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Germainiella clandestina sp. nov. (Bacillariophyta), a new species in a little known diatom genus

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Abstract: A species new to science, *Germainiella clandestina*, was discovered during experiments performed in an artificial freshwater channel. This species is described based on valve morphology typical for the genus *Germainiella* Lange–Bertalot et Metzeltin. The major features conforming to *Germainiella* are the valve face as well as a part of the mantle covered by a conopeum and the presence of very small apertures corresponding to a subconopeum canal system close to the raphe fissure. These characters clearly differentiate this taxon from the two more related genera, namely *Fallacia* Stickle et Mann and *Pseudofallacia occulta* (Krasske) Y. Liu, J.P. Kociolek et Q.X. Wang. Moreover, two chloroplasts could be observed for the first time in *Germainiella*, providing an additional feature to separate *Germainiella* and both genera cited above. Some information is given on its ecology.

Key words: Diatoms, taxonomy, conopeum, subconopeum system, artificial channel

INTRODUCTION

Previously all placed within a variety of subgroups in the genus *Navicula sensu lato*, most of small–cell naviculoid diatoms are now classified in a large number of genera. Some of them are monotypic such as *Labellicula* Van De Vijver et Lange–Bertalot (Van De Vijver et al. 2005) or *Pseudofallacia* Liu, Kociolek et Wang (Liu et al. 2012). Others include only a few species such as *Lecohuia* Lange–Bertalot (Rumrich et al. 2000), *Navigiolum* Lange–Bertalot, Cavacini, Tagliaventi et Alfinito (Lange–Bertalot et al. 2003), *Lacuneolimna* Tudesque, Le Cohu et Lange–Bertalot (Tudesque et al. 2015) or *Veigaludwigia* Lange–Bertalot et Rumrich (Metzeltin & Lange–Bertalot 2007). Till now, *Germainiella* included two species with *G. enigmaticoides* Lange–Bertalot et Metzeltin (Metzeltin et al. 2005) as generitype. It was found in several samples from Rio de la Plata (Uruguay). The second species, *G. enigmatica* (Germain) Lange–Bertalot et Metzeltin, previously described under the name *Navicula enigmatica* by Germain (1980), was discovered in a green–house of the botanical garden in Angers (France). It was recorded later in Tahiti (Coste & Ricard 1990), in cultured material from the Weterau plains near Frankfurt on the Main River, in Germany (Werum & Lange–Bertalot 2004) and in a river situated in Northern Bavaria (Reichardt 2006). A third species, *Germainiella clandestina* sp. nov., was detected in an artificial freshwater channel where this species thrived. The aim of this article is to provide a detailed morphological description of *G. clandestina* using light microscopy, scanning and transmission electron microscopy and to give information on its ecology.

MATERIAL AND METHODS

Samples were collected on artificial substrates (polythene microplates) in an artificial freshwater channel. This mesocosm functioned in closed circuit and the circulating water was rich in nutrients with 71 mg.l^{-1} NO3–N and 207 µg.l^{-1} PO4–P (see Barthès et al. 2014). Two experiments were carried out. On the one hand, clean artificial substrates were submerged during a 14–days period. On the other hand, biofilms on artificial substrates were exposed to a drying period of one month followed by a 13–days rewetting. At the end of these periods, the biofilms were scraped with a cutter blade and used for the study of diatom community. Samples were oxidized with hydrogen peroxide, rinsed three times with deionized water and mounted in Naphrax. Light microscope observations were conducted using an Olympus BX51 microscope with Nomarski mode (DIC) and a SC 30 digital camera. The valve number measured was 200. For scanning electron microscopy examinations, cleaned diatoms were dried onto glass coverslips attached to aluminium stubs using nail varnish and sputter–coated with a thin layer of platinum. The observations were performed using a FEGFEI Quanta 250 SEM. For transmission electronic microscopy, cleaned diatom frustules were transferred to carbon–coated grids with a micropipette and air–dried. The preparations were examined with a Hitachi HT 7600 TEM.

