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Two new *Navicula* species (Bacillariophyceae) from ~~western~~ Western Europe

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Abstract

During a survey of some springs in the French Central Massif, a new *Navicula* species (Bacillariophyceae) was found: *Navicula sanctamargaritae* sp. nov. A second new *Navicula* species was observed during a routine biomonitoring project in Flanders: *Navicula flandriae* sp. nov. The new species are formally described using light and scanning electron microscopy. Both species present a unique set of morphological characters including the structure of the central raphe endings, the striation pattern, the valve dimensions and outline, which allows their separation from similar *Navicula* taxa such as *N. korzeniewskii*, *N. recens* or *N. cincta*. The ecological preferences of each species are briefly discussed.

Keywords: *Navicula*, new species, taxonomy, thermal springs, rivers, France, Flanders,

Introduction

The genus *Navicula* Bory (1822: 128) *sensu stricto* was described in 1822 for a group of species showing a narrowly to broadly lanceolate naviculoid valve outline with capitate, rostrate or bluntly rounded apices. The central sternum is thickened in a more or less asymmetric way. The raphe branches are straight and filiform with unilaterally slightly deflected proximal and strongly hooked distal raphe endings. The striae are uniseriate and composed of apically elongate areolae, hence called ‘lineolae’ (Round *et al.* 1990).

The initial rather broad species concept was modified several times (Patrick 1959, Cox 1979, Round *et al.* 1990). Based on the neotypus generis *Navicula tripunctata* (O.F. Müller 1786: 52) Bory (1827: 563), only members of the section *Lineolatae* Cleve (1895: 10), were included within *Navicula sensu stricto* (Cox 1979). To date, more than 130 described taxa (including subspecies and varieties) of *Navicula sensu stricto* have been recorded from freshwater (excluding brackish-water) habitats of Europe (Lange-Bertalot 2001, Werum & Lange-Bertalot 2004, Levkov *et al.* 2007, Van de Vijver & Lange-Bertalot 2009, Van de Vijver *et al.* 2010, Mertens *et al.* 2014). The genus is a dominant constituent of the temperate and tropical river diatom flora forming often important and highly diverse populations and hence plays a major role in the European biomonitoring of rivers (Werum & Lange-Bertalot 2004, Metzeltin & Lange-Bertalot 2007).

During a survey of the diatom flora in France and Belgium, two *Navicula* species were found that could not be identified using the currently available literature. Following detailed light (LM) and scanning electron microscopy (SEM) observations and comparisons with previously described species, both unknown taxa are described as new to science. A first new species, *Navicula sanctamargaritae* Beauger, sp. nov., was found in the thermal Tennis spring of Sainte-Marguerite (France) and in other thermal springs of the same area of the French Massif Central, influenced by the occurrence of deep CO₂ sources. A second species, *N. flandriae* Van de Vijver & A.Mertens, sp. nov. was found in several Flemish rivers (Belgium).

A comparison is made with the most similar taxa to facilitate the distinction of these two species. The ecology of both species is also addressed.

Material and Methods

Study sites:—On the banks of the river Allier between the villages Longues (Vic-le-Comte) and Sainte-Marguerite (St Maurice-ès-Allier) several well-known springs are found (Figure 1). This geographic area is part of the French Massif Central and more precisely of the southern part of the Limagne d'Allier basin where the emerging mineral waters are strongly influenced by their deep circulation in the crystalline basement and the occurrence of deep CO₂ sources (Fouillac 1983). At Sainte Marguerite, situated on the right bank of the river, different springs are present such as the Tennis spring. The sparkling mineral water of Sainte Marguerite was used from the Antiquity till the 19th century as drinking water. On the left bank, different springs are also present such as the Tambour and Petit Saladis springs.

The second species was found in several rivers and canals such as the Leopoldkanaal and the Oostpolderkreek in Flanders, the northern part of Belgium, in a region called 'Meetjesland'. Most localities where the new species was observed are situated in the vicinity of the village of Sint-Laureins (prov. of East-Flanders).

