Morphological examination and biogeography of the *Gomphonema rosenstockianum* and *G. tergestinum* species complex (Bacillariophyceae)

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Abstract: The type material of *Gomphonema rosenstockianum* Lange–Bert. et E. Reichardt from La Gomera (Canary Islands, Spain) and epilithic material of rivers from several European countries were examined using light and scanning electron microscopy in order to improve the knowledge on the taxonomical status of the *G. rosenstockianum* and *G. tergestinum* (Grunow) M. Schmidt species complex. Two other *Gomphonema* species, *G. supertergestinum* E. Reichardt and *G. angustius* E. Reichardt, recently described and belonging to the same group, are also presented in detail. After the analysis of several populations of *G. rosenstockianum* and *G. tergestinum* it was possible to define several reliable criteria to allow morphological differentiation of both species under LM and SEM as well. A geometric morphometric analysis clearly demonstrated the separation of these taxa considering valve outline, size and shape of the central area and position of the stigma. Apart from the morphological analysis, compilation of the results of this study has also revealed differences in ecological preferences of the two species. World and European distribution maps of the four *Gomphonema* taxa are presented.

Key words: diatoms, geometric morphometry, *Gomphonema*, morphology, type material, ultrastructure

Introduction

The biraphidaceous, heteropolar diatom genus *Gomphonema* was erected by Ehrenberg in 1832, and is very common in freshwater haptobenthic communities (Round et al. 1990). Within this genus, the taxonomy of *Gomphonema tergestinum* (Grunow) M. Schmidt and *G. rosenstockianum* Lange–Bert. et E. Reichardt is considered problematic to the point that these two species are commonly misidentified at first sight, especially under light microscopy (LM).

Gomphonema rosenstockianum was described and illustrated by light and scanning electron microscopy (SEM) in 1993 by Lange–Bertalot & Reichardt from La Gomera, Canary Islands, Spain (Lange–Bertalot 1993). Its occurrence, according to Lange–Bertalot (1993), is limited to several slightly alkaline waters from Canary Islands (La Gomera and Tenerife) with moderate conductivity and oligosaprobic to β–mesosaprobic waters.

Gomphonema tergestinum was originally described from Trieste (Italy) by Grunow as *Gomphonema semiapertum* var. *tergestina* Grunow in Van Heurck 1880. Afterwards, M. Schmidt drew 8 figures of *G. tergestinum* Grunow in Schmidt et al. (1902), based on the analysis of samples collected in Cresswell (United Kingdom) and in Gülzow, Mecklenburg–Vorpommern (North of Germany). These initial mentions of the taxon (Grunow's and Schmidt's) were only presented by drawings, without providing descriptions. The first morphological description of *G. tergestinum* was published by Hustedt (1930), based on samples collected in standing waters in Gülzow and in Jezioro Śremskie (Poland). Later Krammer & Lange–Bertalot (1986) presented a more complete description of its ultrastructure, distribution and ecology. According to Krammer & Lange–Bertalot (1986), *G. tergestinum* has been mainly found in oligotrophic to low mesotrophic lakes with high conductivity (e.g. Lago di Garda in the calcareous Southern Alps). More recently, Van Dam et al. (1994) considered it to be a euryhaline, alkaliphilous, oligosaprobous, oligo-mesotraphentic and nitrogen–autotrophic taxon, tolerating very low concentrations of organically bound nitrogen. Afterwards, Lange–Bertalot (1996) suggested *G. tergestinum* as a presumably endangered taxon in Germany, occurring in oligotrophic and mainly calcareous water bodies. Distribution of *G. tergestinum* was not entirely known until 1986, since it has only been found in a few sites in Europe, Asia and North America (Krammer & Lange–Bertalot 1986). However, considering the currently available references on the occurrence of this taxon, there is a vast set of publications referring to the distribution of this *Gomphonema* from numerous freshwater sites all around the world (see Fig. 294, world distribution map for detail).

Two other taxa belonging to the same group and whose identification can be problematic due to similarities with *G. rosenstockianum* and *G. tergestinum* are *G. angustius* E. Reichardt and *G. supertergestinum* E. Reichardt. *Gomphonema angustius* was recently described from Germany (Reichardt 2009). To date, the occurrence of *G. angustius*, according to Reichardt (2009), is limited to highly calcareous streams in the Franconian Jura, in the Northern Alps of Switzerland and in Sicily. *G. supertergestinum* was described from Germany (Reichardt 2009); this is considered as an independent species although it has already been represented by several authors, e.g. in Schmidt et al. (1902), Hustedt (1930), and Van der Werff & Huls (1957–1974); nevertheless, according to Reichardt (2009), in those works it has not been distinguished from *G. tergestinum*.

The main aim of this study is to increase the knowledge about the taxonomy of the *Gomphonema rosenstockianum* – *G. tergestinum* species complex and to document the morphological differences of the species. To achieve this aim we relied on the analysis in LM and SEM of epilithic diatom samples collected in rivers from different European countries and the type material of *G. rosenstockianum* from Canary Islands, on a valve shape analysis by means of geometric morphometry, and on the verification of the stability of the morphological characters throughout the life cycle of *G. rosenstockianum* through the study of monoclonal cultures from samples collected in South of Portugal streams. Additionally, *G. angustius* and *G. supertergestinum* are characterized and illustrated in detail by LM and SEM with additional references to the distribution of both species. Furthermore, it is aimed to present the world distribution of the four species studied through the analysis of bibliographic information and the epilithic samples studied.

Materials and methods

Observations are based on the original type material of *Gomphonema rosenstockianum* obtained from the Lange–Bertalot Collection, Eu–E 56, Botanik Institut Universität Frankfurt am Main, collected by H. Grasmück (March 1988) in Bosque del Cedro, La Gomera, Canary Islands, Spain.

The type material of *G. semiapertum* var. *tergestina* Grunow has not been found so far, neither at the Grunow Diatom Collection at the Naturhistorisches Museum Wien (Curator: Dr. Anton Igersheim) nor at the National Botanic Garden of Belgium in Meise (Curator: Dr. Bart Van de Vijver). Thus, Grunow's drawing in plate 25, fig. 40 in Van Heurck (1880) represents the only available information about the type of this species. The drawing of the type of *G. semiapertum* var. *tergestina* made by A. Grunow, M. Schmidt's illustrations of *G. tergestinum* presented in plate 234, figs 41–43 in Schmidt et al. (1902), and the LM and SEM images shown in Reichardt & Lange–Bertalot (1991) were the basis used to define the concept of the species.

Moreover, epilithic diatom samples from Bulgaria, France, Hungary, Italy, Portugal, Slovakia and Spain were also analysed in LM and SEM. Details of the sampling sites are presented in Table 1. Additionally, monoclonal cultures of *G. rosenstockianum* were obtained from three samples collected in December 2006 from streams of the Algarve, South of Portugal.

Type material, field samples and monoclonal cultures were treated using hot hydrogen peroxide (120 vols.) and diluted HCl (37%), in order to obtain

Table 1. Sampling sites characteristics. Coordinates referred to the WGS84 datum.

Species / Sites	Sampling date	Latitude	Longitude	Altitude (m a.s.l.)	Conductivity µS cm^{-1}	pH
G. rosenstockianum Lange-Bert. et E. Reichardt						
Arão Stream, Pereira, Algarve (Portugal)	06/05/2006	37º 09’ 31” N	8º 36’ 13” W	24	303	8.9
Algibre Stream, Tôr, Algarve (Portugal)	17/04/2006	37º 11’ 23” N	8º 01’ 37” W	124	236	8.4
Son Brull Stream, Pollença, Mallorca Island (Spain)	10/12/2005	39º 52’ 55” N	3º 01’ 11” E	55	733	7.7
Puigpunyent Stream, Puigpunyent, Mallorca Island (Spain)	19/02/2006	39º 37’ 18” N	2º 31’ 38” E	215	1174	7.9
G. tergestinum (Grunow) M. Schmidt						
Cant Stream, Biancot, Cuneo (Italy)	29/11/2006	44º 20’ 57” N	7º 10’ 26” E	1470	692	8.1
Germanasca Stream, Ghigo di Prali, Torino (Italy)	07/12/2006	44º 53’ 31” N	7º 02’ 53” E	1450	167	8.0
Payant Stream, Bobbio Pellice, Torino (Italy)	05/12/2006	44º 48’ 20” N	7º 07’ 05” E	734	112	8.2
Drôme River, Charens, Rhone Alps (France)	15/09/1997	44º 32’ 18” N	5º 30’ 33” E	849	398	8.3
Danube River, Karlova Ves (Slovakia)	10/04/2006	48º 08’ 39” N	17º 03’ 30” E	140	496	8.4
Lomnica River, Juskova Vola (Slovakia)	16/04/2004	48º 52’ 43” N	21º 34’ 07” E	253	18	7.7
Isuela River, Cálcena, Zaragoza (Spain)	27/07/2003	41º 39’ 15” N	1º 43’ 03” W	817	No data	No data
Araquil River, Asiaín, Navarra (Spain)	16/08/2003	42º 49’ 52” N	1º 47’ 17” W	394	375	8.5
Esca River, Sigües, Zaragoza (Spain)	17/08/2003	42º 37’ 48” N	1º 00’ 55” W	506	300	8.5
Arga River, Huarte, Navarra (Spain)	18/08/2003	42º 49’ 52” N	1º 35’ 09” W	443	223	8.3
G. angustius E. Reichardt						
Arba de Biel River, Luna, Zaragoza (Spain)	29/07/2003	42º 09’ 32” N	0º 55’ 42” W	447	450	7.9
G. supertergestinum E. Reichardt						
Nela River, Cigüenza, Villarcayo Burgos (Spain)	16/08/2003	42º 56’ 33” N	3º 34’ 27” W	597	250	8.0
Danube main arm, Göd (Hungary)	08/02/2006	47º 41’ 36” N	19º 07’ 47” E	103	582	8.6

