (<1 %).

According to the physical and chemical data of water in both reservoirs during 2015–2016 and the accompanying species, *A. druartii* and *A. straubianum* can be considered as alkaliphilous and meso-eutraphentic. The two species have been found together also in France, in the samples in which *A. druartii* was described and approximately with the same average abundance of 6–7 % (Rimet & al. 2010).

Further researches are needed in the future on these and other reservoirs in Bulgaria, as well as in the rivers in their catchments to find out more about the distribution of these species.

Acknowledgements. This research was carried out within the framework of the investigation *Update of the typology and classification system for surface water evaluation of the categories "river", "lake" and "transitional waters" in the period of the first River Basin Management Plan,* with Bulgarian Ministry of Environment and Water as contracting authority and SI Eco – EI as a contractor during 2015–2016. Physical and chemical data of water in both reservoirs during the period of studies was provided by SI Eco – EI.

References

Cantonati, M. 1998. Diatom communities of springs in the southern Alps. – Diatom Res., **13**: 201-220.

EN 13946:2014. Water quality – Guidance for the routine sampling and preparation of benthic diatoms from rivers and lakes. European Committee for Standardization, Brussels, 19 pp.

Guiry, M.D. in Guiry, M.D. & Guiry, G.M. 2017. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. – http://www.algaebase.org (accessed 28.03.2017).

Hasle, G. & Fryxell, G. 1970. Diatoms: cleaning and mounting for light and electron microscopy. – Trans. Amer. Microscop. Soc., **89**(4): 469-474.

Hlúbiková, D., Ector, L. & Hoffmann, L. 2011. Examination of the type material of some diatom species related to *Achnanthidium minutissimum* (Kutz.) Czarn. (Bacillariophyceae). – Algol. Stud., **136/137**: 19-43.

Hofmann, G., Werum, M. & Lange-Bertalot, H. 2011. Diatomeen im Süßwasser-Benthos von Mitteleuropa. A.R.G. Gantner Verlag K.G., Rugell, Koeltz Scientific Books, Königstein, 908 pp.

Krammer, K. & Lange-Bertalot, H. 1986–1991. *Bacillariophyceae* 1-4. – In: **Ettl, H., Gerloff, J., Heynig, H. & Mollenhauer, D. (eds)**, Süßwasserflora von Mitteleuropa 2/1-4, Gustav Fisher Verl., Stuttgart.

Lange-Bertalot, H. 1999. Neue Kombinationen von Taxa aus *Achnanthes* Bory (sensu lato). – Iconogr. Diatomol., **6**: 276-289.

Lange-Bertalot, H. 2001. *Navicula* sensu stricto. 10 genera separated from *Navicula* sensu lato. *Frustulia*. – In: **Lange-Bertalot, H.** (ed.), Diatoms of Europe 2, A.R.G. Gantner-Verl., Ruggell, Liechtenstein, 526 pp.

Lange-Bertalot, H. & Krammer, K. 1989. *Achnanthes* eine Monographie der Gattung mit Definition der Gattung *Cocconeis* und Nachtragen zu den *Naviculaceae*. – Biblioth. Diatomol., **18**: 1-393.

Lange-Bertalot, H. & Metzeltin, D. 1996. Oligotrophie-Indikatoren. 800 Taxa in drei ökologisch diversen Seen-Typen. In: LANGE-BERTALOT, H. (ed.), Iconogr. Diatomol. Annotated Diatom Iconographs, **2**: 1-390.

Novais, M., Jüttner, I., Van de Vijver, B., Morais, M., Hoffmann, L. & Ector, L. 2015. Morphological variability within the *Achnanthidium minutissimum* species complex (Bacillariophyta): Comparison between the type material of *Achnanthes minutissima* and related taxa, and new freshwater *Achnanthidium* species from Portugal. – Phytotaxa, **224**(2): 101-139.

Pérès, F., Barthès, A., Ponton, E., Coste, M., Ten–Hage, L. & Le–Cohu, R. 2012. *Achnanthidium delmontii* sp. nov., a new species from French rivers Fottea. – Olomouc, **12**(2): 189-198.

Potapova, M. 2011. *Achnanthidium druartii*. – In: Diatoms of the United States. – http://westerndiatoms.colorado.edu/taxa/species/achnanthidium_druartii (accessed 27.03.2017).

Rimet, F., Couté, A., Piuz, A., Berthon, V. & Druart, J.-C. 2010. *Achnanthidium druartii* sp. nov. (*Achnanthales*, *Bacillariophyta*), a new species invading European rivers. Vie et Milieu. – Life Environm., **60**: 185-195.

Wojtal, A. 2004. New or rare species of the genera *Achnanthidium* and *Psammothidium* (*Bacillariophyceae*) in the diatom flora of Poland. – Polish Bot. J., **49**(2): 215-22.

Wojtal, A., Ector, L., Van de Vijver, B., Morales, E., Blanco, S., Piatek, J. & Smieja, A. 2011. The *Achnanthidium minutissimum* complex (*Bacillariophyceae*) in southern Poland. – Algol. Stud., **136/137**: 211-238.