Revision of the *Nitzschia sigma* complex (Bacillariophyta), a frequent cosmopolitan species in disguise with the description of two new species

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Abstract

The original material of four taxa in the *Nitzschia sigma* complex has been studied and revised based on light and scanning electron microscopy observations. *Nitzschia sigma*, in this paper lectotypified based on the original drawing in Kützing (1844) and epitypified using original de Brébisson material from Courseulles (Calvados, France), is a large marine-brackish species with a distinct striation pattern and structure. Several of the studied taxa, originally considered as varieties of *N. sigma*, showed sufficient morphological differences to be raised to species level or be described as new species, due to nomenclatorial issues. *Nitzschia rigidula* (Grunow) stat. nov., a freshwater species, originally described as *N. sigma* var. *rigidula* from the region around Brussels (Belgium) is the smallest of the new taxa. Analysis of another, former *N. sigma*-variety, proved to be based on an illegitimate, superfluous Kützing taxon, showed that two independent species were included under the same name, *N. sigma* var. *rigida*, that are described in this paper as two new, brackish to freshwater species: *Nitzschia neorigida* sp. nov. and *N. pararigida* sp. nov. All species are morphologically characterised and illustrated and their ecological preferences based on the associated diatom flora in the samples, are better defined.

Keywords: morphology, new species, *Nitzschia*, type material

Introduction

Nitzschia sigma (Kütz.) W.Sm., originally described as *Synedra sigma* Kütz. in 1844, proves as an ambiguous name used for different independent species, both in marine and freshwater habitats. Following Algaebase (Guiry and Guiry 2022), more than 20 infraspecific taxa (forms and varieties) were described based on *N. sigma*, most of them in the late nineteenth century. Van Heurck (1881, plates 65 & 66) illustrated in his Atlas of the Synopsis des Diatomées de Belgique several of these infraspecific taxa, many of them described by Grunow. However, morphological analysis made clear that these taxa often only have a faint resemblance to the nominate *Nitzschia sigma* and should be separated on species level.

Species in the *Nitzschia sigma* complex are characterized by a sigmoid valve outline, robust valves (length often > 100 µm), a typical striation pattern usually distinctly visible in LM and around 10 fibulae in 10 µm. Krammer and Lange-Bertalot (1988, plates 23 and 24) illustrate a lot of valves in this complex showing, however, a large morphological variability. Some of the illustrated valves are grouped under '*Nitzschia sigma sensu lato*' (plate 23), whereas others are named 'Falsche *Nitzschia sigma*-Sippen' (plate 24). This morphological variability together with a broad ecological preference range for the species clearly shows the necessity for a thorough revision.

This paper presents the results of the taxonomic and morphological analysis of several type materials containing *Nitzschia sigma* and some of its presumed varieties such as *N. sigma* var. *rigidula* Grunow in Van Heurck. The results of this analysis not only better defined the identity of *N. sigma* sensu stricto, but also showed that some of these varieties were actually never validly described. Based on the morphological comparison of all historic populations, two new species are described: *Nitzschia neorigida* Lange-Bert. & Van de Vijver, sp. nov. and *N.*

pararigida Lange-Bert. & Van de Vijver, sp. nov. One investigated variety is raised to species level as *Nitzschia rigidula* (Grunow in Van Heurck) stat. nov. All species are documented using both light (LM) and scanning electron (SEM) microscopy and their ecological preferences are determined based on the associated diatom flora (=ecological profiling).

Material & Methods

In the present study, four historic samples present in the Van Heurck diatom collection (BR, Meise Botanic Garden, Belgium) were investigated to clarify the taxonomic identity of some taxa in the *Nitzschia sigma* complex.

- de Brébisson sample #133 (Courseulles, Calvados, Normandy, France) (BR-4764)
- Rabenhorst sample 2527 (Bad Dürrenberg, Kötzschau bei Leipzig, Germany leg. Paul Gerhard Richter) (BR-4765)
- Walker Arnott sample 814 (Harwich, England, UK) (BR-4766)
- Delogne sample 187 (Rouge Cloître, Oudergem, Brussels, Belgium) (BR-4767)

A sub-sample of each of the selected materials was prepared for LM and SEM observations following the method described in van der Werff (1955). Small sub-samples were cleaned by adding 37 % H_2O_2 and subsequently heated to 80 °C for about 1–2 h. The reaction was completed by addition of saturated $KMnO^4$. Following digestion and centrifugation (three times 10 minutes at 4500 × rpm), the resulting cleaned diatom material was diluted with distilled water to avoid excessive concentrations of diatom valves on a slide and mounted in Naphrax®. Slides were analysed using an Olympus BX53 microscope at x1000 magnification (UPLanFL N 100x objective, N.A. 1.30), equipped with Differential Interference Contrast (Nomarski) optics and the Olympus UC30 Imaging System. For each taxon, the number of specimens, measured at random

on the type slide, is indicated (n=X). To assess the associated diatom flora in each sample, at least 200 valves were enumerated and identified on random transects.