a suspension of clean frustules. Permanent slides were mounted with Naphrax®. LM observations and morphometric measurements were performed using a Leica® DMRX light microscope with 100x oil immersion objective and light microscopy photographs were taken with a Leica® DC500 camera. Samples selected for scanning electron microscopy analysis were filtrated through polycarbonate membrane filters with a pore diameter of 3 µm, mounted on stubs, sputtered with gold (40 nm) with Modular High Vacuum Coating System (BAL–TEC MED 020) and studied with a Leica® Stereoscan 430i, operated at 20 kV.

For the valve shape analysis, 15 landmarks were placed along the valve outline and at the curvature extremes, at the end of the striae delimiting the central area, and on the stigma (Fig. 1) and digitized using tps Dig2 software (Rohlf 2004). The Cartesian coordinates of the cells were aligned (translated, rotated and scaled) by the Procrustes generalized orthogonal least-squared superimposition procedure (Generalized Procustes Analysis, GPA, Rohlf & Slice 1990). Thin–plate spline deformations in landmark configuration relative to a theoretical average configuration representing the consensus form of the valve shape were calculated. A Principal Component Analysis (PCA) was carried out on the tangent space Procrustes coordinates by means of the software Past version 1.78 (Hammer et al. 2001). Afterwards a Hotelling's T–square statistic was performed on the PCA scores of the specimens in the first two axes to account for significant morphological differences among the *a priori* established groups, this test being a generalization of Student's t statistic that is used in multivariate hypothesis testing (Hotelling 1931). We evaluated 50 light microscope photographs of *G. rosenstockianum* for the valve shape analysis, including the type material and the populations of the South of Portugal and Mallorca Island (Spain) and 50 light microscope photographs of *G. tergestinum* from Italy, Slovakia and Spain.

Based on the bibliographic information available and on the distribution of the taxa present in the epilithic river samples analysed in this study, a world distribution map of *G. rosenstockianum*, *G. tergestinum*, *G. angustius* and *G. supertergestinum* has been performed using GIS software.

Fig. 1. Light micrograph of *Gomphonema tergestinum* showing the position of the 15 landmarks on the valve outline used to perform the morphometric analysis. Scale bar 5 µm.

Results

Gomphonema rosenstockianum Lange-Bert. et E. Reichardt in Lange–Bert. 1993 Figs 2–69: LM, Figs 70–79: SEM

References and illustrations

The type of the species has only been illustrated by Lange–Bertalot (1993, p. 71, pl. 76, figs 1–8: LM, figs 9, 10: SEM).

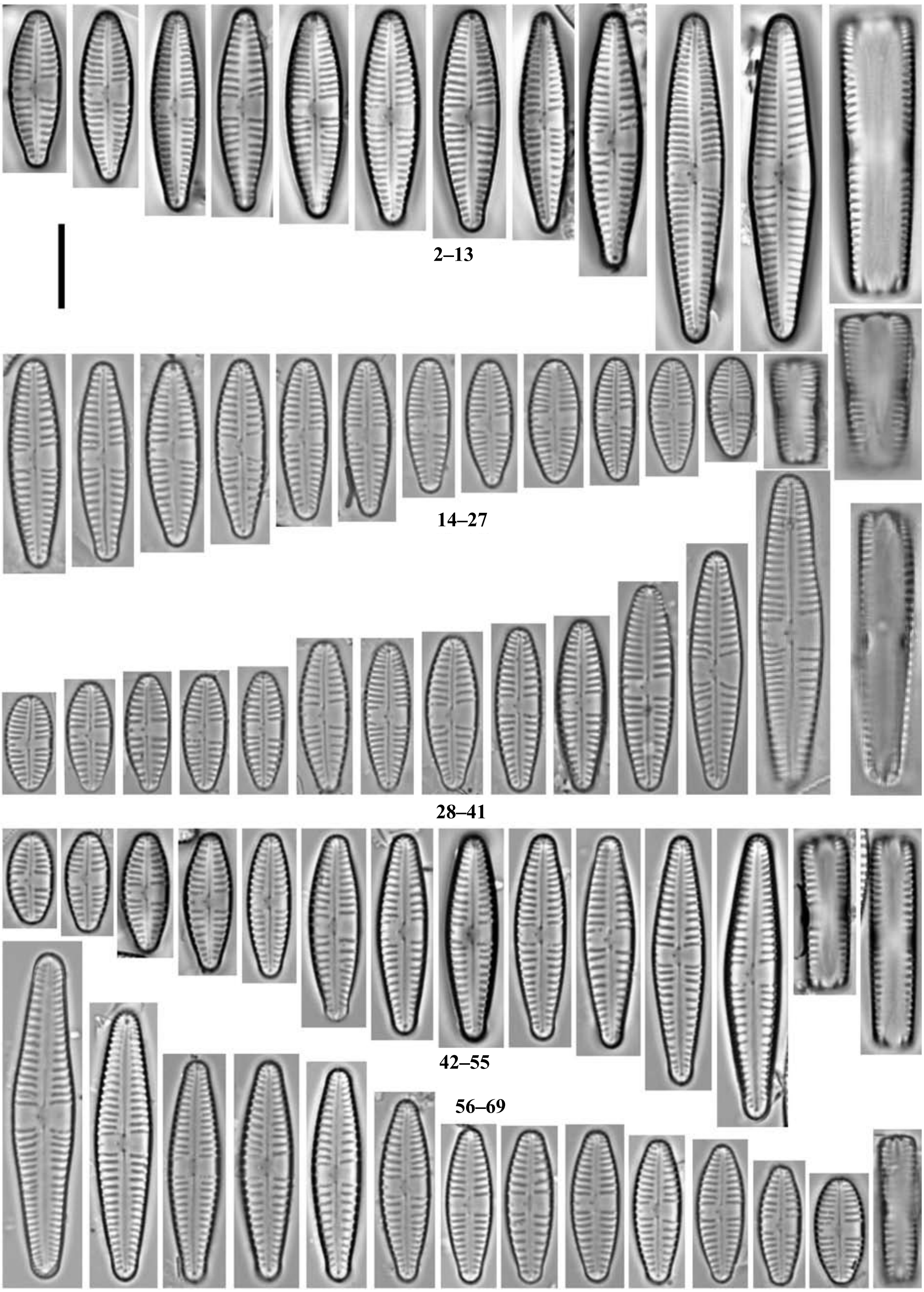
Morphological examination

Type material: Figs 2–13: LM, Figs 70–74: SEM

Populations from river epilithic samples: Figs 14–69: LM, Figs 75-79: SEM

Specimens of *G. rosenstockianum* were abundant in the type material from Canary Islands and the examination of the type population allowed the definition of the following features: valve outline is always sublinear–elliptic to elliptic in smaller individuals, striae are slightly radiate to almost parallel in the proximity of the central area (Figs 2–13). Only one short stria is located on the stigma–bearing side, on the opposite side to the unilaterally expanded central area (Figs 2–13). The ranges of width, length, and density of striae are given in Table 2. Observed in SEM, the stigma is covered by a papilla and does not present a collar-like ring around its aperture in internal view (Fig. 74), striae consist of one row of areolae although sometimes the areolae can be arranged in a double row in the proximity of the raphe (Fig. 70). Areolae are covered by papillae (Fig. 72).

