Observations on two taxa of the section Nitzschiae Lanceolatae (Bacillariophyceae): *Nitzschia blankaartensis* sp. nov. and *N. bulnheimiana*

by

Luc Denys

Department of Biology (Arctic Ecology, Limnology and Paleobiology Unit), Universitair Centrum Antwerpen (RUCA), Groenenborgerlaan 171, B-2020 Antwerpen, Belgium

and

Horst Lange-Bertalot

Botanisches lnstitut der J.W. Goethe-Universität, Siesmayerstrasse 70, D-60323 Frankfurt a. M., Deutschland

With 30 figures and 2 tables

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Abstract: A new freshwater diatom, *Nitzschia blankaartensis*, is described from shallow stagnant waters in Belgium and The Netherlands. The species belongs to a larger group of taxa with Iinear valves and more distantly placed central fibulae which often present identification problems. Although it can be distinguished from most other species in the light microscope by its valve shape and dimensions as well as structural details, it can be separated from *N. hantzschiana* only by the single rows of areolae at the keel, the lower number of transapical costae connected to each fibula and its ecological distribution. Differences with several similar species are discussed. As inferred from contemporary and palaeolimnological observations, the species seems to thrive best in polluted waters with a high content of electrolytes. Electron microscopical observations on *Nitzschia frustulum* var. *bulnheimiana* from Bad Nauheim (Germany) indicate that its raphe structure and areolation differ from that of *N. frustulum*, inclusive of *N. inconspicua*. Therefore, this taxon should again be considered as a separate species: *N. bulnheimiana*.

Introduction

Members of the section Nitzschiae Lanceolatae Grunow occur in nearly every diatom assemblage from eutrophic fresh or brackish water. The pollution tolerance of many of them extends over a distinct range, making them important for water quality assessment. Such applications, however, rely heavily on correct identificatio?. Unfortunately, the lanceolate *Nitzschia* taxa are among the most troublesome diatoms to recognize, especially in the light microscope (LM) where the finer stct1ral details remain invisible. The large number of taxa in this group, the rather hm1ted number of characteristics and the notoriously large variability of the Jatter are he main reasons for this. In spite of numerous recent additions to the enus and exhaustive revision (e.g. Lange-Bertalot 1977, 1980; Lange-Bertalot & Snnone 178; Lange-Bertalot & Krammer 1987; Krammer & Lange-Bertalot 1988), it is _hkely that many taxa still remain unnamed. In part, this may be because - in the hht of the previously mentioned morphological variability - most workers tend to attnbute_ Iess markedly differentiated forms to well-known and widely distributed taxa, especially if they have only little material at hand. Here a new species which may well prove to be of more wide-spread occurrence - *Nitzschia blankaartensis* Denys et Lange-Bertalot - is described from shallow stagnant waters in Belgium and The Netherlands. Furtherrnore, scanning electron microscope (SEM) observations on *N. frustulwn* var. *bulnheimiana* (Rabenh.) Grun. are discussed in relation to its taxonomie status.

