External Quality Assessment Trials Phytoplankton

First German Benthic Diatom Intercalibration Exercise

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1. Introduction

Diatoms occur in almost all aquatic habitats, either attached as part of the benthos or as plankton in the open water (Round et al. 1990). For several decades the suitability of this species-rich algal group to indicate water quality is well known (Battarbee et al. 2001). Next to macrophytes diatoms play an important role for implementing the European Water Framework Directive on a national level. In Germany diatoms are part of the corresponding water quality evaluation called PHYLIB (Schaumburg et al. 2006, 2007, 2011a, 2011b).

The part diatoms of the PHYLIB-method include the sampling of several substrates at the margin of lakes or in rivers and the analysis of the benthic diatom assemblages within these samples. The responsible German authorities usually contract out the sampling and analysis (identification of the diatom assemblages) of the samples to private firms or consulting agencies. The ecological evaluation of each water body is based on the identified species assemblage by calculating various indices that finally evaluate the ecological state of a water body (Schaumburg et al. 2011a).

The use of diatoms for water monitoring usually requires the identification on a species level or higher resolution. Also, diatoms are a very species-rich taxonomic group. Thus, in practise analyst-induced mistakes of identification are a substantial source of the variability of diatom results (Kelly & Lewis 1996, Prygiel et al. 2002, Besse-Lototskaya et al. 2006, Kahlert et al. 2009). Consequently, these analyst-differences may lead to distinct differences in the evaluation of water bodies based on diatoms (Stevenson et al. 2010). Thus, to asses and use diatom-based evaluations in praxis it is essential to know and to minimize the analyst-dependent variability of the primary diatom results (Kelly & Lewis 1996).

Diatom samples should only be analysed by trained specialists and it is known that there are analyst-dependent differences in quality (Besse-Lototskaya et al. 2006, Kahlert et al. 2008, Stevenson et al. 2010). Thus, it is necessary to ensure quality assurance in relation to both diatom results and diatom analysts. Consequently, several measures are recommended, such as intercalibration exercises, workshops, exchange of samples and a permanent communication among diatom analysts (Kelly & Lewis 1996, Prygiel et al. 2002, Kahlert et al. 2009).

Currently, there is no uniform EU-wide standard for quality assurance for diatom analysis used for monitoring, despite a first European Workshop on Diatom Taxonomy in 2009 and one European intercalibration exercise conducted in 2009 (Kahlert et al. 2012). Similarly, some European countries (e.g. Great Britain, Sweden, Hungary and Netherlands)

conduct various measures such as intercalibration exercises and workshops (e.g. Kahlert et al. 2012, Kelly 2013).

In Germany authorities explicitly point out the importance of quality assurance in relation to implementing the European Water Framework Directive (WFD) (Blondzik et al. 2006) and diatoms play an important role in the German procedure for analysing water bodies according to the WFD (Schaumburg et al. 2006, 2007, 2011a). Still, there are no uniform and only sporadic measures for quality assurance in Germany. However, Blondzik et al. (2006) write:

„Assessing the quality of data must not be neglected either in the chemical or biological domain. Data are only assessed as reliable, if they are verified by appropriate quality assurance. This also applies to the monitoring of water bodies according to the EU-WFD (2000), which requires the employment of cost-efficient measures to attain the postulated environmental aim of a 'good' ecological status of the water bodies.“

This "German Benthic Diatom Intercalibration Exercise 2011" is the first German-wide attempt to asses the qualitative variability of diatom results that are used for water quality examinations in praxis based on the counts of different laboratories, i.e. to assess both the quality of the taxonomic analyses of prepared diatom slides and the precision and commensurability of the counted results. Thus, the aim of this intercalibration exercise is not only the evaluation of the quality of each laboratory, but also the assessment of the possible qualitative range in praxis, including the ecological evaluation. Accordingly, the aim of this intercalibration exercise is the taxonomic standardisation of benthic diatoms to improve or to provide a basis for improving the evaluation precision, i.e. the application of the German implementation of the EU-Water Framework Directive (WFD) for running waters (Schaumburg et al. 2006, 2012) and lakes (Schaumburg et al. 2007, 2011 a, b).

Based on the results of the intercalibration and the associated workshop this exercise aims to propose how to avoid certain problems or how to treat certain problems for diatomists, contractors, taxonomists and for the German procedure of water quality assessment.

During and shortly after this intercalibration exercise the German PHYLIB-instructions were amended (Schaumburg et al. 2011a, b and 2012). Important for this intercalibration exercise was the change in mandatory identification literature, which changed from old taxonomy mainly based on Krammer & Lange-Bertalot (1986-2004) to the new taxonomy mainly based on Hofmann et al. (2011). Thus, this exercise was already based on the new taxonomy and the laboratories were assessed according to the new taxonomy. However, the

auditors and some participants additionally counted the samples according to the old taxonomy (this was optional for the laboratories and did not affect the laboratory assessments). A comparison of the counting results according to the new and old taxonomy also aimed to possibly improve the evaluation precision of PHYLIB.

Thirty-seven different laboratories participated in this intercalibration exercise from Germany and other European countries (Belgium, Czech Republic, France, Ireland, Italy, Netherlands, Slovakia, Spain and Sweden). Each laboratory received and analysed four diatom slides. Additionally, three internationally renowned diatomists also analysed slides from the four different samples and served as auditors. The analyses of the slides followed the PHYLIB-instructions (Schaumburg et al. 2006a-2012), where explicit directions are given about the necessary literature for diatom identification.

Authors of this study that either chose the water bodies, took and prepared the samples, send the slides to the participants or that evaluated the results did not participate in the intercalibration exercise. They are employees of Rostock University and of Swedish University of Agricultural Sciences, respectively, and are in no way competing with the participating laboratories. The statistical expert of this study does not know, which lab-code refers to which laboratory. The authors of this study are aware and took into account that it is very unlikely that two countings of two different slides (of the same sample) will lead to identical results (Adler et al. 2010).

2. Material and methods

The intercalibration exercise is based on four benthic samples:

- **D 11**: Lake Krossinsee, Northern Germany, Lowland Lake-Type D 11, calcareous, polymictic
- **D 1.1**: Lake Geneva, Switzerland, Alps/Alpine foothills Lake-Type D 1.1, calcareous, dimictic
- **D 12**: River Klepelshagener Bach, Northern Germany, Lowland River-Type D 12, calcareous
- **D 2**: River Drau, Austria, Alps/Alpine foothills River-Type D 2, siliceous

Samples were taken and prepared according to the German instructions for implementing the European Water Framework Directive (Schaumburg et al. 2006a, b, 2007a, b, 2011). Periphyton was scratched from several stones per site into a 500 ml bottle and alcohol was added. Diatom samples were oxidised and prepared with HCl, H_2O_2, H_2SO_4, $KMnO_4$ and $C_2H_2O_4$ following modified Kalbe & Werner (1974). The slurries of each sample were filled into one 4 ml bottle. This same bottle per sample was used for making the diatom slides for all participants and auditors. Several drops of diatom slurry were dried overnight on cover slips in one round for all slides per sample. The slurry was shaken repeatedly during the procedure. Dried slurries containing the diatoms were then mounted with Naphrax® (refraction index 1.73) onto slides. Each slide from the four samples contained two cover slips, each with a different diatom density. They were prepared by one person (Rostock University) and then send to the participants. Participants could ask for additional slides, if, for example, the diatom density was too low or too high. The slides were labelled with the diatom water body type, i.e. participants knew the region and lake or river-type. However, participants did not know which lake or river exactly they were counting during the intercalibration exercise. The slides for the auditors were taken from the same batch of prepared slides as for the participants.

The auditors are diatom-specialists with more than 20 years of experience of analysing diatom slides. All samples were counted three times on three different slides by the following auditors: (1) Dr. Gabriele Hofmann: three lake samples (one sample twice, i.e. two slides from one sample) and two running water samples, (2) Dr. Thomas Hübener: three lake samples (one sample twice) and two running water samples, (3) Dr. Peter Pfister: two running water samples. Thus, the lake samples were counted by two auditors and the running water samples by three auditors. Accordingly, each lake sample was counted twice by one auditor using two prepared slides to enable an assessment of the variability among slides per sample.

Participants and auditors were instructed to base their counts on the most current (slightly revised) instructions from 2011, i.e. on Schaumburg et al. (2011) (lakes) and Schaumburg et al. (2006a, b) (rivers) and using the instructions in the letter accompanying the samples according to the new and upcoming Schaumburg et al. (Schranz, personal communication) (running waters). The most recent instructions were only available in German. Thus, participants were instructed to mandatorily use the instructions given in Schaumburg et al. (2006a, b) and (2007a, b) (both in English) and additionally the new and relevant changes of instructions given in Schaumburg et al. (2011) (Schranz pers. comm), which were translated and send to the participants in the letter accompanying the samples. As written in the instructions, 400 (running waters) or 500 (lakes) diatom-objects were supposed to be counted per sample. Each frustule within a chain of girdle bands is one object (Hofmann, personal communication). Valves at the end of the chains are also one object (Hofmann, personal communication).

For all samples, the new identification literature was mandatory and the list of all planktonic, pennate diatoms that must be excluded during the count. Accordingly, the standard identification literature was Hofmann et al. (2011). Additionally, identification had to be based on the supplementary books Krammer & Lange-Bertalot (1986-2004), Lange-Bertalot (1993, 2001), Lange-Bertalot & Moser (1994), Lange-Bertalot & Metzeltin (1996), Krammer (1997a, 1997b, 2000, 2002, 2003), Reichardt (1999), Witkowski et al. (2000) and Levkov (2009). All participants and auditors received detailed counting instructions and a list of the mandatory identification literature with a letter that accompanied the slides.

Participants and auditors entered the counting results via the EQAT-webpage (www.planktonforum.eu) into the EQAT entry mask using the laboratory code that was given to each person with the slides. The analyses and presentation of the results are solely based on the laboratory codes. Participants and auditors had to enter the number of identified objects together with the associated most current German DV-number (Mauch et al. 2003, version 2011), a list that was available on the EQAT-webpage.

In addition to the counting results further (mandatory) information had to be entered, which may be relevant for the quality of a count, i.e. used magnification, lens (type, aperture), optical illumination technique, number of years of experience with counting diatoms, number of samples counted per year, regional origin of most samples counted so far. A mixed-effect model (Pinheiro & Bates 2000) was used to assess, if lens type, experience, optical illumination technique, regional origin of samples counted or number of samples counted per

year had an influence on the Bray-Curtis dissimilarity distance (see below) to the auditors. The distance to the auditors was the dependent variable and the prompted facts the independent variable (each fact was one factor). The laboratory code was the random variable.

First, the relative abundances were calculated from the number of counted diatom objects. The number of identified taxa and the number of taxa determined with uncertainty (spec., cf., aff., pennates) were identified to evaluate all data (participants and auditors) and for assessing the counting results of the participants (part taxonomy). Then, the similarity of counting results per sample were determined using both Bray-Curtis dissimilarity distance and a multivariate graph (Detrendet Correspondence Analysis = DCA). These are two independent methods that were used to confirm and ensure the assessment of the counting results of each laboratory. For both methods problematic taxa were pooled into groups (see below) and the data were square root transformed to downweight rare species (function „downweight“ in the package "vegan"; Oksanen et al. 2012), as the identification of rare taxa (relative abundance < 1 %) strongly depends on chance (Adler et al. 2010).

Based on the relative abundance data we calculated the average, standard deviation and corresponding 95 %-confidence interval of the Bray-Curtis dissimilarity distances of the three auditors per sample, representing the similarity of the results of the auditors. If the Bray-Curtis distance of a laboratory is inside of this confidence interval around all three auditors, then the result of the laboratory is as similar to the auditors as the auditors among each other. A laboratory was marked with a green circle (DCA), if the Bray-Curtis distance of the laboratory was outside of the standard deviation of two auditors and with a red circle, if the laboratory was outside the standard deviation of all three auditors. Subsequently, we visually examined, if the Bray-Curtis distances (coloured circles) correspond to the results of the DCA figure (if appropriate also in the third dimension) (for results see Chapter 3.1).

Please note that the calculation of confidence intervals with only three samples is only a rough estimate, as the sample size is very small. Some diatom identifications and corresponding relative abundances differed distinctly among auditors, resulting in rather large confidence intervals. Thus, the evaluation of each laboratory result based on these confidence intervals was rather advantageous for the laboratories. Additionally, the advantageous or optimistic evaluation of the counting results was enhanced, as only laboratory results were classified as "unsuccessful" (on the certificates), if the Bray-Curtis distances were outside the confidence intervals of all three auditors.

Some taxa were pooled to groups for the statistically evaluable samples D 11, D 12 and D 2, when statistically comparing the results of the participants with the auditor results (Bray-Curtis and DCA), i.e. when evaluating the laboratories. These taxa were either taxonomically not distinctly different and consequently can often not be unambiguously distinguished during a routine count using the light microscope or taxa that can be easily distinguished, but where the identification literature lists different, partly contradictory names for the same taxon or where the species description does not match the corresponding pictures in the literature. Thus, overall most grouped taxa were named differently among auditors or were identified to a different level of taxonomic resolution by the auditors. These groups were created to ensure a realistic evaluation of the participating laboratories, i.e. to avoid an unfavourable evaluation due to above taxonomic problems. Thus, only serious taxa confusion or misidentifications were affecting the calculation of the Bray-Curtis distances. The pooled taxa and groups are listed and discussed in detail in Chapters 3.1 and 3.2.

In contrast to the DCA and Bray-Curtis evaluations, taxa were not pooled to groups for the detailed discussion of taxonomic problems, i.e. when presenting the results of each laboratory compared to each auditor (Chapter 3.2) to also identify and present these problems. Thus, each laboratory can see the variation among laboratories but also among auditors for each taxonomic group. Similarly, taxa were *not* pooled to groups calculating the ecological status classes based on the diatom assemblage according to the PHYLIB-methods for lakes and running waters (see below). Instead, the data was used in the way they were delivered by each laboratory and auditor. Thus, the effect of the different counts of the same sample on the results of the PHYLIB water assessment could be examined.

Results from diatom counts are only well comparable, if the results are based on the same counting methods and the same identification literature next to a qualitative comparable taxonomic resolution. Thus, participating laboratories were also evaluated based on deviations from the given official German instruction protocol (Schaumburg et al. 2006a, b, 2011) (as far as possible) as well as on the counting results of the three statistically evaluable samples. Both results were noted on the certificate. The evaluation to what extent a laboratory followed the protocol was solely based on the following accountable facts; (1) number of counted objects were distinctly too low, (2) slides were not scanned for rare taxa in the lake samples and (3) taxa names given did not corresponded to the names given in the requested literature, i.e. the names were either outdated or wrong due to a new taxon concept. Laboratories could have either no (0), minor (1) or substantial deviations (2) from the instruction protocol. Minor

deviations were noted, if the lake samples were not scanned for rare taxa as written in the German instruction protocol or if the number of counted objects were distinctly lower than ask for or if planktonic diatoms were included in the count. Substantial deviations were noted, if at least two or more of the following criteria pertain: if the lake samples were not scanned for rare taxa, if distinctly too few objects were counted, if planktonic diatoms were included in the count and if the taxa-names did not corresponded to the ones given in the mandatory identification literature.

The ecological status of each sample was assessed based on the diatom assemblages using the PHYLIB-Software 4.1 (for results see Chapter 3.3). For each lake sample the Diatom-Index ($DI_{Seen/Lakes}$) was calculated to determine the ecological status class. The Diatom-Index is composed of the module „Trophic-Index“ (TI) and the module „Quotient of Reference Species“ (RAQ) (Schaumburg et al. 2011). The TI accounts for the relative abundances of each taxon and their a priori identified total phosphorus-optima, while the RAQ only uses presence-absence data, which is weighted with an ecological class, i.e. if a taxon represents a type specific reference species or a type specific degradation indicator (or none) (Schaumburg et al. 2007). For each running water sample the $DIÖZ_{Fließgewässer}$ (Diatom Indicted Ecological $Status_{running\ waters}$) was calculated to determine the ecological status class. The $DIÖZ_{Fließgewässer}$ is composed of the modules „Species Composition and Abundance“ and „Trophic Index“ (Schaumburg et al. 2006a, b).

Diatom pictures for this report were taken with the camera ProgRes® SpeedXTcore3 (Jenoptik) attached to an Axioplan light microscope (Zeiss) (differential interference contrast (DIC)), 100x oil-immersion objective Plan-Apochromat, aperture 1.4) at an overall magnification of 1000x. Valves were measured using the software analySIS® (Soft Imaging System GmbH).

3. Results and discussion

3.1 Species assemblages, counting results and evaluations of the laboratories

3.1.1 Lake Krossinsee, North German Lowlands, sample D 11

The sample D 11 from Lake Krossinsee (North German Lowlands) was dominated by *Achnanthidium minutissimum* var. *minutissimum* (Kützing) Czarnecki, *Cocconeis placentula* var. *lineata* (Ehrenberg) van Heurck and *Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot (Tab. 1). Additionally, *Amphora indistincta* Levkov, *Amphora pediculus* (Kützing) Grunow, *Cocconeis pseudolineata* (Geitler) Lange-Bertalot, *Gomphonema pumilum* (Grunow) Reichardt & Lange-Bertalot, *Navicula antonii* Lange-Bertalot, *Navicula cryptotenella* Lange-Bertalot and *Nitzschia fonticola* (Grunow) Grunow were relatively abundant according to the results of the auditors (Tab. 3.1.1-1).

Tab. 1: Relative abundances, mean and standard deviation (stdev) (%) of the dominant and abundant diatom taxa in sample D 11 (North German lowland Lake Krossinsee) based on the results of the auditors (L39-42). For more information see text.

Taxon	L 39	L 41	L 42	mean	stdev
A. minutissimum var. *minutissimum*	15.4	22.6	9.4	15.8	6.6
C. placentula var. *lineata*	2.2	25.6	23.5	17.1	12.9
Rhoicosphenia abbreviata	19.3	11.6	19.8	16.9	4.6
Amphora indistincta	0.0	7.3	3.8	3.7	3.6
Amphora pediculus	7.8	1.2	0.9	3.3	3.9
Cocconeis pseudolineata	0.7	1.4	3.3	1.8	1.3
Gomphonema pumilum	5.2	0.4	1.3	2.3	2.6
Navicula antonii	3.9	0.0	0.8	1.6	2.1
Navicula cryptotenella	5.9	10.0	8.8	8.3	2.1
Nitzschia fonticola	3.2	1.8	0.9	2.0	1.1

Several standard deviations of the above mentioned taxa abundances that are based on the results of the auditors are relatively high (Tab. 1), because they are only based on three values (see Chapter 2). Additionally, taxonomic uncertainties and the inherent variance among the slides increased the standard deviations. This inherent variance stems from the method, as

each participant and auditor received their own prepared slide (i.e. not the same slide was counted), despite using the same method for each slide and despite using the same slurry for each slide (of one sample). This variance is part of all results and directly leads to small variations among counting results and thus contributes a certain amount to the standard deviation among auditors. Thus, this variance is accounted for, as each sample was counted three times by the auditors using three slides. For example, in sample D 11 *Rhoicosphenia abbreviata* is a good species to indicate the standard deviation due to the variation among slides, as there are no taxonomic problems with this species. All three auditors identified *R. abbreviata* in somewhat similar abundances with an average of 16.9 % and a standard deviation of 4.6 %.

Some amount of the sometimes high standard deviations among auditors in Tab. 1 was due to taxonomic problems, as the auditors named some taxa differently to each other, mainly taxonomically difficult diatoms. Thus, this result strongly indicates that the most recent taxonomy of diatoms is not entirely resolved in some cases or partly very complicated, potentially leading to deviating counting results even among very experienced diatomists. For example, two auditors identified mainly *Cocconeis placentula* var. *lineata* in sample D 11, while another auditor identified most of these objects as *C. placentula* var. *euglypta* (Ehrenberg) Grunow (for more details see Chapter 3.2.3). Therefore and for other reasons (see Chapter 2) some taxa were grouped for both the results of the auditors and the participating laboratories, prior to comparing the results of the participants with the results of the auditors (groupings see below).

Some taxa were only identified by two auditors, but not by the third auditor (e.g. *Navicula antonii* and *Amphora indistincta* in sample D 11, for more details see Chapters 3.2.2 and 3.2.12), also leading to a relatively high standard deviation. Overall, these results indicate that universally valid statements can not be generated from the averages and standard deviations of the auditors, but that they need to be looked at in detail.

As mentioned above, some taxa were pooled to groups prior to calculating the Bray-Curtis-Distances (Tab. 2) and presenting the results in the DCA (Fig. 1) (see also Chapter 2). For sample D 11 the following groups were used: **Group 1** (*Cocconeis placentula, C. placentula* var. *euglypta, C. placentula* var. *lineata*), **Group 2** (*Encyonema silesiacum, E. silesiacum* var. *silesiacum*), **Group 3** (*Fragilaria brevistriata*, *F. brevistriata* var. *brevistriata*, *F. brevistriata* var. cf. *brevistriata*, *Staurosira brevistriata*), **Group 4** (*Fragilaria vaucheriae, F. capucina* var. *vaucheriae*), **Group 5** (*Gomphonema olivaceum, G. olivaceum* var. *olivaceum*), **Group 6**

(*Gomphonema pumilum* var. *pumilum*, *G. pumilum* var. *elegans*, *G. pumilum* var. *rigidum*, *G. pumilum* var. cf. *rigidum*), **Group 7** (*Karayevia clevei*, *K. clevei* var. *clevei*, *K. clevei* var. cf. *clevei*, *K. clevei* var. *rostrata*), **Group 8** (*Navicula radiosa*, *N. radiosa* var. *radiosa*), **Group 9** (*Nitzschia dissipata*, *N. dissipata* ssp. *dissipata*, *N. dissipata* var. *media*), **Group 10** (*Nitzschia palea*, *N palea* var. *palea*, *N. palea* var. *debilis*,*N. palea* var. *tenuirostris*) and **Group 11** (pennales, cf. pennales).

The most important taxonomic problems revealed in sample D 11 are discussed in detail in Chapter 3.2. Participants and to a distinctly lesser extend auditors (without any significant statistical effect on the whole counting results of the auditors) had major taxonomic problems when identifying *Achnanthidium minutissimum* var. *minutissimum* (Chapter 3.2.1), small *Amphora*-species (*A. pediculus* and *A. indistincta*) (Chapter 3.2.2), the varieties *euglypta* and *lineata* of *Cocconeis placentula* and similar species (*Cocconeis placentula* and *C. pseudolineata*) (Chapter 3.2.3) and *Navicula cryptotenella* (Chapter 3.2.12) in sample D 11. Additionally, significant taxonomic problems occurred for *Eolimna minima* (Grunow) Lange-Bertalot & Schiller (Chapter 3.2.6), *Gomphonema pumilum* (Chapter 3.2.10), *Navicula antonii* (Chapter 3.2.12), *Navicula reichardtiana* Lange-Bertalot (Chapter 3.2.14) and *Nitzschia fonticola* (Chapter 3.2.15).

Seven of 37 participating laboratories and one auditor identified all taxa unambiguously in sample D 11 (Tab. 2), i.e. they did not indicate identification problems for any of their listed taxa. For thirteen laboratories and two auditors the number of taxa that could not be determined (spec, pennate) and/or could not unambiguously be determined (cf., aff.) exceeded 5 %, with a maximum of 19.2 % (laboratory 12) (Tab. 2). The number of these ambiguous taxa was not part of the evaluation of the laboratories during this intercalibration exercise. Taxonomic uncertainties (cf., aff., spec.) are further discussed in Chapters 3.3 and 5.

The similarity among counting results (relative abundances of the diatom objects) was assessed using the Bray-Curtis-distance. The average Bray-Curtis-distance of the counting results among auditors was 0.3862 with a standard deviation of 0.0607 and thus a 95 %-confidence interval of 0.1187 to 0.5049 in sample D 11. If the Bray-Curtis-distance of a laboratory to all three auditors is within this confidence interval, then the result of the laboratory is as similar to the auditor results as the auditor results among each other. If a Bray-Curtis-distance of a laboratory is outside the confidence interval of an auditor, the distance is marked red and bold in Tab. 2. For more information see Chapter 2.

Tab. 2: Relevant counting parameters of each count from the participants (lab 1-37) and auditors (lab 39-42, shaded in green) for sample D 11, i.e. number of counted diatom-objects (**objects**), number of identified taxa during the count (**NTC**), during the search for rare taxa after the count (**NTS**) and with an abundance >1 % (**NT>1**) and sum of the relative abundance of all ambiguously determined diatom-objects, i.e. taxa labelled with cf., aff., spec or pennates (**%cf**). Also given are the Bray-Curtis-distances of the participants compared to auditor 39 (**Diff 1**), 41 (**Diff 2**) and 42 (**Diff 3**) for sample D 11. Red and bold Bray-Curtis-distances were outside the 95 %-confidence interval. **lab** = laboratory-code. For more information see text.

lab	objects	NTC	NTS	NT>1	%cf	Diff 1	Diff 2	Diff 3
1	500	28	0	16	0	0.2898	0.4627	0.3846
2	394	42	0	15	15.5	0.4265	**0.5158**	0.4800
3	517	37	2	15	1.9	0.4087	**0.5471**	0.4799
4	505	49	6	13	1.6	0.3261	0.4595	0.4300
5	524	37	0	13	2.1	0.3945	**0.5837**	0.5044
6	500	45	0	13	0.0	0.2931	0.4677	0.3893
7	498	69	20	18	1.6	0.4106	**0.5526**	**0.5287**
8	504	51	10	16	2.0	0.3623	**0.5136**	0.4548
9	507	50	9	12	0.8	0.2803	0.4416	0.4126
10	367	31	0	15	1.4	**0.6177**	**0.6605**	**0.6277**
11	499	36	0	12	0.2	0.3012	0.4882	0.4392
12	496	45	4	17	19.2	0.4501	**0.6094**	**0.5372**
13	513	51	8	13	5.7	0.3363	0.4696	0.4697
14	586	51	0	7	0.0	0.2530	0.4735	0.4130
15	408	24	0	6	1.0	**0.6403**	**0.8110**	**0.7331**
16	495	47	1	15	5.1	0.3912	0.4950	0.4498
17	513	42	11	12	0.2	0.4171	**0.5754**	0.4914
18	500	40	8	16	6.4	0.3368	0.4026	0.3651
19	508	21	0	11	11.8	**0.6411**	**0.6823**	**0.6725**
20	499	37	4	18	0.0	**0.5224**	**0.6354**	**0.6069**
21	500	29	0	14	5.0	0.4651	**0.6138**	**0.5141**
22	312	31	0	11	7.7	0.4682	**0.5741**	**0.5886**
23	509	51	15	10	3.9	**0.6222**	**0.7255**	**0.6972**
24	494	51	13	16	5.5	0.3235	0.4789	0.4661
25	506	47	0	18	11.3	0.4628	0.4938	0.4674
26	501	33	5	13	0.0	0.4156	**0.5254**	0.4904
27	535	39	8	11	0.0	0.2985	0.4927	0.4335
28	375	17	0	12	14.4	**0.5795**	**0.6597**	**0.6579**
29	523	53	11	17	0.6	0.3274	0.3838	0.4109
30	491	59	10	19	6.1	0.4073	**0.5425**	**0.5395**
31	528	62	13	16	9.1	0.4606	**0.6232**	**0.5296**
32	531	39	9	8	0.9	0.2790	0.3938	0.4170
33	502	39	5	14	0.2	0.2743	0.4428	0.4325
34	561	69	11	13	8.4	0.2920	0.4313	0.4152
35	478	48	6	17	0.4	0.4291	**0.5744**	**0.5238**
36	806	40	0	11	0.0	0.4994	**0.5635**	**0.5397**
37	500	50	7	14	3.0	0.4594	**0.6216**	**0.5497**
39	539	48	8	12	0.0	0.0	0.4300	0.4115
41	508	42	4	11	5.7	0.4300	0.0	0.3170
42	638	53	8	13	12.9	0.4115	0.3170	0.0

The evaluation of the counting results of each laboratory is based on the Bray-Curtis-distances. Only laboratories that were outside of the confidence intervals of all three auditors were assessed as inadequate (marked "unsuccessful" on the certificate) to tentatively interpret the limits of the confidence intervals and to account for the slight variation among auditor results. Thus, the counting results of the six laboratories 10, 15, 19, 20, 23 and 28 had to be marked as "unsuccessful" on the certificates for sample D 11 (Tab. 2).