A comparison between the epilithic samples from South of Portugal (Figs 14–41: LM, Figs 75–79: SEM) and Mallorca, Balearic Islands, Spain (Figs 42–69: LM) with the type material (Canary Island, Spain) showed a constancy of morphological characteristics. Nevertheless, the populations from the river epilithic samples presented smaller individuals and higher density of striae than the type material (Table 2). LM and SEM examination of the monoclonal cultures from stream samples of South of Portugal allowed the observation of the stability of the characteristics



Figs 2–69. Light micrographs of *Gomphonema rosenstockianum*: (2–13) type material, La Gomera, Canary Islands, Spain; (14–27) Arão Stream, Pereira, South of Portugal; (28–41) Algibre Stream, Tôr, South of Portugal; (42–55) Son Brull Stream, Pollença, Mallorca, Spain; (56–69) Puigpunyent Stream, Puigpunyent, Mallorca, Spain. Scale bar 10 µm.

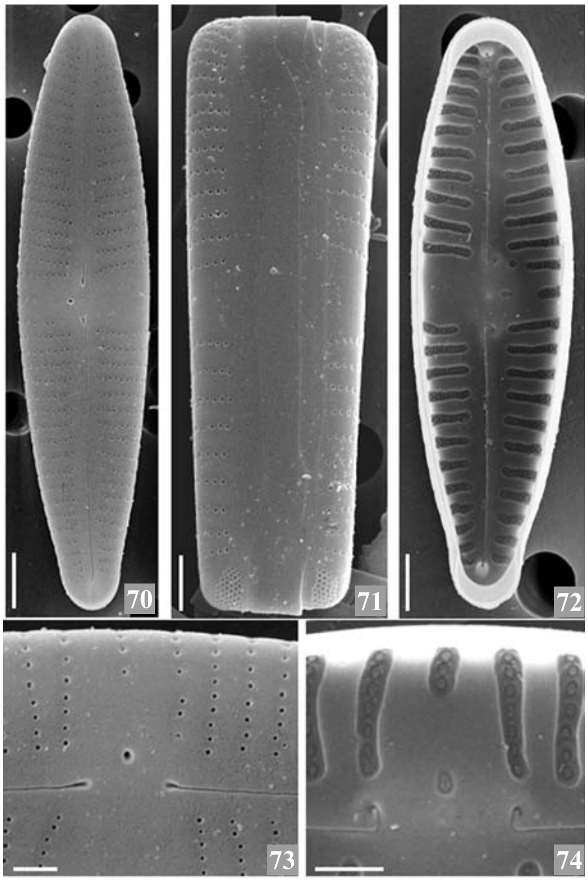
throughout the life cycle. Namely, in LM the valve outline was always sublinear–elliptic to elliptic in smaller individuals, striae were slightly radiate to almost parallel in the proximity of the central area and the presence of only one short stria on the stigma–bearing side, opposite to the unilaterally expanded central area. In SEM, the stigma was covered by a papilla and lacked a collar–like ring around its aperture (in internal view). The striation was formed by one range of areolae internally covered by papillae.

Ecological data

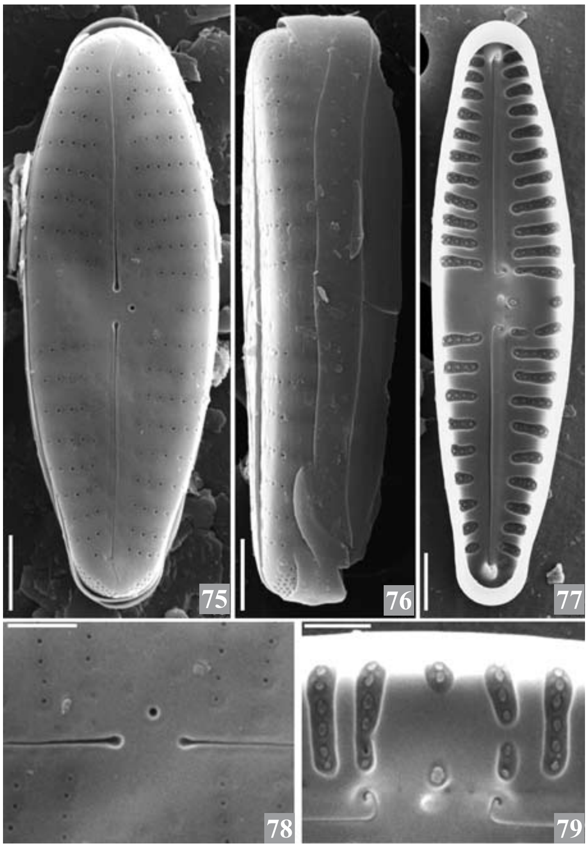
The geographical and chemical characterization of the sites from where the populations of *Gomphonema rosenstockianum* illustrated in this study were collected, is presented in Table 1. This epilithic diatom was found with abundance over 2.5% in several Portuguese streams with a range of pH between 6.5 and 9.2, conductivity between 146 and 600 µS cm^{-1} and dissolved oxygen between 6.6 and 13.0 mg l^{-1}, while the two streams of Mallorca Island (Spain) with presence of *G. rosenstockianum* are characterized by a pH between 7.5 and 8.0, a conductivity between 732–1601 µS cm^{-1} and dissolved oxygen between 6.2–10.4 mg l^{-1}.

Gomphonema tergestinum (Grunow in Van Heurck) M. Schmidt in Schmidt et al. 1902 Figs 1, 80–242: LM, Figs 243–252: SEM

Basionym: *Gomphonema semiapertum* var. *tergestina* Grunow in Van Heurck 1880, pl. 25, fig. 40
Nomenclatural synonyms: *Gomphonema parvulum* var. *tergestina* (Grunow in Van Heurck) Cleve 1894,



Figs 70–74. Scanning electron micrographs of the type material of *Gomphonema rosenstockianum*, La Gomera, Canary Islands, Spain: (70) valvar view (external view); (71) girdle view (external view); (72) valvar view (internal view), showing the uniseriate striation and the areolae covered by papillae; (73, 74) details of the central area in external (Fig. 73) and internal (Fig. 74) views. Scale bars (Figs 70–72) 2 µm, scale bars (Figs 73, 74) 1 µm.



Figs 75–79. Scanning electron micrographs of *Gomphonema rosenstockianum* from Arão Stream, Pereira, South of Portugal: (75) valvar view (external view); (76) girdle view (external view); (77) valvar view (internal view); (78, 79) details of the central area in external (Fig. 78) and internal (Fig. 79) views. Scale bars (Figs 75–77) 2 µm, scale bars (Figs 78, 79) 1 µm.

p. 181; *G. lanceolatum* f. *tergestina* (Grunow) Cleve–Euler 1955, v. 5 (4): p. 185

Infraspecific taxa: *Gomphonema tergestinum* f. *subrostrata* Manguin 1964, p. 91; pl. 21, fig. 4; *Gomphonema tergestinum* var. *arcaliae* Robert 1969, v. 14 (2): p. 44; fig. 4a, b; these two infraspecific taxa (Manguin 1964, Robert 1969) are not similar to the nominate variety and are not included in this study.

References and illustrations

Van Heurck (1880, pl. 25, fig. 40: drawing, as *G. semiapertum* var. *tergestina* Grunow), Schmidt et al. (1902, pl. 234, figs 41–43: drawings, as *G. tergestinum* Grunow), Carter (1960, pl. 2, figs 3–6: drawing, as *G. tergestinum* Grunow), Robert (1969, p. 44, fig. 3: LM, as *G. tergestinum* (Grunow) Fricke), Reichardt & Lange–Bertalot (1991, pl. 10, figs 14–16: LM, fig. 17: SEM, as *G. tergestinum* Fricke), Güttinger (1992, pl. 2.05.24–12, figs 1–6: SEM, as *G. tergestinum* Fricke), Lange–Bertalot & Metzeltin (1996, p. 314, pl. 98, fig. 19: LM, as *G. tergestinum* Grunow), Sims (1996, pl. 109, fig. 18: drawing, as *G. tergestinum* (Grunow in Van Heurck) Fricke in A. Schmidt), Hürlimann & Niederhauser (2007, pl. 21, figs 24–27: LM, as *G. tergestinum* (Grunow) M. Schmidt), Levkov et al. (2007, p. 486, pl. 166, fig. 19: LM, as *G. tergestinum* (Grunow) Fricke), Reichardt (2009, fig. 8: SEM, figs 82–87, 89–92: LM, as *G. tergestinum* (Grunow) M. Schmidt).