For SEM, parts of the oxidized suspensions were filtered through a 5-µm Isopore™ polycarbonate membrane filter (Merck Millipore). Filters were air-dried and pieces were affixed to aluminum stubs. The stubs were sputter-coated with a platinum layer of 10 nm and studied using a JEOL-JSM-7100F field emission scanning electron microscope operated at 2 kV and 4 mm working distance (Meise Botanic Garden, Belgium). Slides, samples and stubs analysed in this study are stored at the BR-collection (Meise Botanic Garden, Belgium). Plates were prepared using Photoshop CS5.

Terminology used for the description of the various structures of the siliceous cell wall is based on Ross et al. (1979, areola structure), Cox and Ross (1981, stria structure), Krammer and Lange-Bertalot (1988, *Nitzschia* genus features), and Round et al. (1990, *Nitzschia* genus features).

For typification of the new species, we chose to use the entire slide as the type following article 8.2 of the International Code for Botanical Nomenclature (Turland et al. 2018). An appropriate image is linked to the designated type by stating "Fig. X illustrates the …type", ensuring that the identity of the species can be fixed.

Results

Division Heterokontophyta/Ochrophyta Cavalier-Smith

Phylum Diatoms (Diatomeae, Bacillariophyceae s.l., Bacillariophyta)

Class Bacillariophyceae Haeckel

Subclass Bacillariophycidae D.G.Mann

Order Bacillariales Hendey

Family Bacillariaceae Ehrenberg

Genus *Nitzschia* Hassall

Nitzschia sigma (Kütz.) W.Sm. (Figs 1 & 2)

Basionym

Synedra sigma Kützing 1844, *Die kieselschaligen Bacillarien oder Diatomeen*, p. 67, pl. 30, fig. 14

Lectotype (here designated)

Kützing (1844) Die Kieselschaligen Bacillarien oder Diatomeen, plate 30, figure 14

Epitype (here designated for the above designated lectotype of Nitzschia sigma)

BR-4764, Courseulles, Calvados, France, (de Brébisson sample #133), Fig. 1D illustrates the epitype.

Registration

http://phycobank.org/103536

Light microscopy (Fig. 1). Valves sigmoid with linear to weakly linear-lanceolate central part due to parallel margins, gradually tapering towards the slightly inflated apices, curved into opposite directions. Valve dimensions (n=20): length 100–160 µm, width 9–10 µm. Fibulae distinct, transapically elongated, shortly continuing onto the valve face, irregularly spaced, 7–9 in 10 µm, separated by rectangular to squarish interspaces. Striae clearly visible in LM, parallel throughout the entire valve, 20–21 in 10 µm. Striae distinctly punctate, ca 20 areolae in 10 µm, areolae less clear towards the fibulae.

Scanning electron microscopy (Figs 1F–1I, 2). Striae uniseriate (Figs 1F–1I) composed of transapically elongated, oval to rounded areolae. Areolae near the raphe keel connected by a

shallow, transapically elongated groove (Figs 1F, 1H). Near the raphe-bearing margin, a continuous narrow slit present running almost entirely from apex to apex (Figs 1F, 2A–2C). Slit separating valve face from the raphe keel (Figs 1F, 2A–2C), bordered at both sides by a broad zone ornamented with a regular pattern of oval markings and irregular ridges, running parallel with the keel (Figs 2A, 2C). Areolae on both sides of the slit with much larger foramina, internally covered by a porous plate (Figs 1H, 2A, 2B). Areolae loculate, inside the areolar tube closed by a perforated plate, distinctly visible at the areolae near the keel (Figs 2A, 2B). Internally, fibulae rather thin, connected to max. 2 striae (Figs 1I, 2D). Fibulae irregularly distributed along the entire valve length (Fig. 1G). Internal terminal raphe endings terminating onto distinct helictoglossae (Fig. 2D).

***Nitzschia neorigida* Lange-Bert. & Van de Vijver, sp. nov.** (Figs 3 & 4)

Holotype

BR-4765 (Meise Botanic Garden, Belgium), Fig. 3D illustrates the holotype.