Sampling and physical and chemical analysis:—In France, three samples belonging to three different springs (Tennis, Tambour and 'Petit Saladis') were taken from the rims of the springs by brushing the stone. In Flanders, samples were collected by scraping off submerged helophyte vegetation such as reed stems.

At the sampling sites (when possible due to logistic constraints), dissolved oxygen (%), pH, conductivity ($\mu\text{S cm}^{-1}$) and water temperature ($^{\circ}\text{C}$) were measured using a WTW Multiline P4. Water samples were collected for further chemical analysis in the laboratory. Water samples from the sources in France were filtered using Whatmann GF/F filters before ionic chromatography (Dionex ICS 1500) analysis. The concentrations in lithium, sodium, ammonium, potassium, magnesium, calcium, fluoride, chloride, bromine, nitrate, phosphate, sulphate and carbonate were measured (mg l^{-1}) (Table 1). The chemical parameters from the Flemish localities were analysed in the laboratories of the Vlaamse Milieu Maatschappij (VMM) and values were added to Table 1.

Small sub-samples of the French material were prepared for LM observation following the method described in Prygiel & Coste (2000). Samples were cleaned using H₂O₂ and dilute hydrochloric acid, and rinsed several times. The Flemish material was prepared following the method described in Van der Werff (1955). Small parts of the sample were cleaned by adding 37% H₂O₂ and heating to 80 $^{\circ}\text{C}$ for about 1h. The reaction was completed by addition of KMnO₄. Following digestion and centrifugation (three times 10 minutes at 3700 g), cleaned material was diluted with distilled water to avoid excessive concentrations of diatom valves on the slides. Cleaned diatom material was mounted in Naphrax®. The slides were analyzed using an Olympus BX53 microscope, equipped with Differential Interference Contrast (Nomarski) and the Olympus UC30 Imaging System. Samples and slides are stored at the BR-collection, property of the Belgian federal government and given in permanent loan to the Botanic Garden Meise (Belgium) and at the Herbiers Universitaires de Clermont-Ferrand (France).

The slide made and annotated by Van Heurck of *N. gracilis* var. *schizonemoides* Van Heurck (1885: 83), present in the Van Heurck collection housed in the Botanic Garden Meise, Belgium (V-12-B7) was used to compare the species with the two new taxa in this paper.

For scanning electron microscopy (SEM), parts of the oxidized suspensions were filtered through a 1 μm Isopore™ polycarbonate membrane filter (Merck Millipore). The stubs were sputter-coated with a Au-Pd layer of 20 nm and studied in a Quanta FEG 250 scanning electron microscope at 5 kV at the Medical Faculty of the University of Toulouse (France) and a Hitachi S-4500 at 5–15 kV (Frankfurt-am-Main, Germany).

Diatom terminology follows Ross *et al.* (1979) (stria/areola structure), Round *et al.* (1990) (raphe structure) and Lange-Bertalot (2001) (genus morphology).

Observations

Division Bacillariophyta
Class Bacillariophyceae
Subclass Bacillariophycidae
Order Naviculales
Family Naviculaceae
Genus *Navicula*

***Navicula sanctamargaritae* Beauger, sp. nov. (Figs 1–40)**

LM (Figs 1–36): Valves lanceolate in longer specimens to elliptic-lanceolate in shorter valves. Margins usually linear to weakly convex. Apices only weakly wedge-shaped, moderately acutely to bluntly rounded. Occasionally, strictly lanceolate valves with convex margins and acutely rounded apices observed. Valve dimensions (n=45): length 19.0–51.5 μm , width 5.5–7.5 μm . Axial area narrow, linear, slightly widening close to the central area. Sternum weakly but still visibly developed. Central area small, rounded to transapically rectangular, bordered by usually one, rarely 2–3 irregularly shortened and more distantly spaced striae. Raphe filiform with enlarged proximal raphe endings, deflected towards the secondary side. Striae radiate becoming parallel and even weakly convergent close to the apices, 10–16 in 10 μm . Lineolae almost not discernible in LM. SEM (Figs 37–40): External raphe sternum distinctly raised, less developed towards the apices, irregularly widened near the central area (Figs 37, 39). Proximal raphe endings drop-like enlarged, clearly deflected to the secondary side (Fig. 39). Distal raphe fissures hooked, continuing onto the mantle (Fig. 37). Striae composed of slit-like lineolae. Virgae larger than the lineolae (Figs 37, 39). Lineolae ca. 35–40 in 10 μm . Internally, raphe running weakly lateral of the raphe sternum with the raphe slit opening on the side, but with central raphe endings in the middle of the central area (Figs 38, 40).