Taxonomical remarks

In Schmidt et al. (1902), the asterisks situated near the illustrations of *G. tergestinum* (figs 39-43) in the plate 234 (Tafel 234. Herausgegeben von Dr. Friedr. Fricke) indicate that M. Schmidt is the author of these drawings in A. Schmidt's Diatom Atlas. For this reason, the correct name is *G. tergestinum* (Grunow) M. Schmidt and not *G. tergestinum* (Grunow) Fricke, as stated in many references, e.g. Hustedt (1930) and Levkov et al. (2007).

Morphological examination

The ranges of length, width, and density of striae of *G. tergestinum* from the available information on the type material and the river epilithic samples are presented in Table 2. Morphological characteristics of the species, based on the literature and river epilithic populations studied, can be summarized as follows: a large central area expanded unilaterally to the valve-margin and presence of a short stria on the opposite side of the central area; an isolated stigma positioned almost in between the proximal raphe ends (surrounded by a narrow collar–shaped ring in internal view in SEM); transapical striae radiate, mainly constituted by one row of areolae covered by papillae (clearly discernible in SEM).

The characteristics of the river epilithic populations studied (Figs 80–242: LM) correspond better to the illustrations of Grunow and M. Schmidt than to the description and illustration of Hustedt (1930) and the description of Krammer & Lange–Bertalot (1986), regarding the valve outline and ranges of length and width. The SEM micrographs (Figs 243–252) correspond well to the internal view presented by Reichardt & Lange–Bertalot (1991) regarding to the presence of the opening of the stigma surrounded by a collar-shaped ring and the striae formed by one row of areolae covered by papillae. Nevertheless, the natural populations studied are slightly different from Grunow's iconotype because of the presence of one short stria in the stigma–bearing side instead of two not shortened striae.

Geometric morphometric analysis

A geometric morphometric analysis was performed in order to justify the separation of *Gomphonema rosenstockianum* and *G. tergestinum* based on the valve outline, the relative dimensions of the central area and the position of the stigma. The results of the Principal Component Analysis on landmark normalized coordinates clearly demonstrated the differentiation between the two taxa, along the second PC axis (Fig. 293), considering the dimensions of the central area and the position of the stigma, proving the validity of these morphological criteria. The explained variance for the first axis (PCA1) is 74.71% and is 9.15% for the second axis (PCA2). Comparing the morphometric data, there are highly significant differences between both species (Hotelling's multivariate discriminant test T^2; $p < 0.001$).

Gomphonema angustius E. Reichardt 2009
Figs 253–265: LM, Figs 283–287: SEM

References and illustrations

This species has recently been described from Germany (Kurzenaltheim, Lkr. WUG, Bavaria) and is illustrated in Reichardt (2009, figs 9–26: LM, figs 27–30: SEM).

Morphological examination

The population of *G. angustius* from Arba River, Zaragoza, Spain (Figs 253–265: LM, Figs 283–287: SEM) corresponds well to the description of the type from Bavaria, Germany by Reichardt (2009). Nevertheless, the Spanish specimens are bigger, presenting a higher length (14.8–35.0 µm), a wider range of valve width (4.2–6.9 µm) and showing a lower density of striae (8–10 in 10 µm). The SEM analysis showed that the striae are composed by a double row of areolae with a certain tendency to be single–rowed in the proximity of the raphe near the central area (Figs 283, 284); the striae are formed by round, dot–like areolae in external view; the areolae are not covered by papillae in internal view (Figs 285, 287) and the internal opening of the stigma is surrounded by a collar–shaped ring (Fig. 287). These ultrastructural characteristics are also present in the illustrations of the German type material provided by Reichardt (2009). The morphometric characteristics of the river epilithic population studied are presented in Table 2.

During the present study, *G. angustius* has been found in Spain and Italy (Sicily) where it was never a dominant species and co–existed with *G. tergestinum*. In LM it can be distinguished either from *G. rosenstockianum* or from *G. tergestinum* by the valve outline and the lower density of striae and in SEM by the areolae not covered by papillae, arranged in a double row and by the opening of the stigma surrounded by a collar–shaped ring. This latter characteristic helps to distinguish this species only from *G. rosenstockianum*, being also a typical character to *G. tergestinum*.

Gomphonema supertergestinum E. Reichardt 2009

Figs 266–282: LM, Figs 288–292: SEM

References and illustrations

This species has only recently been described from Germany (Hardenbecker Haussee in Boitzenburg, Uckermark, Brandenburg) and illustrated by Reichardt (2009, figs 66–81: LM, figs 93–96: SEM).

Morphological examination

In this study, *Gomphonema supertergestinum* has been found in epilithic samples from several European watercourses from Bulgaria, Hungary, Slovakia and Spain, co–existing in some of them with *G. tergestinum.*

The populations of *G. supertergestinum* from Nela River (Burgos, Spain) (Figs 266–274: LM, Figs 288–292: SEM) and Danube River main arm (Göd, Hungary) (Figs 275–282: LM) correspond well to the description of the species by Reichardt (2009). However, the populations from Spain and Hungary are smaller (25.2–38.7 µm length, 6.7–8.5 µm width) than presented in the diagnosis of the species and show a narrower range of density of striae (9–11 in 10 µm). Additionally, a row of poroids in the cingulum is visible in girdle view (Fig. 274).

In SEM some characteristics referred to and illustrated by Reichardt (2009) are visible, like the pseudoseptum in the headpole (Fig. 290), the striae composed by a double row of areolae with some tendency to be uniseriate near the raphe (especially the striae delimiting the central area) (Fig. 291), the areolae not covered by papillae in internal view (Fig. 292), the internal opening of the stigma surrounded by a collar–shaped ring (Fig. 292) and the striae formed by round, dot–like areolae in external view (Fig. 291). The morphometric characteristics of the river epilithic populations studied are presented in Table 2.

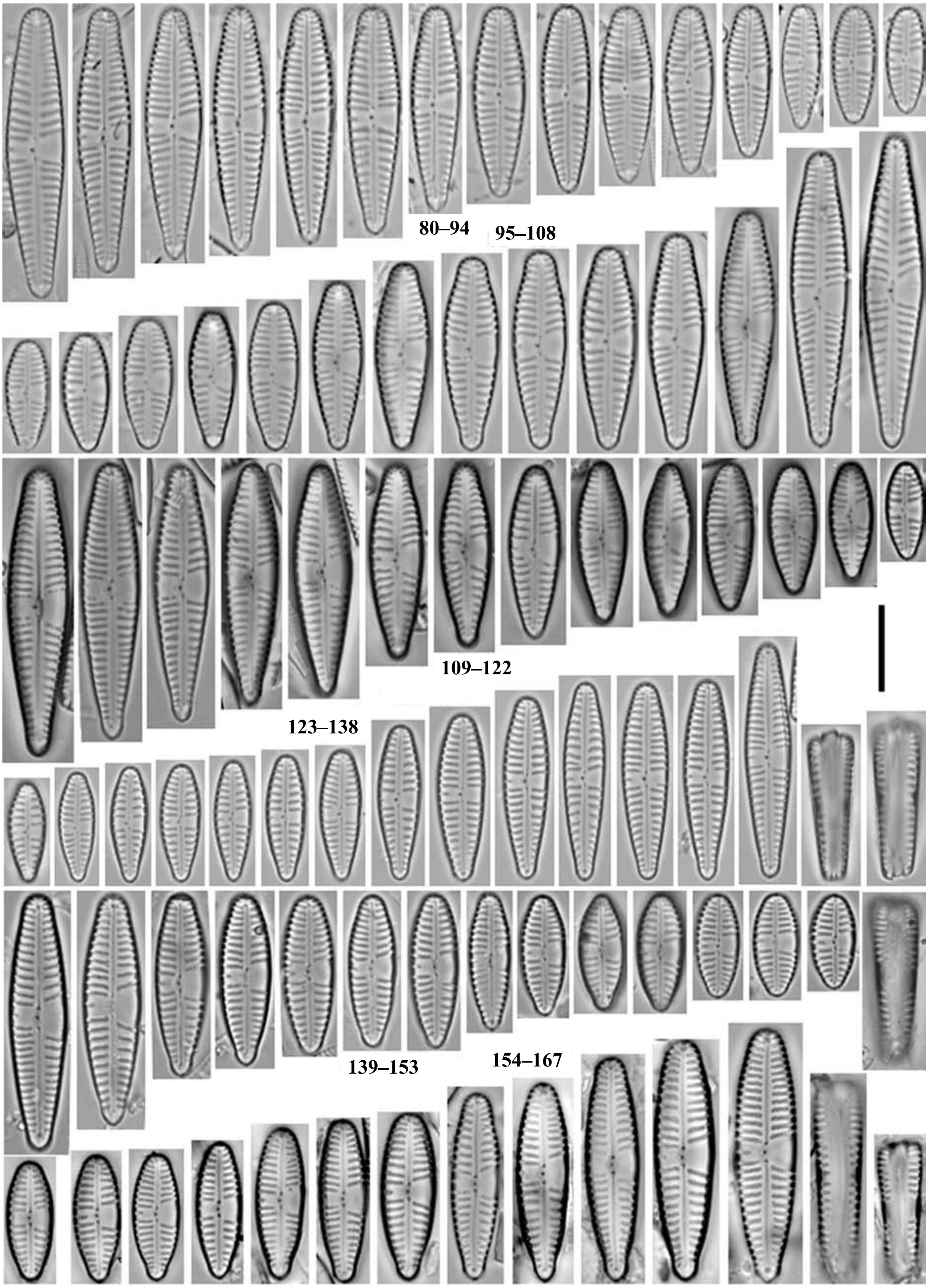
Despite the co–occurrence of *G. supertergestinum* with *G. tergestinum*, it can be easily distinguished from *G. tergestinum* as well as from *G. rosenstockianum* in LM by the more robust valve outline, larger dimensions and lower density of striae in 10 µm in *G. supertergestinum*. In SEM the areolae not–covered by papillae and arranged in double rows can separate it from *G. tergestinum* and *G. rosenstockianum* while the internal opening of the stigma surrounded by a collar–shaped ring can help to differentiate it from *G. rosenstockianum*. The more robust valve outline and larger dimensions can facilitate the discrimination of this species from *G. angustius*. Morphometric and ultrastructural characteristics that allow the distinction between these four taxa are presented in Table 3.