Isotype

Slide 418 (University of Antwerp, Belgium)

Type locality

Bad Dürrenberg, Kötzschau bei Leipzig, Germany (Rabenhorst sample 2527, leg. Paul Gerhard Richter)

Registration

http://phycobank.org/103537

Light microscopy (Fig. 3). Valves distinctly sigmoid with a linear central part due to parallel margins, gradually tapering towards the very elongated, thin apices, clearly curved into opposite directions. Valve dimensions (n=25): length 115–155 µm, width 6.0–6.5 µm, length/width ratio 19–24.5. Fibulae transapically elongated, clearly continuing onto the valve face, irregularly spaced, 9–10 in 10 µm, separated by rectangular to squarish interspaces. Striae vaguely discernible in LM, parallel throughout the entire valve, ca 35 in 10 µm. Areolae only very weakly to not visible in LM.

Scanning electron microscopy (Fig. 4). Striation pattern quite regular throughout the entire valve (Figs 4A, 4C). Striae uniseriate, composed of rounded areolae, becoming weakly transapically elongated towards the raphe keel, 33–36 in 10 µm. At the raphe keel, striae formed by short series of 3–4 small, rounded areolae (Fig. 4C) less distinctly visible at the apices (Fig. 4D). Areola density: 30–35 in 10 µm. Distinct hyaline zone present at the raphe keel between last areolae and valve face/mantle junction (Fig. 4C). Raphe keel weakly thickened, at the apices bordered by a distinct groove and ridge (Fig. 4D), less pronounced but still present at the valve center (Fig. 4C). Internally, fibulae clearly irregularly distributed along the entire valve (Fig. 4B). Internal areola foramina transapically shortly elongated (Fig. 4E). Fibulae robust, connected to 2–3 striae (Figs 4E, 4F). Internal terminal raphe endings terminating onto small helictoglossae (Fig. 4F).

***Nitzschia pararigida* Lange-Bert. & Van de Vijver, sp. nov.** (Figs 5 & 6)

Holotype

BR-4766 (Meise Botanic Garden, Belgium), Fig. 5B illustrates the holotype.

Isotype

Slide 419 (University of Antwerp, Belgium)

Type locality

Harwich, England, UK (Walker Arnott sample 814)

Registration

http://phycobank.org/103538

Light microscopy (Fig. 5). Valves sigmoid with a linear central part due to parallel margins, gradually tapering towards the shortly elongated apices, clearly curved into opposite directions. Valve dimensions (n=30): length 80–115 µm, width 6.5–7.5 µm, length/width ratio 11.5–17.5. Fibulae transapically elongated, clearly continuing onto the valve face, irregularly spaced, 9–11 in 10 µm, separated by rectangular to squarish interspaces. Striae vaguely discernible in LM, parallel throughout the entire valve, ca 35 in 10 µm. Areolae not or only very weakly visible in LM.

Scanning electron microscopy (Fig. 6). Striation pattern quite regular throughout the entire valve (Fig. 6A). Striae uniseriate, composed of transapically elongated; rarely rounded, areolae, becoming longer towards the raphe keel, terminating by a short series of 2–3 small, rounded areolae, 34–36 striae in 10 µm (Figs 6A, 6B), the latter less distinctly visible at the apices (Fig. 6C). Areola density: ca 30 in 10 µm, becoming less dense towards the raphe keel (Fig. 6B). Distinct hyaline zone present at the raphe keel between last areolae and valve face/mantle junction (Fig. 6B). Raphe keel thickened, running along the entire valve face/mantle margin, at the apices bordered by a distinct groove and ridge (Fig. 6C), lower at the valve center (Fig. 6B). Internally, fibulae clearly irregularly distributed along the entire valve (Fig. 6D). Internal areola foramina rounded to transapically shortly elongated (Fig. 6E). Fibulae thin, connected to 1–3

striae (Figs 6D, 6E). Internal terminal raphe endings terminating onto small helictoglossae (Fig. 6F).

***Nitzschia rigidula* (Grunow) Lange-Bert. & Van de Vijver, stat. nov.** (Fig. 7)

Basionym

Nitzschia sigma var. *rigidula* Grunow in Van Heurck, Synopsis des Diatomées de Belgique, Atlas, 1881, plate 66, fig. 8.

Type locality

Rouge Cloître (Brussels, Belgium), Delogne sample 187

Lectotype (here designated)

BR-4767 (Meise Botanic Garden, Belgium), Fig. 7D illustrates the lectotype.

Registration

http://phycobank.org/103540 (registration of the name)

http://phycobank.org/103541 (registration of the lectotypification)

Light microscopy (Figs 7A–7M). Valves distinctly sigmoid with linear central part due to parallel margins, gradually tapering towards the clearly inflated apices, curved into opposite directions. Valve dimensions (n=30): length 55–70 µm, width 4.0–4.5 µm. Fibulae transapically elongated, shortly continuing onto the valve face, irregularly spaced, 10–12 in 10 µm, separated by rectangular to squarish interspaces. Striae discernible in LM, parallel throughout the entire valve, 30–35 in 10 µm. Areolae weakly visible in LM with magnification higher than 1500 times.