Material and methods

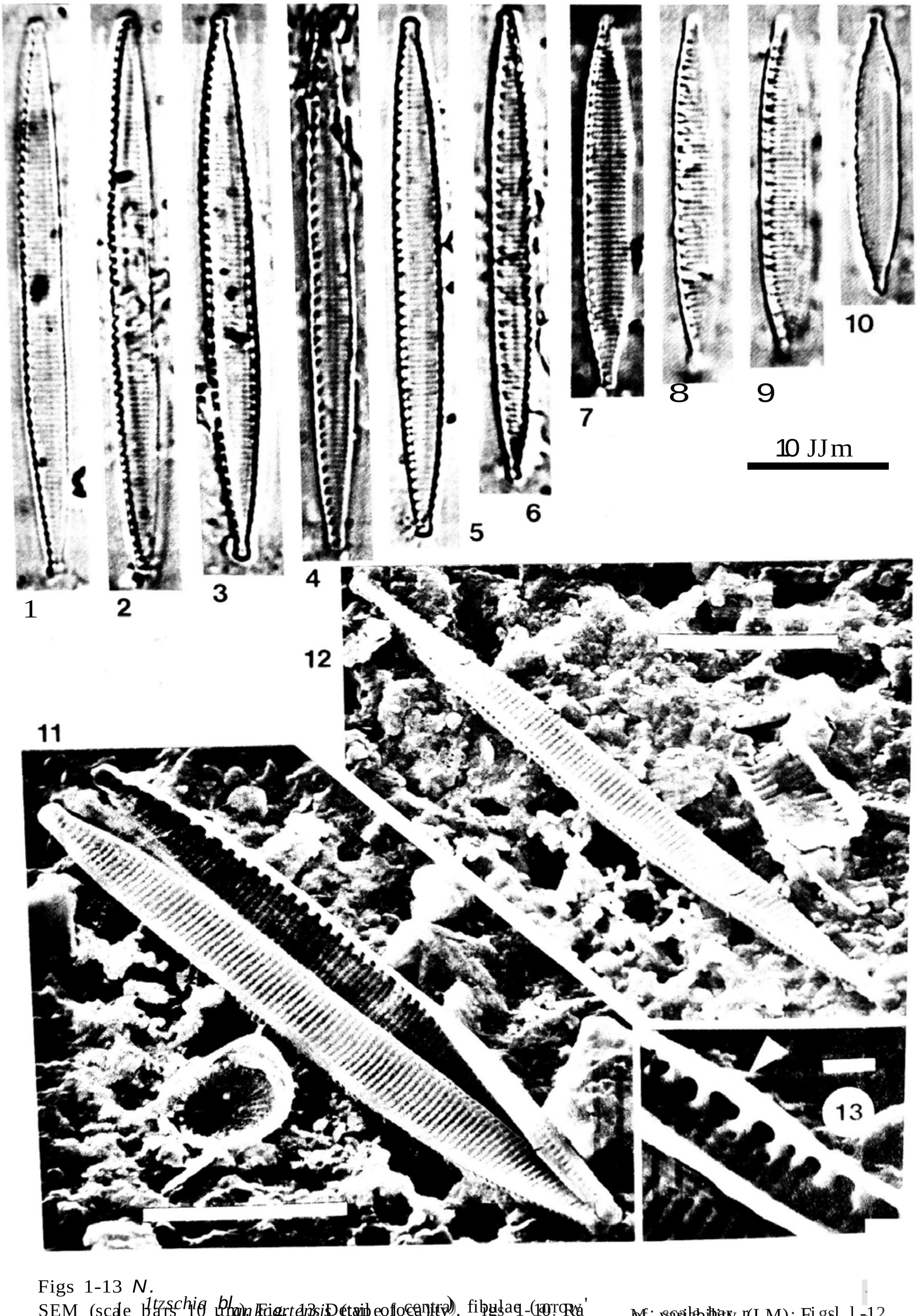
Diatom samples were examined from the Blankaart (Woumen, Belgium), the Fonteintjes (Zeebrugge, Belgium), the Oostvaardersplassen (Lelystad, The Netherlands; coli. H. van Dam) and the German saltworks Bad Nauheim and Bad Kreuznach. Old diatom samples from the Blankaart and the Fonteintjes were retrieved from herbarium macrophytes in the collections of the Nationale Plantentuin, Meise (BR) and the University of Gent (GENT). In the Blankaart, four sediment cores of 35 to 45 cm length were taken in 1993 with a hand-operated piston corer. These were sectioned into 1 cm slices using an extruder, and analyzed for diatom remains at 2 cm intervals. Material for diatom analysis was cleaned with concentrated hydrogen peroxyde. Solutes were removed by repeated washing with distilled water and settling in test tubes. Cleaned residue was mounted in Naphrax. A Philips 515 and a Hitachi S-500 were used for SEM analyses. Relative diatom abundances are based on counts of at least 500 valves.

Observations

Nitzschia blankaartensis Denys et Lange-Bertalot sp. nov. Figs 1-18, 19?

Valvae distin_cte_ lneares olum sub_apices atenuatae cuneiformes - marginibus paullo concavis in media quoad md1v1dua ma1ora et maxima - ap1c1busplerumque subcapitatis vel fere capitatis. Lonoitudo 9-45(55? µm, latitudo 3-_4(.6?) µ. Raphe eet in _carina comparate valde excentrica, simpliciter 1teupta 1n noulo centrhs 1consp1cua;praed1t f1buhs fere crass1s;.duae medi_ae fibulae plusminus ve d1stmcte d1stant1ores pos1tae mter se, (9.)11-13 m 10 µm. Omnes fibulae comunctae cum 1-2 costis transapicalibus._Striae transapi_ ales punctatae apparets (LM), (18?)21-25 in 10 µm. Species nova dtffert a *Nttzschza frustulum* (Kutz.) Grun. et *N. bulnhe1m1ana* (Rabenh.) H. L. Smith et alteris speciebus similaribus singulari complexione signorum typicorum.