Biogeography of *Gomphonema rosenstockianum* and *G. tergestinum* species complex

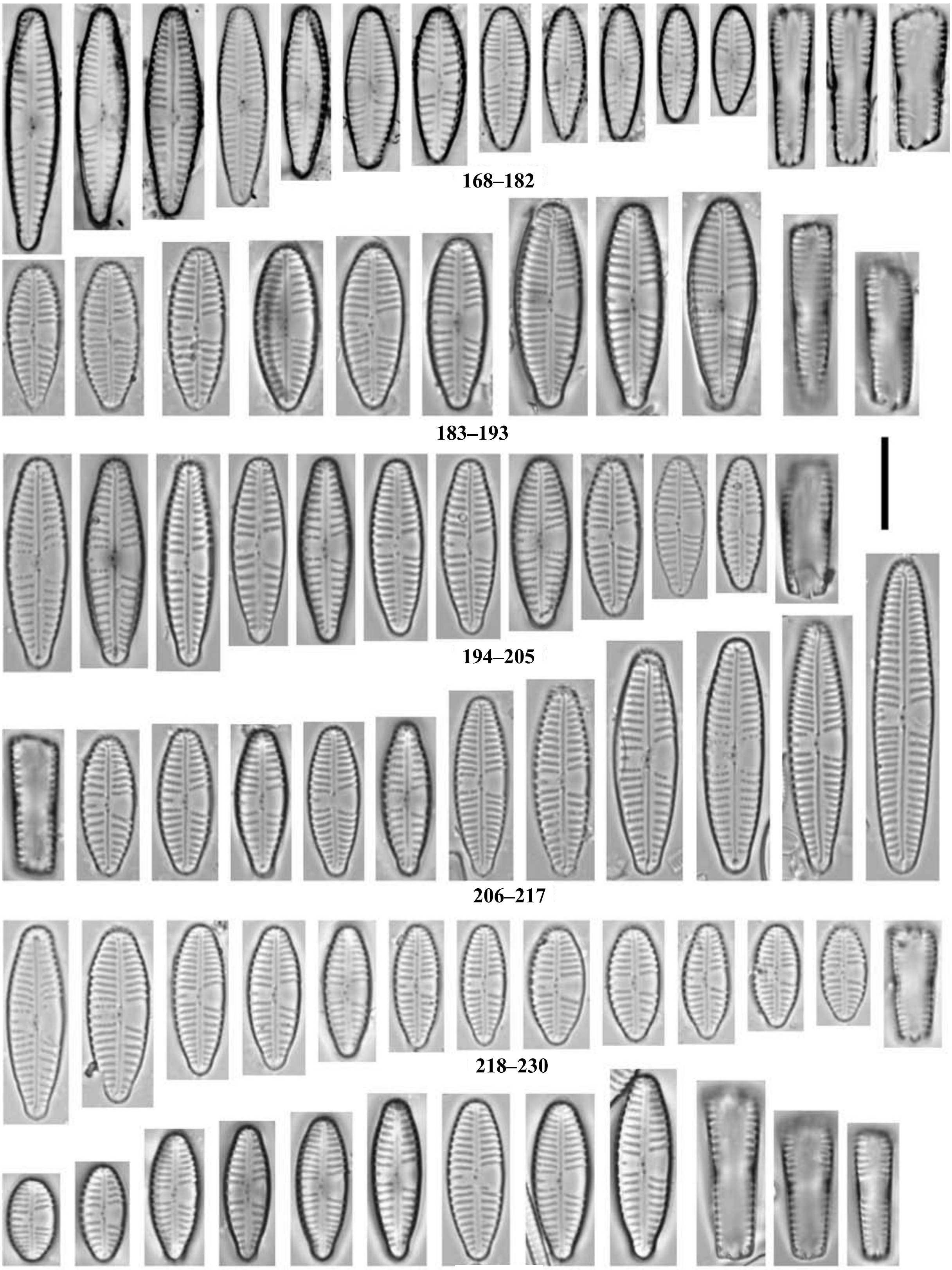
Subsequently to the revision of the literature published about this *Gomphonema* species complex, and taking into account the information provided by the analysis of the samples in this study, maps with world and European distribution of *G. tergestinum*, *G. rosenstockianum*, *G. angustius*, and *G. supertergestinum* have been performed, as shown in Figs 294 and 295.

Discussion and conclusions

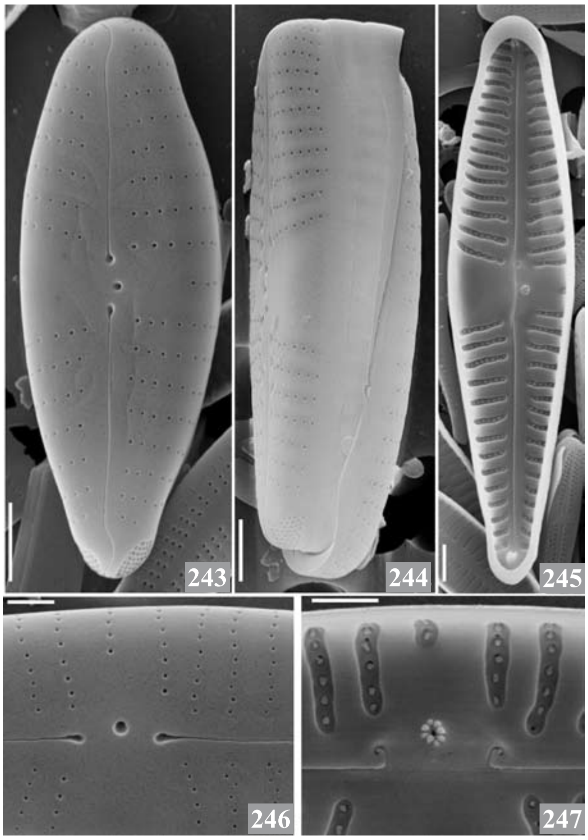
The observations of type material, literature data and field populations from different European rivers made during this study allowed the identification of the following morphological



Figs 80–167. Light micrographs of *Gomphonema tergestinum*: (80–94) Cant Stream, Biancot, Cuneo, Italy; (95–108) Germanasca Stream, Ghigo di Prali, Torino, Italy; (109–122) Payant Stream, Bobbio Pellice, Torino, Italy; (123–138) Drôme River, Charens, Rhone Alps, France; (139–153) Danube River, Karlova Ves, Slovakia; (154–167) Lomnica River, Juskova Vola, Slovakia. Scale bar 10 μm.