The results of the Bray-Curtis-distances were verified with another, independent method, a multivariate figure (DCA, Detrendet Correspondence Analysis). Fig. 1 displays the first and second axis and confirms the results of the Bray-Curtis-distances when also reviewing the third dimension for sample D 11. The samples of the auditors are the red numbers. The samples of the participants that are not marked agree well with the results of all three auditors based on the Bray-Curtis-distances. The green-rimmed samples of the participants were outside the confidence-intervals of two auditors (Fig. 1). These results were still labelled "successful" on the certificates, even though taxonomic discrepancies were obvious. The red-rimmed samples of the participants were outside the confidence-interval of all three auditors based on the Bray-Curtis-distances (Tab. 2, Fig. 1).

In addition to evaluating the counting results of the participants based on the Bray-Curtis-distances, deviations from the instruction protocol was indicated on the certificates as far as this was possible based on the counting results (for more information see Chapter 2). For example, at least 500 diatom objects had to be counted for the lake sample D 11 according to Schaumburg et al. (2011). However, several laboratories counted distinctly less objects, especially laboratory 22 (312 counted objects), 10 (367), 28 (375), 2 (394) and 15 (408) (Tab. 2). Similarly, the instruction protocol stipulates that the sample must be screened for rare taxa after the regular count of at least 500 diatom objects (Schaumburg et al. 2011) and that these rare taxa need to be indicated with an abundances of „0“ in the result-tables. However, 14 of 37 participating laboratories (1, 2, 5, 6, 10, 11, 14, 15, 19, 21, 22, 25, 28, 36) did not search for rare taxa or did not list any taxa with an abundance of „0“ in the result tables (Tab. 2).

The results of laboratories 2, 8, 10, 15, 19, 23 and 30 clearly indicate that the taxa-denotations did not correspond to the taxa names of the most recent identification literature that was mandatory for this intercalibration exercise according to the relevant instruction protocol.

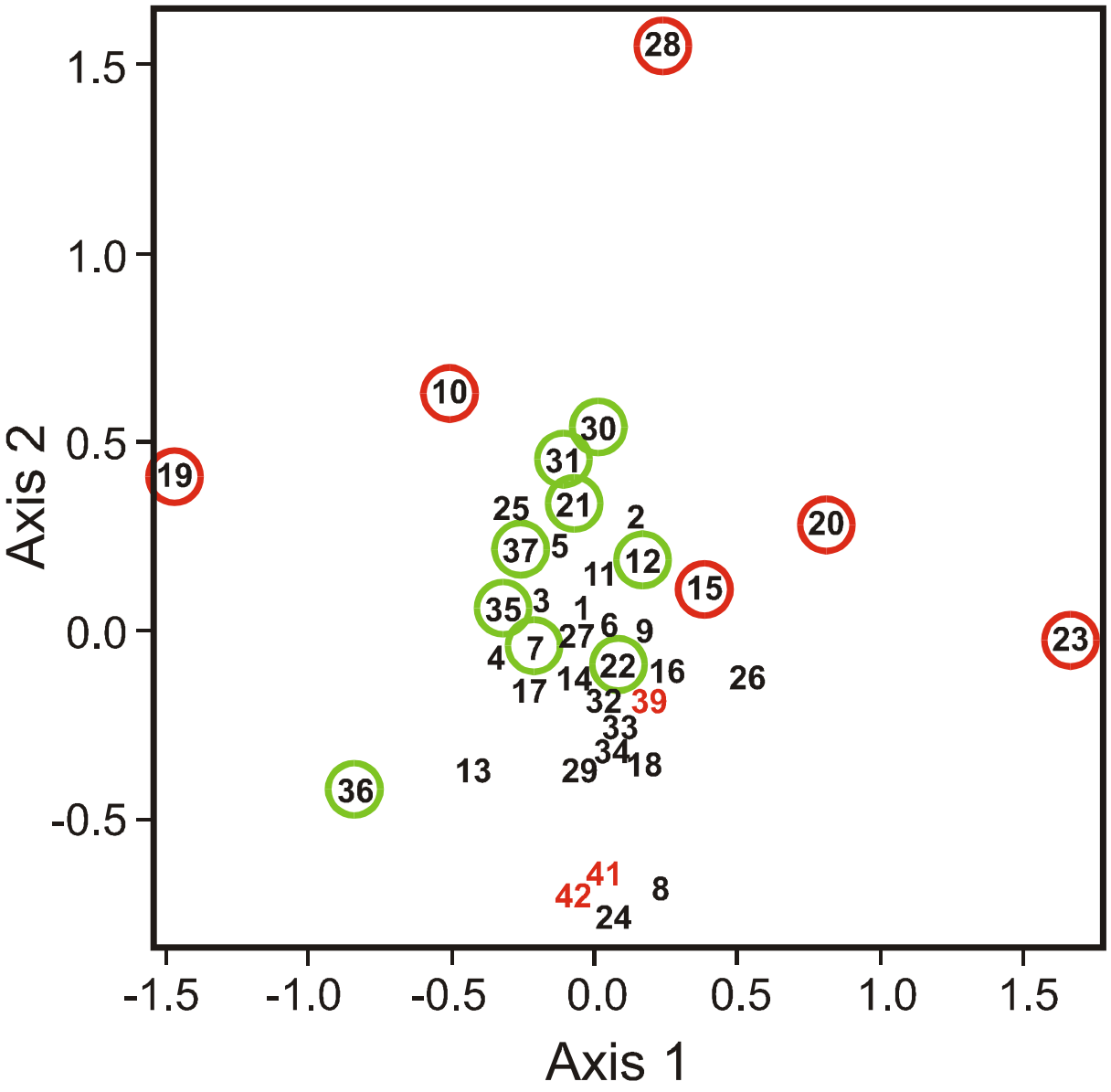


Fig. 1: First and second axis of the DCA based on the diatom results of all participants and auditors of sample D 11 (Lake Krossinsee, North-German lowlands). Numbers correspond to the laboratory-codes. Red numbers = laboratory codes of the auditors. The green-rimmed samples of the participants were outside the 95 % confidence-intervals of two auditors, red-rimmed samples were outside the confidence-intervals of three auditors based on the Bray-Curtis-distances. For more information see text.

Apart from the mentioned taxonomic problems and the deviations from the instruction protocol there was a high variability among counting results based on other parameters. For example, the number of counted taxa varied distinctly with 17 to 69 taxa identified by the participants in sample D 11. The auditors identified 48, 42 and 53 taxa during the regular count (Tab. 2). Similarly, the number of taxa found after the regular count during the search

for rare taxa varied distinctly with one to 20 identified taxa, the auditors found 4-8 taxa during the search for rare taxa (Tab. 2). The number of taxa contributing to more than 1 % relative abundance per count ranges from 6 to 19 taxa (auditors: 11-13; Tab. 2). These parameters further emphasize the variability among results for one sample. However, they were not used for the assessment of the laboratories.

3.1.2 Lake Geneva, Switzerland, Alps and Alpine foothills, sample D 1.1

Sample D 1.1 from Lake Geneva, Switzerland (lake from the Alps/Alpine foothills) was dominated by *Achnanthidium minutissimum* var. *minutissimum* (Kützing) Czarnecki, *Encyonopsis minuta* Krammer & Reichardt and *Encyonopsis subminuta* Krammer & Reichardt (and similar *Encyonopsis* species) and by a small *Gomphonema* Ehrenberg, which looks similar to the *Gomphonema pumilum* ((Grunow) Reichardt & Lange-Bertalot) -complex (Tab. 3). Additionally, *Amphora indistincta* Levkov, *Amphora pediculus* (Kützing) Grunow, *Cymbella excisa* Kützing and *Cymbella parva* (W.Smith) Kirchner, *Fragilaria brevistriata* Grunow (synonymous: *Staurosira brevistriata* (Grunow) Grunow), *Fragilaria pinnata* (Ehrenberg) Williams & Round (see *Staurosirella pinnata* Ehrenberg), *Gomphonema olivaceolacuum* (Lange-Bertalot & Reichardt) Lange-Bertalot & Reichardt, *Navicula cryptotenella* Lange-Bertalot, *Navicula cryptotenelloides* Lange-Bertalot, *Nitzschia lacuum* Lange-Bertalot and *Nitzschia palea* (Kützing) W.Smith were relatively abundant according to the results of the auditors (Tab. 3).

Similar to sample D 11 (see Chapter 3.1.1), some taxa were pooled to groups prior to calculating the Bray-Curtis-Distances (Tab. 4) (see also Chapter 2). For sample D 1.1 the following groups were used based on the different usage of taxa names by the participants and auditors: **Group 1** (*Cocconeis placentula, C. placentula* var. *euglypta, C. placentula* var. *lineata*), **Group 2** (*Cymbella excisa*, *Cymbella excisa* var. *excisa*), **Group 3** (*Cymbella hustedtii*, *Cymbella hustedtii* var. *hustedtii*), **Group 4** (*Encyonema cespitosum*, *Encyonema cespitosum* var. *cespitosum*), **Group 5** (*Encyonema silesiacum*, *E. silesiacum* var. *silesiacum*), **Group 6** (*Fragilaria brevistriata*, *F. brevistriata* var. *brevistriata*, *F. brevistriata* var. cf. *brevistriata*, *Staurosira brevistriata*), **Group 7** (*Fragilaria famelica*, *Fragilaria famelica* var. *famelica*), **Group 8** (*Fragilaria pinnata*, *Fragilaria pinnata* var. *pinnata*), **Group 9** (*Fragilaria vaucheriae*, *F. capucina* var. *vaucheriae*), **Group 10** (*Gomphonema olivaceum*, *G. olivaceum* var. *olivaceum*), **Group 11** (*Karayevia clevei*, *K. clevei* var. *clevei*, *K. clevei*

var. cf. *clevei*, *K. clevei* var. *rostrata*), **Group 12** (*Navicula cryptocephala*, *Navicula cryptocephala* var. *cryptocephala*), **Group 13** (*Navicula reichardtiana*, *Navicula reichardtiana* var. *reichardtiana*), **Group 14** (*Nitzschia dissipata*, *N. dissipata* ssp. *dissipata*, *N. dissipata* var. *media*), **Group 15** (*Nitzschia palea*, *N palea* var. *palea*, *N. palea* var. *debilis*,*N. palea* var. *tenuirostris*), **Group 16 (***Nitzschia fonticola*, *Nitzschia fonticola* var. *fonticola*), **Group 17** (pennates, pennates cf.), **Group 18** (*Planothidium frequentissimum*, var. *frequentissimum*, *magnus*, *minus*) and **Group 19** (*Reimeria sinuata*, var. *sinuata*, var. *sinuata* cf.).

Tab. 3: Relative abundances (%), mean and standard deviation (stdev) (%) of the dominant and abundant diatom taxa in sample D 1.1 (Lake Geneva, Alps/Alpine foothills) based on the results of the auditors (L39-42). For more information see text, Chapter 2 and Chapter 3.1.1.

Taxon	L 39	L 40	L 42	mean	stdev
A. minutissimum var. *minutissimum*	26.7	27.5	25.4	26.5	1.1
Gomphonema pumilum	20.4	21.4	8.4	16.8	7.2
Gomphonema spec.	0.0	0.0	15.7	5.2	9.1
Encyonopsis minuta	13.2	11.7	0.4	8.4	7.0
Encyonopsis subminuta	0.0	0.0	9.2	3.1	5.3
Navicula cryptotenelloides	7.1	7.5	0.0	4.8	4.2
Navicula cf. *cryptotenella*	0.0	0.0	6.5	2.2	3.8
Amphora pediculus	6.9	6.5	0.7	4.7	3.4
Amphora indistincta	0.0	0.0	6.4	2.1	3.7
Gomphonema olivaceolacuum	6.9	4.7	5.0	5.5	1.2
Fragilaria pinnata	3.2	1.9	3.0	2.7	0.7
Fragilaria brevistriata	2.3	1.6	0.6	1.3	0.9
Nitzschia lacuum	2.5	4.7	0.0	2.4	2.3
Nitzschia palea	0.0	0.0	5.6	1.9	3.2
Cymbella parva	1.7	2.6	0.0	1.4	1.3
Cymbella excisa	0.0	0.0	2.2	0.7	1.3

Next to many other considerable taxonomic problems (see below), the occurrence of a small, not yet described *Gomphonema*-species was especially problematic in sample D 1.1 (see Chapter 3.2.10). This taxon contributed about 20 % to the diatom assemblage in sample D 1.1 (in Tab. 3 named *Gomphonema pumilum* or *Gomphonema* spec.). The taxon looks somewhat similar to the *Gomphonema pumilum*-complex. However, when considering the details it is

obvious that this taxon is neither *G. pumilum* nor another similar, already described taxon (see Chapter 3.2.10). Thus, the correct name for this little *Gomphonema*-taxon is *Gomphonema* spec. according to the current state of research (Reichardt, personal communication in 2013).

Two auditors named this taxon *G. pumilum*. One of them pointed out that this taxon is not *G. pumilum*, but the name was still allocated to enable an ecological assessment of the sample (see further discussion in Chapters 3.2.10 and 3.3). Similarly, many participants used *G. pumilum* or a name from a similar taxon for this species. Thus, the group used here included *G. pumilum* and the correctly used names *Gomphonema* spec. or names of the *G. pumilum* group that were labelled with a „cf.“, i.e. not determined with certainty, to avoid a disadvantage during the assessment of each laboratory for participants that labelled the taxon correctly. Consequently, this group included *G.* cf. *pumilum*, *G.* cf. *pumilum* var. *pumilum*, *G.* cf. *pumilum* var. *rigidum*, *G.* cf. *micropumilum*, *G.* cf. *elegantissimum*, *G.* cf. *angustivalva* and *Gomphonema* spec.

None of the other three samples (see Chapters 3.1.1, 3.1.3 and 3.1.4) of this intercalibration exercise revealed such substantial taxonomic problems as sample D 1.1. The problems are obvious in both the results of the auditors and –to an even larger extent- in the results of many participating laboratories. Auditors and participants had considerable problems with identifying the small *Gomphonema* spec. (see above and Chapter 3.2.10), *Encyonopsis minuta* and *Encyonopsis subminuta*, *Navicula cryptotenelloides* and *Navicula cryptotenella*, *Amphora pediculus* and *Amphora indistincta*, *Nitzschia lacuum* and *Nitzschia palea* as well as *Cymbella parva* and *Cymbella excisa*. Additionally, some participants had taxonomic difficulties with identifying *Achnanthidium minutissimum*, *Fragilaria* (*Staurosira*) *brevistriata* and similar taxa, *Fragilaria capucina* and similar taxa, *Gomphonema olivaceolacuum* as well as *Navicula reichardtiana* and *Navicula caterva* (see Chapter 3.2).

The similarity among counting results (relative abundances of the diatom objects) was assessed using the Bray-Curtis-distance. In sample D 1.1 the average Bray-Curtis-distance of the counting results among auditors was 0.46 with a high standard deviation of 0.3 and thus a 95 %-confidence interval that covers the entire possible range from zero to one (in comparison the standard deviations in sample D 11 was 0.06, in sample D 12 0.06 and 0.09 in sample D 2). Thus, the results of the auditors cannot be used to assess the quality of the results of the participating laboratories in sample D 1.1. Consequently, sample D 1.1 could not be evaluated as participants could not be assessed statistically and the results of the participants were not evaluated on the certificates for sample D 1.1. Additionally, the

graphical confirmation of the Bray-Curtis results with another, independent method (DCA, Detrendet Correspondence Analysis) became superfluous (which was conducted for the other three samples, see Chapters 3.1.1, 3.1.3 and 3.1.4). However, the fundamental taxonomic problems that occurred in sample D 1.1 are discussed in detail in Chapter 3.2.

Tab. 4: Relevant counting parameters of each count from the participants (lab 1-37) and auditors (lab 39-42, shaded in green) for sample D 1.1, i.e. number of counted diatom-objects (objects), number of identified taxa during the count (NTC), during the search for rare taxa after the count (NTS) and with an abundance >1 % (NT>1) and sum of the relative abundance of all ambiguously determined diatom-objects, i.e. taxa labelled with cf., aff., spec or pennales (% cf). Also given are the Bray-Curtis-distances of the participants compared to auditor 39 (Diff 1), 40 (Diff 2) and 42 (Diff 3) for sample D 1.1. lab = laboratory-code. For more information see text.

lab	objects	NTC	NTS	NT>1	% cf	Diff 1	Diff 2	Diff 3
1	500	30	0	19	15.0	0.4611	0.4543	0.6673
2	411	38	0	17	23.4	0.5680	0.5774	0.7744
3	524	33	3	15	0.0	0.4386	0.4174	0.7249
4	501	40	1	22	1.2	0.4380	0.4198	0.6438
5	588	38	0	17	4.3	0.5638	0.5571	0.6774
6	500	36	0	17	0.8	0.4250	0.3857	0.5844
7	500	35	11	20	20.6	0.6178	0.6078	0.7609
8	400	37	9	22	2.0	0.4948	0.5237	0.7823
9	558	28	6	13	1.6	0.3257	0.3107	0.5691
10	562	46	0	26	2.8	0.8686	0.8564	0.8711
11	500	31	0	17	0.8	0.3883	0.4091	0.6125
12	499	38	7	24	4.6	0.6627	0.6707	0.8087
13	510	42	9	16	21.4	0.4937	0.4628	0.6608
14	584	44	0	18	0.0	0.3130	0.3125	0.7356
15	396	30	0	15	0.8	0.6887	0.6526	0.8976
16	512	40	10	13	14.3	0.5808	0.5857	0.7350
17	507	24	7	14	0.4	0.4487	0.4248	0.7826
18	495	32	6	19	22.8	0.5220	0.5299	0.5592
19	506	19	0	16	35.0	0.8412	0.8365	0.8939
20	601	35	8	18	0.0	0.6085	0.5924	0.7843
21	507	32	0	15	3.9	0.7786	0.7753	0.7873
22	417	28	0	15	26.4	0.5095	0.4820	0.7374

Continuation of Tab. 4

23	554	37	11	14	26.0	0.7198	0.7139	0.9028
24	500	27	8	16	31.4	0.4727	0.4486	0.7541
25	516	30	3	16	47.5	0.6650	0.6645	0.7215
26	513	22	7	15	0.2	0.4848	0.4595	0.6783
27	528	25	10	17	0.0	0.3315	0.3302	0.6853
28	415	17	0	12	50.4	0.7611	0.7395	0.7916
29	520	34	12	18	0.0	0.3715	0.3526	0.6118
30	591	40	8	19	2.7	0.5267	0.5515	0.6563
31	540	25	12	14	20.9	0.5799	0.5760	0.8258
32	521	30	9	17	0.2	0.5069	0.4876	0.7635
33	500	31	7	18	4.2	0.5143	0.5047	0.7584
34	546	43	12	18	27.8	0.5191	0.5163	0.6132
35	526	34	8	16	0.2	0.6770	0.6705	0.7351
36	529	39	0	15	0.0	0.6818	0.6817	0.7513
37	500	29	12	19	13.2	0.5722	0.5474	0.7823
39	524	27	10	13	0.0	0.0000	0.1129	0.6863
40	429	23	9	15	0.0	0.1129	0.0000	0.6846
42	535	37	8	11	25.0	0.6863	0.6846	0.0000

Six of 37 participating laboratories and two auditors identified all taxa unambiguously in sample D 1.1 (Tab. 4), i.e. they did not indicate (with a cf.) identification problems for any of their listed taxa despite the substantial taxonomic problems that became apparent in sample D 1.1. In contrast, the results of the remaining participants and auditor clearly reflect the substantial taxonomic problems of sample D 1.1. For 15 laboratories and one auditor the number of taxa that could not be determined (spec, pennates) and/or could not unambiguously be determined (cf., aff.) exceeded 5 %, with a maximum of 50.4 % (laboratory 12) (Tab. 4) and an average of 11.5 % (n = 37) when using all participants. Taxonomic uncertainties (cf., aff., spec.) are further discussed in Chapters 3.3 and 5.

In contrast to not evaluating the counting results of the participants based on the Bray-Curtis distances for sample D 1.1 (see above), deviations from the instruction protocol was indicated on the certificates as far as this was possible based on the counting results (for more information see Chapter 2). For example, at least 500 diatom objects had to be counted for the lake sample D 1.1 according to Schaumburg et al. (2011). However, several laboratories

counted distinctly less objects, especially laboratory 15 (396 counted objects), 8 (400), 2 (411), 28 (415), 22 (417) and 40 (429) (Tab. 4). Similarly, the instruction protocol stipulates that the sample must be screened for rare taxa after the regular count of at least 500 diatom objects (Schaumburg et al. 2011) and that these rare taxa need to be indicated with an abundance of „0“ in the result-tables. However, 13 of 37 participating laboratories (1, 2, 5, 6, 10, 11, 14, 15, 19, 21, 22, 28, 36) did not search for rare taxa or did not list any taxa with an abundance of „0“ in the result tables (Tab. 4).

Apart from the mentioned taxonomic problems and the deviations from the instruction protocol there was also a high variability among counting results based on other parameters. For example, the number of counted taxa varied distinctly with 17 to 46 taxa identified by the participants in sample D 1.1. The auditors identified 27, 23 and 37 taxa during the regular count (Tab. 4). Similarly, the number of taxa found after the regular count during the search for rare taxa varied distinctly with one to 12 identified taxa, the auditors found 8-10 taxa during the search for rare taxa (Tab. 4). The number of taxa contributing to more than 1% relative abundance per count ranged from 12 to 26 taxa (auditors: 11-15; Tab. 4).

3.1.3 River Klepelshagener Bach, North German Lowlands, sample D 12

Sample D 12 from River Klepelshagener Bach, northern Germany (running water from the lowlands) was dominated by *Planothidium lanceolatum* (Brébisson) Lange-Bertalot, *Planothidium frequentissimum* (Lange-Bertalot) Round & Bukhtiyarova and *Eolimna minima* (Grunow) Lange-Bertalot & Schiller (Tab. 5). Additionally, *Gomphonema insigniforme* Reichardt & Lange-Bertalot, *Gomphonema micropus* Kützing, *Gomphonema parvulum* (Kützing) Kützing, *Mayamaea permitis* (Hustedt) Bruder & Medlin (synonymous: *Mayamaea atomus* var. *permitis* (Hustedt) Lange-Bertalot), *Meridion circulare* (Greville) Agardh, *Navicula cryptocephala* Kützing, *Navicula cryptotenella* Lange-Bertalot, *Navicula lundii* Reichardt, *Navicula veneta* Kützing, *Sellaphora joubaudii* (Germain) Aboal, *Sellaphora seminulum* (Grunow) Mann and *Stauroneis kriegeri* Patrick were relatively abundant according to the results of the auditors (Tab. 5).

Similar to the other intercalibration exercise samples (see Chapters 3.1.1, 3.1.2 and 3.1.4), some taxa were pooled to groups prior to calculating the Bray-Curtis-Distances (Tab. 6) and

presenting the results in the DCA (Fig. 2) (see also Chapter 2). For sample D 12 the following groups were used: **Group 1** (*Cocconeis placentula*, *C. placentula* var. *euglypta*, *C. placentula* var. *lineata*), **Group 2** (*Eunotia bilunaris*, *E. bilunaris* var. *bilunaris*), **Group 3** (*Fragilaria vaucheriae*, *F. capucina* var. *vaucheriae*), **Group 4** (*Gomphonema acuminatum*, *G. acuminatum* var. *acuminatum*, *G. acuminatum* cf. var. *acuminatum*), **Group 5** (*Meridion circulare*, *M. circulare* var. *circulare*, *M. circulare* var. *constrictum*), **Group 6** (*Navicula cryptocephala*, *N. cryptocephala* var. *cryptocephala*), **Group 7** (*Navicula radiosa*, *N. radiosa* var. *radiosa*), **Group 8** (*Nitzschia dissipata*, *N. dissipata* ssp. *dissipata*, *N. dissipata* var. *media*), **Group 9** (*Nitzschia linearis*, *N. linearis* var. *linearis*), **Group 10** (*Nitzschia palea*, *N palea* var. *palea*, *N. palea* var. *debilis*), **Group 11** (pennates, pennates cf.) and **Group 12** (*Planothidium frequentissimum*, *P. frequentissimum* var. *frequentissimum*, *P. frequentissimum* var. *magnus*, *P. frequentissimum* var. *minus*).

Tab. 5: Relative abundances (%), mean and standard deviation (stdev) (%) of the dominant and abundant diatom taxa in sample D 12 (lowland-river Klepelshagener Bach) based on the results of the auditors (L39-42). For more information see text, Chapter 2 and Chapter 3.1.1

Taxon	L 39	L 41	L 42	mean	stdev
Planothidium lanceolatum	34.6	27.2	44.4	35.4	8.6
Planothidium frequentissimum	7.0	11.7	4.5	7.7	3.6
Eolimna minima	12.8	15.1	4.5	10.8	5.6
Gomphonema insigniforme	1.2	1.1	0.0	0.8	0.7
Gomphonema micropus	0.7	1.1	3.5	1.8	1.5
Gomphonema parvulum	4.8	0.6	2.2	2.6	2.1
Mayamaea permitis	6.3	7.6	0.2	4.7	4.0
Meridion circulare	1.7	3.5	2.2	2.4	0.9
Navicula cryptocephala	0.7	0.6	2.2	1.2	0.9
Navicula cryptotenella	0.7	0.0	2.2	1.0	1.1
Navicula lundii	0.0	2.4	1.2	1.2	1.2
Navicula veneta	2.2	1.5	1.5	1.7	0.4
Sellaphora joubaudii	3.9	1.9	1.3	2.4	1.3
Sellaphora seminulum	5.1	6.7	6.0	5.9	0.8
Stauroneis kriegeri	1.0	2.6	0.8	1.5	1.0

The results of the auditors for sample D 12 agree well with one another (Tab. 5) with only some variance among slides (see Chapter 3.1.1). Thus, the auditor results represent a sound basis for the statistical evaluation of the results of the participants of the intercalibration exercise for sample D 12. For the assessment and discussion of the auditors standard deviations see Chapter 3.1.1.

For the participants taxonomic problems mainly occurred during the identification of *Eolimna minima* and similar taxa (*Sellaphora joubaudii*, *S. seminulum*), *Gomphonema micropus*, *G. parvulum*, *Mayamaea permitis*, *Navicula lundii*, *N. veneta* and *Planothidium frequentissimum* (and *P. lanceolatum*) in sample D 12. The fundamental taxonomic problems that occurred in sample D 12 are discussed in detail in Chapter 3.2.

Nine of 37 participating laboratories identified all taxa unambiguously in sample D 2 (Tab. 6), i.e. they did not indicate (with a cf.) identification problems for any of their listed taxa. For eight laboratories the number of taxa that could not be determined (spec, pennates) and/or could not unambiguously be determined (cf., aff.) exceeded 5 %, with a maximum of 23.0 % (laboratory 28) (Tab. 6). Taxonomic uncertainties (cf., aff., spec.) were not part of the evaluation of the laboratories and are further discussed in Chapters 3.3 and 5.