Type—FRANCE. Sainte-Marguerite: Tennis Spring, sample S1_BA_151114 (45° 40' 06.9" N, 3° 13' 20.3" E), A. Beauger, 15 November 2014 (holotype: CLF!, slide no. 104186; isotypes: CLF! slide no. 104187, Herbiers Universitaires de Clermont-Ferrand, France, and BR! slide no. 4427).

Etymology—The new species is named after the village where it was discovered, Sainte-Marguerite, situated in the French Massif Central.

Ecology and associated diatom taxa—The type population of *N. sanctamargaritae* was found in Tennis spring where it entirely dominated the diatom flora. The new species was also found in two other springs such as Tambour Spring and Petit Saladis Spring. The latter two were co-dominated by *N. sanctamargaritae* and *Crenotia thermalis* (Rabenhorst 1864: 107) Wojtal (2013: 81). *Fragilaria famelica* (Kützing 1844: 64) Lange-Bertalot (1980a: 749), *Nitzschia valdecostata* Lange-Bertalot & Simonsen (1978: 58) and *Rhopalodia acuminata* Krammer in Lange-Bertalot & Krammer 1987: 75 were also identified.

The springs have a circumneutral pH, an elevated conductivity level (>8000 $\mu\text{S cm}^{-1}$) and are enriched with by sodium-chloride and bicarbonate sodium with CO_2 present as a gas phase as observed by other authors in the same area (Negrel *et al.* 1997, Rihs *et al.* 2000, Gal *et al.* 2012). The water temperature ranged between 17.5 and 27.0 °C. The concentrations of the different ions were very similar between the three springs although Lithium ions were present in each spring (8.6 mg l^{-1}) underlining deep water origin (Michard 1990). Tables 1 show the main physical and chemical values for the three springs.

***Navicula flandriae* Van de Vijver & A.Mertens, sp. nov. (Figs 41–65)**

LM (Figs 41–61): Valves narrowly lanceolate to weakly elliptic-lanceolate in smaller specimens. Valve margins linear to slightly convex, gradually narrowing towards the non-protracted, acutely rounded to wedge-shaped apices. Valve dimensions (n=25): length 35–65 μm , width 7.0–9.5 μm . Axial area very narrow, linear, weakly widening near the central area. Central area small, rounded to almost rectangular, usually slightly asymmetric, bordered by several more distantly spaced, irregularly shortened striae. Raphe filiform with almost straight, clearly expanded proximal raphe endings. Striae

radiate and geniculate near the valve center, becoming rapidly parallel and even convergent near the apices, 12–13 in 10 μm . Voigt discordance clearly visible. Lineolae weakly discernible, ca. 32 in 10 μm . SEM (Figs 62–65): Proximal raphe endings almost straight to slightly undulating terminating in clearly expanded, droplike pores (Figs 62, 64). Distal raphe fissures clearly hooked to the secondary side (Fig. 63). Striae composed of lineolae as long as the width of the virgae, 30–34 in 10 μm , clearly longer than the virgae near the apices (Fig. 63). External raphe sternum not raised (Fig. 62). Internally, raphe running weakly lateral of the raphe sternum with the raphe slit opening on the side, but with central raphe endings in the middle of the central area (Fig. 65). Areolae covered by individual hymenes (Fig. 65).