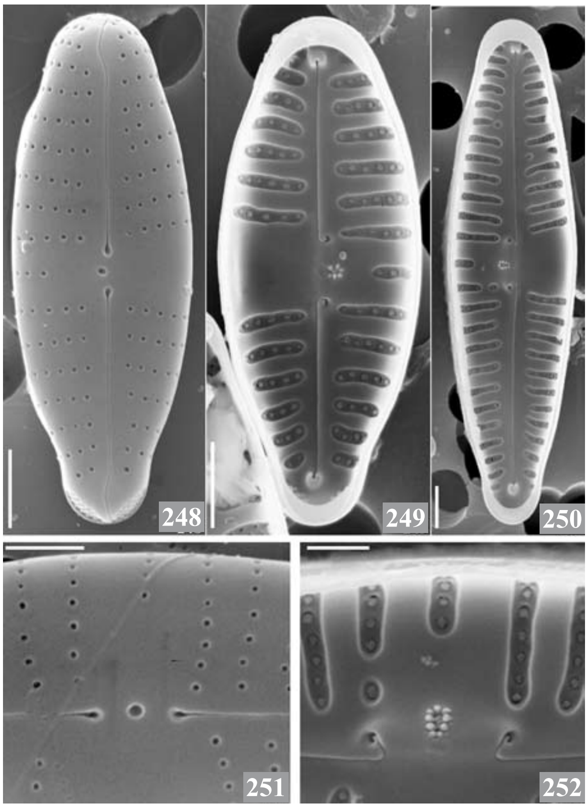
Figs 168–242. Light micrographs of populations of *Gomphonema tergestinum* from Spain: (168–182) Isuela River, Ermita de San Roque, Cálcena, Zaragoza; (183–193) Araquil River, Asiaín, Navarra; (194–205) Esca River, Sigües, Zaragoza; (206–217) Arga River, Huarte, Navarra; (218–230) Ebro River, San Adrián, Navarra; (231–242) Arga River, Embalse Eugui, Navarra. Scale bar 10 μm.



Figs 243–247. Scanning electron micrographs of *Gomphonema tergestinum* from Payant Stream, Bobbio Pellice, Torino, Italy: (243) valvar external view; (244) girdle external view from Fiumedinisi, Sicily, Italy; (245) valvar internal view, showing the uniseriate striation and the areolae covered by papillae; (246, 247) details of the central area in external (Fig. 246) and internal (Fig. 247) view, presenting the stigma surrounded by a collar-shaped ring in internal view. Scale bars (Figs 243–245) 2 μm; scale bars (Figs 246, 247) 1 μm.

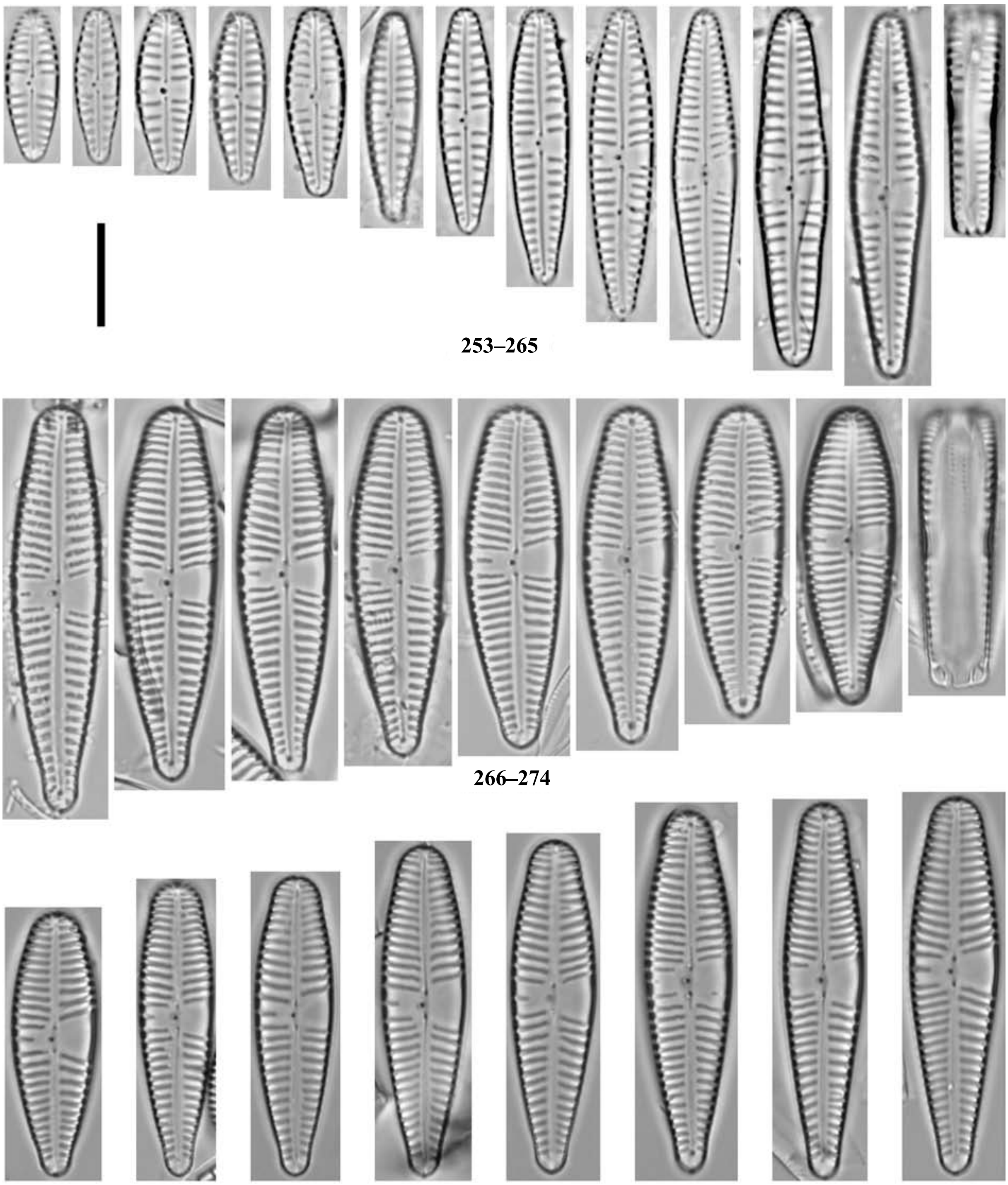
criteria to reliably distinguish between *G. rosenstockianum* and *G. tergestinum*: 1) the presence of a collar–shaped ring around the opening of the stigma in internal view in *G. tergestinum* (only visible by SEM) and its absence in *G. rosenstockianum* (the stigma is instead covered by a papilla); 2) the more central position of the stigma situated almost in between proximal raphe ends in *G. tergestinum* (visible by LM and SEM on external view); 3) the more evident stigma in *G. tergestinum* (LM); 4) the presence of a wider central area in *G. tergestinum* with the striae of the central area more radiate while in *G. rosenstockianum* these striae are usually more parallel.

The geometric morphometric analysis proved to be a useful tool and allowed the validation of these criteria and the subsequent separation of both species. This approach has become a standard tool of taxonomic studies due to its higher capacity to distinguish shapes, and proved to be a useful tool in clarifying difficult species complexes such as *Achnanthidium* Kützing (Potapova et Hamilton 2007) or *Reimeria sinuata* and *Gomphonema tergestinum* (Fránková et al. 2009).



Figs 248–252. Scanning electron micrographs of *Gomphonema tergestinum* from Arga River, Embalse Eugui, Navarra, Spain: (248) valvar external view; (249, 250) valvar internal view; (251, 252) details of the central area in internal (Fig. 251) and external (Fig. 252) view. Scale bars (Figs 248–250) 2 μm; scale bars (Figs 251, 252) 1 μm.

The biogeography of these *Gomphonema* species can also be helpful in distinguishing them, since *G. rosenstockianum* has only been found in the Canary Islands and Mediterranean region (Balearic Islands, Cyprus and South of Portugal) (Fig. 295) up to now. *G. tergestinum* seems to be widespread all over the world, although being mainly a palearctic species (Fig. 294). As regards to the ecology of *G. rosenstockianum*, the analysis of the chemical parameters of the sites where the natural samples were collected allowed the confirmation of the information already provided by Lange–Bertalot (1993): *G. rosenstockianum* is an alcaliphilous species, mainly occurring in oligo- to β–mesosaprobic waters, although it can also be found in α–mesosaprobic waters. In the