Results

Germainiella clandestina **Le Cohu, Ten–Hage et Barthès sp. nov.**

Diagnosis: *Frustula aspectu cingulari rectangulata. In vivis cellulis, adest unus chloroplastus in forma renis situs ex utroque latere raphis. Valvae anguste ellipticae vel lineares – ellipticae vel ample ellipticae. Longitudo : 2.8–7.6 µm; latitudo : 1.3–1.9 µm. Facies valvae hyalina, solum area axialis aspectabilis. In microscopo electronico : facies valvae et limbi pars tectae conopeo. Rami raphis leviter curvati cum extremis proximalibus directis ad primum latus valvae. Ordo minimorum pororum aspectabilium utrimque prope fissuram externam raphis. Striae parallelae; 50–62 in 10 µm, compositae alveolis. Cingulum compositum saltem quartis copulis*

Holotype: PC0534079, Muséum National d'Histoire Naturelle, Laboratoire de Cryptogamie, Paris, France
Isotype: BR – 4402, Belgium National Garden, Département Bryophytes & Thallophytes, Domaine du Bouchont, Meise, Belgium
Iconotype: Fig. 6
Type locality: artificial channel inoculated with biofilms from the Garonne river
Habitat: freshwater, benthic, Garonne river
Etymology: *clandestina* because cryptical in natural samples from Garonne river

LM morphology (Figs 1–4)

Live cells have two diagonally offset plastids, more or less reniform (Figs 1, 2). The valves are from narrowly elliptical, linear elliptical to broadly elliptical (Figs 3–4). The valve face appears hyaline with any structure other than the axial area.

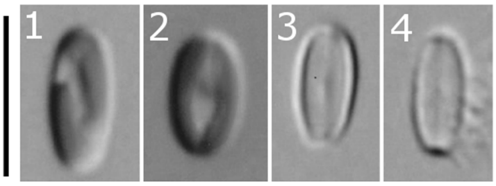
SEM and TEM morphology

The frustules are rectangular in girdle view (Fig. 5). The valve face and a part of the mantle are covered by a conopeum (Fig. 6). The skeleton of this conopeum is formed by two elevated and very silicified marginal ribs and the axial sternum. These three components enclose two thin sheets faintly transparent (Fig. 7). Externally the raphe fissure is slightly curved with hardly enlarged proximal endings pointed towards the primary side of the valve. The terminal fissures are deflected to the secondary side of the valve and terminate on the mantle (Figs 6). Internally, the proximal raphe endings are clearly bended to the same side and the distal raphe endings terminate in poorly developed helictoglosse (Fig. 8). A row of very small apertures (107–128 in 10 µm) lies externally on either side of the raphe very close to it (Fig. arrows, Fig. 9) and corresponds to a subconopeum canal system (Figs 9, 10, arrows). The striae are parallel throughout and seem to have an alveolar structure (Fig. 11). They continue uninterruptedly onto the mantle where they appear as a row of rectangular open "windows" (Figs 5, 6, 10).

The cingulum consists of at least four girdle bands (Fig. 5). The valvocopula is broad; the second and the third level (C1, C2) are composed of two half opposite bands which are closed at the poles of the valves, open near the valve centre and terminating in more or less cuneate endings (Fig. 5, arrow).

Discussion

Based on the presence of a conopeum, Werum & Lange–Bertalot (2004) first placed *Navicula enig-*