Valves distinctly linear or - in the largest individuals - slightly concave in the middle narrowing cuneatly at the ends. Apices generlly subcapitate or capitate. Length 19-14(55) µm, w1dth_ 3-4(4.6?) µm. Raphe straight, situated in a strongly excentric keel and mterrupted m a small central nodule. Fibulae robust connected to 1-2 transapial cosae; 9) 11-13 in 10 µm, the two iddle ones placed further apart. Transap1cal stnae d1stmctly punctate, (18?)21-25 m 10 µm. Differs from *Nitzschia*



Figs 1-13 *N.* *1tzschia bl* ... *nkaartensis* (type locality). Figs 1-10. Range of variability (LM); scale bar represents 10 µm. Figs 11-12. SEM (scale bars 10 µm). Fig. 13. Detail of central fibulae (arrow) ...

frustulum (Kütz.) Grun" *N. b11/11heimia11a* (Rabenh.) H.L. Smith and other similar species by its complex of characters.

Holotype: slide Eh-B 91, Coll. Lange-Bertalot, Botanisches lnstitut, rankfu rt am Main. Isotypes: slide BM 98137 (Natural History Museu m, London); shde BR 1213 (Nationale Plantentuin, Meise).

Tvpe locality: Blankaart, Woumen (Belgium), sediment sample June 1993, core 111, i6-17 cm depth (leg. L. Denys, January 1997). In sediment and epiphyton.