Tab. 6: Relevant counting parameters of each count from the participants (lab 1-37) and auditors (lab 38-42, shaded in green) for sample D 12, i.e. number of counted diatom-objects (objects), number of identified taxa during the count (NTC), during the search for rare taxa after the count (NTS; note that for sample D 12 this search was not required as D 12 was a running water sample) and with an abundance >1.0 % (NT>1) and sum of the relative abundance of all ambiguously determined diatom-objects, i.e. taxa labelled with cf., aff., spec or pennales (% cf). Also given are the Bray-Curtis-distances of the participants compared to auditor 38 (Diff 1), 39 (Diff 2) and 42 (Diff 3) for sample D 12. Red and bold Bray-Curtis-distances were outside the 95 %-confidence intervals. lab = laboratory-code. For more information see text.

lab	objects	NTC	NTS	NT>1	% cf	Diff 1	Diff 2	Diff 3
1	389	27	0	15	2.0	0.3732	0.3906	0.4653
2	413	38	0	15	4.8	0.2862	0.3888	0.4515
3	517	30	0	11	0.4	0.4637	**0.4983**	**0.5301**
4	405	34	0	16	2.2	0.3688	0.3985	0.4193
5	444	36	0	15	2.3	0.3912	0.3992	0.4827
6	388	37	0	17	3.0	0.2935	0.3534	0.4772
7	500	40	14	14	0.0	0.3739	0.3093	0.4484
8	400	39	15	18	0.0	0.4138	0.4279	**0.5433**

Continuation of Tab. 6

9	404	31	0	16	3.5	0.2682	0.2671	0.3728
10	324	59	0	19	0.0	**0.8755**	**0.8790**	**0.8476**
11	395	30	0	13	1.3	0.2898	**0.2433**	0.4836
12	400	34	6	18	0.0	0.4892	0.4667	**0.5437**
13	392	41	0	15	5.4	0.4026	0.3986	0.4835
14	467	45	0	15	0.0	0.3664	0.3554	0.4032
15	416	32	0	12	1.0	**0.6504**	**0.6748**	**0.6726**
16	414	39	7	15	1.9	**0.2615**	0.3179	0.4087
17	405	33	9	15	0.0	0.4031	0.4562	0.4611
18	398	34	17	17	6.0	0.2771	0.3622	0.4334
19	350	28	0	13	16.3	**0.8700**	**0.8973**	**0.8890**
20	716	33	6	17	0.0	**0.6018**	**0.6754**	**0.6491**
21	514	29	0	17	0.4	0.3619	0.4200	0.4732
22	414	35	0	12	2.4	0.403	0.3845	**0.5255**
23	432	35	15	14	3.9	0.3288	0.4376	0.4494
24	377	36	4	16	7.2	0.3651	0.3811	**0.5336**
25	362	37	0	15	11.7	0.4604	**0.4929**	**0.5368**
26	427	26	0	14	1.4	0.3213	0.3622	0.4657
27	421	30	0	15	0.5	0.3692	0.3641	0.4409
28	405	15	0	8	23.0	**0.7513**	**0.7353**	**0.7450**
29	416	40	2	13	0.2	0.3118	**0.2518**	0.3973
30	419	45	3	15	2.1	0.4767	0.4905	**0.4970**
31	490	42	6	15	9.7	0.3782	0.4215	**0.5298**
32	415	32	0	14	0.2	0.2960	0.3067	0.3608
33	387	34	0	18	8.1	0.3503	0.4058	0.4082
34	440	38	0	15	2.2	0.2697	**0.2664**	0.3915
35	442	35	2	13	0.7	0.3394	0.3940	0.4441
36	639	27	0	10	0.0	**0.6260**	**0.5586**	**0.5846**
37	400	32	4	18	0.0	0.3817	0.3729	0.4413
38	413	41	0	13	2.9	0	0.3171	0.3886
39	445	34	3	15	3.9	0.3171	0	0.4305
42	596	49	6	18	4.8	0.3886	0.4305	0

The similarity among counting results (relative abundances of the diatom objects) was assessed using the Bray-Curtis-distance. The average Bray-Curtis-distance of the counting results among auditors was 0.3788 with a standard deviation of 0.0573 and thus a 95 %-confidence interval of 0.2664 to 0.4911 in sample D 12. If the Bray-Curtis-distance of a laboratory to all three auditors is within this confidence interval, then the result of the laboratory is as similar to the auditor results as the auditor results among each other. If a Bray-Curtis-distance of a laboratory is outside the confidence interval of an auditor, the distance is marked red and bold in Tab. 6. For more information see Chapter 2.

The evaluation of the counting results of each laboratory is based on the Bray-Curtis distances. Only laboratories that were outside of the confidence intervals of all three auditors were assessed as inadequate (marked "unsuccessful" on the certificate) to tentatively interpret the limits of the confidence intervals and to account for the slight variation among auditor results. Thus, the counting results of the six laboratories 10, 15, 19, 20, 28 and 36 had to be marked as "unsuccessful" on the certificates for sample D 12 (Tab. 6).

The results of the Bray-Curtis-distances were verified with another, independent method, a multivariate figure (DCA, Detrendet Correspondence Analysis). Fig. 2 displays the first and second axis and confirms the results of the Bray-Curtis-distances when also reviewing the third dimension (not shown in figure) for sample D 12. The samples of the auditors are the red numbers. The samples of the participants that are not marked agree well with the results of all three auditors based on the Bray-Curtis-distances. The green-rimmed samples of the participants were outside the confidence- intervals of two auditors (Fig. 2). These results were still labelled "successful" on the certificates, even though taxonomic discrepancies were obvious. The red-rimmed samples of the participants were outside the confidence-intervals of all three auditors based on the Bray-Curtis-distances (Tab. 6., Fig. 2).

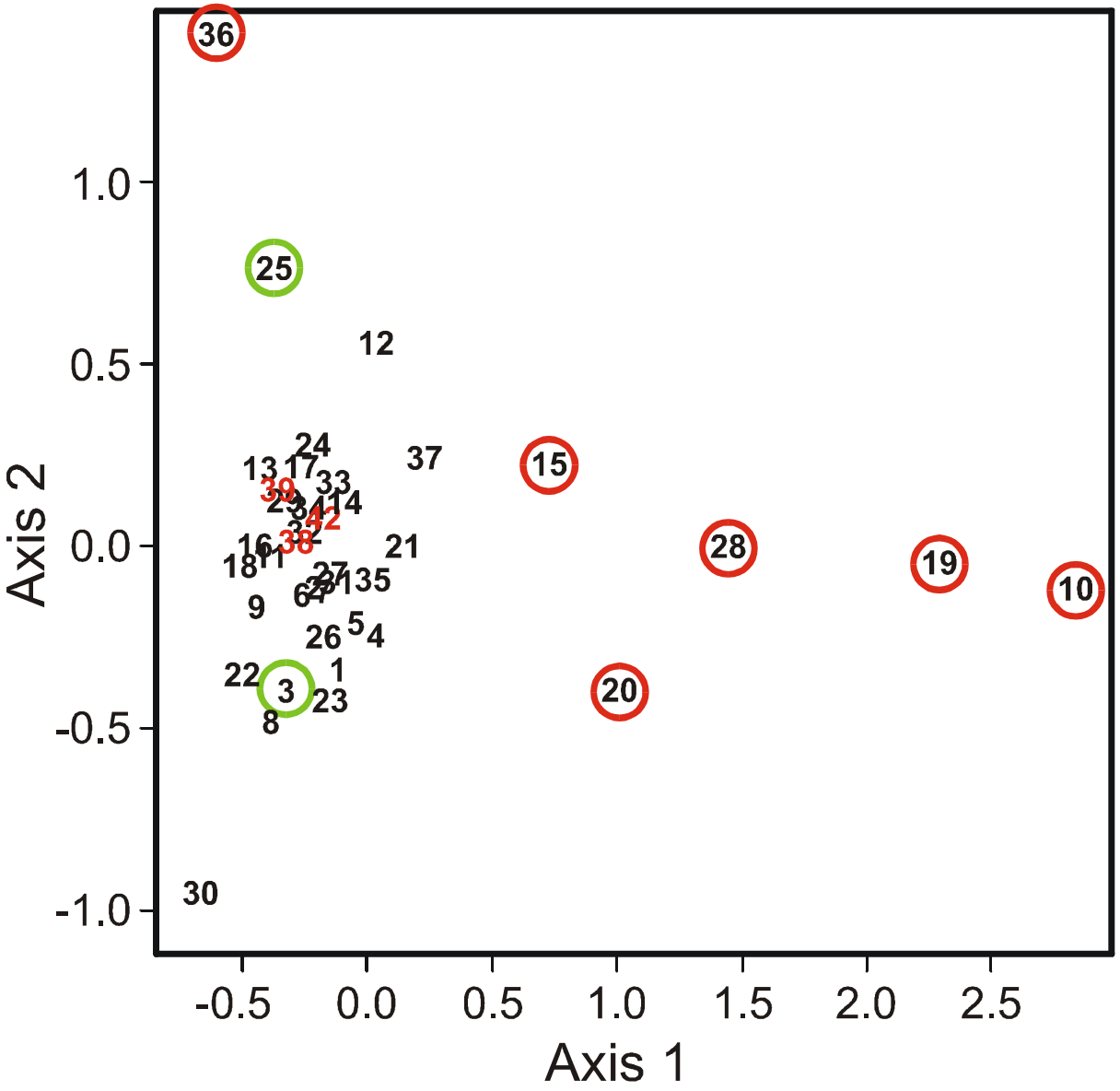


Fig. 2: First and second axis of the DCA based on the diatom results of all participants and auditors of sample D 12 (lowland-river Klepelshagener Bach). Numbers correspond to the laboratory-codes. Red numbers = laboratory codes of the auditors. The green-rimmed samples of the participants were outside the 95 % confidence-intervals of two auditors. Red-rimmed samples were outside the confidence-intervals of three auditors based on the Bray-Curtis-distances.

In addition to evaluating the counting results of the participants based on the Bray-Curtis distances, deviations from the instruction protocol was indicated on the certificates as far as this was possible based on the counting results (for more information see Chapter 2). For example, at least 400 diatom objects had to be counted for the running water sample D 12 according to Schaumburg et al. (2006). However, several laboratories counted distinctly less

objects, especially laboratories 10 (324 objects), 19 (350 objects) and 25 (362 objects) (Tab. 6).

In contrast to lake samples the instruction protocol does **not** stipulate for running water samples that the sample must be screened for rare taxa after the regular count (Schaumburg et al. 2006). However, 14 of 37 participating laboratories did search for rare taxa or listed some taxa with an abundance of „0“ in the result tables (Tab. 6).

The results of laboratories 2, 8, 10, 15, 19, 23, 30 clearly indicate that the taxa denotations did not correspond to the taxa names of the most recent identification literature that was mandatory for this intercalibration exercise according to the relevant instruction protocol. This deviation from the mandatory instruction protocol was also noted on the certificates.

Apart from the mentioned taxonomic problems and the deviations from the instruction protocol there was a high variability among counting results based on other parameters. For example, the number of counted taxa varied distinctly with 15 to 59 taxa identified by the participants in sample D 12. The auditors identified 41, 34 and 49 taxa during the regular count (Tab. 6). Similarly, the number of taxa contributing to more than 1% relative abundance per count ranged from 8 to 19 taxa (auditors: 13-18; Tab. 6). These parameters further emphasize the variability among results for one sample. However, they were not used for the assessment of the laboratories.

3.1.4 River Drau, Austria, Alps and Alpine foothills, sample D 2

The sample D 2 from the River Drau, Austria (running water of the Alps/Alpine foothills) was dominated by *Achnanthidium minutissimum* var. *minutissimum* (Kützing) Czarnecki, *Achnanthidium pyrenaicum* (Hustedt) Kobayasi, *Diatoma ehrenbergii* Kützing, *Encyonema silesiacum* (Bleisch) Mann and *Fragilaria capucina* var. *vaucheriae* (Kützing) Lange-Bertalot (Tab. 7). Additionally, *Diatoma mesodon* (Ehrenberg) Kützing, *Encyonema minutum* (Hilse) Mann, *Fragilaria ulna* (Nitzsch) Lange-Bertalot, *Reimeria sinuata* (Gregory) Kociolek & Stoermer and *Fragilaria* spec. were relatively abundant according to the results of the auditors (Tab. 7).

Tab. 7: Relative abundances (%), mean and standard deviation (stdev) (%) of the dominant and abundant diatom taxa in sample D 2 (Alps/Alpine foothills River Drau) based on the results of the auditors (L38-42). For more information see text, Chapter 2 and Chapter 3.1.1.

Taxon	L 38	L 39	L 42	mean	stdev
A. minutissimum var. *minutissimum*	35.7	42.5	42.7	40.3	4.0
Achnanthidium pyrenaicum	13.8	9.2	10.7	11.3	2.3
Diatoma ehrenbergii	8.1	9.0	12.3	9.8	2.2
Encyonema silesiacum	8.6	9.5	12.9	10.3	2.3
F. capucina var. *vaucheriae*	8.4	9.5	2.8	6.9	3.6
Diatoma mesodon	3.0	3.9	3.2	3.3	0.5
Encyonema minutum	1.5	1.7	0.2	1.1	0.8
Fragilaria ulna	1.5	2.4	0.0	1.3	1.2
Reimeria sinuata	4.4	3.4	2.2	3.3	1.1
Fragilaria spec.	1.0	0.0	6.2	2.4	3.3

Similar to the other intercalibration exercise samples, some taxa were pooled to groups prior to calculating the Bray-Curtis-Distances (Tab. 8) and presenting the results in the DCA (Fig. 3) (see also Chapter 2). For sample D 2 the following groups were used: **Group 1** (*Cocconeis placentula*, *C. placentula* var. *euglypta*, *C. placentula* cf. var. *euglypta* and *C. placentula* var. *lineata*), **Group 2** (*Diatoma moniliformis*, *D. moniliformis* ssp. *moniliformis*, *D. moniliformis* cf. ssp. *moniliformis*, *D. moniliformis* ssp. *ovalis*), **Group 3** (*Encyonema silesiacum*, *E. silesiacum* var. *silesiacum*, *E. silesiacum* cf. var. *silesiacum*, *E. silesiacum* var *latum*), **Group 4** (*Fragilaria vaucheriae*, *F. capucina* var. *vaucheriae*), **Group 5** (*Gomphonema pumilum*, *G. pumilum* var. *pumilum*, *G. pumilum* cf. var. *pumilum*, *G. pumilum* var. *rigidum*) and **Group 6** (*Reimeria sinuata*, *R. sinuata* var. *sinuata*, *R. sinuata* cf. var. *sinuata*).

Tab. 8: Relevant counting parameters of each count from the participants (lab 1-37) and auditors (lab 38-42, shaded in green) for sample D 2, i.e. number of counted diatom-objects (objects), number of identified taxa during the count (NTC), during the search for rare taxa after the count (NTS; note that for sample D 2 this search was not required as D 2 was a running water sample) and with an abundance >1 % (NT>1) and sum of the relative abundance of all ambiguously determined diatom-objects, i.e. taxa labelled with cf., aff., spec or pennales (% cf). Also given are the Bray-Curtis-distances of the participants compared to auditor 38 (Diff 1), 39 (Diff 2) and 42 (Diff 3) for sample D 2. Red and bold Bray-Curtis-distances were outside the 95 %-confidence intervals. lab = laboratory-code. For more information see text.

lab	objects	NTC	NTS	NT>1	% cf	Diff 1	Diff 2	Diff 3
1	400	20	0	15	13.5	0.3675	0.3369	0.4651
2	402	34	0	13	19.9	0.5054	0.5409	**0.6259**
3	515	28	0	15	0.2	0.4600	0.4466	0.5389
4	407	30	2	15	0.7	0.3442	0.3619	0.4790
5	463	35	0	12	2.4	0.4557	0.4449	**0.6344**
6	400	33	0	15	0.0	0.3639	0.3333	0.4440
7	500	35	10	15	3.6	0.4677	0.4992	**0.6421**
8	400	32	9	17	9.3	0.3971	0.4040	0.5345
9	410	23	0	10	1.0	0.2963	0.2327	0.3943
10	639	42	0	16	0.3	**0.8526**	**0.8470**	**0.8560**
11	400	21	0	13	0.0	0.3366	0.2902	0.4122
12	400	25	8	13	11.3	0.5297	0.5461	**0.6569**
13	404	32	0	13	5.7	0.3832	0.4122	0.4982
14	459	36	0	13	0.0	0.3341	0.3314	0.4719
15	402	20	0	10	0.5	**0.6048**	**0.6010**	**0.6559**
16	405	38	6	9	0.7	0.4225	0.4876	0.5507
17	404	24	3	12	0.2	0.4770	0.4869	**0.5880**
18	400	28	3	13	5.3	0.4215	0.4650	0.4968
19	480	22	0	14	20.0	**0.8253**	**0.8312**	**0.8167**
20	444	24	1	16	0.0	**0.5802**	**0.5585**	**0.6309**
21	517	30	0	16	7.7	**0.5921**	**0.6012**	**0.5802**
22	412	24	0	10	8.5	0.3734	0.4084	0.4215
23	407	30	3	10	2.7	0.5109	0.5314	**0.6316**
24	400	26	3	12	4.3	0.3449	0.2774	0.4211
25	431	22	11	8	3.5	0.4409	0.4529	0.4538

Continuation of Tab. 8

26	427	21	0	9	0.0	0.3744	0.3668	0.3782
27	431	25	0	10	0.2	0.3335	0.2516	0.4433
28	402	14	0	8	0.0	**0.7600**	**0.7289**	**0.7966**
29	424	25	5	11	0.0	0.2920	0.2753	0.4756
30	446	28	4	13	0.4	**0.5810**	**0.5757**	**0.7294**
31	548	30	5	14	1.8	0.5499	**0.5760**	**0.6132**
32	407	24	0	9	1.0	0.2792	0.2699	0.4003
33	400	28	0	12	0.0	0.2881	0.2526	0.4716
34	443	39	0	12	4.1	0.2819	0.3180	0.4165
35	435	28	5	10	0.7	0.5389	**0.5529**	**0.6094**
36	487	23	0	13	0.0	**0.5709**	0.5400	**0.6442**
37	400	27	3	13	0.5	0.5068	**0.5523**	**0.6170**
38	406	35	0	11	3.2	0	0.2630	0.4363
39	412	30	6	11	0.5	0.2630	0	0.4092
42	503	22	6	12	15.5	0.4363	0.4092	0

The results of the auditors for sample D 2 agree well with one another (Tab. 7) with only some variance among slides (see Chapter 3.1.1). Thus, the auditor results represent a sound basis for the statistical evaluation of the results of the participants of the intercalibration exercise of sample D 2. For the assessment of the auditors standard deviations see Chapter 3.1.1.

For the participants taxonomic problems mainly occurred during the identification of *Achnanthidium minutissimum* var. *minutissimum*, *A. pyrenaicum*, *Encyonema silesiacum*, *E. minutum* and *Fragilaria capucina* var. *vaucheriae* in sample D 2. The fundamental taxonomic problems that occurred in sample D 2 are discussed in detail in Chapter 3.2.

Nine of 37 participating laboratories identified all taxa unambiguously in sample D 2 (Tab. 8), i.e. they did not indicate (with a cf.) identification problems for any of their listed taxa. For nine laboratories and one auditor the number of taxa that could not be determined (spec, pennates) and/or could not unambiguously be determined (cf., aff.) exceeded 5 %, with a maximum of 20.0 % (laboratory 19) (Tab. 8). Taxonomic uncertainties (cf., aff., spec.) were not part of the evaluation of the laboratories and are further discussed in Chapters 3.3 and 5.

The similarity among counting results (relative abundances of the diatom objects) was assessed using the Bray-Curtis-distance. The average Bray-Curtis-distance of the counting

results among auditors was 0.3695 with a standard deviation of 0.0932 and thus a 95 %-confidence interval of 0.1868 to 0.5522 in sample D 2. If the Bray-Curtis-distance of a laboratory to all three auditors is within this confidence interval, then the result of the laboratory is as similar to the auditor results as the auditor results among each other. If a Bray-Curtis-distance of a laboratory is outside the confidence interval of an auditor, the distance is marked red and bold in Tab. 8. For more information see Chapter 2.

The evaluation of the counting results of each laboratory is based on the Bray-Curtis distances. Only laboratories that were outside of the confidence intervals of all three auditors were assessed as inadequate (marked "unsuccessful" on the certificate) to tentatively interpret the limits of the confidence intervals and to account for the slight variation among auditor results. Thus, the counting results of the seven laboratories 10, 15, 19, 20, 21, 28 and 30 had to be marked as "unsuccessful" on the certificates for sample D 2 (Tab. 8).

The results of the Bray-Curtis-distances were verified with another, independent method, a multivariate figure (DCA, Detrendet Correspondence Analysis). Fig. 3 displays the first and second axis and confirms the results of the Bray-Curtis-distances when also reviewing the third dimension (not shown) for sample D 2. The samples of the auditors are the red numbers. The samples of the participants that are not marked agree well with the results of all three auditors based on the Bray-Curtis-distances. The green-rimmed samples of the participants were outside the confidence- intervals of two auditors (Fig. 3). These results were still labelled "successful" on the certificates, even though taxonomic discrepancies were obvious. The red-rimmed samples of the participants were outside the confidence-intervals of all three auditors based on the Bray-Curtis-distances (Tab. 8, Fig. 3).

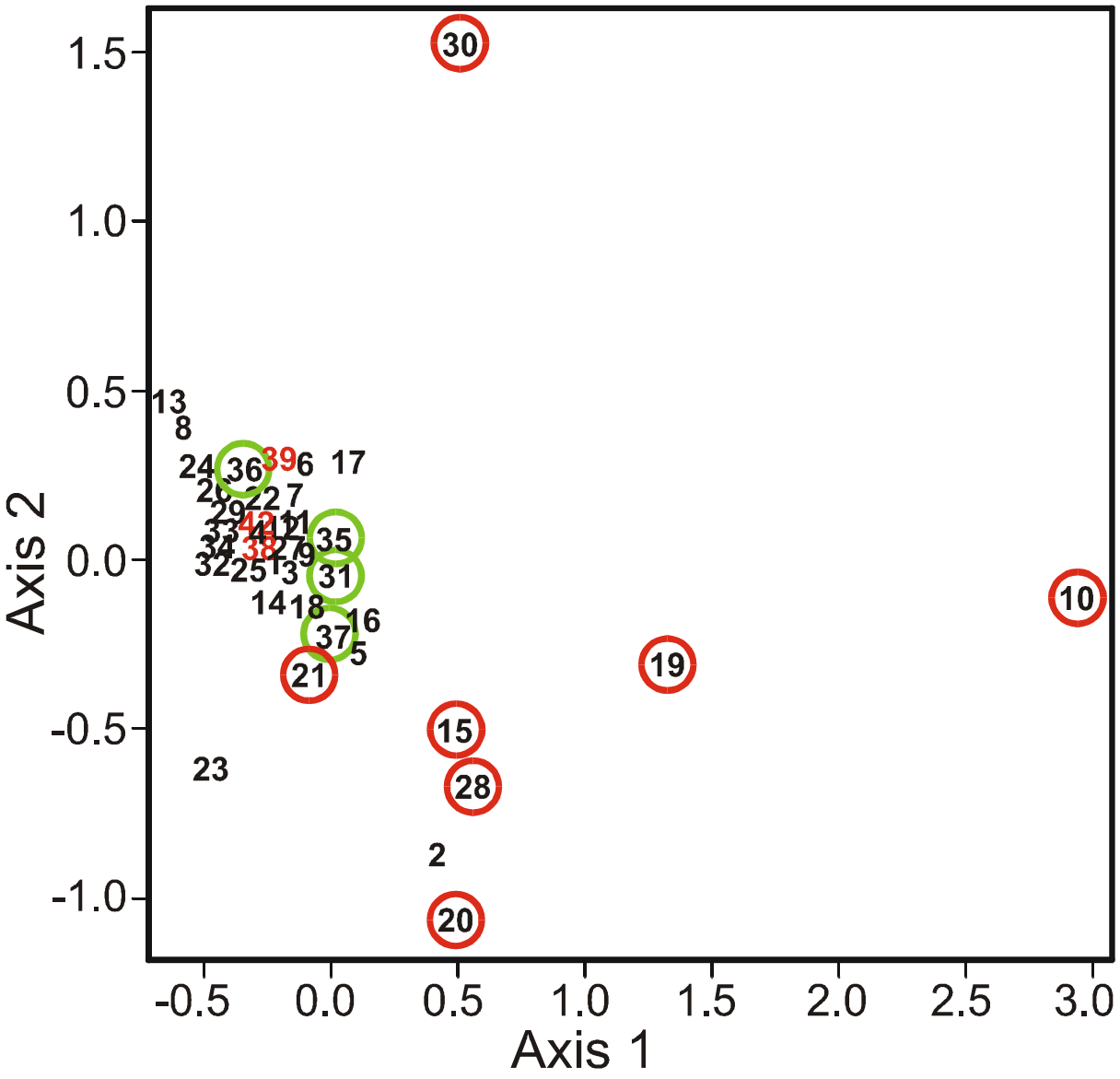


Fig. 3: First and second axis of the DCA based on the diatom results of all participants and auditors of sample D 2 (Alps/Alpine foothills River Drau). Numbers correspond to the laboratory-codes. Red numbers = laboratory codes of the auditors. The green-rimmed samples of the participants were outside the 95 % confidence-intervals of two auditors, red-rimmed samples were outside the confidence-intervals of three auditors based on the Bray-Curtis-distances. For more information see text.

In contrast to lake samples (Schaumburg et al. 2011), the instruction protocol does **not** stipulate for running water samples that the sample must be screened for rare taxa after the regular count (Schaumburg et al. 2006). However, 16 of 37 participating laboratories did search for rare taxa or listed some taxa with an abundance of „0“ in the result tables (Tab. 8). All laboratories counted at least 400 objects as was mandatory according to the instruction protocol (Schaumburg et al. 2006) in sample D 2 (Tab. 8).

The results of laboratories 2, 8, 10, 15, 19, 23, 30 clearly indicate that the taxa denotations did not correspond to the taxa names of the most recent identification literature that was mandatory for this intercalibration exercise according to the relevant instruction protocol. This deviation from the mandatory instruction protocol was also noted on the certificates.

Apart from the mentioned taxonomic problems and the deviations from the instruction protocol there was a high variability among counting results based on other parameters. For example, the number of counted taxa varied distinctly with 14 to 42 taxa identified by the participants in sample D 2. The auditors identified 35, 30 and 22 taxa during the regular count (Tab. 8). Similarly, the number of taxa contributing to more than 1% relative abundance per count ranges from 8 to 17 taxa (auditors: 11-12; Tab. 8). These parameters further emphasize the variability among results for one sample. However, they were not used for the assessment of the laboratories.

3.2 Details of taxonomic problems

3.2.1 *Achnanthidium pyrenaicum* and *Achnanthidium minutissimum* in sample D 2

The results of the 37 participants of the intercalibration exercise indicate that there were some taxonomic problems with identifying *Achnanthidium pyrenaicum* (Hustedt) Kobayasi (Plate 1: 1-4) and *Achnanthidium minutissimum* var. *minutissimum* (Kützing) Czarnecki (Plate 1: 5-8) in sample D 2. The sum of all *Achnanthidium*- and *Achnanthes*-taxa (20.0-70.3 %, average 48.5 %) (Fig. 4) was mainly dominated by *Achnanthidium minutissimum* var. *minutissimum* (including *Achnanthes minutissima* var. *minutissima*) with 0.0-52.2 % (average 21.2 %) (Fig. 5) and *Achnanthidium pyrenaicum* with 0.0-27.6 % (average 10.9 %) (Fig. 6). The auditors identified *Achnanthidium minutissimum* var. *minutissimum* (35.7-42.7 %, average 40.3 %) and *Achnanthidium pyrenaicum* (9.2-13.7 %, average 11.3 %) in good agreement to each other (Figs. 5 and 6).