Scanning electron microscopy (Figs 7N–7Q). Striation pattern quite regular throughout the entire valve (Fig. 7N). Striae uniseriate, composed of rounded to weakly transapically elongated areolae, becoming slightly longer towards the raphe keel, ca 35 in 10 µm. At the raphe keel, striae terminated by short series of 2–3 small, circular areolae (Fig. 7P) less distinctly visible at the apices (Fig. 7O). Areola density: ca 40 in 10 µm. Very narrow hyaline zone present at the raphe keel between last areolae and valve face/mantle junction (Figs 7N, 7P). Raphe keel weakly thickened throughout the entire valve length (Figs 7N–7P). Groove bordering the raphe keel ridge absent (Fig. 7O). Internal areola foramina distinctly transapically elongated (Fig. 7Q). Fibulae relatively thin, window-like, connected to 2 striae (Fig. 7Q).

Discussion

Taxonomy

The exact taxonomic identity of *Nitzschia sigma* remained up to now an enigma. Originally, the taxon was described by Kützing (1844, p. 67, pl. 30, fig. 14) as *Synedra sigma* based on material from the 'Ostsee bei Hofmannsgave', a locality most likely close to the city of Odense on the Danish island Fyn. Apart from a short morphological description, *S. mediocris, sigmoidea, utrinque parum attenuata truncata, margine leviter striolata* [medium-sized *Synedra*, sigmoid-shaped, on both sides only weakly attenuated and truncated, the margins slightly striated], Kützing (1844) also indicated his uncertainty ('und bin in Zweifel' [and I doubt]) about the exact taxonomic position of this taxon. A minority of authors, however, follows William Smith (1853, p. 39, pl. 13, fig. 108) who discussed and depicted in 1853 a specimen from marine and brackish water based on material from Pevensey Beach (southeast England between Hastings and Brighton, UK) collected on the 2nd of September 1850 by himself (Hoover 1976, p. 21) as

'*Nitzschia sigma*, W.Sm.'. In his protologue, Smith mentioned the earlier published taxon *Synedra sigma* Kützing, described from the Baltic Sea, by indicating the drawing Kützing published in 1844 (plate 30, fig. 14). It must be noted that William Smith often published new combinations of older taxa (usually previously described by Kützing, de Brébisson or Greville), but never added the original author in the authorship, which of course most likely confused a lot of later scientists. Smith (1853, p. 4) briefly explained that in case of new combinations, he only wrote 'W.Sm.' behind a species name without adding the original author name. But he (Smith 1853, p. 4) also added that "*the original appellation is given in the list of synonyms*". New species, however, in the same publications (Smith 1853, 1856), such as for instance *Nitzschia obtusa* W.Sm. (1853, p. 39), were always followed by 'sp. nov.' clearly indicating its status as new species (Smith 1853, p. 4). Grunow in Van Heurck (1881, pl. LXV, figs 7 & 8) also notes W. Smith exclusively as author of *Nitzschia sigma* but supplies (*Synedra* Kütz.) in brackets behind the name and author. Even Van Heurck (1885, p. 179 and 1896, p. 396) reported the taxon as *N. sigma* W.Sm. referring to the publication in Smith (1853). In his Treatise, Van Heurck (1896) even confirmed having personally seen the Smith specimens (addition of ! to the name). And also Hustedt (1921 in A.Schmidt Atlas pl. 336, figs 1 (2–6)) notes exclusively William Smith as author for *Nitzschia sigma*, erroneously omitting Kützing's *Synedra sigma* described in 1844. In 1930, Hustedt (1930, p. 420), however, seemed to correct this and reported *N. sigma* (Kütz.) W.Sm.

Most of the original Kützing diatom collection is nowadays housed in three institutes: Natural History Museum (BM, London, UK), Meise Botanic Garden (BR, Belgium) and Naturalis (Leiden, the Netherlands). Unfortunately, type material of *Synedra sigma* from Hofmannsgave, which could clarify whether the Kützing species and the combination William Smith made based