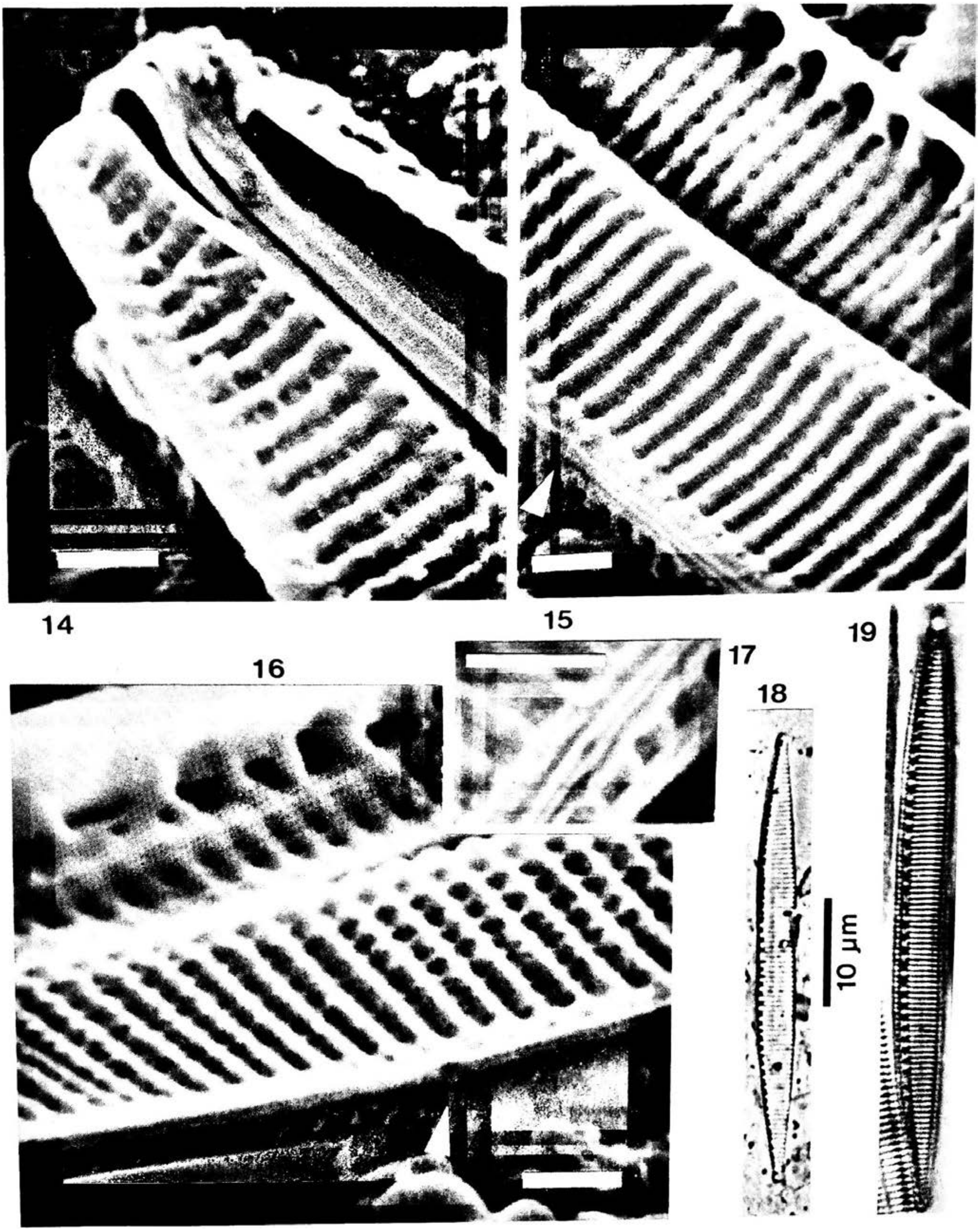
Valve morphology

The valves are linear for most of their length, narrowing gradually over a relatively short distance towards the subcapitate to capitate ends (Figs 1-12, 18). The largest specimens tend to have slightly concave margi ns in the middle. Their length varies from 19 to 45(55?) µm, whereas width ranges from 3.4 to 4(4.6?) µm. The fibulae are conspicuous in the LM, where they appear more or less quadratic. By focussing they can be seen to terminate into one or two interstriae, i.e. transapical ribs (Figs 6-9). With a density amounting to (9?)11-13 in 10 µm, the fibulae often occur at fairly irregular distances from each other. The two median ones are slightly (Fig. 3) to distinctly (Figs 2, 5) set apart from each other. There are (18?)21-25 striae in 10 µm, which generally can be resolved with the LM into rather coarse punctae. The striae are parallel to each other in the middle part of the valve, becoming slightly curved towards the apices.

In the SEM, the valve face appears to be slightly depressed longitudinally (Figs 11-12, 15-16). Valve face and mantle are at straight angles to each other. The raphe canal is situated on an elevated narrow keel at the edge between valve face and mantle (Figs 11-12, 14-16). It is flanked on both sides by a thin rim (Figs 15-17). These rims depart slightly from each other in the middle, forming a small rounded centra!nodule (Fig. 17). The proximal raphe endings remain invisible due to the small size of the central nodu1e. At high magnification, the transapical costae appear knotted (Figs 14, 16). They are linked by thin intercostal ribs at a level below that of the valve face. The single row of areolae between them continues up to a part of the raphe keel (Figs 15, 17). On the mantle a single corresponding row of large elongated areolae is present (Figs 11, 12, 15, 16). From the interior of the valve (Figs 11, 13, 15, 16), the fibulae can be seen to consist of firm tubular to quadrangular struts, reaching inwards over a small distance only and merging with one or two transapical costae. The areolae appear rounded and rather small from the inside.

Distribution and ecology

So far, *Nitzschia blankaartensis* has been found in Belgiu m at two sites, both in the Flemish coastal polders. It was most abundant in the Blankaart, a ca. 30 ha large, very shallow, strongl y polluted and hypertrophic freshwater reservoir created by former peat digging. The species was recorded here during the summer of 1993 in



Figs 14-17. *Nitzschia blankaartensis* (type Jocality; SEM; scale bars represent 1 pm). Fig. 14. Valve apex (note intercostae and intercostal ribs). Figs 15- 16. Centra! parts, external and intern al views. Arrows mark centra] nodule. Note fibulae merging with one or two transapical costae. Fig. 17. Detail of centra! nodule. Fig. 18. Specimen from Keersluisplas (LM). Fig. 19. *Nitzschia* aff. *blankaartensis* from Bad Kreuznach (LM).