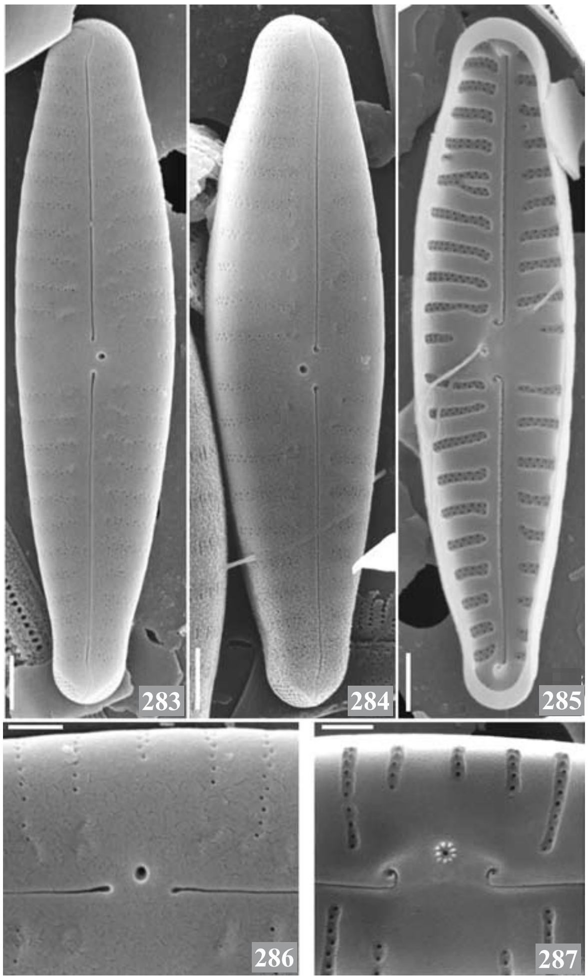


253–265

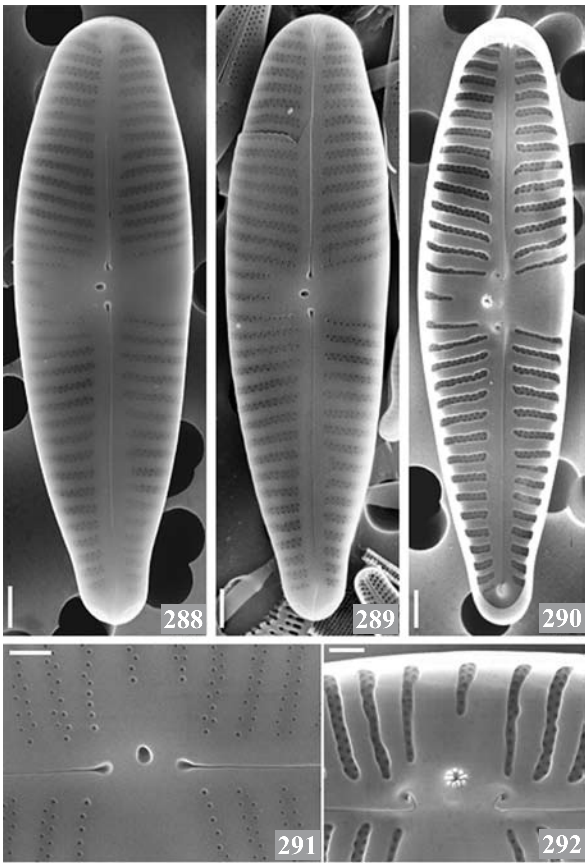
266–274

275–282

Figs 253–265. Light micrographs of *Gomphonema angustius* from Arba de Biel River, Luna, Zaragoza, Spain.
Figs 266–282. Light micrographs of *Gomphonema supertergestinum*: (266–274) Nela River, Cigüenza, Villarcayo, Burgos, Spain; (275–282) Danube main arm, Göd, Hungary. Scale bar 10 µm.



Figs 283–287. Scanning electron micrographs of *Gomphonema angustius* from Arba River, Biel in Luna, Zaragoza, Spain: (283, 284) valvar external view; (285) valvar internal view, presenting the biseriate striation; (286, 287) details of the central area in external (Fig. 286) and internal (Fig. 287) view, showing the areolae not covered by papillae and the stigma surrounded by a collar–shaped ring. Scale bars (Figs 283–285) 2 µm; scale bars (Figs 286, 287) 1 µm.



Figs 288–292. Scanning electron micrographs of *Gomphonema supertergestinum* from Nela River, Cigüenza, Villarcayo, Burgos, Spain: (288, 289) valvar external view, presenting the biseriate striation; (290) valvar internal view; (291, 292) details of the central area in external (Fig. 291) and internal (Fig. 292) view, showing the areolae not covered by papillae and the stigma surrounded by a collar-shaped ring. Scale bars (Figs 288–290) 2 µm; scale bars (Figs 291, 292) 1 µm.

light of the presented results, several pictures of *G. rosenstockianum* referred from Bulgaria in reality belong to *G. tergestinum* (Ivanov et al. 2006a, figs 16–18; Ivanov et al. 2006b, pl. 7, figs 2, 3; Stancheva et al. 2007, pl. 3, figs 1–4).

Furthermore, the examination of the bibliographic references about *G. tergestinum* made clear that also the species *G. supertergestinum* has been previously incorrectly identified as *G. tergestinum* by Schmidt et al. (1902, figs 39, 40), Hustedt (1930, fig. 717), Van der Werff & Huls (1957–1974), Krammer & Lange–Bertalot (1986, pl. 162, figs 6, 7), Ivanov et al. (2006a, p. 332, fig. 19), Levkov et al. (2007, pl. 166, fig. 17), and as *G. rosenstockianum* by Ivanov et al. (2006b, pl. 7, fig. 1) and Stancheva et al. (2007, pl. 3, figs 5, 6). Noticeably, most of these references correspond to lentic habitats, thus confirming the ecological preferences of *G. supertergestinum* for standing waters as noticed by Reichardt (2009). Indeed the samples studied in this work were collected in large rivers, where sometimes the diatoms could only be collected near the margins, consequently in zones with low water flow and with more lentic characteristics.

Gomphonema angustius can be misidentified as *G. angustum* C. Agardh or *G. occultum* E. Reichardt & Lange–Bert. in LM by the valve outline, similar striation pattern and position of the stigma. Although Reichardt (2009) stated that *G. angustius* can be smaller (narrower) than *G. angustum*, the population studied in this work was wider than the type material of *G. angustius*.

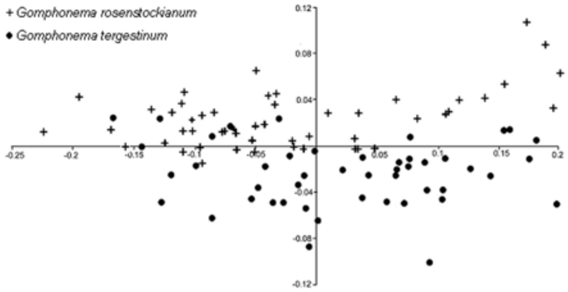


Fig. 293. Principal Component Analysis (PCA) plot of normalized coordinates for the morphological landmarks digitized on LM images of selected populations of *Gomphonema rosenstockianum* (cross) and *G. tergestinum* (circle).

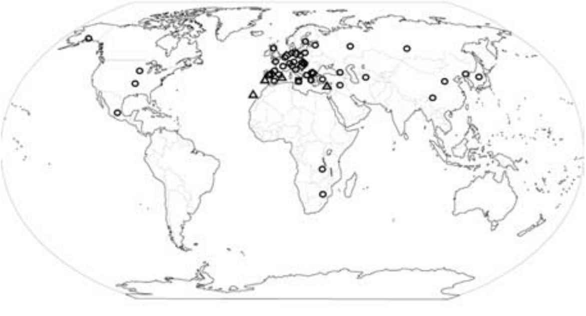


Fig. 294. World distribution map of *Gomphonema rosenstockianum* (triangle), *G. tergestinum* (circle), *G. angustius* (square), *G. supertergestinum* (diamond).