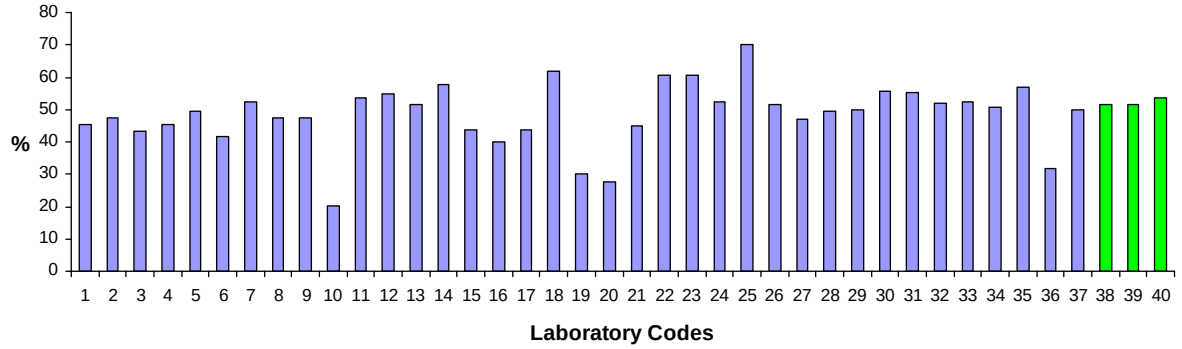


Fig. 4: Sum of the relative abundances of **all *Achnanthes* and *Achnanthidium*-taxa** in sample D 2, as identified by the participants (blue bars) and auditors (green bars).

Of 37 participants thirteen participants identified *Achnanthes minutissima* or *Achnanthidium minutissimum* without any further differentiation of the varieties (2.8-49.5 %, average 32.3 %) instead of *Achnanthidium minutissimum* var. *minutissimum* (Fig. 7). One participant (laboratory 18) counted *Achnanthidium minutissimum* var. *minutissimum* in addition to *Achnanthidium minutissimum* without any further differentiation of the varieties (Fig. 7).

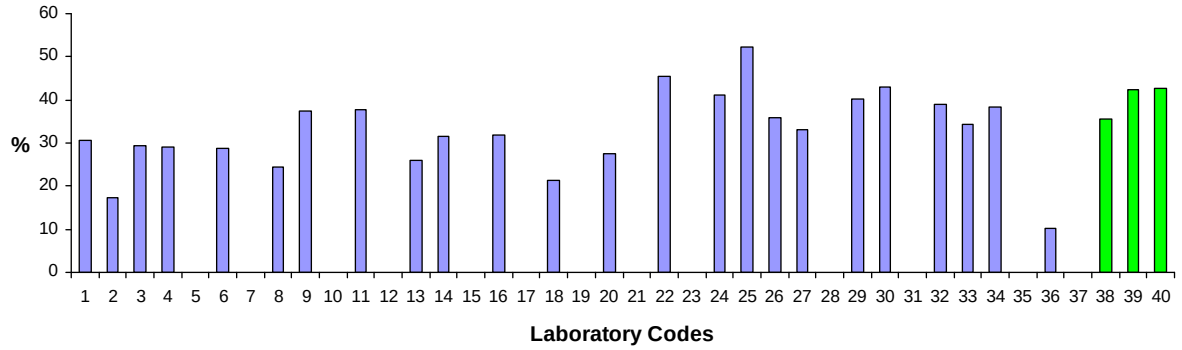


Fig. 5: Sum of the relative abundances of ***Achnanthes minutissima* var. *minutissima*** and ***Achnanthidium minutissimum* var. *minutissimum*** in sample D 2, as identified by the participants (blue bars) and auditors (green bars).

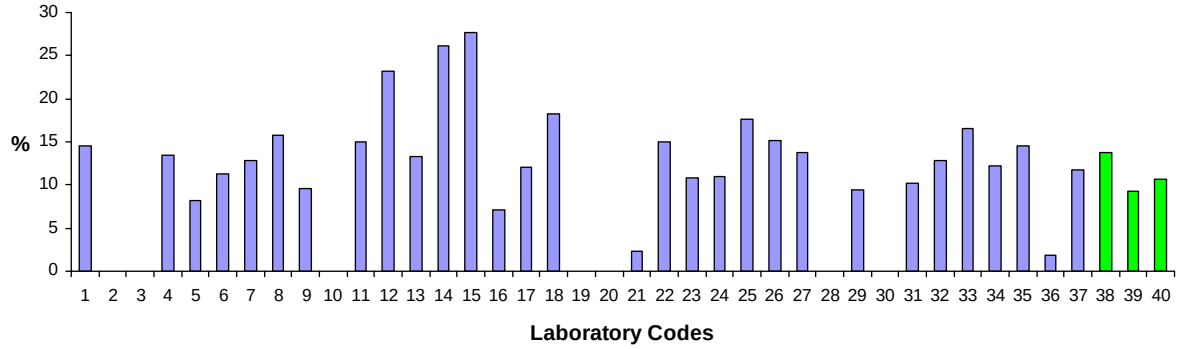


Fig. 6: Relative abundances of ***Achnanthidium pyrenaicum*** in sample D 2, as identified by the participants (blue bars) and auditors (green bars).

30 of 37 participants identified *Achnanthidium pyrenaicum* (1.8-27.6 %, average 13.4 %) (Fig. 6). Two additional participants (laboratory 21 and 36) identified *Achnanthidium pyrenaicum* in abundances that were distinctly below average (Fig. 6). Three laboratories detected *Achnanthes biasolettiana* (11.2 % laboratory 2 and 3.8 % laboratory 10) or *Achnanthes biasolettiana* var. *biasolettiana* (11.7 % laboratory 30) Fig. 8) instead of *Achnanthidium pyrenaicum*. *Achnanthes biasolettiana* var. *biasolettiana* is a synonym of *Achnanthidium pyrenaicum*. According to old literature (e.g. Krammer & Lange-Bertalot 1986-2004) these three laboratories would have named the taxon correctly. However, in the accompanying letter and in the instructions of the German implementation of the EU-water framework directive (Schaumburg et al. 2011) the mandatory identification literature (Hofmann et al. 2011) was listed. Thus, these laboratories deviated from the binding instruction protocol. Additionally, laboratories 2 and 10 did not identify the varieties of *Achnanthes biasolettiana*. This is problematic, because two different species may represent *Achnanthes biasolettiana* (*Achnanthidium pyrenaicum* and *Achnanthidium subatomus*) according to Krammer & Lange-Bertalot (1986-2004), with differing ecological preferences. Thus, the water evaluation based on these results may be impeded or distorted (see Chapter 3.3).

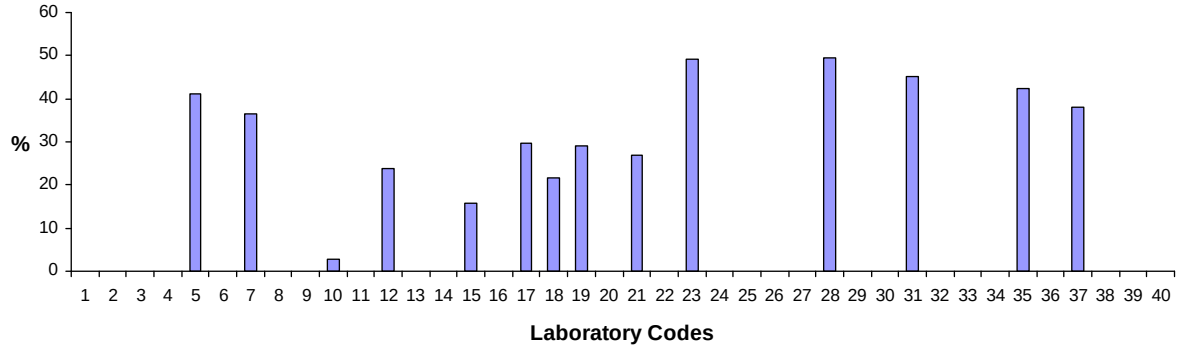


Fig. 7: Sum of the relative abundances of ***Achnanthes minutissima*** and ***Achnanthidium minutissimum*** without any differentiation of the varieties in sample D 2, as identified by the participants (blue bars) and auditors (no. 38-40).

Of the laboratories that did not identify any *Achnanthidium pyrenaicum* or only in very low abundances the laboratories 2, 3, 30 and 36 counted *Achnanthes* spec., *Achnanthidium* spec., *Achnanthes biasolettiana*, *Achnanthes biasolettiana* var. *biasolettiana*, *Achnanthidium eutrophilum* (Lange-Bertalot) Lange-Bertalot, *Achnanthes petersenii* Hustedt, *Achnanthes pusilla* Grunow, *Achnanthidium minutissimum* var. *jackii* (Rabenhorst) Lange- Bertalot and *Achnanthidium* cf. *minutissimum* var. *jackii* (next to *Achnanthidium minutissimum* var. *minutissimum*) (Fig. 8). Thus, *Achnanthidium pyrenaicum* was very likely mistaken for these taxa. Three other laboratories (19, 20, 28) that did not report *Achnanthidium pyrenaicum* only identified *Achnanthidium minutissimum* without any further differentiation of the varieties. Thus, they seem to have counted *Achnanthidium minutissimum* without any further differentiation of the varieties instead of *Achnanthidium pyrenaicum* and *Achnanthidium minutissimum* var. *minutissimum*. Laboratories 10 (no *Achnanthidium pyrenaicum*) and 36 (very sparse *Achnanthidium pyrenaicum*) identified *Achnanthes biasolettiana* (laboratory 10), *Achnanthes petersenii* (laboratory 10), *Achnanthidium minutissimum* var. *jackii* (laboratory 36) and *Achnanthidium minutissimum* (laboratories 10 and 36) without further differentiation. Also, laboratory 10 generally identified distinctly below average abundances of *Achnanthes*- or *Achnanthidium*-taxa.

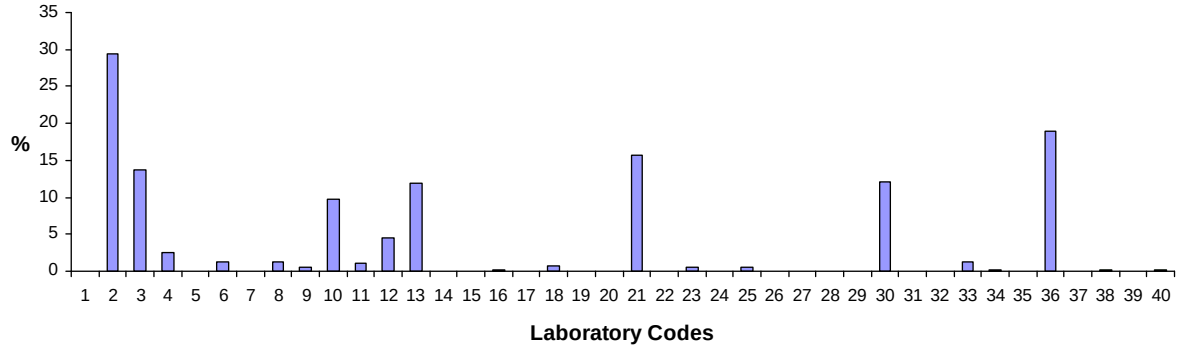


Fig. 8: Sum of the relative abundances of *Achnanthes* spec., *Achnanthidium* spec., all here listed taxa with cf., *Achnanthes biasolettiana*, *Achnanthes biasolettiana* var. *biasolettiana*, *Achnanthidium eutrophilum*, *Achnanthes petersenii*, *Achnanthes pusilla*, *Achnanthidium minutissimum* var. *jackii* and *Achnanthidium* cf. *minutissimum* var. *jackii* in sample D 2, as identified by the participants (blue bars) and auditors (green bars, i.e. no. 38-40).

Overall, only 17 of the 37 participating laboratories identified *Achnanthidium pyrenaicum* and *Achnanthidium minutissimum* var. *minutissimum* in abundances that are in good agreement with the abundances of the auditors. These were the laboratories 1, 4, 6, 8, 9, 11, 14, 16, 22, 24, 25, 26, 27, 29, 32, 33 and 34. Also, laboratories 13 and 18 counted *Achnanthidium pyrenaicum* and *Achnanthidium minutissimum* var. *minutissimum* in similar abundances to the auditors. However, they also identified *Achnanthidium minutissimum* var. *jackii* (laboratory 13) and *Achnanthidium minutissimum* without indicating a further differentiation of the varieties (laboratory 18). The remaining 18 laboratories seem to have substantial identification problems with the two discussed species with possible faulty ecological assessments as a consequence (Chapter 3.3). For example, laboratories 2, 3, 20 and 30 only identified *Achnanthidium minutissimum* var. *minutissimum*, but no *Achnanthidium pyrenaicum*, while laboratories 5, 7, 12, 15, 17, 21, 23, 31, 35 and 37 detected *Achnanthidium pyrenaicum*, but no *Achnanthidium minutissimum* var. *minutissimum*. Laboratories 10, 19 and 28 had the biggest problems, as they did not find either *Achnanthidium pyrenaicum* or *Achnanthidium minutissimum* var. *minutissimum*.

Thus, this intercalibration exercise determined at least two problematic areas for identifying *Achnanthidium pyrenaicum* and *Achnanthidium minutissimum* var. *minutissimum*. For one, *Achnanthidium pyrenaicum* was mistaken for *Achnanthidium minutissimum* var. *minutissimum* or other taxa (Fig. 8) and secondly the differentiation of the varieties of *Achnanthidium minutissimum* were taxonomically problematic. Additionally problematic was

the use of old taxonomic concepts by some participants (e.g. *Achnanthes* instead of *Achnanthidium*), which was probably a consequence of using old identification literature (e.g. Krammer & Lange-Bertalot 1986-2004). However, the binding identification literature was clearly stated in the accompanying letter and the instruction protocol (Schaumburg et al. 2011). As the standard reference was Hofmann et al. (2011) *Achnanthidium pyrenaicum* must be named as such and not labelled *Achnanthes biasolettiana* as done by laboratories 2, 10 and 30.

In the following we discuss how to differentiate *Achnanthidium pyrenaicum*, *Achnanthidium minutissimum* var. *minutissimum*, *Achnanthidium eutrophilum*, *Achnanthes petersenii*, *Achnanthes pusilla* and *Achnanthidium minutissimum* var. *jackii* as this was problematic in sample D 2. Additionally we refer to *Achnanthidium subatomus* that was not identified in this intercalibration exercise, because *Achnanthidium subatomus* is very similar to *Achnanthidium pyrenaicum*. *Achnanthidium straubianum* will be mentioned, as it occurred with very low abundances in sample D 2 and as it may also be confused with the taxa discussed here (Tab. 9).

It is possible to confuse the two most abundant taxa of sample D 2 with one another, i.e. *Achnanthidium pyrenaicum* and *A. minutissimum* var. *minutissimum*. Their size-range overlap and thus small valves of *Achnanthidium pyrenaicum* may be mistaken for big valves of *A. minutissimum* var. *minutissimum*. However, it should be possible to keep the confusion to a feasible minimum, if all characteristics are taken into account, especially the increasing striae density towards the poles in *Achnanthidium pyrenaicum*.

Tab. 9: Characteristic features of *Achnanthidium pyrenaicum* and *Achnanthidium minutissimum* var. *minutissimum* and similar or potentially associated taxa. Length and width in µm; R = raphe valve, RL = rapheless valve, striae density was identical between R and RL, if only one measure is given. For further explanations see text.

	length	width	striae/10µm	comment
Achnanthidium pyrenaicum	6-35	3-6	R: (15) 20-27 (40); RL: ø 18-24	striae-density
Achnanthidium subatomus	6-35	3-6	R: (15) 20-27 (40); RL: ø 18-24	shape, areolae visible
Achnanthidium minutissimum var. *minutissimum*	5-25	2.5-4	~30	
Achnanthidium minutissimum var. *jackii*	5-25	2.5-4	~26	
Achnanthidium eutrophilum	4-19	3-5	23-27	shape, length to width ratio
Achnanthidium straubianum	6-10	3-4	25-29	shape, length to width ratio
Achnanthes petersenii	8.5-42.5	4-5	26-36	shape, striae orientation
Achnanthes pusilla	8.5-18	3.5-4.5	18-23	shape, striae orientation

Achnanthidium subatomus differs from *A. pyrenaicum* by their elliptic to linear-elliptic shape without protracted ends and by their visible areolae on the striae (using a light microscope), at least on the rapheless valve (not visible on *A. pyrenaicum*).

Achnanthidium minutissimum var. *minutissimum* is still seen as a heterogeneous taxonomic complex (Hofmann et al. 2011), which will continue to cause identification problems. Hofmann et al. (2011) list some of the similar taxa. *Achnanthidium minutissimum* var. *jackii* differs from the type variety mainly by striae that are further apart. *Achnanthidium eutrophilum* has a higher length to width ratio compared to *Achnanthidium minutissimum* var. *minutissimum*, a more rhombic shape and always an isolated stria in the middle of the raphe valve. Within this taxonomic complex *Achnanthidium straubianum* is comparatively easy to identify, because it is better defined with a hardly varying shape and a narrowly defined length to width ratio (Tab. 9).

Achnanthes petersenii and *Achnanthes pusilla* were also mentioned in the intercalibration exercise that can be confused with *Achnanthidium linearioides*. All three taxa are characterised by a linear-elliptic shape with broadly rounded ends. Their striae density differ with *Achnanthes petersenii* having 26-36 striae/10µm, *Achnanthes pusilla* with 18-23

striae /10 µm and *Achnanthidium linearioides* with 24-28 striae/10. Additionally *Achnanthes petersenii* is characterised by a wide central area that usually reaches the margin on the raphe valve.

Many other related taxa could be discussed here. However, we will just mention a few exemplary taxa in the following that might be problematic as well: *Achnanthidium saprophilum* (Kobayasi & Mayama) Round & Bukhtiyarova, *Achnanthidium affine* (Grunow) Czarnecki, *Achnanthidium exile* (Kützing) Heiberg, *Achnanthidium kranzii* (Lange-Bertalot) Round & Bukhtiyarova, *Achnanthidium rivulare* Potapova & Ponader and *Achnanthidium pfisteri* Lange-Bertalot.

Conclusively, we can generally suggest to always incorporate the entire combination of characteristics when identifying the here mentioned taxa. Hofmann et al. (2011) recommend to refrain from differentiating *Achnanthidium minutissimum* var. *minutissimum* and *Achnanthidium minutissimum* var. *jackii* for the water quality assessment. However, this is problematic when using the Phylib-Software, because both species have different indicator values (see Chapter 3.3). We recommend to differentiate as much as possible when counting such a sample and to document both taxa with pictures. This will enable an adjusted assessment of the critical samples later, i.e. once e.g. ecological preferences or taxonomic differences are clarified.

Hofmann et al. (2011) recommend to use the water quality or the composition of the associated diatom assemblage as an additional tool to identify *Achnanthidium eutrophilum*. We consider this to be a circular argument and in practice very difficult, as the diatomist usually either does not know the water quality or is just trying to identify the water quality by using the diatom assemblage. Also, the associated diatom-taxa do not always permit a clear allocation to the water quality. Here too, we suggest to photographically document the valves that are especially difficult to allocate to one species to enable a later assessment of the samples and to label these valves with cf.

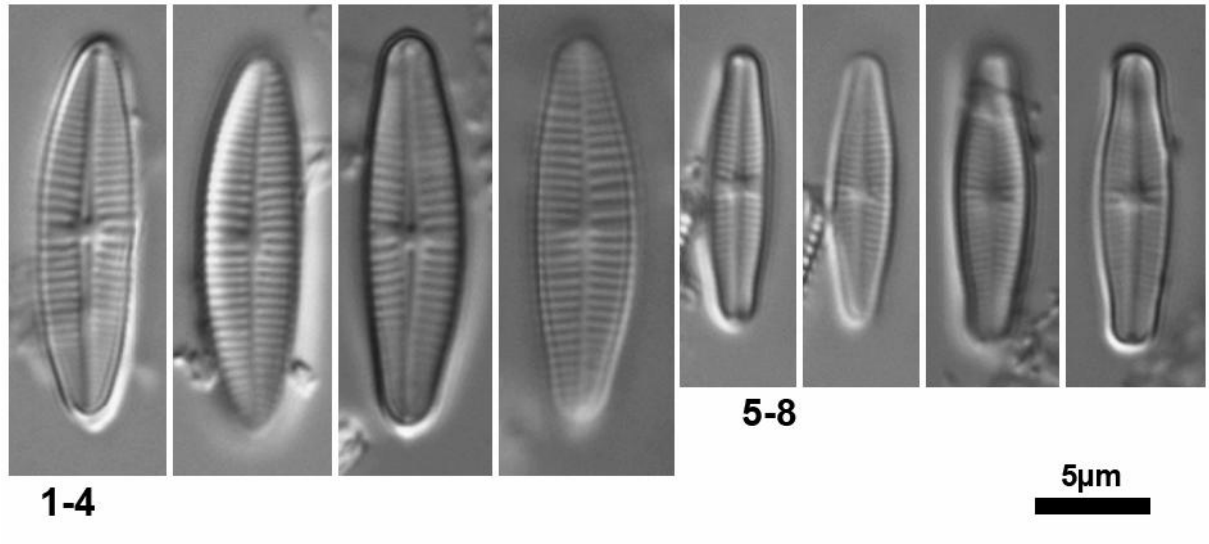


Plate 1: Light microscopic images of *Achnanthidium pyrenaicum* (1-4) and *Achnanthidium minutissimum* var. *minutissimum* (5-8) from sample D 2 (River Drau, Austria).

3.2.2 Small *Amphora*-species in sample D 11

Considerable problems occurred with identifying small species of the genus *Amphora* Ehrenberg (here *A. inariensis* Krammer, *A. indistincta* Levkov and *A. pediculus* (Kützing) Grunow) in samples D 11 and D 1.1. As the counting results of these *Amphora* species were very similar between samples D 11 and D 1.1, we only present and discuss the results of sample D 11 in the following.

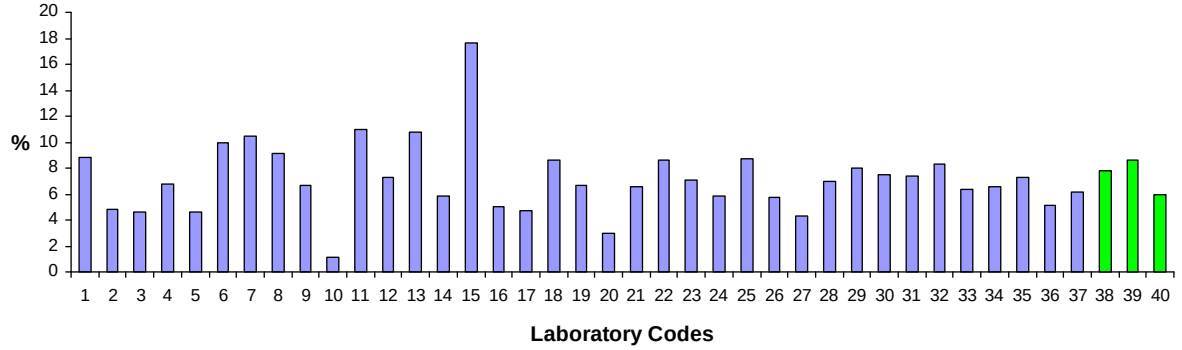


Fig. 9: Sum of the relative abundance of *Amphora inariensis*, *A. indistincta* and *A. pediculus* from sample D 11 (including spec. and cf.) from the participants (blue bars) and the auditors (green bars).

Overall, the sum of small *Amphora*-species were similar among participants (1.1-17.6 %, average 7.2 %) and auditors (6.0-8.7 %, average 7.5 %) and also among auditors in sample D 11 (Fig. 9). Just the results of laboratory 10 (1.1 %) and laboratory 15 (17.6 %) deviated distinctly from the rest. However, distinct differences occurred when each species is looked at by itself. All participants (0.8-17.6 %, average 6.6 %) and auditors (average 3.3 %) identified *Amphora pediculus* in sample D 11 (Fig. 10). However, relative abundances of *A. pediculus* of the three auditors varied distinctly with 7.8 %, 1.2 % and 0.9 %, respectively. Complementary, the auditors identified none, 7.3 % and 3.8 % of *A. indistincta* in sample D 11. Of the participants only five laboratories detected *A. indistincta* in sample D 11 (Fig. 11).

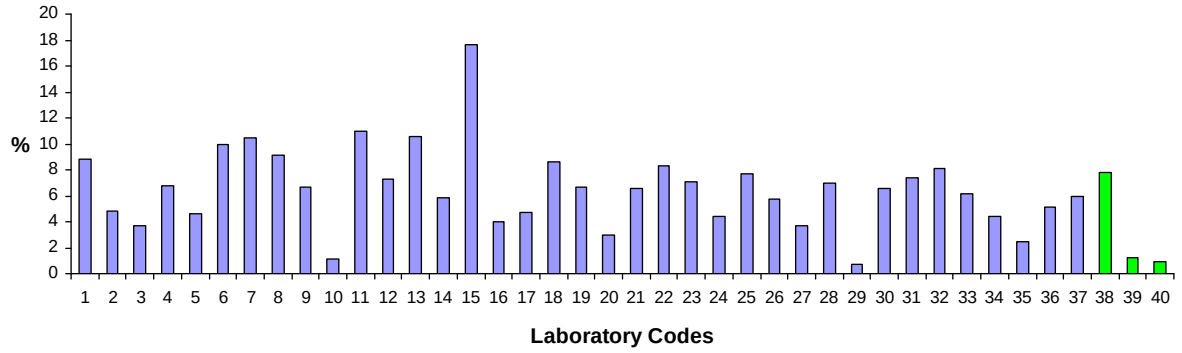


Fig. 10: Relative abundance of ***Amphora pediculus*** from sample D 11 as counted by each participant (blue bars) and auditor (green bars).

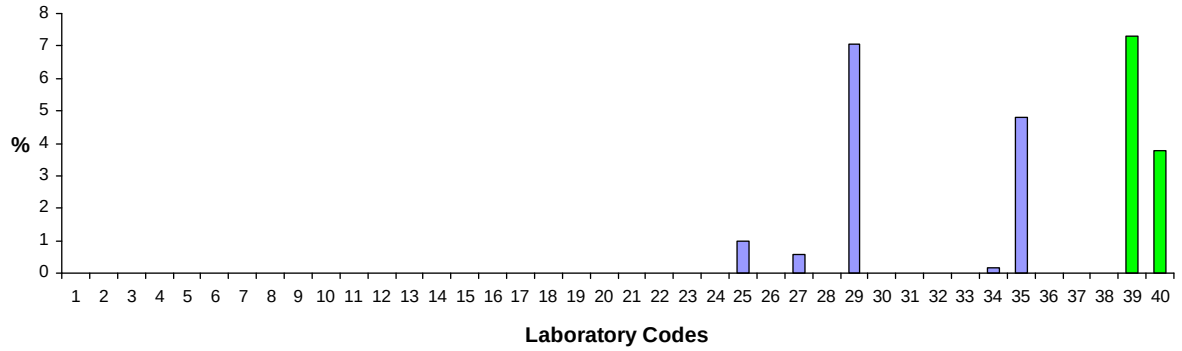


Fig. 11: Relative abundance of ***Amphora indistincta*** from sample D 11 as counted by each participant (blue bars) and auditor (green bars).