Type—BELGIUM. Stenenschuurbrug: Leopoldkanaal, sample VMM70-2010 (51° 15' 59.24' [SN](#), 03° 39' 39.09' E), [VMM Vlaamse Milieu Maatschappij](#), 28 June 2010 (holotype: BR! slide no. 4428; isotype: PLP! slide no. 291, University of Antwerp, Belgium).

Etymology—The species is named after Flanders (*Flandria*), the northern region in Belgium where the new species was observed in several rivers (Leopoldkanaal, Oostpolderkreek).

Ecology and Distribution—*Navicula flandriae* was observed in several canals and rivers in Flanders, the region occupying the northern part of Belgium. The largest population was found in the Leopoldkanaal presenting an alkaline pH (8.7), high conductivity (7270 $\mu\text{S cm}^{-1}$) and chloride (1750 mg l^{-1}) levels and moderate nutrient levels (nitrate: 4 mg l^{-1}). Accompanying taxa include *Nitzschia liebetruthii* Rabenhorst (1864: 157), *Rhoicosphenia abbreviata* (C.Agardh 1831: 34) Lange-Bertalot (1980b: 586), *Tabularia fasciculata* (C.Agardh 1812: 35) D.M.Williams & Round (1986: 326) and *Cyclotella meneghiniana* Kützing (1844: 50). The species has recently been observed in several other rivers and canals along the Belgian coast (Van de Vijver, unpublished results).

Discussion

Navicula sanctamargaritae and *N. flandriae* both belong to the section *Navicula* within the genus *Navicula*, characterized in having proximal raphe endings (sometimes slightly) deflected to the secondary side (where the Voigt discordance is) (Lange-Bertalot 2001). Although both species show a faint resemblance to each other (most visible in the smaller valves of *N. flandriae* and the larger valves of *N. sanctamargaritae*), then can be easily separated. *Navicula sanctamargaritae* has lower valve dimensions, especially a lower valve width (5.5–7.5 vs. 7.0–9.5 μm). The valve margins in *N. flandriae* are more linear and only slightly convex, even in smaller specimens, whereas in *N. sanctamargaritae*, valve margins tend to be more convex with more bluntly, broadly rounded apices. *Navicula flandriae* has acutely rounded, even pointed apices. Finally, *N. sanctamargaritae* has a higher stria density (10–16 in 10 μm) compared to *N. flandriae* that has a less variable stria density (12–13 in 10 μm), although in the latter feature, there is a clear overlap.

Navicula sanctamargaritae presents a rather important variability in valve outline. Within the type population, both lanceolate valves with more acutely rounded apices (Figs 25–36) and elliptic-lanceolate valves with more bluntly rounded apices (Figs 1–24) can be observed. Since this is the only morphological feature that could be used to discriminate between both forms, i.e. all other morphological features show absolutely no variability, we consider this variability to be insufficient to justify the separation of two independent taxa and include both within *N. sanctamargaritae*.

Both new species belong to the group of taxa around shows similarities to the group of taxa related to *Navicula tripunctata* such as *N. recens* (Lange-Bertalot 1980a: 37) Lange-Bertalot in Krammer & Lange-Bertalot (1985: 91), *N. margalithii* Lange-Bertalot (1985: 79) *N. korzeniewskii* Witkowski *et al.* (2000: 287) and *N. cincta* (Ehrenberg 1854: figs 10/2, 6a-e) Ralfs in Pritchard (1861: 901). The combination of a relatively low stria density, the more distantly placed central striae, the valve outline and the non-protracted apices separate both species from all other *Navicula* taxa.