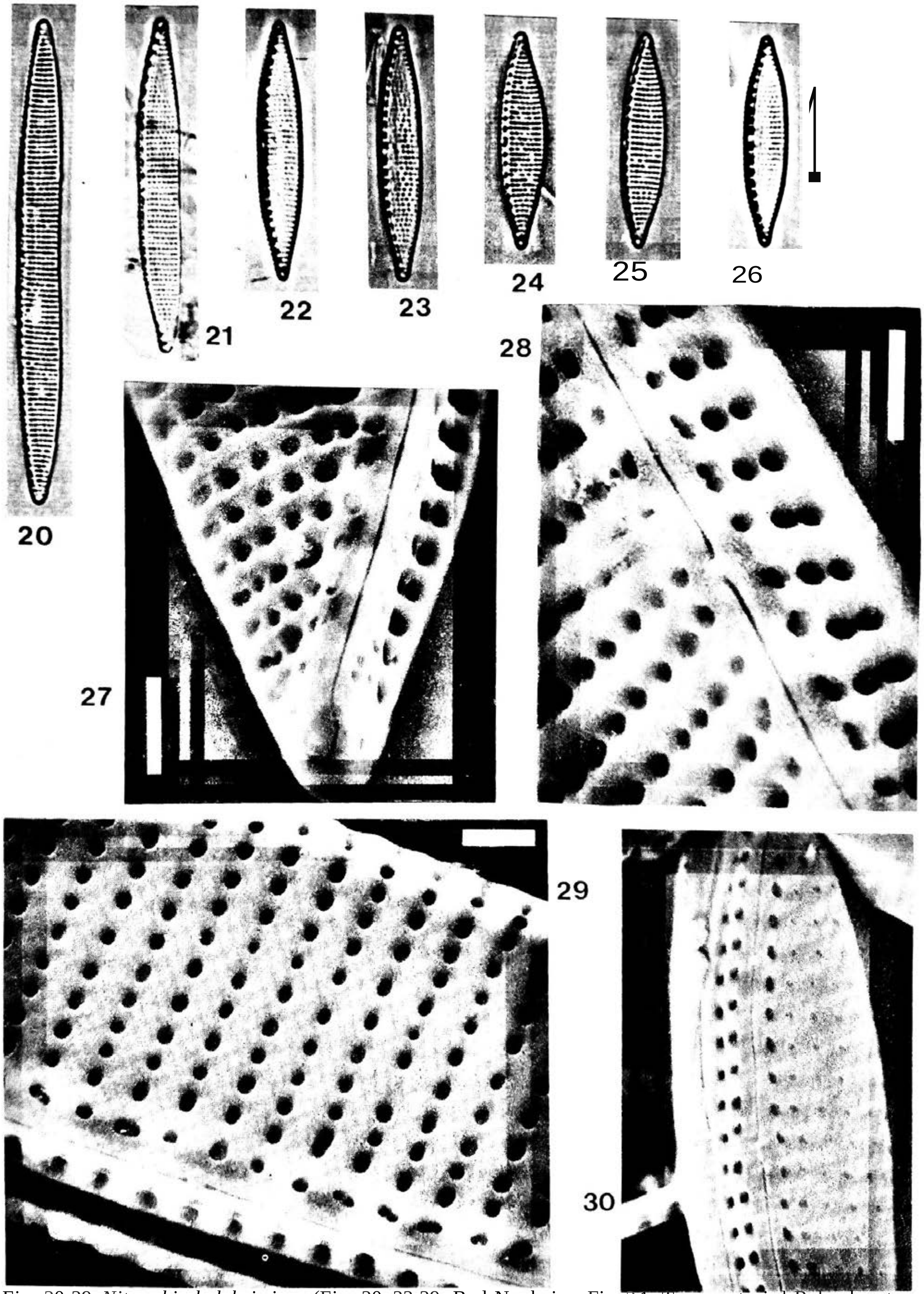
Table 1. Average and range (in brackets) of some water quality parameters at the outflow of the Blankaa rt reservoir for the years 1977-1978 and 1993 (data Vlaamse Maatschappij voor Watervoorziening).