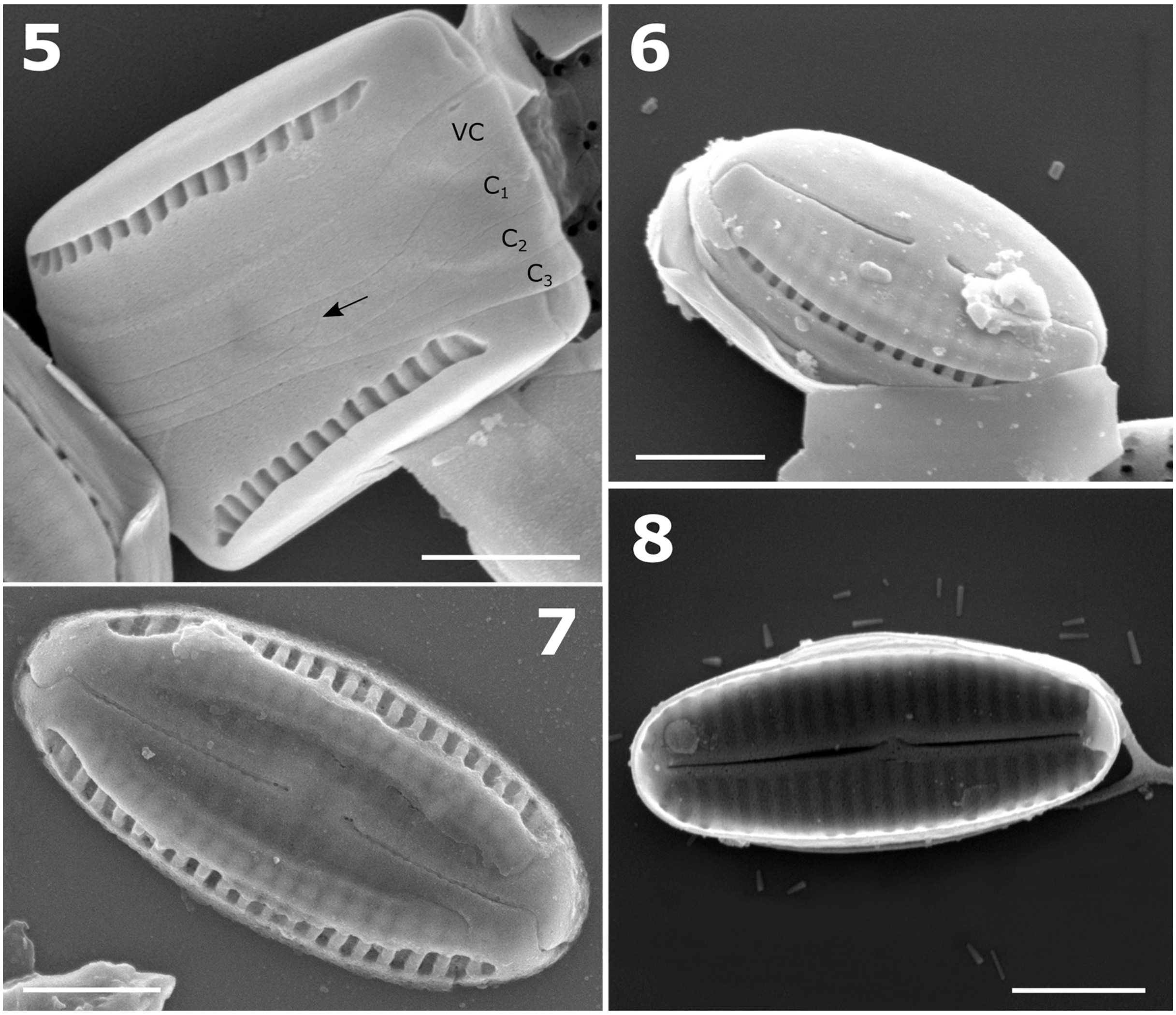
Figs 1–4. *Germaniella clandestina*: (1–2) live cells showing the two plastids; (3–4) light micrographs showing the frustule morphology of type population. Scale bars 10 µm.

Table 1. Morphological comparison of *Germainiella* species including *G. clandestina* sp. nov.

	G. clandestina **sp. nov.**	***G. enigmaticoides***	***G. enigmatica***
Length (µm)	2.8–7.6	6.5–11.5	6–15
Width (µm)	1.3–1.9	1.9–2.5	2–2.5
Striae number (in 10 µm)	50–62	73–79	50

matica Germain in the genus *Fallacia*. Later, describing the new genus *Germainiella*, Lange–Bertalot et Metzeltin (Meltzeltin et al. 2005) transferred this taxon to the new genus and highlighted the differences between *Fallacia* and *Germainiella* wich are closely related each other. They listed the diagnostic features of *Germainiella*, namely a conopeum covering the valve face and a part of the mantle, presence of canal apertures externally on either side of the raphe and the alveolar structure of the striae. However, Van de Vijver & Cox (2015) have observed in *Fallacia emmae* Van de Vijver et Cox a similar striae structure as in *Germainiella*. Consequently, it means that the striae structure is not a discriminating feature between *Fallacia* and *Germainiella*. On the other hand, *Germainiella* has two diagonally offset plastids. *Fallacia* presents a single H–shaped chloroplast (Cox 1996). A conopeum covering the valve face as well as a part of the mantle, the presence of typical canal apertures on either side of the raphe and the morphology and the number of chloroplasts are the features distinguishing *Germainiella* from *Fallacia*. *Germainiella clandestina* clearly belongs to the genus *Germainiella* showing all the diagnostic genus features cited above. It has to be noted that the type of the cingulum organization in *G. clandestina* is similar to that observed in *Encyonopsis* (Le Cohu et al. 2014) and reminiscent of that found in *Urosolenia* (Round et al. 1990).