Another substantial problem was that 33 of the 37 participants and one auditor did not brand any valves of the small *Amphora*-species as „spec.“ or „cf.", i.e. according to their results they identified the species with certainty. Just four laboratories and two auditors branded some

valves as „spec.“ or „cf." (Fig. 12), i.e. they were uncertain about the identity of some valves from this taxonomic group. Several valves of the small *Amphora*-species were definitively present on many slides that do not fit any *Amphora*-taxa description in Hofmann et al. (2011) or Levkov (2009) in sample D 11 (see Plate 2 and see below).

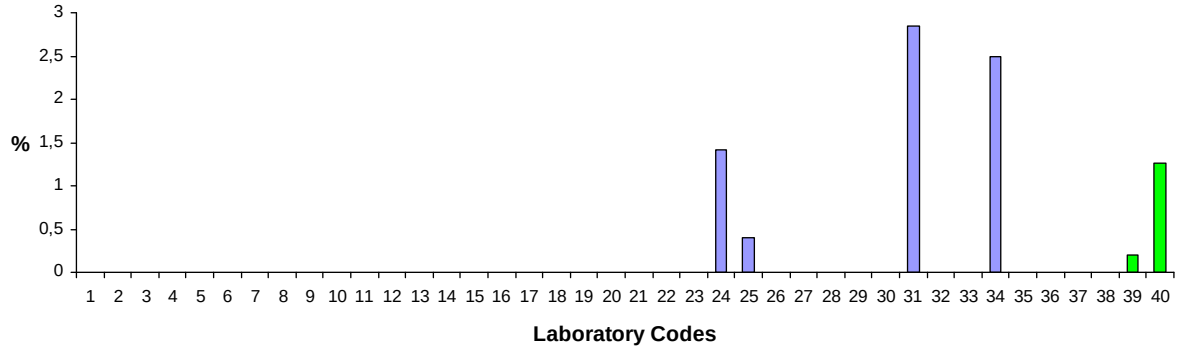


Fig. 12: Sum of the relative abundance of ***Amphora* spec**. and all small ***Amphora***-taxa that were labelled with "**cf.**" from sample D 11 as counted by the participants (blue bars) and auditors (green bars).

Several participants identified other small *Amphora*-species (e.g. *A. inariensis*, *A. eximia* Carter and *A. aequalis* Krammer) than the above described taxa in sample D 11. However, these taxa were rare, i.e. they occurred to less than one percent. Thus, the presence or absence during a routine count is dependent on chance and can not be attributed to an identification or counting error.

Overall, some *A. indistincta* may have been mistaken for *A. pediculus* and the results were potentially presented with more certainty than in reality present. For example, 30 participating laboratories identified solely valves of *A. pediculus* in sample D 11 without indicating any uncertainties of identification. See Chapter 5 for a discussion on how to deal with taxonomic uncertainties (cf., aff. and spec.)

Differentiating the small *Amphora*-species:

The striae from *A. pediculus* are visibly punctuated under the light microscope in contrast to *A. indistincta*, *A. inariensis* and *A. subatomus* (Tab. 10). The ends of *A. indistincta* are never bend ventrally, the ends of *A. pediculus* are rarely bend ventrally. The branches of the raphe are bend towards the central area in *A. indistincta* and almost straight (and not centered) in

A. pediculus. However, the latter trait is not always clearly visible when using the light microscope.

A. inariensis and *A. minutissima* have a lower striae density and a ventrally bulbous shape in contrast to the other three discussed *Amphora*-species (Tab. 10). *A. subatomus* resembles *A. inariensis* in many traits, but is smaller and has a higher striae density. *A. indistincta* and *A. subatomus* differ in shape, e.g. *A. subatomus* has pointy, rounded and ventrally bend ends (Tab. 10).

Next to the here discussed small *Amphora*-species there are other taxa in Central Europe that need to be considered when identifying *Amphora*-species, especially as they are not included in Hofmann et al. (2011) (see Hofmann et al. 2011 and Levkov 2009).

Tab. 10: Measureable dimensions and some comments for differentiating small *Amphora*-species relevant to the results of the intercalibration exercise. Given length and width (in µm) describe measures of one valve, not of one frustule. Measures according to Hofmann et al. (2011) and Levkov (2009).

taxon	length	width	dorsal striae/10µm	comment
A. inariensis	15-28	3.5-6	15-17	ventrally bulbous
A. minutissima	13-20	3.5-4.5	17-18	ventrally bulbous, pointy ends
A. indistincta	6-20	3-4	18-22	shape
A. pediculus	7-15	2.5-4	18-24	areolae visible on dorsal striae
A. subatomus	9-20	2-3	18-22	size, shape

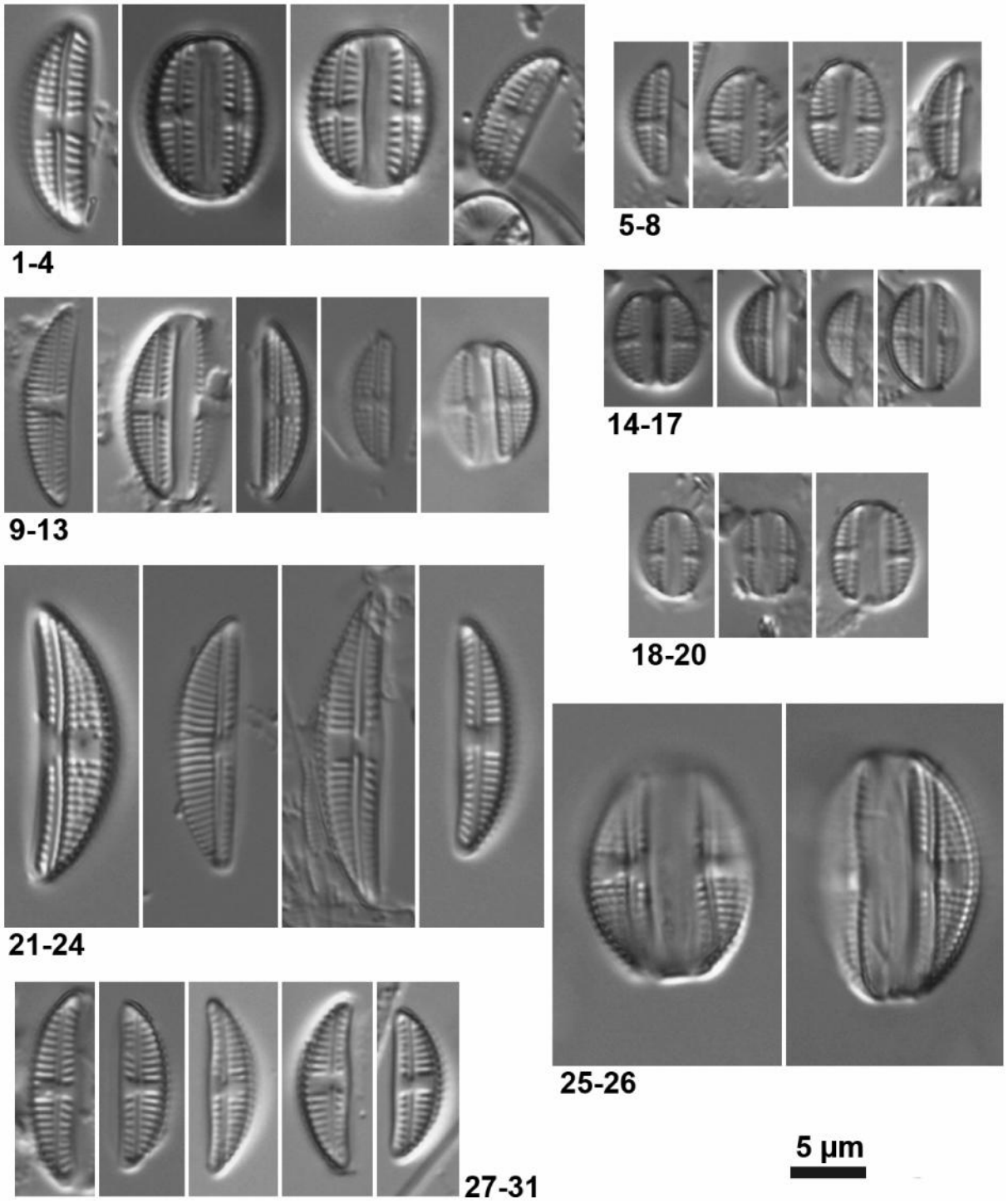


Plate 2: Images of small *Amphora*-taxa from sample D 11 taken with a light microscope (Lake Krossinsee, Northern Germany). **1-4**: *A. indistincta*; **5-8**: *A.* cf. *indistincta*; **9-13**: *A. pediculus*; **14-17**: *A.* cf. *pediculus*; **18-20**: *Amphora* spec.; **21**: *A.* cf. *copulata*; **22**: *A. eximia*; **23**: *A. inariensis*; **24**: *A.* cf. *subatomus*; **25**: *A. minutissima*; **26**: *A.* cf. *minutissima*; **27-31**: *A.* cf. *subatomus*.

On Plate 2 valves 5-8 are labelled *Amphora* cf. *indistincta*, as all traits mainly resemble *A. indistincta* (Plate 2: 1-4), but most valves are too small (1.9-2.9 µm instead of 3.0-4.0 µm) and the dorsal striae density is too high (e.g. mainly 24-25 instead of 18-22 striae/10 µm) for an *A. indistincta* according to the species description given in Levkov (2009) and Hofmann et al. (2011). Similarly, *A.* cf. *pediculus* (Plate 2: 14-17) is morphologically similar to *A. pediculus* (Plate 2: 9-13), as even the areolae on the dorsal striae are visible in the light microscope despite their small size. However, valves are too small (5.8-6.9 µm long instead of 7.0-15.0 µm and 1.9-2.3 µm wide instead of 2.5-4.0 µm) and striae density is too high (e.g. ≥25 dorsal striae/10 µm instead of 18-24) for an *A. pediculus*.

Valves 18-20 in Plate 2 are even smaller than 5-8 (*Amphora* cf. *indistincta*) and 14-17 (*A.* cf. *pediculus*) and further deviate from the species descriptions of *A. indistincta* and *A. pediculus*. Thus, they are labelled *Amphora* spec., as an allocation to a species is difficult.

Valves 21-26 (Plate 2) are also from sample D 11 and are mainly shown for comparison. Sometimes, it may be possible to confuse these taxa (*A.* cf. *copulata*, *A. eximia*, *A. inariensis*, *A.* cf. *subatomus*, *A. minutissima* and *A.* cf. *minutissima*) with the discussed small *Amphora*-species (see Tab. 10). However, distinction should be possible, if all traits are taken into account. The valve 21 (*A.* cf. *copulata*) (Plate 2) was marked with a „cf“, because the valve is slightly too short (18.7 µm long instead of 19-42µm) and has too many striae in 10 µm (dorsally 18 instead of 14-16) for an *A. copulata*. Similarly, the valve 26 (*A.* cf. *minutissima*) (Plate 2) was labelled with a „cf.“, as the valve is slightly too wide (with of valve = 4.8 µm instead of 3.5-4.5 µm), striae density is too high (dorsally 20 instead of 17-18) and the central area differs compared to *A. minutissima* (Levkov 2009).

All morphological traits and measurable dimensions (using a light microscope) of the valves 27-31 in Plate 2 correspond to *A. subatomus*. However, no electron microscope analysis was conducted and this species has so far only been found in Central Africa in Lake Tanganyika (Levkov 2009). Thus, for now we named the taxon *A.* cf. *subatomus*. The valve 24 (Plate 2) was also named *A.* cf. *subatomus* for the same reasons and because this valve is additionally slightly too wide (3.5 µm instead of 2.0-3.0 µm wide).

Overall, it seems very likely that the *Amphora*-taxa discussed here have wider morphological ranges than given in the identification literature (Levkov 2009, Hofmann et al. 2011), especially *A. indistincta* and *A. pediculus*, and to some extend also *A. copulata*, *A. inariensis* and *A. minutissima*. Thus, the valves 5-8 (*A.* cf. *indistincta*), 14-17 (*A.* cf. *pediculus*) and also 21 (*A.* cf. *copulata*) and 26 (*A.* cf. *minutissima*) (Plate 2) may actually belong to their

respective species. However, as long as further taxonomic and morphological examinations do not clarify the dimensional ranges, these valves should be counted with a "cf." and be documented with a picture. Also, they may be pooled (these species with and without cf.), but only when conducting the ecological assessment (see Chapters 3.3 and 5 for a further discussion).

The valves 18-20 (*Amphora* spec.) (Plate 2) remain problematic, as a reasonable allocation to a species is not possible due to their strongly deviating measurable dimensions. Here too, further morphological and taxonomical investigations are necessary for a definite identification. Also, the recent situation for the valves 24, 27-31 (*A.* cf. *subatomus*) and 26 (*A.* cf. *minutissima*) (Plate 2) are problematic. Both taxa are not contained in the most current Phylib-Software 4.1 (despite the common distribution of *A. minutissima* in Europe according to Levkov 2009). Consequently, they do not contribute to calculating the ecological status class (see Chapter 3.3 for a detailed discussion). However, both taxa are common in rivers and lakes of the North-German lowlands, i.e. the depicted valves are not rare findings (Dreßler, Werner; unpublished data). Thus, hopefully both taxa will be included in the Phylib-Software and their distribution and ecology will be further investigated (see Chapters 3.3 and 5). This would be particularly important for *A. subatomus* for clarifying, if for example the valves 24 and 27-31 (Plate 2) really belong to *A. subatomus* or if they represent a taxon that has not been described yet.

Recommendations:

Especially for differentiating small *Amphora*-species it is essential to consider the entire combination of characteristics. Similar to the discussed sample D 11 several different small-celled species of the genus *Amphora* can be present in the same sample. Thus, it may be necessary to repetitively validate the identity of several valves, if small *Amphora*-taxa are present in higher abundances. It is not sufficient to measure and identify just a few valves and allocate somewhat similar valves to these species, which some participants report as common in practise. Additionally, a lot of work has been done on the taxonomy of the genus *Amphora* during the past years. Hofmann et al. (2011) give a coarse overview of the genus *Amphora*, although not all taxa occurring in Germany are presented. Thus, it is necessary to use additional literature for identifying small *Amphora*-species (e.g. Levkov 2009).

Some authors recommend to use the ecological preferences of a species as an additional trait to help to identify a species (e.g. Hofmann et al. 2011) However, we recommend not to use the ecological preferences that are given in books, e.g. *A. indistincta* occurs in nutrient-

poor water, while *A. pediculus* has a wide trophic state amplitude according to Hofmann et al. (2011). These preferences are often not sufficiently verified and *A. indistincta* and *A. pediculus* often occur together despite the different ecological amplitudes given in Hofmann et al. (2011). Additionally, we use the diatom assemblage to infer the ecological status of the water body. Thus, using the ecology to identify species and then using the ecological preferences of the species to identify the ecological status would be a circular argument. Similarly to ecology, taxa distribution (water body or lake/river-type) according to literature should be ignored when identifying the species. Data on geographic distribution are as uncertain or possibly incomplete as the ecological preferences given in literature.

Another problem that is not restricted to small *Amphora*-species is the fact that some pictures do not correspond to the written descriptions of the species in the identification literature, as the given traits are not visible or do not match the description. For example, the visible areolae on the dorsal striae distinguish *A. pediculus* from other similar species. However, on most pictures in Hofmann et al. (2011) the areolae are not visible (see page 785). Similarly, *A. inariensis* is supposed to be at least 15 µm long. However, the picture on Plate 91, Figure 9 in Hofmann et al. (2011, p. 785) is only 11.3 µm long and often individual valves that look identical to *A. inariensis* are shorter than 15 µm. Thus, it is not sufficient to only use measurable values such as length and width of the valves and the pictures to identify diatoms. Verbal descriptions should also and always be considered during identifications.

3.2.3 *Cocconeis placentula*-aggregate and similar taxa in sample D 11

The biggest difficulty with the identification of *Cocconeis placentula* Ehrenberg in sample D 11 was the differentiation between the varieties *euglypta* (Ehrenberg) Grunow and *lineata* (Ehrenberg) van Heurck. Some problems also occurred relating to the type variety.

All 37 laboratories identified *C. placentula* including the varieties *euglypta*, *lineata* or *placentula* or just *C. placentula* without further differentiation (12.9-44.3 %, average 24.4 %) (Fig. 13). 19 laboratories detected *C. placentula* var. *lineata* (0.5-31.8 %, average 9.9 %) (Fig. 14) and 22 laboratories identified *C. placentula* var. *euglypta* (0.2-42.8 %, average 14.8 %) (Fig. 15). 14 laboratories detected *C. placentula* without further differentiation (2.8-33.9 %, average 21.9 %) (Fig. 16). Just three of these 14 laboratories indicated that they

detected at least one of the here mentioned varieties of *C. placentula*. Eleven laboratories identified *C. placentula* var. *placentula* (0.18-29.7 %, average 7.6 %) (Fig. 17), of which two (laboratories 15 and 23) did not detect any other varieties of *C. placentula*.

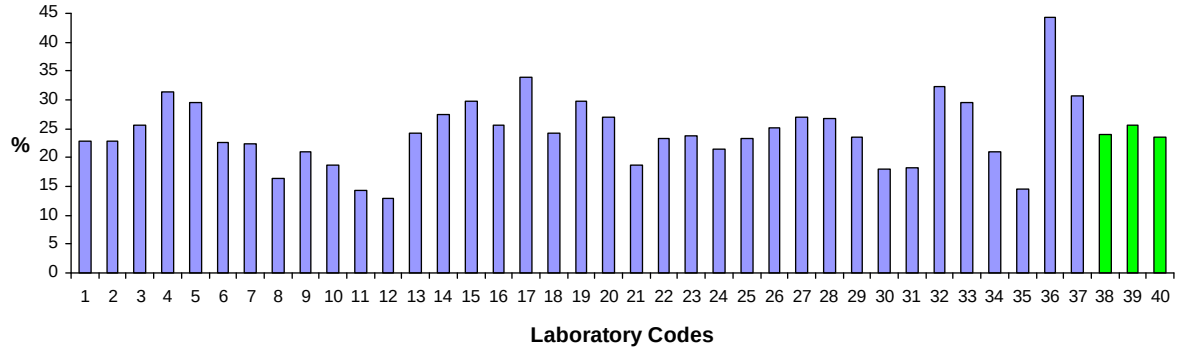


Fig. 13: Sum of the relative abundances of ***Cocconeis placentula* var. *lineata*, var. *euglypta*, var. *placentula*** and ***C. placentula*** without differentiation of the varieties identified in sample D 11 by the participants (blue bars) and the auditors (green bars).

The results of the auditors suggest that *C. placentula* was truly dominated by the varieties *euglypta* and/or *lineata* in sample D 11. Two auditors exclusively identified *C. placentula* var. *lineata* (25.5 % and 23.5 %, respectively). The third auditor detected 21.7 % *C. placentula* var. *euglypta* and 2.2 % *C. placentula* var. *lineata*. Other varieties than *euglypta* and *lineata* were not identified by the auditors in sample D 11.

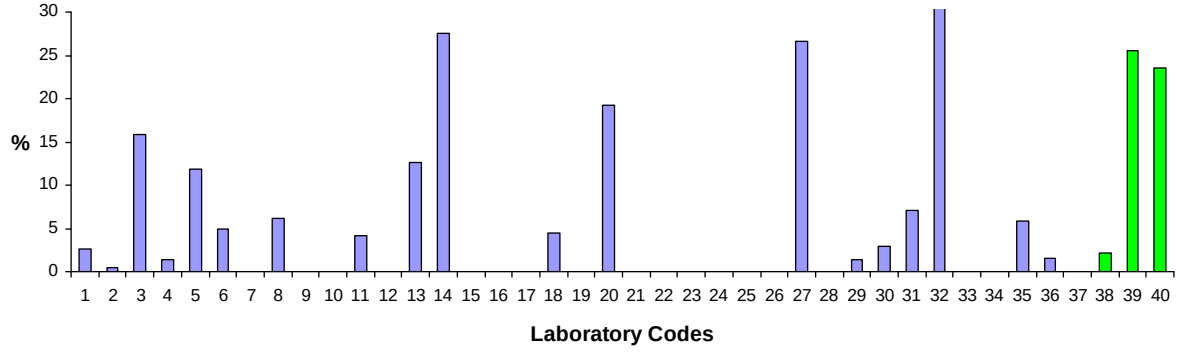


Fig. 14: Relative abundances of ***Cocconeis placentula* var. *lineata*** identified in sample D 11 by the participants (blue bars) and the auditors (green bars).

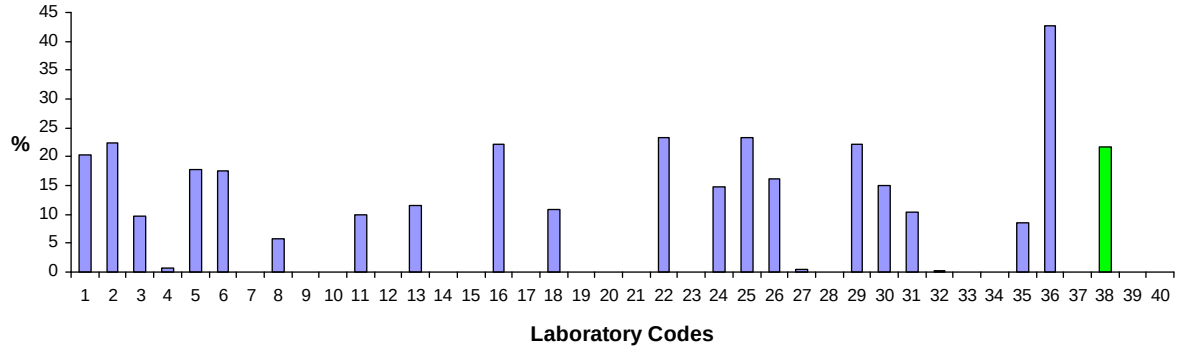


Fig. 15: Relative abundances of ***Cocconeis placentula* var. *euglypta*** identified in sample D 11 by the participants (blue bars) and the auditors (green bars).

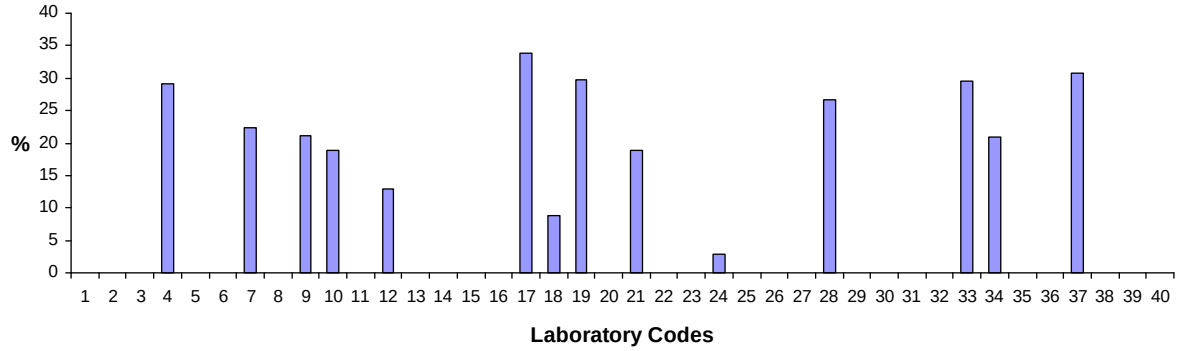


Fig. 16: Relative abundances of ***Cocconeis placentula*** without further differentiation of the varieties, identified in sample D 11 by the participants (blue bars) and the auditors (No. 38-40).

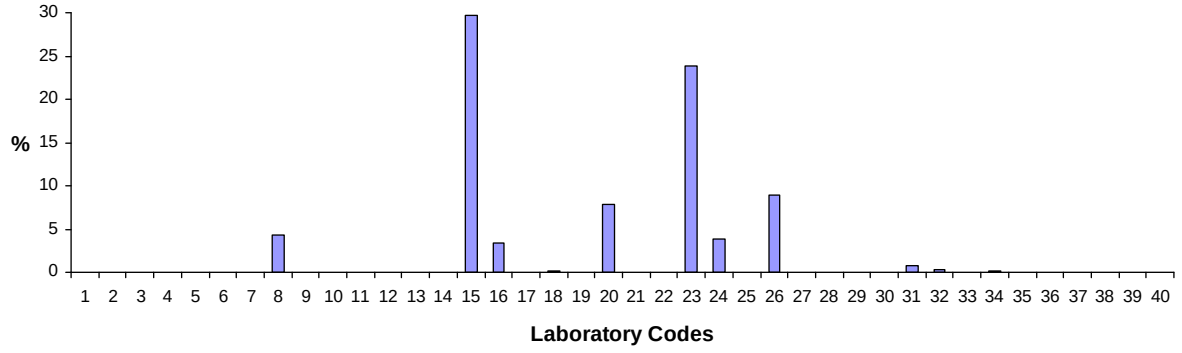


Fig. 17: Relative abundances of ***Cocconeis placentula* var. *placentula*** identified in sample D 11 by the participants (blue bars) and the auditors (No. 38-40).

Thus, the results suggest substantial problems with differentiating between the varieties *euglypta* and *lineata*. Additionally, some laboratories probably mistook *C. placentula* var. *placentula* for the varieties *euglypta* and/or *lineata*. These will very likely be

misidentifications, especially by laboratories 15 and 20, as the latter exclusively identified *C. placentula* var. *placentula*.

Problems with the taxonomy of the varieties of *C. placentula* are far reaching. One source of the problems is that several authors have described the varieties based on different traits (e.g. Ehrenberg 1838, Grunow 1884, Van Heurck 1885, Geitler 1927). Another source is Geitler, who described most of the varieties, who also used life-form, mode of auxospore development and other traits to differentiate the varieties, next to morphometric characteristics (Geitler 1958, 1982). Interestingly, Geitler mainly used valves from just one body of water to describe his varieties and he did not work with clone cultures (Geitler 1982). Also, it is not resolved, if the presence of different modi of auxospore development that Geitler described may just be controlled by various environmental factors (Mann 1999). The problem is that Geitlers system of classification was applied to *C. placentula* findings in all of Europe and world-wide. Additionally, there are different morphological concepts for differentiating the varieties of *C. placentula* (see e.g. Hustedt 1930, Krammer & Lange-Bertalot 1986-2004, Jahn et al. 2009, Romero & Jahn 2013) that are simultaneously used in practise today, which adds to the problem of identifying the varieties.

Hofmann et al. (2011) refer to Krammer & Lange-Bertalot (1986-2004) for differentiating the varieties of *C. placentula*. Additionally, they recommend not to differentiate the varieties of *C. placentula* for now, when using the diatom assemblage for water quality assessments (exception: *Cocconeis pseudolineata* (Geitler) Lange-Bertalot). In addition to the morphological problems this recommendation was probably the reason, why 14 laboratories identified just *Cocconeis placentula* without indicating the varieties in sample D 11. However, when using the German method for water quality assessment (Schaumburg et al. 2006, 2007) the varieties should be separated, as the associated Phylib-Software (2012) allocates different trophic values and corresponding weightings to the varieties (see Tab. 11 and Chapter 3.3). Thus, the water body assessments will differ with different use of the varieties.