	1977-1978	1993
Temperature (°C)	12.8 (3.2-24)	9.7 (3.1-18.8)
Oxygen saturation (%)	99.3 (21-180)	59.7 (34.3-95.6)
BOD (mg 0/1)	15.8 (2.4-40.8)	6.9 (3.6-10.2)
pH	7.9 (7.7-9.2)	7.6 (6.9-8.5)
Conductivity (µSlem)	919 (648-1475)	877 (736-1096)
COD (mgO/l)	72.7 (39.6-117)	56.8 (37.5-82.4)
Chl-a (µg/l)	144 (8.3-224.3)	70 (l.5-196)
P-tot (mg P/l)	6.9 (l.1-25.2)	2.8 (0.8-6.5)
Nitrate (mg N/l)	67.7 (4.4-171)	58.3 (0-115)
Ammonium (mg N/l)	2.6 (0.2-16.7)	0.8 (0-1.9)
Calcium (mg/l)	102 (82.4-153.6)	96 (71-110.7)
Magnesium (mg/l)	20.5 (13.1-27.2)	77.4 (16-110.7)
Chloride (mg/l)	120.7 (83-303)	97.1 (65-181)
Sulphate (mg/l)	175.4 (77-286)	104.7 (59-150)
Silica (mg/l)	15.2 (1.5-27.6)	13.4 (1.1-21)

each of 16 periphyton samples from *Phragmites* and *Typha* with a mean abundance of 0.7%. *Nitzschia blankaartensis* was absent in 7 samples from herbarium specimens of *Ceratophyllum*, *Hydrocharis, Nymphoides, Utricularia*, *Scirpus* and *Typha* that were collected in the reservoir between 1914 and 1923 (Denys in press). The analysis of the sediment cores, however, apparently indicates its presence throughout this century, although its abundance remained very low until the sixties, when pollution was already considerable (Louis & Beernaert 1969). It is a characteristic component of the younger sediment assemblages, reaching its maximum abundance - up to 10% - at levels corresponding to the seventies. In the sediment cores the rise of *Nitzschia blankaartensis* coincides with a very strong development of reputed indicators of increased electrolyte concentrations, strong eutrophication and organic pollution, e.g. *Cyclotella atomus* Hust., *C. meneghiniana* Kütz" *Nitzschia paleacea* Grun., *N. supralitorea* Lange-Bertalot, *Stephanodiscus hantzschii* Grun., *S. parvus* Stoermer et Häkansson and *Thalassiosira pseudonana* Hasle et Heimdal, leaving no doubt that the new species is favoured by such conditions as well. In the most recent sediments, *Nitzschia blankaartensis* attains an abundance of 1.7 to 4.3%. In Table I some results of water analyses on the outflow of the reservoir u ndertaken in 1977-1978 (corresponding to the period of maximum abu ndance) and in 1993 are shown, indicating (very) high loads of organic matter, n utrients, conservative ions and sulphate, as well as slightly alkaline conditions and a rather poor oxygen balance.

A single specimen was also recorded on herbariu m material of *Ceratophyllum submersum* collected in 1975 from a eutrophic pool (known as Struweelfonteintje) with a high electrolyte content (conductivity about 1500 *µSlem)* in the nature reserve The Fonteintjes, situated near the village of Blankenberge at the Belgian coast.

In the Netherlands, *Nitzschia blankaartensis* has been found in low numbers in the nature reserve Oostvaardersplassen (Zuid Flevoland), a series of large shallow waters



Figs 20-29. *Nitz.schia hulnheimiono* (Figs 20, 22-29. Bad Nauheim: Fig. 21. Type material Rabenhorst 1301). Figs 20-26. LM. Figs 27-29. SEM; scale bars represent 1 prn. Figs 27-29. Note dou bli ng of areolae on the keel and straight proxirnal raphe endings. Fig. 30. *N itzschia inconsp icua* (SEM; scale bar represents l µm). Note deflected proximal raphe endings and single rows of areolae.

that have formed after redamation of the polder in 1968. by va 1 Dam Menens t 1995) who recorded it under the name of *N. .fi11st11/11111.* The spectL'S was tmmli hl'l in the Keersluisplas which holds water that is alkaline. eutrophtc a1d VL'ry -1ch 11 electrolytes (ahout 1300 1S/cm). The accompanying assemblage cons1 tcd na111ly lt *Frailaria co11strue11s* f. *ve11ter* (Ehrenb.) Hust" *F. pimwta* Ehrenh" *N11-;.scl11a md11-baldii* Large-Bertalot and *N. p11111ila* Hust.

Finally, a very similar diatom (Fig. 19), albeit with a somewhat coarser structure (about 18 striae and 9 fibulae / 10 µm) occurs in the saltworks of Bad Kreuznad1, Germany. The identity of this form requires further examination hut conspeciti̇̈city with *N. bla11kaarte11sis* appears likely.