Apart from *Fallacia*, *Germainiella* is related to the newly created and monotypic genus *Pseudofallacia* based on *Navicula occulta* Krasske (Liu et al. 2012). Both genera share a few features such as two chloroplasts and a conopeum. The chloroplasts are different in the morphology and the arrangement. SEM observations provide a clear difference in the conopeum structure. In *Pseudofallacia occulta* (Krasske) Y. Liu, J.P. Kociolek et Q.X. Wang, a narrow conopeum runs along the raphe on either side. The striae are interrupted at the conopeum whereas they are completely covered by the conopeum in *Germainiella*. Additional



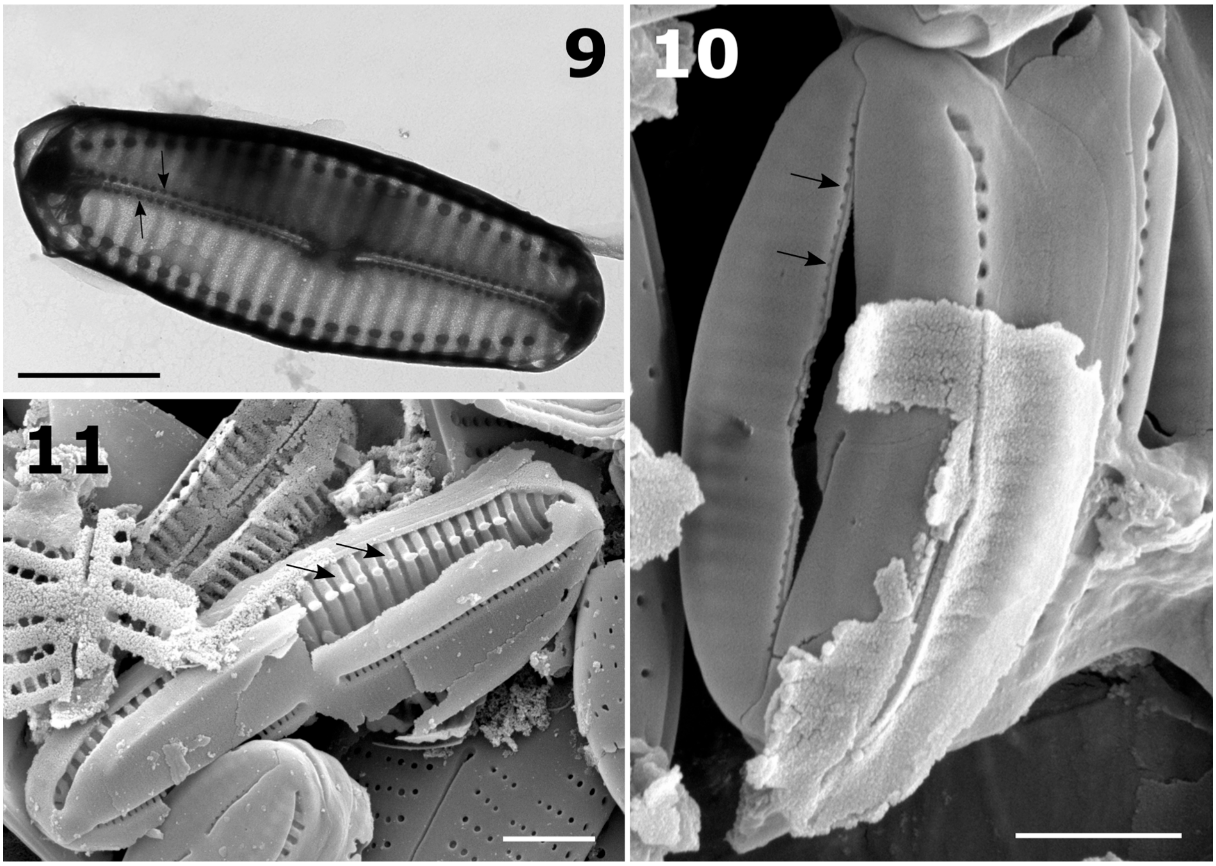
Figs 5–8. *Germaniella clandestina*: (5) SEM, girdle view showing the cingulum (VC: valvocopula. C_1, C_2 and C_3: copulae); (6) SEM, external valve view showing the conopeum and the raphe fissure; (7) SEM, external view showing the skeleton composed of marginal ribs and the sternum enclosing two thin sheets hardly transparent; (8) SEM, internal view with proximal raphe ending curved to the same side. Scale bars 1 µm.