on his Pevensey Bay population are conspecific, could not be found in either London, Leiden or Meise (D.M. Williams, pers. comm. & B. Van de Vijver, pers. obs.). Kützing (1849, p. 45) mentioned in 1849 under his taxon, *Synedra sigma* three localities where the species was found: *Sinus Codanus* (old Latin name for the Kattegat and the Baltic Sea), *Canalis Brittanicus* (Channel between the UK and France) and specimens sent to him by his friend Alphonse de Brébisson originating from *ad oras Calvadosii* (the coast of the Calvados department, Normandy, France). In London (BM) and Meise (BR), Calvados material is present as Kützing samples 1489 (= slide BM 18302) and 1491 (= slide BM 18303). In the personal diatom sample collection Henri Van Heurck put together and conserved in BR, two de Brébisson samples from Courseulles (Calvados, Normandy, France) containing *N. sigma* were found, one lacking a sample number and one with an original de Brébisson number (#133). Additionally, in the original collection of William Smith (Hoover 1976), now also part of the Van Heurck diatom collection at BR, five samples containing *N. sigma* are conserved, four originating from Pevensey Beach (England, UK; collected in 1850 and 1851 by W. Smith) and one sent by de Brébisson and listed as #77 from Courseulles. In the present paper, one of the Pevensey Beach populations and the de Brébisson sample 133 have been studied for the first time by means of light and scanning electron microscopy. Since all original Kützing marial from Ostsee bei Hofmannsgave is lost, we opt for a lectotypification of *N. sigma* by choosing the only original drawing of the species in Kützing (1844, plate 30, fig. 14). The de Brébisson sample #133 (Calvados, France) is designated as epitype for the designated lectotype of *Synedra sigma* syn. *Nitzschia sigma*, as this Calvados population was one of the localities mentioned by Kützing in 1849 in accordance with ICN Art. 9.8 (Turland et al. 2018).

By far most records of *Nitzschia sigma* in freshwater diatom literature do not concern the authentic *Nitzschia sigma* s.str., which is an exclusively marine (or brackish) species. The overall confusion is due to a widely neglected, today almost forgotten taxon, *Nitzschia sigma* var. *rigida* Grunow (1878). Grunow mentioned the name for the first time in 1878 when discussing the complex of species related to *Nitzschia sigma*, following the description of *N. sigma* var. *intercedens* Grunow, the latter observed in the Caspian Sea (Grunow 1878, pp. 118–119). Grunow added that according to Walker Arnott the variety *rigida* could be identical to *Amphipleura sigmoidea* W.Sm., a new name Smith gave in 1853 for *Amphipleura rigida* Kütz. The latter species was originally described by Kützing (1844, p. 104, pl. 4, fig. 30), from the North Sea island of Wangerooge, belonging to the East Friesean Isands (Germany), situated parallel and very close to the German mainland. Type material is still present but unfortunately damaged by preparation processes and therefore can no longer be analysed in detail. Grunow (1878) transferred *Amphipleura rigida* to the genus *Nitzschia* and combined it infraspecifically with *N. sigma* (Kütz.) W.Sm. as *Nitzschia sigma* var. *rigida* (Kütz.) Grunow without actually using the type material of the basionym from Wangerooge. A revision of *Amphipleura rigida*, however, showed that Kützing's name has to be considered an illegitime name as it was an unwarranted name change for a species described in 1838 as *Sigmatella subrecta* Bréb. (Brébisson 1838). Van de Vijver & Kusber (2022) analysed the type material of the latter and transferred it to the genus *Gyrosigma*. This means that all later transfers of *A. rigida* to the genus *Nitzschia* are most likely valid, but superfluous as they are based on a species that does not belong to the genus *Nitzschia*. Therefore, *Nitzschia sigma* var. *rigida* sensu Grunow has to be described as a new species. Van Heurck (1881, plate 66) illustrated 2 valves identified as *N. sigma* var. *rigida*, however, taken from different populations. The original drawings on this plate

are kept partly in the Van Heurck collection (BR, Belgium) and partly in the Grunow collection at the Naturhistorisches Museum in Wien (W, Austria). The Grunow collection not only conserves all drawings Grunow made during his career, but also possesses an annotated copy of Van Heurck's Atlas with handwritten notes next to the species' drawings mentioning the samples used for the drawings. Analysis of these drawings allowed us to trace the origin of the populations Grunow used to document the varieties in Van Heurck (1881, plate 66). Van Heurck's plate 66, fig. 2 originates from Harwich (England, UK) based on Walker Arnott sample 814 (Figs 8A, 8B, 8H) whereas his fig. 5 comes from the saline spring of Bad Dürrenberg, a spa resort in Saxony Anhalt (eastern Germany) (Figs 8C, 8D, 8G). This variety (sensu Grunow) differed mainly from the *N. sigma* nominate variety, by its distinctly higher stria and areola density, 33–36 striae in 10 µm (versus 20–21 in 10 µm in *N. sigma*) and differences in valve dimensions (Table 1). The revision of both populations illustrated in Van Heurck (1881), showed that they not only differ from the nominate *N. sigma*, but also cannot be considered conspecific and therefore should be described as two independent species: *N. neorigida* Lange-Bert. & Van de Vijver, sp. nov., and *N. pararigida* Lange-Bert. & Van de Vijver, sp. nov. Both differ in several aspects justifying the separation of both as independent species: 1. valve dimensions (see Table 1) with *N. pararigida* being much shorter for a similar valve width; 2. valve outline with *N. neorigida* possessing more elongated apices; and 3. structure of the striae and the areolae (typically rounded in *N. neorigida* and transapically elongated in *N. pararigida*).