Tab. 11: Saprobic (S) and trophic (T) value with corresponding weighting (G) of some varieties of *Cocconeis placentula* in lakes and rivers, as currently given and used in the Phylib-Software (2012).

taxon	running waters				lakes		
	S	G	T	G	TI North	TI South	G
C. placentula aggregate	1.8	2.0	2.6	2.0	3.45	-	-
var. *euglypta*	-	-	2.3	2.0	-	-	-
var. *lineata*	-	-	2.3	2.0	2.93	-	-
var. *placentula*	1.8	2.0	2.6	2.0	3.45	-	-
var. *tenuistriata*	-	-	-	-	-	-	-

According to Krammer & Lange-Bertalot (1986-2004) the differentiation of the varieties should be based on the fine structures of the rapheless valves that are visible in the light microscope (Tab. 12). Accordingly, the varieties *placentula* and *tenuistriata* can be easily distinguished based on the striae density (Tab. 12). Problems occur with the most common varieties, i.e. *lineata* and *euglypta*, as the striae density and number of areolae per stria of *euglypta* are entirely within the range of *lineata*. The areolae of *euglypta* are supposed to be more robust compared to *lineata*, whereas the striae of *lineata* are supposed to appear distinctly dotted (Krammer & Lange-Bertalot 1986-2004). However, the differences between robust and distinct areolae remain unclear. The variety *lineata* is supposed to often have slit-like areolae which appear somewhat isolated from one another and an irregular or zigzag pattern of the longitudinal lines of areolae (areolae along the apical axis).

Overall, it is difficult to clearly differentiate the varieties *euglypta* and *lineata* based on these rather vague characteristics. This may also explain the contradictory results of the intercalibration exercise with respect to the varieties *euglypta* and *lineata* (see Figs. 14 and 15). The pictures of the varieties *euglypta* and *lineata* in Krammer & Lange-Bertalot (1986-2004) add to the problem, as they do not entirely match the verbal descriptions. For example, the pictures on Plate 53, Figures 17-18 (page 354) depict *C. placentula* var. *euglypta* in Krammer & Lange-Bertalot (1986-2004; Band 2/4), but show distinctly more than five areolae per stria and a pattern of longitudinal lines of areolae that can be called irregular. Please note that there are also several other varieties of *C. placentula* that we do not address here.

Tab. 12: Selected traits for differentiating varieties of *Cocconeis placentula* using the rapheless valves. Source: Krammer & Lange-Bertalot (1986-2004; Band 2/4). Rows = longitudinal lines of areolae.

taxon	length	striae/10µm	areolae/stria	comment
C. placentula aggregate	7.5-98	13-36		
var. *euglypta*	10-46	19-22	3-5	even rows
var. *lineata*	10-80	16-23	3-10	uneven rows (zigzag)
var. *placentula*		24-26		delicately dotted
var. *tenuistriata*	12-38	26-32(38)		very delicately dotted

Based on the current inadequate state of information about the taxonomy and ecology of the varieties of *C. placentula*, it is difficult to make recommendations for the praxis. Further taxonomic and ecological work is necessary and thus the recommendations about the problems may change anytime.

In European waters the varieties *euglypta* and *lineata* are the most abundant taxa and to a certain degree also the varieties *placentula* and *tenuistriata*. In addition, *C. pseudolineata*, now separated from the other varieties on a species level, occurs regularly. In general, all varieties should be separated as much as possible, which should be achievable without problems for the varieties *placentula*, *intermedia*, *rouxii*, *tenuistriata*, *klinoraphis* and for *C. pseudolineata* based on the rapheless valves. The varieties *euglypta* and *lineata* may be pooled for the water assessment evaluation, but should be counted separately. Pictures should document the separation and should be supplemented with a brief explanatory text. Thus, later changes and adjustments to the taxonomy and ecology will still be applicable to the dataset in hindsight. For the German water quality assessment according to Schaumburg et al. (2006, 2007) we recommend as a provisional, accountable and pragmatic solution to pool the varieties *euglypta* and *lineata* and enter them as *C. placentula* var. *euglypta*, because both varieties have identical indicator values or *euglypta* has no indicator value where *lineata* does (Pylib-Software from 2012) (see Tab. 11).

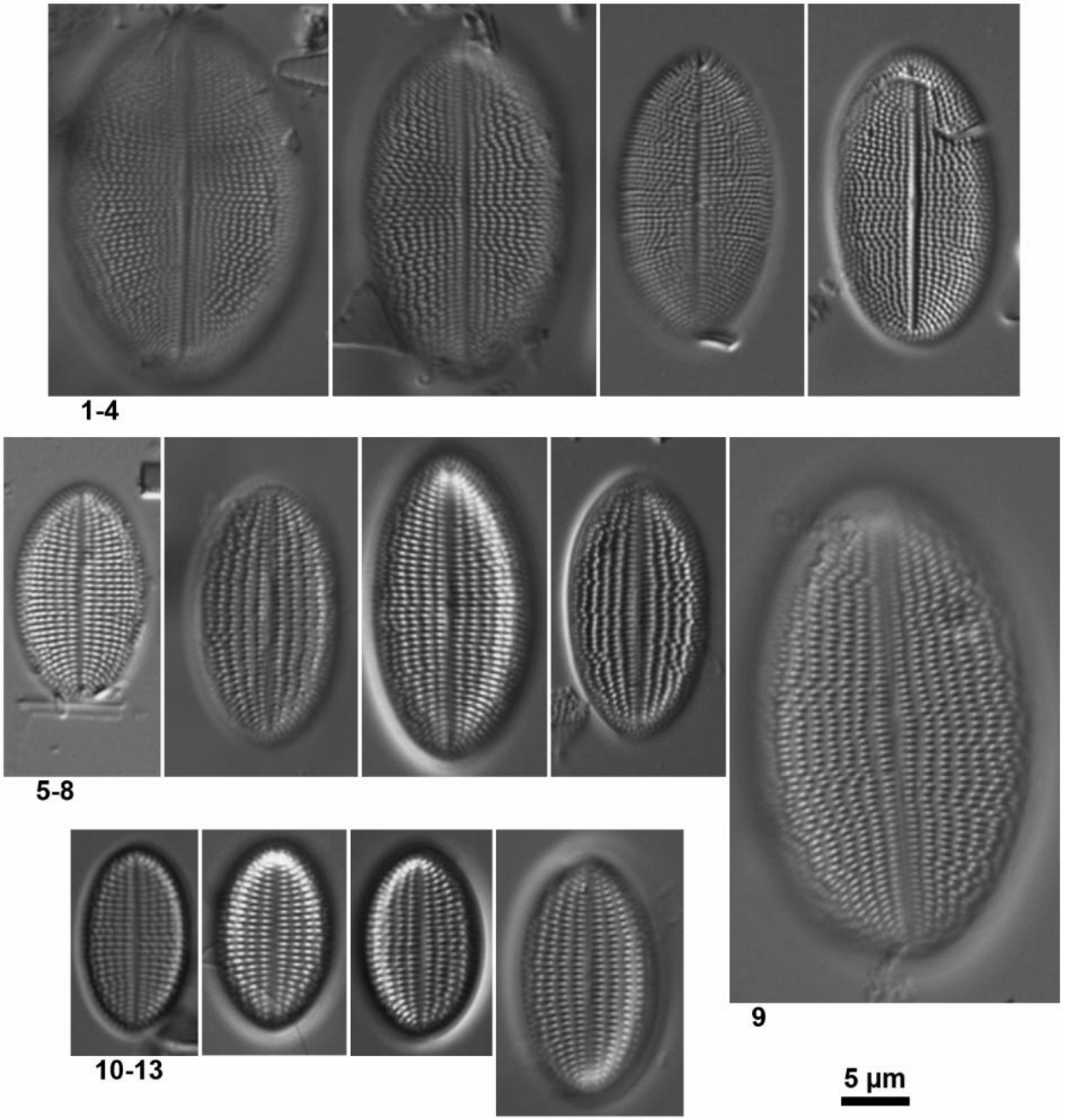
Another problem is that most varieties of *C. placentula* cannot be distinguished on the basis of their raphe-valves (the only exception is var. *klinoraphis*). We recommend to count the raphe-valves separately and subsequently allocate these valves to the identified varieties (based on the rapheless valves) according to their relative abundances. Here, *C. pseudolineata*

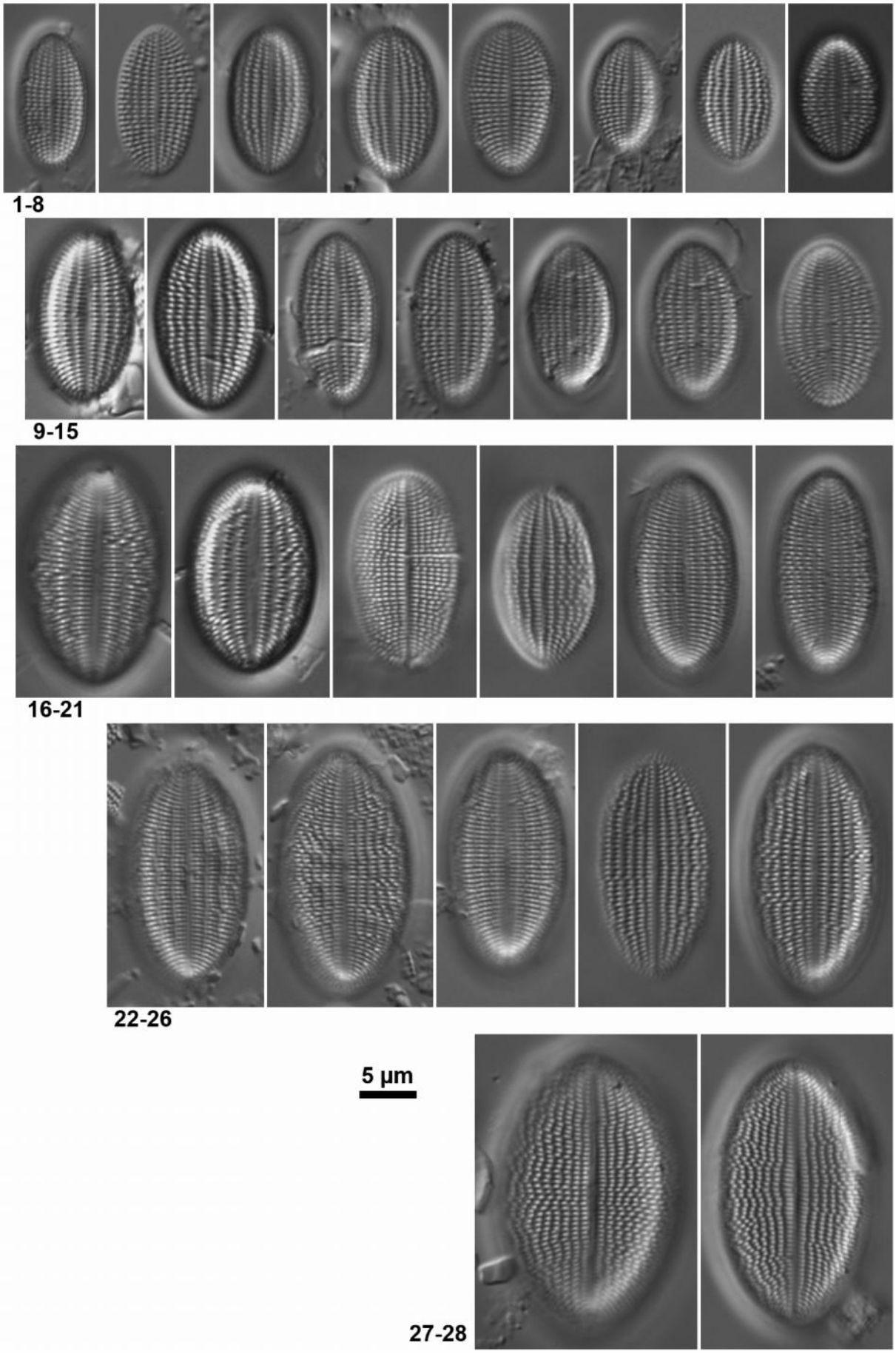
needs to be included, as their raphe-valves also do not differ from the raphe-valves of the *C. placentula* varieties.

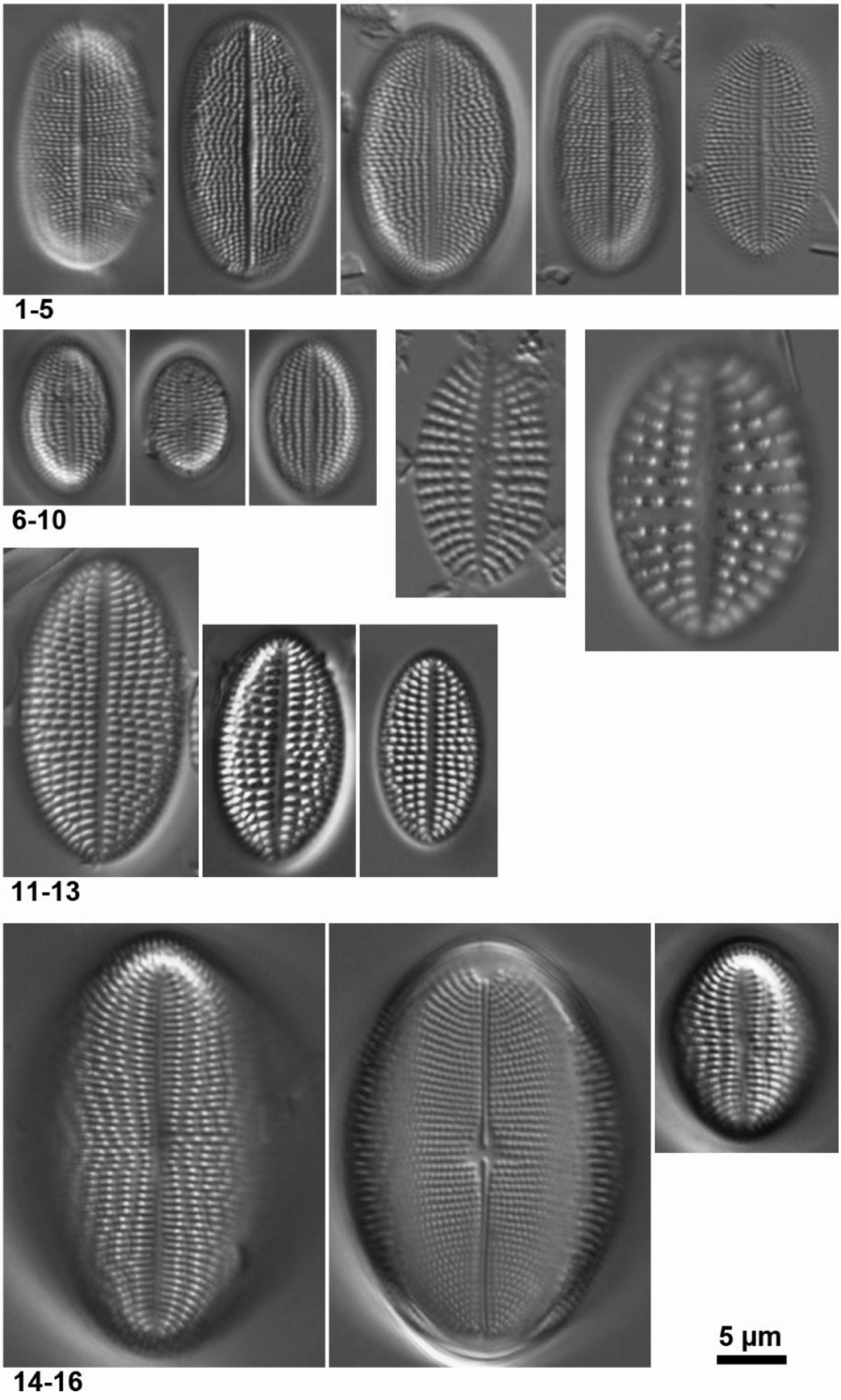
Plate 3 (page 56): Raphe-less valves of *Cocconeis placentula*-varieties according to the taxonomic concept of Krammer & Lange-Bertalot (1986-2004) from sample D 11 (Lake Krossinsee, Northern Germany). **1-4**: *C. placentula* var. *placentula*; **5-9**: *C. placentula* var. *lineata*; **10-13**: *C. placentula* var. *euglypta*.

Plate 4 (page 57): Raphe-less valves of *Cocconeis placentula* from sample D 11 (Lake Krossinsee, Northern Germany) with a striae-density of 24-26 /10 µm. Based on their striae density these valves (1-28) should be called *C. placentula* var. *placentula* following Krammer & Lange-Bertalot (1986-2004). However, the arrangement and shape of the areolae rather lead to the designation *C. placentula* var. *euglypta* (**1-14**) or *C. placentula* var. *lineata* (**15-28**) according to Krammer & Lange-Bertalot (1986-2004). For more information see text.

Plate 5 (page 58): **1-5**: *C. placentula* var. *tenuistriata*; **6-8**: *C. placentula* cf. var. *tenuistriata*; **9**: *C. neodiminuta*; **10**: *C. disculus*; **11-13**: *C. pseudolineata*; **14-16**: *C. pediculus*. Valves 1-8 and 14-16 are from sample D 11 (Lake Krossinsee, Northern Germany), valves 9-13 (*C. neodiminuta*, *C. disculus* and C. *pseudolineata*) are not from sample D 11. Valves 9-16 are presented for comparison. These taxa can hardly be mistaken for *C. placentula*, if all characteristic traits are taken into account. Valves 1-8 have 28-32 striae in 10 µm. Thus, valves 6-8 have the correct striae density but mismatching shape and arrangement of areolae for *C. placentula* var. *tenuistriata* according to Krammer & Lange-Bertalot (1986-2004). Names of depicted valves were given according to details given in Krammer & Lange-Bertalot (1986-2004) and Hofmann et al. (2011).


1-4
5-8
9
10-13
5 µm


1-8
9-15
16-21
22-26
5 µm
27-28


1-5
6-10
11-13
14-16
5 µm

3.2.4 *Encyonema silesiacum* and similar taxa in sample D 2

Sample D 2 indicates some taxonomic problems related to the identification of *Encyonema lange-bertalotii* Krammer, *E. minutum* (Hilse) Mann, *E. reichardtii* (Krammer) Mann, *E. silesiacum* (Bleisch) Mann and *E. ventricosum* (Agardh) Grunow. Based on the results of the participants, the sum of above taxa (0.2-16.9 %, average 11.2 %) (Fig. 18) was dominated by *E. silesiacum* (0.0-14.6 %, average 7.1 %) (Fig. 19) and *E. minutum* (0.0-13.7 %, average 3.4 %) (Fig. 20). Correspondingly, the auditors identified *E. silesiacum* (8.6-12.9 %, average: 10.3 %) (Fig. 19) and *E. minutum* (0.2-1.6 %, average 1.1 %) (Fig. 20) in good agreement.

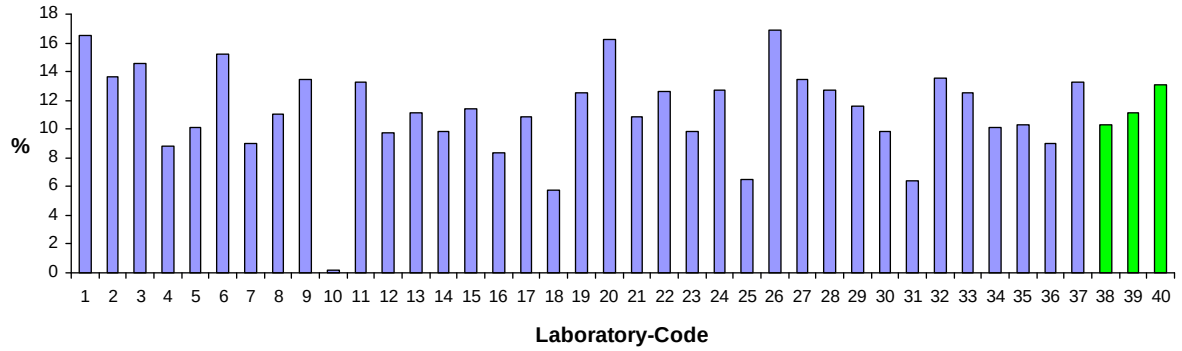


Fig. 18: **Sum** of the relative abundances of ***Encyonema lange-bertalotii, E. minutum, E. reichardtii, E. silesiacum*** **and** ***E. ventricosum*****, including** ***E.*** **cf.** ***silesiacum*** (with very low abundances) as identified by the participants (blue bars) and auditors (green bars) in sample D 2. All varieties of *E. silesiacum* are included in this figure.

32 of 37 participants identified *E. silesiacum* in sample D 2, the most abundant taxon of this group (Fig. 19). Five laboratories (2, 10, 20, 25, 28) did not identify any *E. silesiacum* in sample D 2 (Fig. 19). Instead, these laboratories detected above average abundances of *E. minutum* (laboratory 2, 20, 25, 28) or of *E. minutum* and *E. reichardtii* (laboratory 20) (Figs. 20 and 22). Laboratory 10 identified a total of only 0.2 % of this taxonomic group. Seven additional laboratories (8, 15, 16, 18, 27, 30, 31) detected below average abundances of *E. silesiacum* compared to the auditors (Fig. 19).

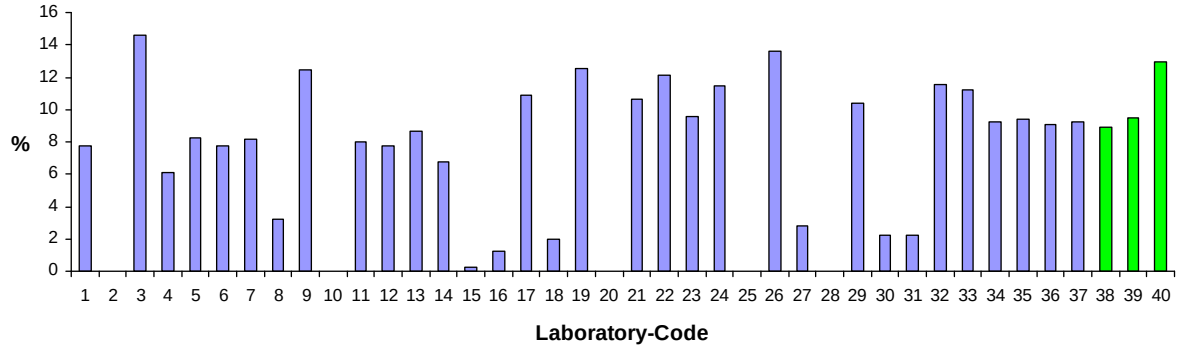


Fig. 19: Relative abundances of ***Encyonema silesiacum*** (including cf.) as identified by the participants (blue bars) and auditors (green bars) in sample D 2. All varieties of *E. silesiacum* are included in this figure.

E. minutum, the second most abundant species of this taxonomic group in sample D 2, was identified by 33 of 37 participants (Fig. 20), with above average abundances compared to the auditors by 15 laboratories (1, 2, 6, 8, 11, 16, 18, 2, 25, 26, 27, 28, 30, 31, 37) (Fig. 20). One auditor and ten participants also identified the relatively rare *E ventricosum* in sample D 2 (participants: 0.2-2.0 %, average 0.8 %, n=10) (Fig. 21). Additionally, four other laboratories detected *E. lange-bertalotii* and *E. reichardtii* in sample D 2 (Fig. 22), with two of these laboratories stating very high abundances of *E. lange-bertalotii* (laboratory 15) or *E. reichardtii* (laboratory 20).

Thus, several misidentifications occurred among the taxonomic *E. silesiacum*-group, although apart from *E. reichardtii* all other mentioned taxa were in fact present in sample D 2 (Plate 6). However, only *E. silesiacum* was dominant or relatively abundant and *E. minutum* was subdominant. *E. lange-bertalotii* and *E. ventricosum* occurred only very seldom and may or may not be encountered during a single, regular count.

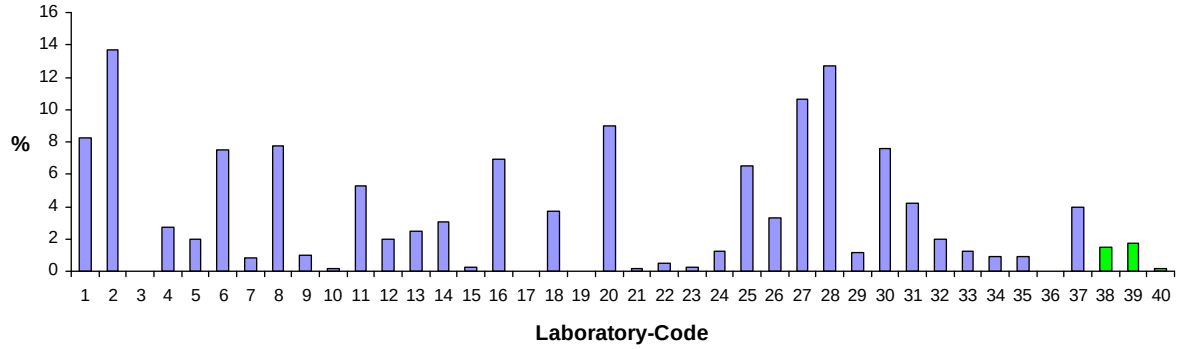


Fig. 20: Relative abundances of ***Encyonema minutum*** as identified by the participants (blue bars) and auditors (green bars) in sample D 2.

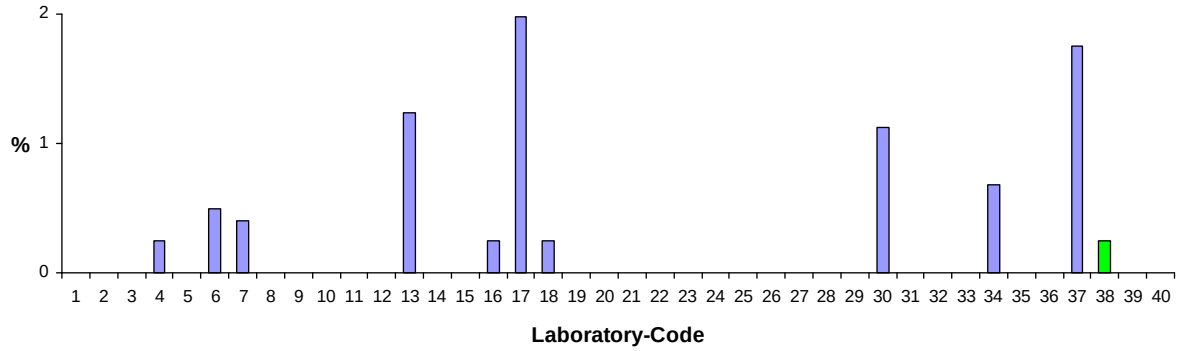


Fig. 21: Relative abundances of ***Encyonema ventricosum*** as identified by the participants (blue bars) and auditors (green bars) in sample D 2.

Fig. 22: Relative abundances of ***Encyonema lange-bertalotii*** (laboratories 1, 15 and 16) and ***E. reichardtii*** (laboratory 20) as identified by the participants (blue bars) and auditors (no. 39-40) in sample D 2.

There are several varieties and morphotypes of the here discussed species (Tab. 13) that are often difficult to distinguish from one another (see Krammer 1997a, 1997b). However, the results of this intercalibration exercise suggest that there are already significant problems with

distinguishing the species. Thus, in the following we will only discuss the differences among the species but not among the varieties and morphotypes.