Navicula korzeniewskii has wider valves (valve width 9.5–11.5 μm vs. 5.5–7.5 μm), more elongated, linear-lanceolate valves with acutely rounded apices and a different striation pattern compared to *N. sanctamargaritae*. *Navicula tripunctata* has a more regularly spaced, only weakly radiate to even parallel striation pattern with typically equidistant striae in the central area, a feature never observed in *N.*

sanctamargaritae. The valves in *N. tripunctata* are usually also larger (up to 70 µm) with strictly parallel margins, only narrowing towards the pointed apices. The most similar species are *N. recens* and *N. cincta*. *Navicula recens* has a similar valve outline, though often more wedge-shaped, less acutely rounded apices and comparable valve dimensions (although often a broader valve width, up to 9 µm). Both species can however be separated based on their striation pattern in the central area. In *N. sanctamargaritae*, only one, occasionally 2–3 irregularly shortened striae can be observed that are always clearly more distantly spaced than the other striae. This feature has never been observed in *N. recens* populations all over the world. Consequently, the central area in *N. recens* is smaller than in *N. sanctamargaritae*. *Navicula cincta* shows a similar striation pattern in the central striae but has a lower stria density (8–12 vs. 10–16 in 10 µm) and presents usually an abrupt change from radiate to convergent striae near the Voigt discordance (Lange-Bertalot 1980a, 2001). The axial sternum is more raised in *N. cincta*, making it very well discernible in LM (Lange-Bertalot 2001), whereas in *N. sanctamargaritae*, it is present but less discernible.

Based on valve outline, *N. flandriae* could be confused with *N. radiosa* Kützing (1844: 91) although the latter has wider valves (8–12 µm vs. 6.5–9.5 µm) and a different striation pattern with more strongly radiate striae becoming only convergent near the apices whereas *N. flandriae* has parallel (and convergent) striae in a large part of the valve. *Navicula recens* can be easily distinguished based on its more elliptic-lanceolate and less linear-lanceolate valve outline with convex margins and a different striation pattern in the central area (Lange-Bertalot 1980a). In *N. flandriae*, several (3–6) striae are irregularly shortened and show a more radiate orientation whereas in *N. recens*, only 2, rarely 3, are shortened and more or less parallel (Lange-Bertalot 1980a, 2001). *Navicula margalithii* shows less acutely rounded apices and more parallel margins, only narrowing close to the apices whereas *N. flandriae* shows parallel margins near the central area and a gradual tapering towards the more acutely rounded to wedge-shaped apices. The number of shortened striae in the central area in *N. margalithii* is lower (up to 2–3) and the striae have a more parallel orientation (Krammer & Lange-Bertalot 1985, Lange-Bertalot 2001). Moreover, *N. margalithii* also presents a clearly asymmetrical central area, contrary to *N. flandriae* that has a more or less symmetrical central area. Probably the most similar taxon is *N. korzeniewskii*, described in 2000 from the Lower Jordan River in Israel. Although the striation pattern and orientation are comparable, *N. korzeniewskii* however, has more convex valve margins, has always much wider valves (width 9.5–11.5 µm vs. 6.5–9.5 µm) and a slightly lower stria density (max. 11 in 10 µm) (Witkowski *et al.* 2000). Finally, another taxon that shows some similarities is *Navicula tripunctata* var. *schizonemoides* (Van Heurck 1885: 83) Patrick (1959: 106). The species was described in 1885 by Van Heurck as *N. gracilis* var. *schizonemoides* and transferred in 1959 by Patrick to *N. tripunctata* when it became clear that *N. gracilis* Ehrenberg (1832: 64) was a younger synonym of *N. tripunctata*. Analysis of a slide made and annotated by Van Heurck of this taxon (present in the Van Heurck collection at BR, no. V-12-B7) revealed important differences between both taxa. The valve outline in *N. tripunctata* var. *schizonemoides* is strictly lanceolate (Figs 66–67) with less parallel margins compared to *N. flandriae*. The observed valves of *N. tripunctata* var. *schizonemoides* also showed a higher valve width (9.5–10.0 µm) with a lower stria density (up to 10 in 10 µm). The combination with *N. tripunctata* for this variety is however doubtful as the latter shows rather parallel striae (even in the central area) and a different valve outline (*N. tripunctata* has a more linear valve outline) making a raise to species level more likely. Lange-Bertalot (2001) discussed a possible conspecificity between *N. tripunctata* var. *schizonemoides* and *N. recens*. Given the clear differences in valve outline (*N. recens* is more elliptic-lanceolate) between both species, this is however less likely. Based on the above observations, the following new combination (and name) is proposed:

Navicula neoschizonemoides Van de Vijver & A.Mertens, *stat. nov., nom. nov.*

Replaced synonym: *Navicula tripunctata* var. *schizonemoides* (Van Heurck) Patrick. A new name is proposed since the name *Navicula schizonemoides* Kisselev was already used in 1930 by Kisselev.