Grunow described a second *Nitzschia sigma*-variety in Van Heurck (1881, plate 66, fig. 8, see our Figs 8E & 8F) based on a sample (n° 187) he received from Charles-Henri Delogne, collected at the Rouge Cloître (Red Monastery, Oudergem, Belgium) near Brussels. This freshwater sample contained a large population of what Grunow described as *Nitzschia sigma*

var. *rigidula* Grunow in Van Heurck. Krammer & Lange-Bertalot (1988, plate 23, figs 8–9) included the taxon within *N. sigma sensu lato*, illustrating two valves from Van Heurck's Types du Synopsis des Diatomées de Belgique exsiccata slide n°396. The latter slide was based on Delogne material from Rouge Cloître, although the original Delogne sample number was not indicated on the vial Van Heurck conserved for slide 396. However, based on the annotated Van Heurck Atlas, kept in W (Austria), we know that Grunow used sample 187 (Fig. 8E). Analysis of the material showed that the taxon differs sufficiently from *N. sigma* s. str. to justify its raise to species level as *Nitzschia rigidula*. The latter has smaller valve dimensions (max. length 65 µm), short, clearly inflated valve apices, a higher stria density (30–35 in 10 µm versus 20–21 in 10 µm in *N. sigma*), and a different structure of the striae (lacking the typical grooves at the keel). *Nitzschia rigidula* can also be separated from both *N. neorigida* and *N. pararigida* (Table 1) based on valve dimensions, especially the valve width, being 4.0–4.5 µm in *N. rigidula* but at least 6 µm in *N. neorigida*) and even 6.5–7.0 µm in *N. pararigida*.

The *Nitzschia-sigma* complex counts a lot more varieties such as for instance *N. sigma* var. *intercedens* Grunow (Grunow 1878) and *N. sigma* var. *fonticola* Hustedt, described in 1937 from a freshwater spring on Sumatra and later in 1998 recombined as *Nitzschia kanakarum* Gerd Moser et al. as it was clear that this variety showed important morphological differences with the *N. sigma*-complex (Moser et al. 1998, p. 210). The illustrations in Simonsen (1987, plate 360) and Moser et al. (1998, plat 68, fig. 1) indeed show a species with a very fine striation and thick, robust fibulae, clearly different from all species described or discussed in the present paper.

Ecology and Distribution

The new species, split off *Nitzschia sigma* sensu stricto were often erroneously misidentified as *Nitzschia sigma*. Under this mask of incorrect identification, these species were recorded (as *N. sigma* var. *rigida*) during the last 40 years in brackish and freshwater diatom literature by many authors from all continents, making its distribution considered as being cosmopolitan. Based on these records, its autecology is also very broad, ranging from marine, brackish to freshwater habitats with medium to low alkalinity. The species was even reported from oligosaprobic environments, there occasionally in association with acidophilous diatom taxa. Analysis of these populations will be necessary to allow for a correct identification. Table 2 lists a large number of records of *N. sigma sensu lato* together with their geographical locality. Some of these identifications can be corrected based on the series of the published LM and SEM pictures, especially when European populations are considered. Non-European populations such as those illustrated in Metzeltin et al. (2005, plate 205, figs 5–7) from Uruguay should be reinvestigated in more detail before making statements about their identity. It is possible that some of these can be identified as one (or more) of the newly described and separated species from the current paper, but pseudocryptic diversity cannot be ruled out.

The *N. sigma* valves illustrated in Reichardt (2018, plate 376: figs 1–12) most likely represent both *N. pararigida* (figs 1–9, 12) and *N. rigidula* (Figs 10–11). Several of the valves in Krammer and Lange-Bertalot (1988, plate 23, figs 8–9), on the other hand represents *N. rigidula*. Other populations are less clear. Bey and Ector (2013, p. 1075, figs 1–2) show two short valves with a distinct striation pattern that cannot be identified based on the species described in this paper.

The revision of the different populations allows for a better ecological characterisation of this complex. *Nitzschia sigma* is a marine to brackish species as shown by the lectotype material of