differences are observed such as the presence of two elongate pores in each terminal area in *Pseudofallacia*. In LM, it should be noted that both genera can be easily separated by the number of the striae clearly visible in *Pseudofallacia* whereas they cannot be resolved in the tree species of *Germainiella*.

The discussion about genus delimitation and validity is related to the question of the value of each morphological feature or molecular data (Williams 2013; Van de Vijver & Cox 2015). For instance, over the past 15–years, several new small genera were created from parts of the genus *Navicula*, including *Lacunicula* Lange–Bertalot et al. (2003). The latter species was rejected as an independant genus by Morales & Le (2003), who stated that only the presence of deep grooves next to the raphe is not sufficient to separate it from the genus *Craticula* Grunow. Another example is *Synedra berolinensis* Lemmerman whose the genus name has changed several times during the last twenty years but it is still very hard to know what is the scientific evidence to adopt a definitive name (Williams 2013). Van de Vijver & Cox (2015) have observed intermediate features of *Fallacia emmae* between *Fallacia* and *Germainiella* such as a structure of the striae but other characters can differentiate both genera (see above). Intermediate taxa, such as *F. emmae* combining features of two closely related genera could help in establishing better boundaries to prevent the proliferation of new, often monotypic, genera. However the problem is always the same, namely what are the criterions to select the predominant key feature or the combination of features (morphological or molecular) which differentiate two genera.

Within the genus *Germainiella*, *G. clandestina* sp. nov. can be differenciated from *G. enigmaticoides* and *G. enigmatica* by the valve morphology (respectively pl. 54, figs 8–21, Metzeltin et al. 2005 and pl. 2, figs 14–19, Germain 1980), the striae density (Table 1), the dimensions and secondarily the slight external curvature of the raphe. It has to be noted that the very delicate pores of the conopeum observed in *G. enigmaticoides* (Metzeltin et al. 2005) and *G. enigmatica* (Fig. 30, Germain 1989) could not be detected in *G. clandestina*, even in TEM.

In the studies concerning the diatoms of the Garonne River (Eulin & Le Cohu 1998; Ameziane et al. 2003; Tekwani et al. 2013), *G. clandestina* was overlooked probably because of its rarity with environmental factors which not favoured its growth but also given its very small dimensions and its hyaline valve surface. *G. clandestina* seems to be associated with an environment rich in nitrogen (71 mg.



Figs 9–11. *Germaniella clandestina*: (9) TEM, valve view with small apertures along the raphe (arrows); (10) SEM, valve view showing the subconopeum canal system (arrows); (11) SEM, valve view with remains of the conopeum and showing the alveolar structure of the striae (arrows). Scale bars 1 µm.

l^{-1} NO_3–N) and phosphorus (207 µg.l^{-1} PO_4–P) and a temperature around 15°C. *G. clandestina* as *G. enigmaticoides* shows some affinity for a high electrolyte content, respectively 1330 µS. cm^{-2} and around 600 µS. cm^{-2} (Metzeltin et al. 2005). In the experiments with cleaned substrates submerged during a 14–days period, *Germainiella clandestina* was dominant with a relative abundance of 64 %. It was accompanied by *Achnantidium exiguum* (Grunow) Czarnecki (15 %), *A. aff. minutissimum* (Kützing) Czarnecki (13%) and *Sellaphora seminulum* (Grunow) D.G. Mann (8%). In the experiments with biofilms on artificial substrates exposed to a drying phase of one month followed by a 13–days rewetting, *G. clandestina* was always dominant with a relative abundance of 52 %. It was again accompanied by *A. exiguum* (14%), *A. aff. minutissimum* (22%) and *Sellaphora seminulum* (12%). In the two experiments, the relative abundance of *G. clandestina* is at least four times higher than the subdominant species. Presumably, *G. clandestina* has a cell division rate higher than those of the three other species, what could explain why *G. clandestina* is dominant.

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