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Table & Figure captions

TABLE 1. Chemical variables (when measured) of the sampling localities ((DO = dissolved oxygen).

FIGURES 1–36. *Navicula sanctamargaritae* Beauger, *sp. nov.*, LM. Type population from Tennis spring. 1–24. Valves with more bluntly rounded ends. 25–36. Valves with acutely rounded apices from the same population. Scale bar = 10 µm.

FIGURES 37–40. *Navicula sanctamargaritae* Beauger, *sp. nov.*, SEM. Type population from Tennis spring. 37. External view of an entire valve showing the raphe structure and the striae. 38. Internal view of an entire valve. Note the rather low raphesternum. 39. External detail of the central area. Note the typical enlarged proximal raphe endings. 40. Internal detail of the central area showing the typical areola coverings, the proximal raphe endings and the almost central running raphe branches. Scale bar = 10 μm (Figs 37, 38), 5 μm (Figs 39, 40).

FIGURES 41–61. *Navicula flandriae* Van de Vijver & A.Mertens, *sp. nov.*, LM. Type population from Leopoldkanaal. Scale bar = 10 μm .

FIGURES 62–65. *Navicula flandriae* Van de Vijver & A.Mertens, *sp. nov.*, SEM. Type population from Leopoldkanaal. 62. External view of an entire valve showing the raphe structure and the striae. 63. External detail of the apex with the typical hooked distal raphe ending. 64. External detail of the central area. Note the typical weakly deflected proximal raphe endings. 65. Internal detail of the central area showing the typical areola coverings, the proximal raphe endings and the laterally running raphe branches. Scale bar = 10 μm (Figs 62, 63), 5 μm (Figs 64, 65).

FIGURES 66–67. *Navicula neoschizonemoides* Van de Vijver & A.Mertens, *stat. nov. nom. nov.* Illustrations from the slide made and annotated by Van Heurck of *Navicula gracilis* var. *schizonemoides* Van Heurck (slide number V-12-B7 in the Van Heurck collection). Scale bar = 10 μm .

	Conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$)	pH (pH units)	Temperature ($^{\circ}\text{C}$)	DO (%)	Li ⁺ (mg l ⁻¹)	Na ⁺ (mg l ⁻¹)	NH ₄ ⁺ (mg l ⁻¹)	K ⁺ (mg l ⁻¹)	Mg ²⁺ (mg l ⁻¹)	Ca ²⁺ (mg l ⁻¹)	F ⁻ (mg l ⁻¹)	Cl ⁻ (mg l ⁻¹)	Br ⁻ (mg l ⁻¹)	NO ₃ ⁻ (mg l ⁻¹)	PO ₄ ³⁻ (mg l ⁻¹)	HCO ₃ ⁻ (mg l ⁻¹)	SO ₄ ²⁻ (mg l ⁻¹)
Tennis spring	8490	6.95	27	42	8.6	1664.0	2.5	184.4	135.4	337.4	0.9	1797.3	4.5	0.2	0.7	2960.0	116.8
Tambour spring	8390	6.94	17.5	44.7	8.6	1612.5	2.4	178.4	130.8	298.3	1.1	1722.1	4.8	0.2	0.2	2820.0	108.4
Petit Saladis spring	8540	6.89	20.7	42.3	8.6	1628.2	2.4	179.9	132.0	327.1	1.1	1752.1	5.1	0.3	0.3	2840.0	112.6
Leopold-kanaal	7290	8.20	24.8	-	-	-	<0.08	-	-	-	0.77	2900	-	<0.2	1.0	-	450
Oostpolderkreek	5780	8.50	26.8	-	-	-	<0.08	-	-	-	-	1600	-	<0.4	0.4	-	185