Courseulles (France) that is almost entirely dominated by *N. sigma* with lower frequencies of *Pleurosigma angulatum* (Quekett) W.Sm. and several *Tryblionella* species. On the other side of the spectrum, *N. rigidula* is situated, observed in a typical freshwater habitat, showing meso- to eutrophic conditions and with higher electrolyte contents as indicated by the species composition (Lange-Bertalot et al. 2017). Delogne sample 187, the lectotype sample for *N. rigidula*, is dominated by a handful of taxa such as *Navicula menisculus* Schumann, *Nitzschia dubia* W.Sm., *Gyrosigma acuminatum* (Kütz.) Rabenhorst, *Cymatopleura solea* (Bréb.) W.Sm., *Campylodiscus hibernicus* Ehrenb., *Nitzschia recta* Hantzsch and *N. rigidula*. Other frequent taxa include *Hippodonta capitata* (Ehrenb.) Lange-Bert. et al., *Frustulia vulgaris* (Thwaites) De Toni, *Stauroneis smithii* Grunow and *Surirella kuetzingii* var. *brebissonii* Krammer & Lange-Bert. The sample was taken, according to a handwritten label prepared by Delogne from a small brook near the red monastery (Van de Vijver, pers. obs.). In between, we can situate both *N. neorigida* and *N. pararigida*, as representatives of more brackish to even saline conditions. The type sample for *N. pararigida* is dominated by brackish taxa such as *Petrodictyon gemma* (Ehrenberg) D.G.Mann, *Navicula supergregaria* Lange-Bert. & U.Rumrich, *N. digitoradiata* (Greg.) Ralfs in Pritchard and *Fallacia pygmaea* (Kütz.) D.G.Mann & Stickle, all known to prefer salt- to brackish water conditions or very electrolyte-rich freshwaters (Lange-Bertalot et al. 2017), often found in the estuaries of larger rivers (Jahn and Kusber 2004). *Nitzschia neorigida* was observed in a very low frequency in a sample dominated by *Navicula salinarum* Grunow in Cleve & Grunow, *Ctenophora pulchella* (Ralfs ex Kütz.) D.M.Williams & Round and *Sellaphora saugerresii* (Desm.) C.E.Wetzel & D.G.Mann. According to Lange-Bertalot et al. (2017) this species composition is typically found in the intertidal zone of rivers, tide-influenced marshes and naturally salt-enriched freshwater habitats. The Solestadt (=natural brine city) Bad

Dürrenberg (located in central Germany), where the sample was taken, has a long history of saltmining, which explains the natural saline conditions of the type material and the resulting saline diatom composition.

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Author contributions

Horst Lange-Bertalot

Contribution: provision of literature, analysis of the observations, discussion of results, writing, revision and editing of the manuscript.

Bart Van de Vijver

Contribution: generation and analyses of LM and SEM materials, discussion of results, development, writing, revision and editing of the manuscript.

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Table 1. Comparison table of the morphometric data of all discussed *Nitzschia sigma*-complex species.

	Nitzschia sigma (Kützing) W.Sm.	*Nitzschia neorigida* sp. nov.	*Nitzschia pararigida* sp. nov.	*Nitzschia rigidula* (Grunow) stat. nov.
valve length (µm)	100-160	115-155	80-115	55-70
valve width (µm)	9-10	6.0-6.5	6.5-7.5	4.0-4.5
stria density (in 10 µm)	20-21	33-36	34-36	30-35
areola density (in 10 µm)	ca 20	30-35	ca 30	ca 40
number of fibulae (in 10 µm)	7-9	9-10	9-11	10-12

Table 2. List of alledged *N. sigma* records

Geographic location	**reference**
East Australia	Foged (1978, p. 110, pl. 46, figs 3 & 4)
North Island, New Zealand	Foged (1979, p. 19, pl. 43, fig. 16)
Öland, Sweden	Foged (1980, p. 73, pl. 44, fig. 2)
Uruguay	Metzeltin et al. (2005, figs 205: 5-7)
Okinawa Island, Japan	Nakai (1997, p. 265-269, fig. 52)
Alsea Bayn Oregon, USA	Sawai and Nagumo (2003, p. 36, fig. 49)
South West France	Germain (1981, p. 368, pl. 139, figs 1-6)
Brussels, Belgium	Krammer and Lange-Bertalot (1988, p. 32, pl. 23, figs 5-7)
South Africa	Taylor et al. (2007, pl. 149, figs 1-8)
East Canada	Lavoie et al. (2008, pl. 62, figs 1-4)
Swan River Estuary, West Australia	John (2012, p. 185, figs 133 J & K)
South-east France	Bey and Ector (2013, p. 1074-1075, figs 1, 2 (non 3,4)
South Germany	Reichardt (2018, p. 239, figs 376: 112, SEM fig. 391: 11)

Figure captions

Figure 1. *Nitzschia sigma* (Kütz.) W.Sm. LM and SEM images taken of the epitype material (BR-4764, Courseulles, Calvados, France, leg. de Brébisson sample #133). Figs 1A–1E. LM views of a size diminution series. Fig. 1F. SEM external view of an entire valve. Fig. 1G. SEM internal view of an entire valve. Fig. 1H. SEM external detail of the valve surface near the central area. Note the special structure with short grooves at the raphe keel and the irregular pattern of ridges. Fig. 1I. SEM internal detail of the fibulae and the areolae. Scale bars represent 10 µm except for Figs 1H–1I where scale bar = 1 µm.