Tab. 13: Selected characteristics according to Hofmann et al. (2011) and Krammer (1997a, 1997b) for differentiating the *Encyonema*-species that were counted by the participants and auditors in sample D 2.

	length (µm)	**width (µm)**	**striae/10µm**	**areolae/10µm**	**comment**
E. lange-bertalotii	16-38	6.2-11	12-16	27-31	protracted ends
E. ventricosum	9-29	4.5-6.9	(12)14-19	33-39	protracted ends
E. reichardtii	6.7-14.5	3.2-4	18-22	35-42	central area
E. minutum	7-23	4.2-6.9	15-18	34-38	size
E. silesiacum-aggregate	14-44(48)	5.9-9.6(11)	11-14	(24)28-32	stigmata

In contrast to the other taxa listed in Tab. 13 *Encyonema silesiacum* has a clearly visible, isolated stigma located dorsally near the central stria. *E. reichardtii* is distinctly smaller than *E. silesiacum* and is also the only species from Tab. 13 with a small central area on the ventral side and dorsally less dense striae in the middle compared to the ends. *E. minutum* is relatively well distinguished from *E. silesiacum* by its smaller size, denser striae and denser areolae (Tab. 13). For single valves it may be impossible or difficult to tell *E. minutum* and *E. silesiacum* apart, while it should be mostly possible if the species are sufficiently abundant (even if they co-occur). In contrast to the just mentioned species, *E. lange-bertalotii* and *E. ventricosum* have distinctly protracted ends that are bend ventrally. On average *E. lange-bertalotii* is bigger compared to *E. ventricosum* with coarser areolae-density. Here too, a differentiation may be impossible, if there are just single valves. Apart from the here discussed species, there are other *Encyonema*-species similar to *E. silesiacum* that are not mentioned here. For more details see Hofmann et al. (2011) and Krammer (1997a, 1997b).

Recommendations:

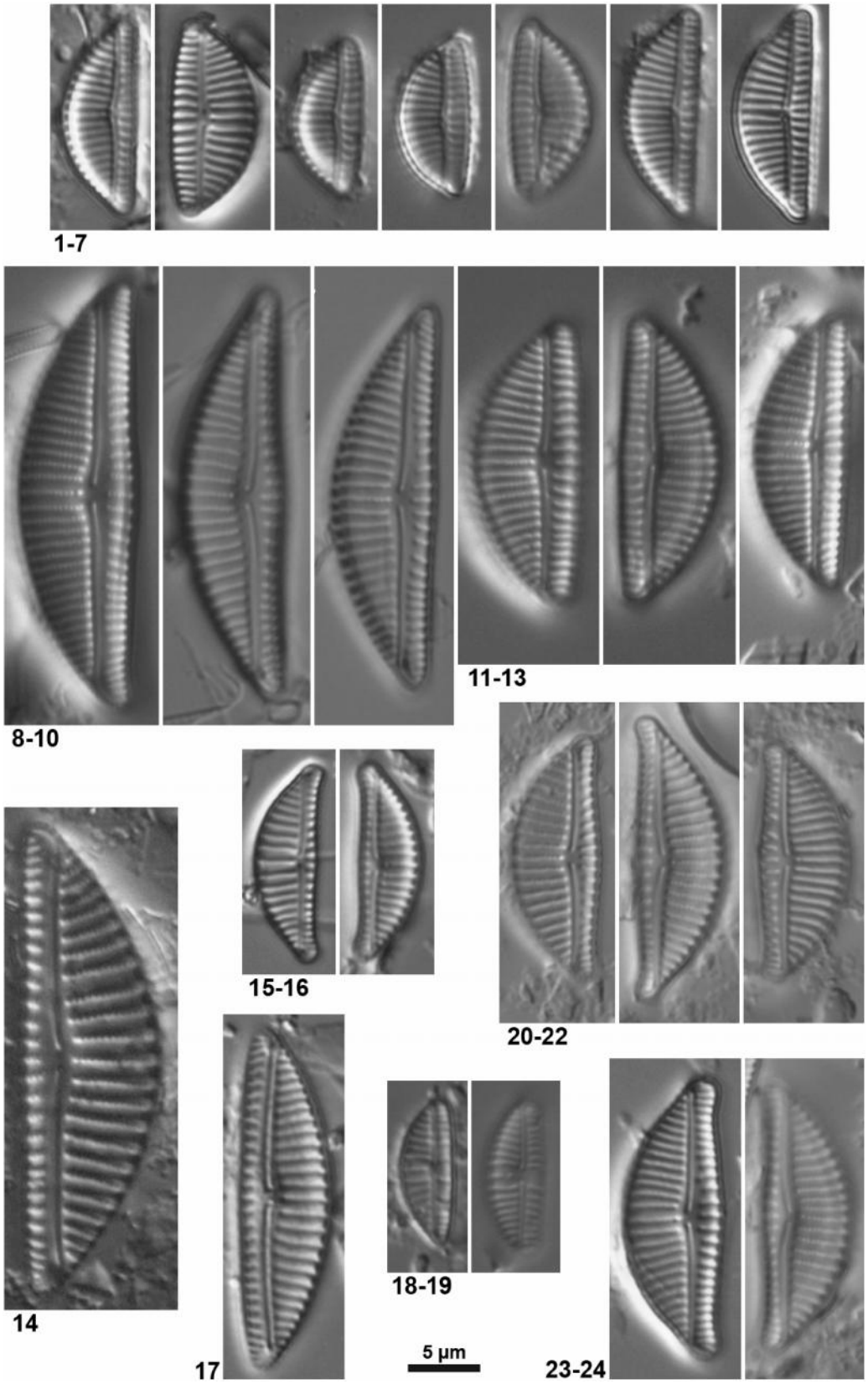
Similar to the other discussed species (Chapter 3.2) it is essential to use the whole combination of traits to identify taxa of the *Encyonema silesiacum* complex. Additionally, it is especially important to base identification on as many valves of the occurring population as possible, instead of just using a few objects to allocate names to all valves. Many similar looking but different species may co-occur, such as *E. silesiacum* and *E. minutum* in sample D 2. For single finds or few valves of this species complex chances for misidentification may

be relatively high. Those valves should be labelled with a cf. and be documented with a picture.

For a reliable identification of the here discussed *Encyonema*-species Hofmann et al (2011) is often insufficient, as only the most common species are included in this book. A necessary supplement is Krammer (1997a, 1997b). However, these books are out of print. If a laboratory has no access to Krammer (1997a, 1997b), then scrutiny is necessary to decide, if the given valves correspond in all traits to the species given in Hofmann et al. (2011). Otherwise such valves should be labelled with a cf. or spec. and be documented with a picture. If such valves are abundant, we recommend to exchange pictures with colleagues that have the Krammer-books (1997a, 1997b).

Similar to Hofmann et al. (2011) we recommend not to distinguish between the varieties of *E. silesiacum* when using the Phylib-method (Schaumburg et al. 2011, 2012) for now, as this intercalibration exercise demonstrates that it is already very problematic to distinguish the here mentioned species and distinguishing varieties is even more difficult. Also, the varieties of *E. silesiacum* that are included in the recent Phylib-Software (*var. distigmatum, var. distinctepunctatum, var. excisum, var. latareum, var. latestriatum, var. latum, var. silesiacum and var. ventriforme*) have identical indicator values to *E. silesiacum*. Thus, currently distinguishing the varieties would not make a difference in the water quality assessment, but may increase the chances of misidentifications.

Plate 6 (next page): Light microscopic pictures of *Encyonema minutum* (1-7), *E. silesiacum* (8-13), *Encyonema vulgare* Krammer (14), *E. ventricosum* (15-16), *Encyonema hebridiforme* Krammer (17), *E. reichardtii* (18-19) and *E. lange-bertalotii* (20-24). Valves 1-13, 15-17 and 23 are from sample D 2 (River Drau, Austria). Valves 14, 18-22 and 24 are from various running waters from northern Germany and are depicted for comparison. For more explanations see text.


1-7
8-10
11-13
15-16
20-22
14
17
18-19
5 µm
23-24

3.2.5 *Encyonopsis subminuta* and similar taxa in sample D 1.1

The results suggest extreme difficulties with identifying the species that were formerly called *Cymbella microcephala* Grunow and now belong to the genus *Encyonopsis* Krammer. Great problems occurred with differentiating *Encyonopsis microcephala* (Grunow) Krammer, *E. minuta* Krammer & Reichardt, *E. subminuta* Krammer & Reichardt and *E. krammeri* Reichardt in sample D 1.1.

35 of 37 participants identified species of this group (1.1-18.4 %, average 10.7 %) (Fig. 23) including *Cymbella microcephala*. The latter name was used by two laboratories (10 and 23) (Fig. 27) that very likely used only old identification literature (which is insufficient, especially for this taxonomic group). Two laboratories (10 and 32) identified distinctly below average abundances of this group. Just two laboratories (19 and 20) did not find any *Encyonopsis* or similar taxa (Fig. 23).

E. minuta was detected by 27 participants (0.7-13.4 %, average 7.3 %) (Fig. 24), *E. subminuta* by 21 (1.2-8.5 %, average 3.4 %) (Fig. 25) and *E. microcephala* by 15 participants (0.2-11.6 %, average 4.2 %) (Fig. 26). Furthermore, laboratory 2 identified *E. krammeri* with 10.2 %, laboratories 25 and 31 detected *Encyonopsis* spec. (12.2 % and 8.5 %, respectively) and laboratories 10 and 23 named *Cymbella microcephala* with 1.1 % and 9.7 %, respectively (Fig. 27).

Similar to the participants, the results of the auditors also suggest potential problems with identifying the taxa of this group in sample D 1.1. All three auditors detected *E. minuta*, however, with considerable differences in the relative abundances (13.2 %; 11.7 % and 0.4 %) (Fig. 24). In contrast, only one auditor identified *E. subminuta* with 9.2 % (Fig. 25). The other aforementioned taxa were not detected by any of the auditors. Another scrutiny of sample D 1.1 identified *E. minuta* and *E. subminuta* with about the same abundances and very sparse occurrences of *E. microcephala* and *E. tavirana* Krammer (Plate 7).

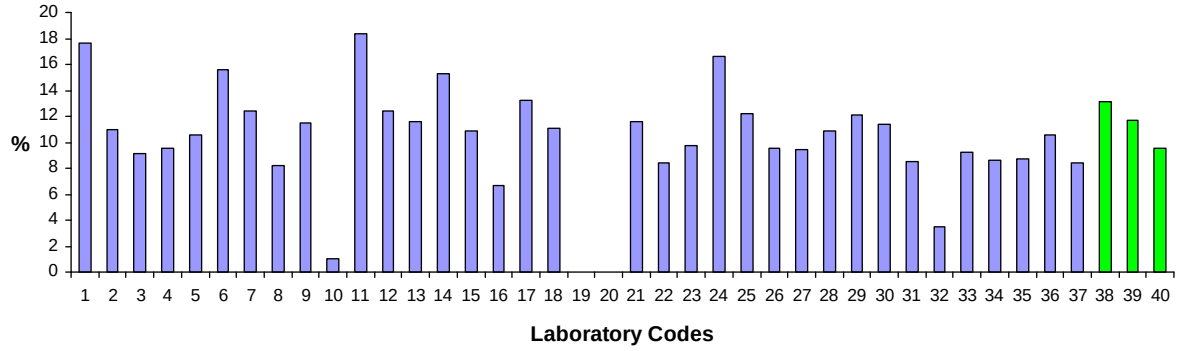


Fig. 23: **Sum** of the relative abundances of ***Encyonopsis* spec.**, ***E. microcephala, E. minuta, E. subminuta, E. krammeri*** and ***Cymbella microcephala*** including these taxa with cf. (that occurred in only very low abundances) as identified by the participants (blue bars) and auditors (green bars) in sample D 1.1.

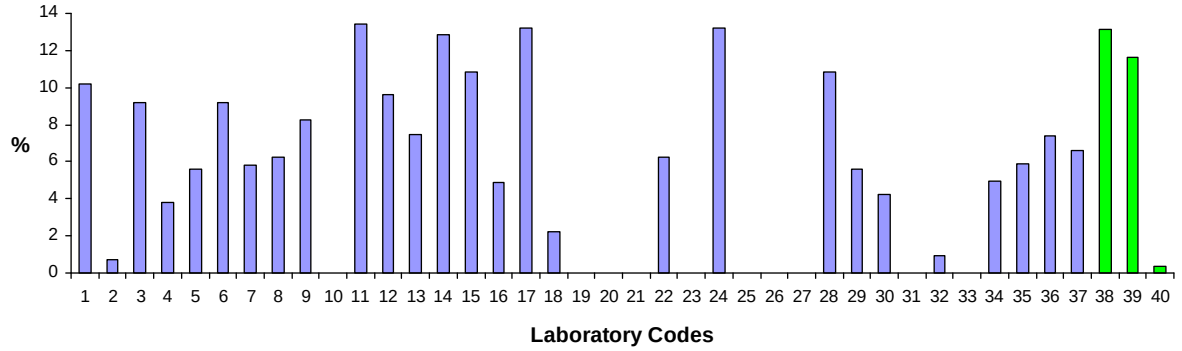


Fig. 24: Relative abundances of ***Encyonopsis minuta*** as identified by the participants (blue bars) and auditors (green bars) in sample D 1.1.

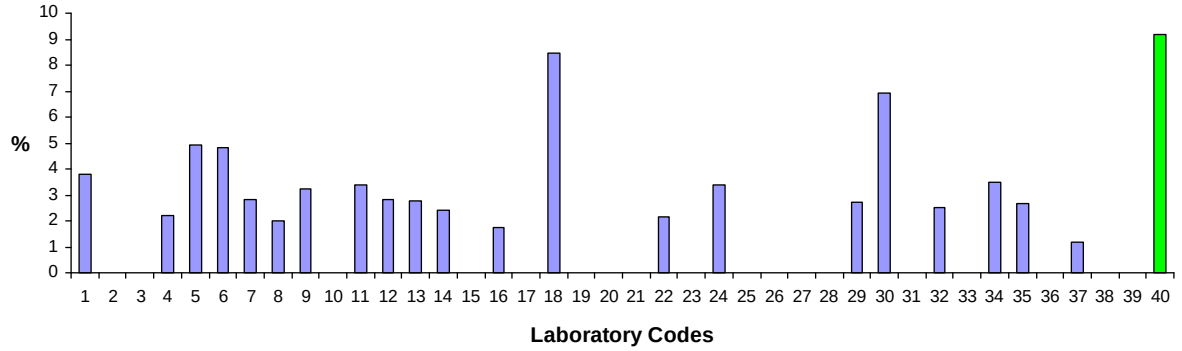


Fig. 25: Relative abundances of ***Encyonopsis subminuta*** as identified by the participants (blue bars) and auditors (green bars) in sample D 1.1.

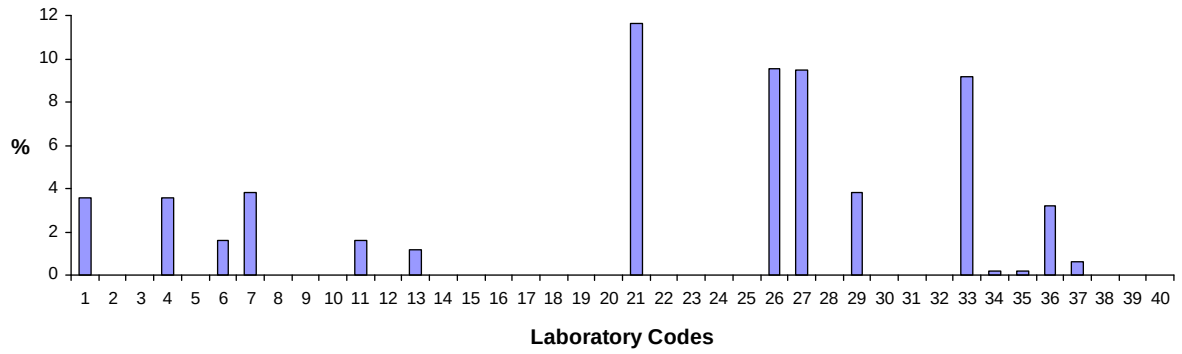


Fig. 26: Relative abundances of ***Encyonopsis microcephala*** as identified by the participants (blue bars) and auditors (no. 38-40) in sample D 1.1.

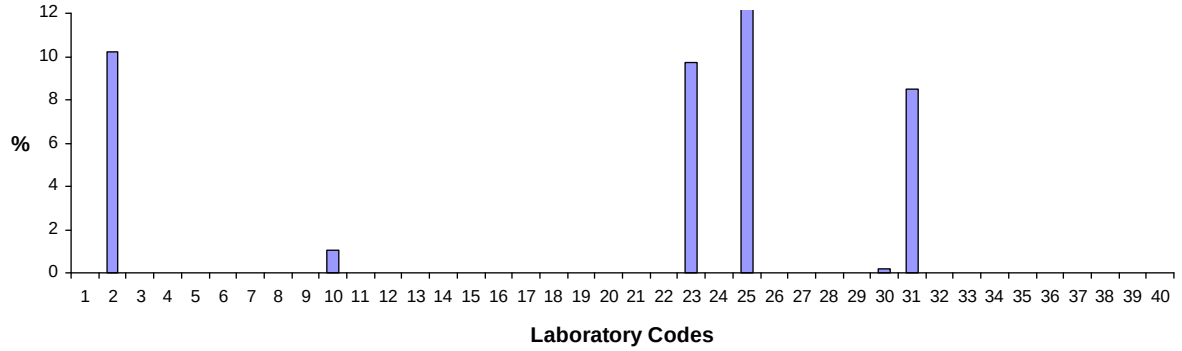


Fig. 27: Relative abundances of ***Encyonopsis krammeri*** (laboratory 2), ***Encyonopsis spec.*** (laboratories 25 and 31) and ***Cymbella microcephala*** (laboratories 10 and 23) as identified by the participants (blue bars) and auditors (no. 38-40) in sample D 1.1.

For identifying the here discussed species it is essential to use the entire combination of characteristics (Tab. 14), even more than for other diatoms. Here, the hardly variable shape is of special importance.

E. krammeri has a lanceolate and weakly dorsiventral shape. Both sides are slightly to distinctly convex, the ends are protracted and capitate. *E. krammeri* is well distinguished from *E. microcephala, E. minuta* and *E. subminuta* by a higher striae density (Tab. 14).

E. microcephala has a linear to linear-elliptic shape and is symmetrical to weakly dorsiventral. The sides are parallel to convex. The ends are protracted and capitate with distinct shoulders that are missing in *E. krammeri, E. minuta* and *E. subminuta*.

E. minuta has an elliptical to elliptical-lanceolate shape and is symmetrical to weakly dorsiventral. Both sides are distinctly convex. The ends are more or less capitate without distinct shoulders. *E. minuta* has a smaller width compared to *E. microcephala, E. subminuta* and *E. tavirana* (Tab. 14). The width-range of *E. minuta* overlaps with the range of

E. krammeri and *E. alpina* Krammer & Lange-Bertalot. However, their shape and striae density are distinctly different and thus they should not be confused with one another.

E. subminuta has an elliptical to lanceolate shape and is symmetrical to weakly dorsiventral. In contrast to the other taxa, both sides are distinctly convex. The ends are protracted and small-capitate, but have no shoulders. In contrast to the other here discussed taxa *E. subminuta* is almost symmetrical.

E. alpina and *E. tavirana* are similar to *E. microcephala*, but have a higher striae density. In contrast to *E. tavirana*, *E. alpina* is more elongated (higher length to width ratio) and has on average a higher striae density (Krammer 1997b; Tab. 14, Plate 7).

Tab. 14: Selected characteristics for differentiating *Encyonopsis alpina*, E. krammeri, E. microcephala, E. minuta, E. subminuta* and *E. tavirana**. Source: Hofmann et al. (2011) and *Krammer (1997b). max. L/W = maximum length to width ratio.

	length, µm	width, µm	striae/10µm	max. L/W	comment
E. microcephala	10-23	3.5-4.2	23-25	5.4	shape
E. minuta	8-17	2.8-3.5	24-25	4.9	shape, width
E. subminuta	10-25	3.4-4.5	23-26	~6	shape, symmetrical
E. tavirana	10-18	3.4-4.0	28-30	4.5	striae density
E. krammeri	11.5-23	2.6-3.8	(27)28-30(32)	7	striae density, shape
E. alpina	11-20	3.0-3.8	28-32	5.3	striae density, shape

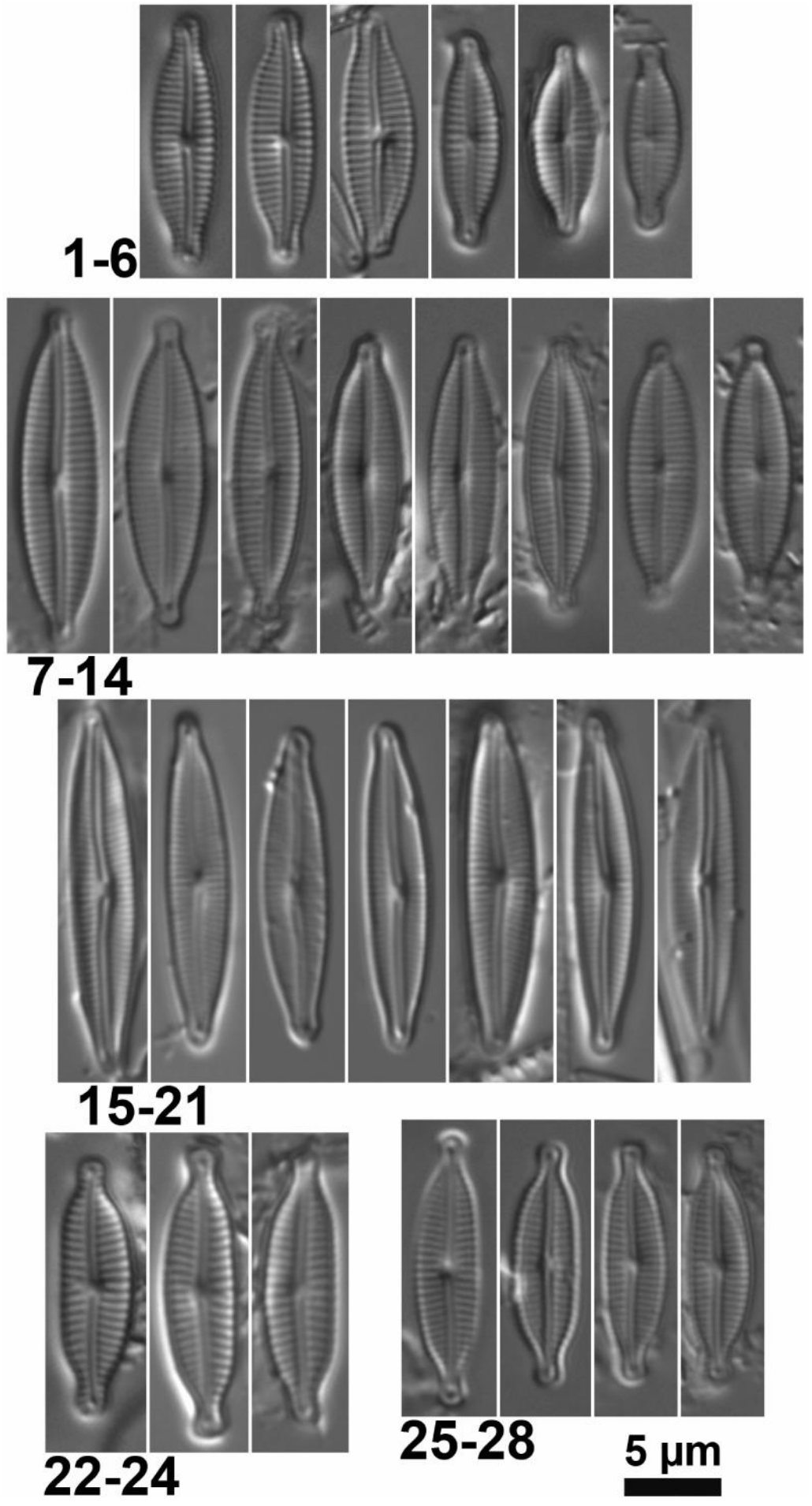
Please note that there are many other similar taxa of the genus *Encyonopsis* which are not discussed here, but which may also occur in northern Germany. For more information and extensive explanations of the identification traits see Krammer (1997b) and Hofmann et al. (2011). One problem is that Krammer (1997b) is out of print and that Hofmann et al. (2011) is constrained to the most abundant taxa, i.e. not all similar taxa are listed. Thus, if only Hofmann et al. (2011) is used, all the characteristics of each taxon must be very carefully considered, cf. should be used if a taxon does not entirely fit and pictures should document the critical taxa. If the critical taxa are abundant, colleagues that possess the Krammer-books should be contacted with the pictures with a request to help with the identification.

In general, it is taxonomically and ecologically necessary to separate the here discussed species, especially as these taxa can usually be well distinguished from one another when considering all characteristics (among others the shape is very important). Furthermore, it is

important to use as many valves of a population as possible to identify the species, instead of just identifying a few valves and allocating this name to all similar valves. Not all similar valves belong to the same species, as several similar species may co-occur, such as *Encyonopsis minuta* and *E. subminuta* in sample D 1.1. Single finds or only few valves can easily be misidentified. Thus, these objects should be labelled with a cf. and should be documented with a picture.

Another problem with the *Encyonopsis*-taxa is that the relevant taxa were not yet described when the training set of the Phylib-method was generated. Then, these taxa were all called *Cymbella microcephala*. When adjusting the Phylib-Software according to the now mandatory new taxonomy, *E. alpina*, *E. minuta*, *E. subminuta*, *E. microcephala* and *E. krammeri* have identical indicator-values, according to *C. microcephala* (Software 4.1 02.10.2012; *E. tavirana* is not yet included). Thus, we recommend to re-count the Phylib-training set according to the new taxonomy (i.e. using the identification literature stipulated in Schaumburg et al. 2011) to generate appropriate indicator values. Only thus the new, mainly well manageable taxonomy can be used effectively (for further discussion see Chapter 3.3).

Plate 7 (next page): Light microscopic pictures of *Encyonopsis minuta* (1-6), *E. subminuta* (7-14), *E. krammeri* (15-21), *E. microcephala* (22-24) and *E. tavirana* (25-28). Valves 1-14 and valves 22 and 26 are from sample D 1.1 (Lake Geneva, Switzerland). All other valves are from various lakes and rivers from northern Germany and are depicted for comparison. For more information see text.


1-6
7-14
15-21
22-24
25-28
5 µm

3.2.6 *Eolimna minima* and similar taxa in sample D 12

The auditors counted 11.8-21.8 % (average: 19.1 %) of the group *Eolimna minima* (Grunow) Lange-Bertalot & Schiller and similar species in sample D 12, while 34 of 37 participants identified 0.2-29.8 % (average 17.6 %) (Fig. 28). Laboratories 19, 20 and 28 did not detect any of the here addressed taxa and laboratories 3, 10, 15 and 36 only identified very low abundances (0.2-1.4 %; Fig. 28).

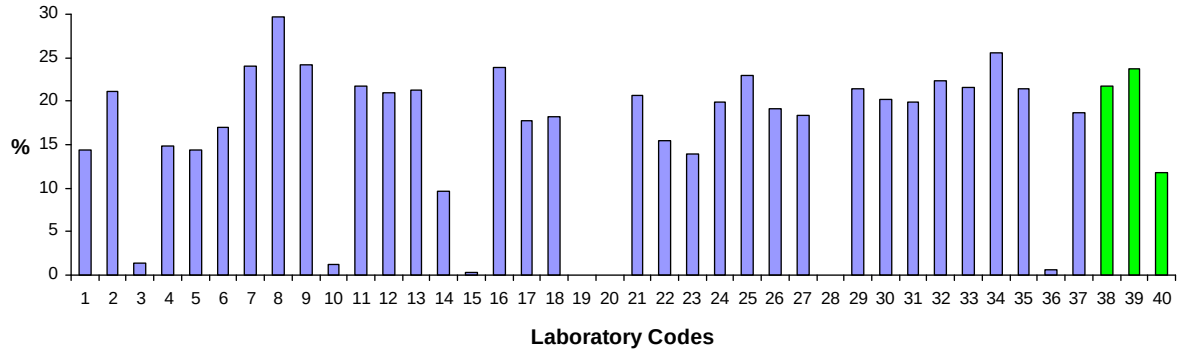


Fig. 28: Sum of the relative abundance of *Eolimna* (*Navicula*) *minima* (including cf.), *Sellaphora* (*Navicula*) *seminulum* (including cf.) and *Sellaphora* (*Navicula*) *joubaudii* from sample D 12 as identified by participants (blue bars) and auditors (green bars).