From the present observations it follows that *N. blcmkaartensis* occurs in more or less alkaline, (strongly) eutrophic waters with a high electrolyte content and tolcrates considerable organic pollution.

Nitzschia bulnheimiana (Rabenh.) H.L. Smith — Figs 20-29

Synonym: *Nit:schia fr11st11fum* var. *b11f11heimia11a* (Rabnhorst) Grunow in Van Heurck 1881.

Rabenhorst's material from Bad Sulza (Rabenhorst 1862, 1964, Nr. 1301) was extensiveJy illustrated by Lange-Bertalot (1977) and Krammer & Lange Bertalot (1988) and is onJy represented here by Fig. 21 to allow comparison. Specimens from the saltworks of Bad Nauheim appear to be identical to this taxon (Figs 20, 22-26). SEM observations on the Jatter show that there are notable differences with *Nitz.schia frustulum* or *N. inconspicua* Grun" which have an identical ultrastructure and presumabJy represent conspecific morphotypes (Lange-Bertalot & Simonsen 1978; Krammer & Lange-Bertalot 1988; Wendker, 1990). Firstly, in *N. b11!11hei111iana* the rows of areoJae are often doubled adjacent to the raphe, either on the mantle (Figs 27-28) or the valve face part of the keel (Fig. 29), which is never the case for *N. frustulum / N. inconspicua*. Secondly, the proximal raphe endings are straight in the former (Figs 28-29) and not bent towards the mantle as in the Jatter (Fig. 30).

Discussion

In Table II some valve characteristics of *N. blankaartensis* and of taxa with similar appearance in the LM are compared. Differences in valve width are noted with *Nitzschia radicula* Hust. and *N. incognita* Krasske which are always narrower. When larger populations are available, *N. blankaartensis* may be distinguished from these species also by its constantly linear outline, whereas only longer specimens of *N. .fossilis* Grun" *N. radicula, N. incognita, N. frustulum* and *N. bulnheimiana* tend to have linear valves. In combination with its generally more capitate valve endings, this feature nevertheless lends the new species a rather typical habitus. Some quantitative differences in the density of striae and fibulae also seem to exist. *Nitzschia radicula* and *N. incognita* have more striae in 10 µm, whereas *N. fossilis* and *N. bulnheimiana* have slightly less; moreover, the fibulae are further apart in *N. fossilis*.

Table Il. Valve characternti:s for *Nitzschia blankaartensis* (dimensions of specimens from Bad Kreuznach in brackets), *N. frustulum*, *N. bulnheimiana* and sOJre resembling Nitzschiae lanceolatae with distant rredian fibulae.

	N. blankaartensis	*N. hantzschiana*	*N. fossilis*	*N. radicula*	*N. incognita*	*N. frustulum*	*N. bulnheimiana*
Jength (µm)	19-45(55?)	8-50	30-85	33->70	20-70	3-45	12-60
width (µm)	3.4-4(4.6?)	3-4(5)	3.5-5	2.5-3	2-3	2-4.5	4-4.7
valve outline	linear, tendency to concave margins	lanceolate to linear	lanceolate to linear (rapidly narrowing)	narrowly lanceolate, tending to linear	narrowly lanceolate, tending to linear	lanceolate to linear	lanceolate to linear
valve endings	(sub)capitate	slightly capitate to subrostrate	slightly cpitate to subrostrate	slightly capitate to subrostrate	slightly capitate	rmstly subrostrate, rarely subcapitate	subrostrate
fibulae density (in 10 µm)	(9?)11-13	7-12.5	7-9	10-13	I0-15	12-16	8-13
striae density (in 10 µm)	(I8?)21-25	20-26	18-21	28-30	28-30	22-30	19-22
rows of aeroles at keel	single	regularly doubled	?	?	single	single	regularly doubled
transapical costae/fibula	1-2	2-3	2-3	?	2	2-3	2-3
external centra! raphe endings	invisible	invisible	?	?	?	visible	visible