Figure 2. *Nitzschia sigma* (Kütz.) W.Sm. SEM images taken of the epitype material (BR-4764, Courseulles, Calvados, France, leg. de Brébisson sample #133). Fig. 2A. SEM external view of the valve apex in oblique view showing the areolae on the mantle. Fig. 2B. SEM external view of the valve apex. Note the typical groove. Fig. 2C. SEM external view of the valve apex showing the terminal raphe fissure. Fig. 2D. SEM internal view of the valve apex showing the window-like fibulae and the helictoglossa. Scale bars represent 10 µm except for Fig. 2D where scale bar = 1 µm.

Figure 3. *Nitzschia neorigida* sp. nov. LM images taken of the holotype material (BR-4765, Bad Dürrenberg, Kötzschau bei Leipzig, Germany, Rabenhorst sample 2527, leg. Paul Gerhard Richter). LM views of a size diminution series. Scale bar represents 10 µm.

Figure 4. *Nitzschia neorigida* sp. nov. SEM images taken of the holotype material (BR-4765, Bad Dürrenberg, Kötzschau bei Leipzig, Germany, Rabenhorst sample 2527, leg. Paul

Gerhard Richter). Fig. 4A. SEM external view of an entire valve. Fig. 4B. SEM internal view of an entire valve. Fig. 4C. SEM external detail of the valve surface near the central area. Note the small, circular areolae near the raphe keel. Fig. 4D. SEM detail of the valve apex. Fig. 4E. SEM internal detail of the fibulae and the areolae. Fig. 4F. SEM internal detail of the valve apex showing the small helictoglossa. Scale bars represent 10 µm except for Figs 4C–4F where scale bar = 1 µm.

Figure 5. *Nitzschia pararigida* sp. nov. LM images taken of the holotype material (BR-4766, Harwich, England, UK, Walker Arnott sample 814). LM views of a size diminution series. Scale bar represents 10 µm.

Figure 6. *Nitzschia pararigida* sp. nov. SEM images taken of the holotype material (BR-4766, Harwich, England, UK, Walker Arnott sample 814). Fig. 6A. SEM external view of an entire valve. Fig. 6B. SEM external detail of the valve surface near the central area. Note the small, circular areolae near the raphe keel. Fig. 6C. SEM detail of the valve apex. Fig. 6D. SEM internal view of an entire valve. Fig. 6E. SEM internal detail of the fibulae and the areolae. Fig. 6F. SEM internal detail of the valve apex showing the small helictoglossa. Scale bars represent 10 µm for Figs 6A & 6D and 1 µm for the other figures.

Figure 7. *Nitzschia rigidula* (Grunow) Lange-Bert. & Van de Vijver, stat. nov. LM and images taken of the lectotype material (BR-4767, Rouge Cloître, Brussels, Belgium, Delogne sample 187). Figs 7A–7M. LM views of a size diminution series. Fig. 7N. SEM external view of an entire valve. Fig. 7O. SEM detail of the valve apex. Fig. 7P. SEM external detail

of the valve surface near the central area. Fig. 7Q. SEM internal detail of the fibulae and the areolae. Scale bars represent 10 µm, except for Figs 7O–7Q where scale bar = 1 µm.

Figure 8. Historic drawings and handwritten notes accompanying some of the historic samples. Fig. 8A. Cut-out remainder of the original Grunow drawing for *Nitzschia sigma* var. *rigida* (= *N. pararigida*) from Walker Arnott sample 814. Fig. 8B. Original drawing Grunow made for *Nitzschia sigma* var. *rigida* (= *N. pararigida*), used on Van Heurck (1881) plate 66, fig. 2. Fig. 8C. Cut-out remainder of the original Grunow drawing for *Nitzschia sigma* var. *rigida* (= *N. neorigida*) from the Dürrenberg sample. Fig. 8D. Original drawing Grunow made for *Nitzschia sigma* var. *rigida* (= *N. pararigida*), used on Van Heurck (1881) plate 66, fig. 5. Fig. 8E. Cut-out remainder of the original Grunow drawing for *Nitzschia rigidula* from Delogne sample 187. Fig. 8F. Original drawing Grunow made for *Nitzschia sigma* var. *rigida* (= *N. pararigida*), used on Van Heurck (1881) plate 66, fig. 8. Fig. 8G. Original handwritten notes from the Grunow accession book for sample 1698 collected by Paul Richter in Dürrenberg (= Rabenhorst 2527). Fig. 8H. Original handwritten notes for sample 814 from the Walker Arnott catalogue, conserved in the Van Heurck collection (BR, Belgium).