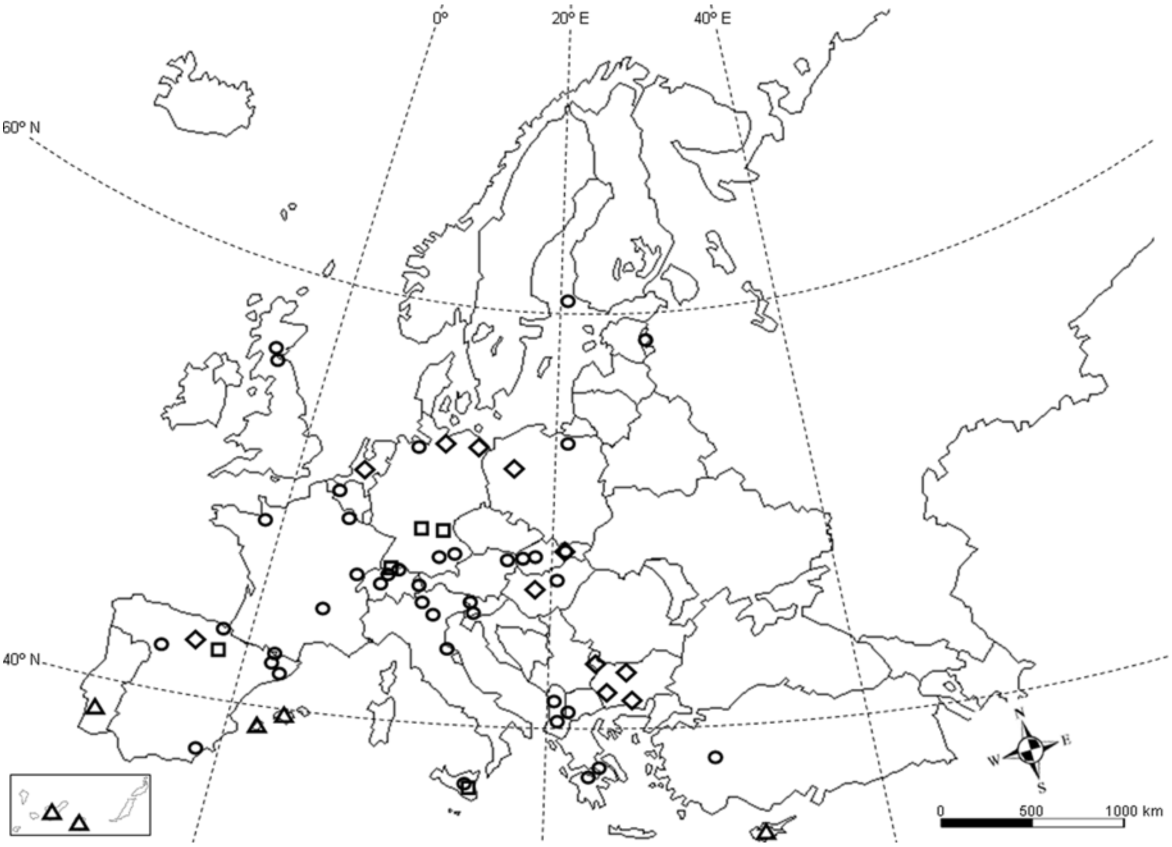


Fig. 295. European distribution map of *Gomphonema rosenstockianum* (triangle), *G. tergestinum* (circle), *G. angustius* (square), *G. supertergestinum* (diamond).

Therefore, we conclude that these three species can only be distinguished with certainty in SEM. *G. angustius* differs from *G. angustum* and from *G. occultum* by the areolae not covered by papillae and arranged in double rows; additionally *G. angustius* is distinguished from *G. angustum* by the more recurved hook–shaped proximal raphe ends (Reichardt & Lange–Bertalot 1991).

Regarding the general morphology of the species complex presented, mostly biseriate striation of *G. angustius* (Figs 283–285) and *G. supertergestinum* (Figs 288–290) could induce questions whether the placement of these species in the genus *Gomphonema* is justified. The taxonomic position of the double punctate species within the genera *Gomphonema* or *Gomphoneis* without longitudinal lines has already been discussed in the last decades, allowing different opinions. According to Dawson (1974) and Tuji (2005), species presenting double rows of simple

Table 2. Morphometric data of the *Gomphonema* studied (underlined = type material).
Sample size: *G. rosenstockianum*, *n* = 61; *G. tergestinum*, *n* = 148; *G. angustius*, *n* = 12; *G. supertergestinum*, *n* = 16.

Species / Sites	**Length (µm)**	**Width (µm)**	**Number of striae in 10 µm**
G. rosenstockianum Lange-Bert. & E. Reichardt			
La Gomera, Canary Islands (Spain)	16.7–34.0	4.9–6.3	10–13
Arão Stream, Algarve (Portugal)	10.1–29.6	4.8–5.7	12–16
Algibre Stream, Algarve (Portugal)	10.1–32.8	4.5–6.1	12–15
Son Brull Stream, Mallorca Island (Spain)	9.7–29.7	4.6–5.8	11–15
Puigpunyent Stream, Mallorca Island (Spain)	10.9–34.0	4.4–6.2	10–17
G. tergestinum (Grunow) M. Schmidt			
Trieste (Italy)	14.0	3.5	14
Cant Stream, Cuneo (Italy)	11.1–30.1	4.1–6.0	11–14
Germanasca Stream, Torino (Italy)	11.4–32.7	4.3–6.3	10–16
Payant Stream, Torino (Italy)	11.1–30.1	4.1–6.0	11–14
Drôme River, Charens, Rhone Alps (France)	11.6–26.6	4.0–5.3	11–15
Danube River (Slovakia)	9.5–26.4	4.4–5.7	11–15
Lomnica River (Slovakia)	12.3–26.4	4.9–6.0	10–16
Isuela River, Zaragoza (Spain)	9.9–23.3	3.6–5.4	10–14
Araquil River, Navarra (Spain)	13.4–20.1	5.0–6.5	11–15
Esca River, Zaragoza (Spain)	12.6–20.1	4.2–5.9	10–14
Arga River, Huarte, Navarra (Spain)	13.3–29.7	5.1–6.0	12–14
Ebro River, San Adrián, Navarra (Spain)	10.0–20.7	4.7–6.3	12–14
Arga River, Embalse Eugui, Navarra (Spain)	8.7–20	4.7–5.8	9–14
G. angustius E. Reichardt			
Kurzenaltheim, Lkr. WUG, Bavaria (Germany)	10.3–26.0	4.0–5.6	10–12
Arba de Biel River, Luna, Zaragoza (Spain)	14.8–35.0	4.2–6.9	8–10
G. supertergestinum E. Reichardt			
Boitzenburg, Uckermark, Brandenburg (Germany)	22.0–52.0	7.0–9.8	9–12
Nela River, Burgos (Spain)	28.1–38.7	7.0–8.0	10–11
Danube main arm, Göd (Hungary)	25.2–36.2	6.7–8.5	9–11

pores instead of the reniform poroidal structure typical of *Gomphonema* should be placed in the genus *Gomphoneis*. Merino et al. (1994) also suggested the placement in the genus *Gomphoneis* of *Gomphonema rhombicum* M. Schmidt, based on the presence of septa and pseudosepta in the headpole and striae composed by double rows of single pores; however, the latter species lacks the longitudinal lines. Other authors, such as Iserentant & Ector (1996) and Reichardt (2007), state that the arrangement of areolae in double rows is more common than has been suggested and can be found in all groups of the genus *Gomphonema*, even in taxa closely related

Table 3. Ultrastructural and total range of morphometric data of the studied *Gomphonema*.

Species	**Length (µm)**	**Width (µm)**	**Number of striae in 10 µm**	**Stigma**	**Striation**	**Areolae**
G. rosenstockianum LANGE–BERT. & E. REICHARDT	9.7–34.0	4.4–6.3	10–17	Covered by a papilla in internal view (SEM) Absence of collar–like ring in internal view (SEM)	Uniseriate on the mantle with tendency to biseriate on the proximity of the raphe	Covered by papillae in internal view (SEM)
G. tergestinum (GRUNOW) M. SCHMIDT	9.5–32.7	3.5–6.5	10–16	Collar–like ring in internal view (SEM)	Uniseriate on the mantle with tendency to biseriate on the proximity of the raphe	Covered by papillae in internal view (SEM)
G. angustius E. REICHARDT	10.3–35.0	4.0–6.9	8–12	Collar–like ring in internal view (SEM)	Biseriate	Not covered by papillae in internal view (SEM)
G. supertergestinum E. REICHARDT	22.0–52.0	6.7–9.8	9–12	Collar–like ring in internal view (SEM)	Biseriate	Not covered by papillae in internal view (SEM)

to the generic type. Furthermore, *G. angustius* (Fig. 283), *G. supertergestinum* (Figs 288, 291) and also other species such as *Rhoicosphenia abbreviata* (C. Agardh) Lange–Bert. can even present both uniseriate and biseriate striae within the same valve (Lange–Bertalot 1980), which evokes doubts about the taxonomic relevance of this character on the generic level. Therefore we consider the criterion of double punctuation as not sufficient to place these species in the genus *Gomphoneis*.

Apart of double rows of simple pores, *G. supertergestinum* also presents a pseudoseptum in the bluntly rounded headpole; nevertheless, in our opinion, this species should be kept in the genus *Gomphonema*. The genus *Gomphoneis* is still not clearly defined since it is a rather heterogeneous group lacking reliable and clear characters to allow its differentiation from the genus *Gomphonema*, as pointed out by Reichardt (2007).

From our study it becomes clear that there are recognizable differences in the ecological preferences of the taxa within this group, which can also serve as a helpful tool for the identification. It would thus be interesting to perform a similar study at a wider geographical scale, in order to increase and to support the current knowledge about the taxonomy, autoecology and biogeography of this species complex. Furthermore, it would be very useful to include the investigation of other species belonging to the same group, such as *G. angustum* and *G. occultum*.

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