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Care should however be taken in interpreting these LM characters seprately, b ause in this group of diatoms each one of them is renowned for high variatI?n.coff1c1ents (e.g. Wendker & Geissler 1988; Wendker 1990). In the LM, he d1stmct10n from *Nitzschia hantzschiana* Rabenh. is probably the most problematlc, as the gross morphology of larger specimens of this species is almost identical to that of *N. blankaartensis.* A further set of diagnostic criteria is provided by ultrastruct ral details conceming the areolation pattern near the raphe, the number of transap1cal costae associated to each of the fibula, and whether the fibulae merge more or less gradually with the transapical costae or not. At this level, the small central nodule, which hides the external central raphe endings, is one the most distinctive featu res of *N. b/ankaartensis.* A similar structure is - again - only observed in *N. hantzschiana.* In fact, the SEM reveals only few diagnostic criteria with regard to the Jatter species. In *N. hantzschiana,* the rows of areolae are regularly doubled near the keel (Germain 1981; Krammer & Lange-Bertalot 1988; personal commu nication P. Hamilton 1997), whereas this is never the case in *N. blankaartensis.* Also, as in *N.fossilis, N. frustulum* and *N. bulnheimiana,* most of the fibulae are connected to two or three transapical ribs in *N. hantzschiana,* whereas they merge with only one or two costae in *N. blankaartensis.* With some effort, this can be made out in the LM, where the fibulae of *N. hantzschiana* appear somewhat more sturdy, as well. Also the fibulae tend to be slightly further apart in this species than in *N. blankaartensis.* Since the morphology of the keel and the associated areolation pattern appear to be particularly valuable for taxonomie differentiation in this group (e.g. Lange-Bertalot & Krammer 1987; Krammer & Lange-Bertalot 1988), these few but constant differences are presently considered sufficient for a distinction at the species level. Such a separation is supported entirely by the strong divergence in the autecology of *N. blankaartensis* and its morphologically closest relative, *N. hantzschiana.* In contrast to the new species, the Jatter occurs especially in mesotrophic and oligosaprobic waters with a rather low electrolyte content, a slightly acid to circumneutral pH, and high oxygen saturation (Germain 1981; Krammer & Lange-Bertalot 1988; van Dam et al. 1994). It is quite unlikely that it could even support the conditions prevailing in the Blankaart reservoir, let alone attain a sizeable population here. Confusion may furthermore arise with *N. diversa* Hust., which is also extremely similar to *N. blankaartensis* in its general appearance, but in contrast regularly presents equidistant median fibulae. Finally, *N. gesssneri* Hust. differs more markedly by regular double rows of areolae at the keel and fibulae with gradually tapering bases, next to a higher striae density.

In view of its considerable pollution tolerance it seems likely that *N. blankaartensis* may prove to be of more widespread occurrence and that it has been confused in the past with "popular" taxa, such as *N.frustulum* or *N. hantzschiana.* In cases where the latter species plays a key role in water quality assessment a check of its identity by means of EM appears recommendable.

N. frustulum var. *bulnheimiana* (Rabenh.) Grun., originally described as *Homoeocladia bulnheimiana* Rabenhorst 1862 (Rabenhorst 1861-1879), was synonymized with *Nitzschia frustulum* by Lange-Bertalot & Simonsen (1978). Krammer & Lange-Bertalot (1988) considered it again as a variety of this species, specifying that it differs from the nominate variety by a generally larger size, coarser structure and the formation of mucilaginous tubes. Table II allows a comparison of some valve characteristics of this taxon with those of *N. frustulum.* Both are distinct not only in life form (tube-forming versus free-living) and quantitative morphological features (mainly fibulae and striae density), but also in ultrastructural details. The latter include the disposition of areolae at the keel and the shape of the central raphe ends. In agreement with Reimer (1966), who considered only the LM aspect of both taxa (the resolution of the puncta), it is therefore clearly more appropriate to reinstate a distinction at the species level. According to the nomenclatural rules, *Nitzschia bulnheimiana* (Rabenh.) H.L. Smith 1874, introduced by Smith's (1874-1879, no. 689) transfer of the taxon to *Nitzschia,* is the correct name to use in this case. *N. bulnheimiana* appears to have a considerably narrower ecological range than *N. frustulum* and occurs predominantly, or perhaps even exclusively, in saline springs and waters in contact with such mineral sources. *N. frustulum,* however, is widely distributed from the marine littoral to various inland waters, and not restricted to sites with higher electric conductivity. When both occur sympatrically, such as in the catchment area of the River Werra, Germany (observations L.-B.), a clear morphological separation remains. The complete absence of intermediate forms in such cases further corroborates their separation as species.

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