The auditors identified 12.8 %, 15.1 % and 4.5 % (average 10.8 %) of *E. minima* in sample D 12, which thus represents the species with the highest abundances from this group (Fig. 29). 33 of 37 participants detected 0.3-22.5 % (average 11.3 %) of *E. minima* (partly named *Navicula minima*), while laboratories 15, 19, 20 and 28 did not detect any *E. minima* and laboratories 3, 10 and 36 counted *E. minima* only in very low relative abundances (Fig. 29).

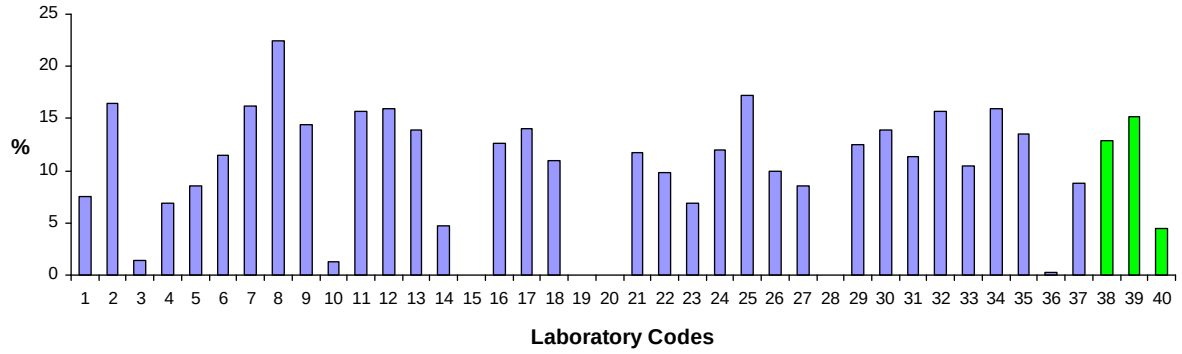


Fig. 29: Relative abundance of ***Eolimna*** (*Navicula*) ***minima*** (including cf.) from sample D 12 as identified by participants (blue bars) and auditors (green bars).

Sellaphora seminulum (Grunow) Mann (partly named *Navicula seminulum* by the laboratories) was identified with 5.1 %, 6.7 % and 6.0 % (average 5.9 %) by the auditors and with 0.2-9.3 % (average 4.5 %) by 31 of 37 participants (Fig. 30). Six participants (laboratories 3, 10, 19, 20, 28 and 36) did not detect any *S. seminulum* and laboratory 15 counted 0.2 % of *S. seminulum* (Fig. 30).

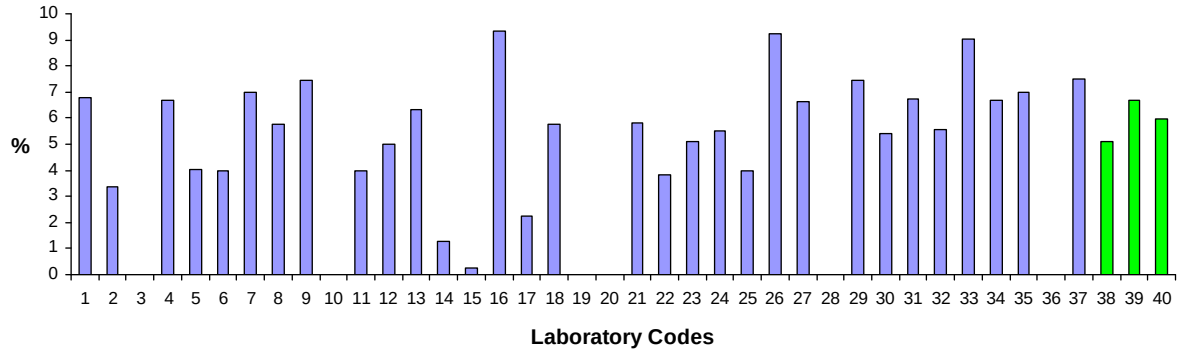


Fig. 30: Relative abundance of ***Sellaphora*** (*Navicula*) ***seminulum*** (including cf.) from sample D 12 as identified by participants (blue bars) and auditors (green bars).

Sellaphora joubaudii (Germain) Aboal (partly named *Navicula joubaudii* by the participants) was identified with 3.9 %, 1.9 % and 1.3 % by the auditors (average 2.4 %) and with 0.31-3.6 % (average: 1.3 %) by 28 of the 37 participants (Fig. 31). Nine participants of the intercalibration exercise did not detect any *S. joubaudii* in sample D 12 (Fig. 31).

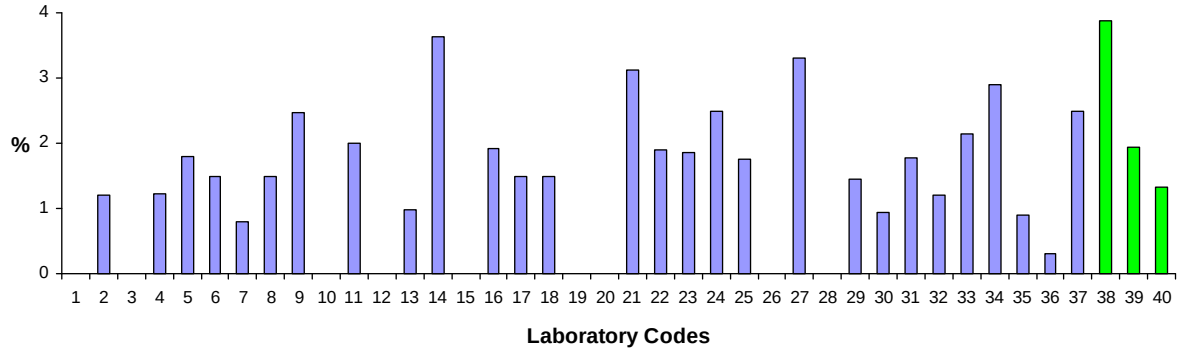


Fig. 31: Relative abundance of ***Sellaphora*** (*Navicula*) ***joubaudii*** (including cf.) from sample D 12 as identified by participants (blue bars) and auditors (green bars).

Altogether the relative abundances of *Eolimna minima*, *Sellaphora seminulum* and *Sellaphora joubaudii* agree relatively well among auditors in sample D 12. In contrast, the results of laboratories 1, 3, 10, 12 and 36 suggest that the three species may have been confused with

one another, while other results (laboratories 3, 10, 15, 19, 20, 28 and 35) suggest that these small-celled diatoms may have been entirely or mainly overlooked as they were not detected at all or only in very low abundances. The reasons are not clearly identifiable with the present results. Theoretically, it is possible that the technical equipment was insufficient (aperture, magnification) or that participants lacked experience with diatom identification. However, we can exclude these reasons for the results of this exercise based on the additionally given information by the participants. The objective type, aperture, experience with counting diatoms, optical illumination technique, regional origin of samples commonly counted by the participants or number of samples counted per year did not significantly influence the statistical distance (see Chapter 3.1) of the participants results to the results of the auditors (mixed-effect model according to Pinheiro & Bates (2000), $p<0.05$).

Tab. 15: Selected characteristics for differentiating *Eolimna minima, Sellaphora seminulum, S. joubaudii* and *Navicula utermoehlii* as identified in sample D 12 and similar species. *Naviculadicta raederae* Lange-Bertalot and *Naviculadicta schaumburgii* Lange-Bertalot & Hofmann were not detected in sample D 12, but are present in the North-German lowland and may be confused with the here mentioned species. Details according to Hofmann et al. (2011).

taxon	length (µm)	width (µm)	striae/10µm	comment
Eolimna minima	5-18	2-4.5	25-30	
Sellaphora seminulum	3-21	3-5	18-22	
Sellaphora joubaudii	5-15	3-4.5	18-20	shape, striae bend
Navicula(*dicta*) *utermoehlii*	8-12	4.5-6	24-36	striae continuously radial
Naviculadicta raederae	7-9	3.5-4	26-29	central area variable
Naviculadicta schaumburgii	8.5-13	5.5-6.6	18-20	width, striae-density

Length, width, striae density, central area and shape all need to be considered for identifying *Eolimna minima* and similar taxa with certainty. Additionally, the species descriptions and pictures of similar species need to be looked at, e.g. by using Hofmann et al. (2011).

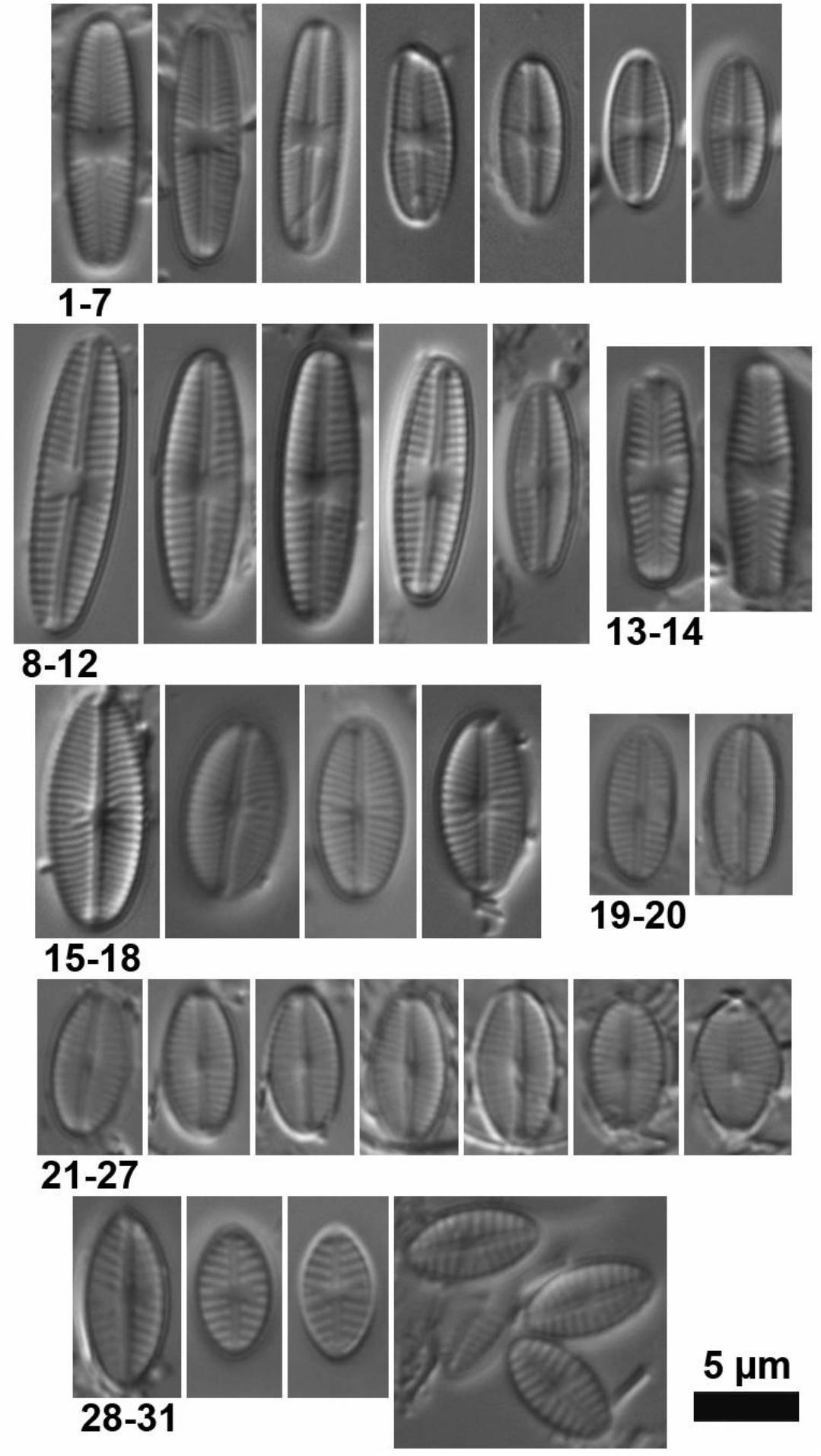
Sellaphora joubaudii is probably the easiest to identify from the species listed in Tab. 15, as the striae are arched and the central area is big and shaped like a butterfly (Hofmann et al. 2011). Also, the shape of *S. joubaudii* distinctly differs from the shape of the other species of this group, as *S. joubaudii* is linear-elliptically shaped with slightly protracted and broadly rounded ends. *Eolimna minima* distinctly differs from *S. joubaudii* due to a higher striae density, not-arched striae and a shape with only rarely protracted ends (Tab. 15).

Sellaphora seminulum (18-22 striae/10 µm) and *E. minima* (25-30 striae/10 µm) also differ in striae density. However, shape and size (length, width) do not distinguish these two species of this group very well (see Plate 8; Tab. 15).

Generally, *Navicula utermoehlii* can be identified on the basis of its elliptical shape with broadly rounded ends and its continuously radial striae. However, some valves of *E. minima* and *S. seminulum* may also be elliptical with broadly rounded ends. This emphasises the need to use all characteristic traits (length, width, striae density and shape) for an exact identification. For example, here *S. seminulum* is well defined by its striae density.

Naviculadicta raederae clearly differs from *Navicula utermoehlii* by its small width (Tab. 15). *Naviculadicta schaumburgii* has a coarser striae density than *Navicula utermoehlii* and *E. minima* (Tab. 15). *Naviculadicta schaumburgii* is also wider than *E. minima, S. seminulum* and *S. joubaudii* (Tab. 15). However, differentiating *Naviculadicta raederae* from *E. minima* may be difficult as the ranges of length, width and striae density overlap.
Girdle band views of taxa from the *E. minima*-group can only be distinguished by length and striae density (if visible). This will not always suffice for a clear allocation to the species, but should be tried in any case (see Tab. 15).

Plate 8 (next page): Light microscopic pictures of *Eolimna minima* (1-7), *Sellaphora seminulum* (8-12), *Sellaphora joubaudii* (13-14) and *Navicula utermoehlii* (15-18) from sample D 12 (Lowland River Klepelshagener Bach, Northern Germany). The following exemplary similar species are depicted for comparison; these species were sampled from other running waters from the North-German Lowlands: *Fallacia lucinensis* (19-20), *Naviculadicta raederae* (21-27) and *Navicula crassulexigua* (28-31). There are many other similar small-celled diatoms from various genera that need to be taken into account when identifying given species. Each pair of the pictures 6 and 7, 22 and 23, 24 and 25, 26 and 27 and 29 and 30 represent both valves of the same frustule. For more information see text.


1-7
8-12
13-14
15-18
19-20
21-27
28-31
5 µm

3.2.7 *Fragilaria* (*Staurosira*) *brevistriata* and similar taxa in sample D 1.1

Currently there are several different taxonomic systems and opinions about the classification and naming of small fragilarioid diatoms (see e.g. Williams & Round 1987, Lange-Bertalot 1989, Krammer & Lange-Bertalot 1986-2004, Sato et al. 2008, Hofmann et al. 2013, Williams 2013), which are simultaneously in use. As the opinions still differ greatly and the discussion about how to separate the fragilarioid diatoms discussed here is still in progress (also on the genus level), we name the taxa according to Hofmann et al. (2013) for practical reasons for now (as this is the main identification literature postulated by the German method; Schaumburg et al. 2011). For more information about this taxonomic discussion see, for example, Haworth (1975), Williams & Round (1987), Lange-Bertalot (1989), Round et al. (1990), Witkowski et al. (1995), Krammer & Lange-Bertalot (1986-2004), Morales (2001, 2002, 2006), Morales et al. (2003, 2010), Morales & Manoylov (2006), Sato et al. (2008), Cejudo-Figueiras et al. (2011) and Williams (2013).

The following taxa were identified in good agreement among the three auditors in sample D 1.1 (see also Plate 9): ***Fragilaria brevistriata*** **Grunow** (see e.g. *Staurosira brevistriata* (Grunow) Grunow, *Pseudostaurosira brevistriata* (Grunow) Williams & Round) and ***Fragilaria pinnata*** **Ehrenberg** (*Staurosirella pinnata* (Ehrenberg) Williams & Round, *Staurosira mutabilis* (W. Smith) Pfitzer). Addititionally, one auditor also determined ***Fragilaria construens*** **f.** ***venter*** **(Ehrenberg) Hustedt** (*Staurosira venter* (Ehrenberg) Kobayasi, *Fragilaria venter* Ehrenberg, *Staurosira venter* (Ehrenberg) Cleve & Moeller, *Fragilaria construens* var. *venter* (Ehrenberg) Grunow, *Staurosira venter* (Ehrenberg) Grunow, *Staurosira construens* var. *venter* (Ehrenberg) Hamilton) and ***Fragilaria leptostauron*** **(Ehrenberg) Hustedt** (*Staurosirella leptostauron* (Ehrenberg) Williams & Round, *Staurosira pinnata* Ehrenberg), but in very low abundances (<0.8 %). During an intensive search of sample D 1.1 ***Fragilaria parasitica*** **var.** ***parasitica*** **(W. Smith) Grunow** (*Odontidium parasiticum* W. Smith, *Synedrella parasitica* (W. Smith) Round & Maidana and *Pseudostaurosira parasitica* (W. Smith) Morales) was also detected.

The average relative abundances of all *Fragilaria brevistriata* and similar taxa coincided well between the 37 participants (average 4.6 %) and auditors (5.5, 3.5 and 4.5 %) (Fig. 32). However, the maximum (11.4 %, laboratory 15) and minimum (0.4 %, laboratory 24) differ distinctly from the average of the participants.

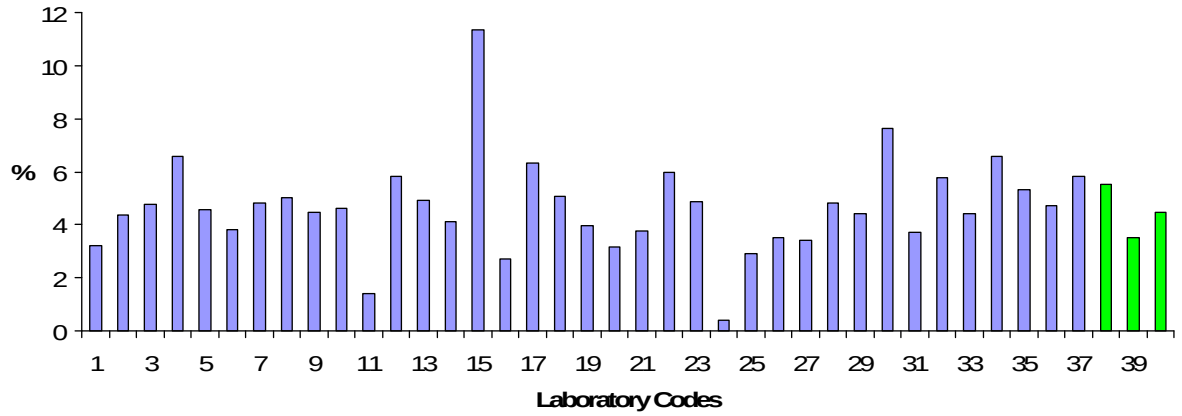


Fig. 32: Sum of the relative abundance of *Fragilaria brevistriata*, *F. brevistriata* var. *brevistriata*, *F. construens*, *Fragilaria construens* f. *construens*, *F. construens* f. *venter*, *F. elliptica*, *F.* cf. *leptostauron*, *F. leptostauron* var. *dubia*, *F. leptostauron* var. *leptostauron*, *F. martyi*, *F. oldenburgiana*, *F. parasitica*, *F. pinnata*, *F.* cf. *pinnata*, *F. pinnata* var. *pinnata*, *Opephora mutabilis* and *Staurosira brevistriata* from sample D 1.1. Blue bars: participants, green bars: auditors.

From the sum of small fragilarioid diatoms above (Fig. 32) *Fragilaria brevistriata* was detected with 0.6 %, 1.6 % and 2.3 % by the auditors (mean: 1.5 %) and "not at all" (nine laboratories) to a maximum of 4.0 % (laboratory 12) by the participants (mean: 1.0 %) (Fig. 33).

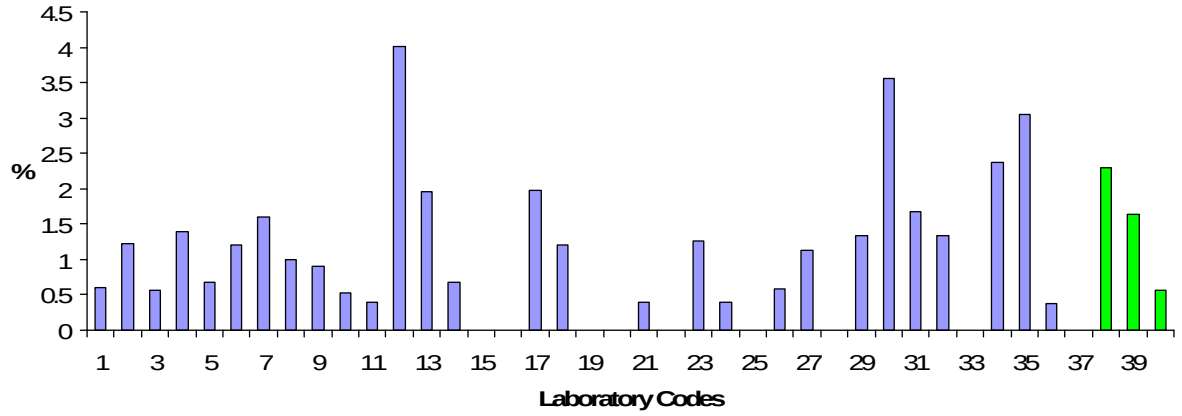


Fig. 33: Sum of the relative abundance of *Fragilaria brevistriata*, *F. brevistriata* var. *brevistriata* and *Staurosira brevistriata* as detected by the participants (blue bars) and auditors (green bars) in sample D 1.1.

Fragilaria construens including the forms *construens* and *venter* was identified by one auditor with 0.8 % (Fig. 34). More than half of the participants (n = 23) did not detect any

taxa from this group, possibly due to a certain variability among slides and not necessarily due to identification problems, as relative abundances were low. *Fragilaria construens* and forms were detected with minimal abundances of 0.2 % (laboratory 20) and 0.4 % (laboratory 35) by the participants and with maximal abundances of 5.3 % (laboratory 15) and 4.2 % (laboratory 3) (average of all 37 participants: 0.5 %) (Fig. 34).

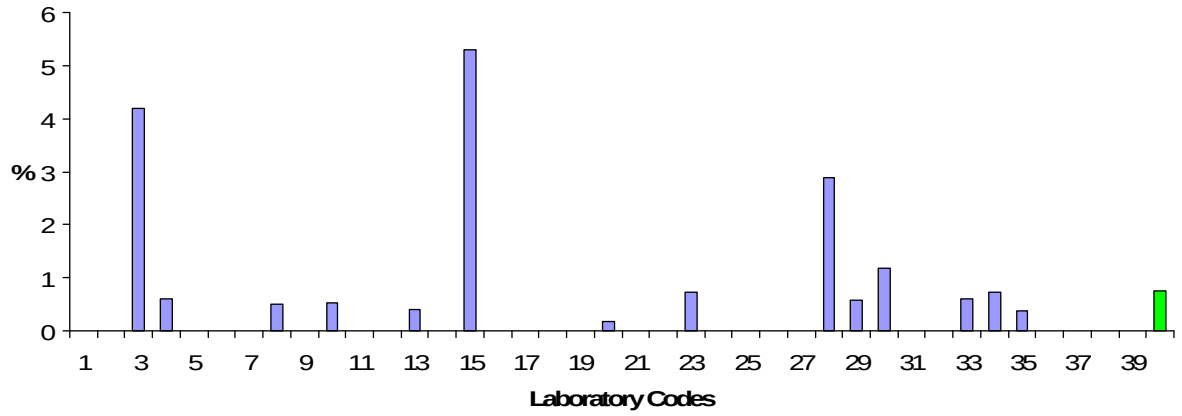


Fig. 34: Sum of the relative abundances of *Fragilaria construens*, *Fragilaria construens* f. *construens* and f. *venter* in sample D 1.1. Blue bars: participants, green bars: auditors.

The auditors identified *Fragilaria pinnata*, *F. pinnata* var. *pinnata* and *Fragilaria* cf. *pinnata* with 1.9 %, 3.0 % and 3.2 %, while four participants did not detect any taxa from this group in sample D 1.1 (Fig. 35). Participant abundances of the *Fragilaria pinnata*-group ranged from 1.0 % (laboratory 11) and 1.8 % (laboratory 12) to 5.8 % (laboratories 15 and 37) (average 2.8 %) (Fig. 35).

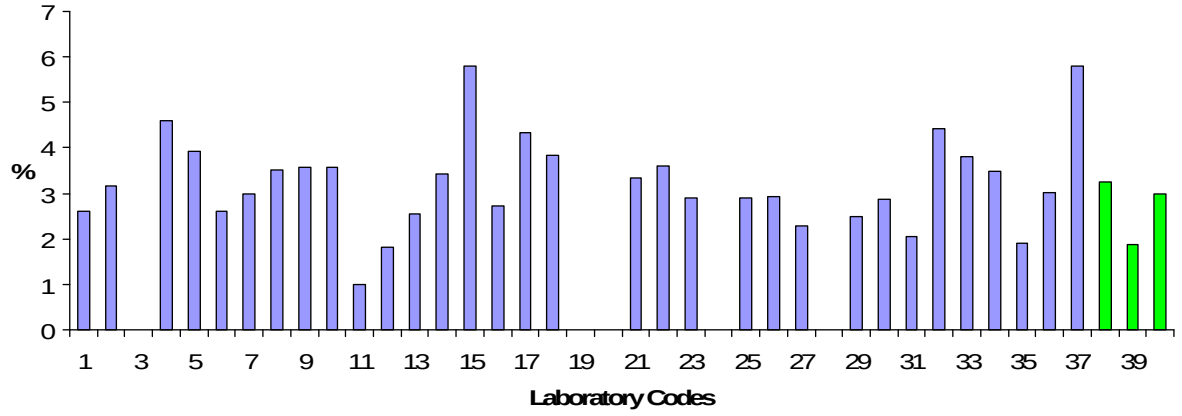


Fig. 35: Sum of the relative abundances of *Fragilaria pinnata*, *Fragilaria pinnata* var. *pinnata* and *Fragilaria* cf. *pinnata* from sample D 1.1. Blue bars: participants, green bars: auditors.

Fragilaria leptostauron var. *leptostauron*, *F. leptostauron* var. *dubia*, *F.* cf. *leptostauron* and *F. martyi* were detected with a sum of 1.9 % by one auditor in sample D 1.1 (Fig. 36). Most participants did not find any valves from this group. Only laboratory 15 detected the *Fragilaria leptostauron*-group with 0.3 %, laboratory 19 with 4.0 %, laboratory 20 with 3.0 % and laboratory 36 with 1.3 % (Fig. 36).

Additionally, *Fragilaria parasitica* was identified by laboratory 28 with 1.9 % and *Fragilaria elliptica* by laboratory 22 with 1.0 % (not shown in figures).

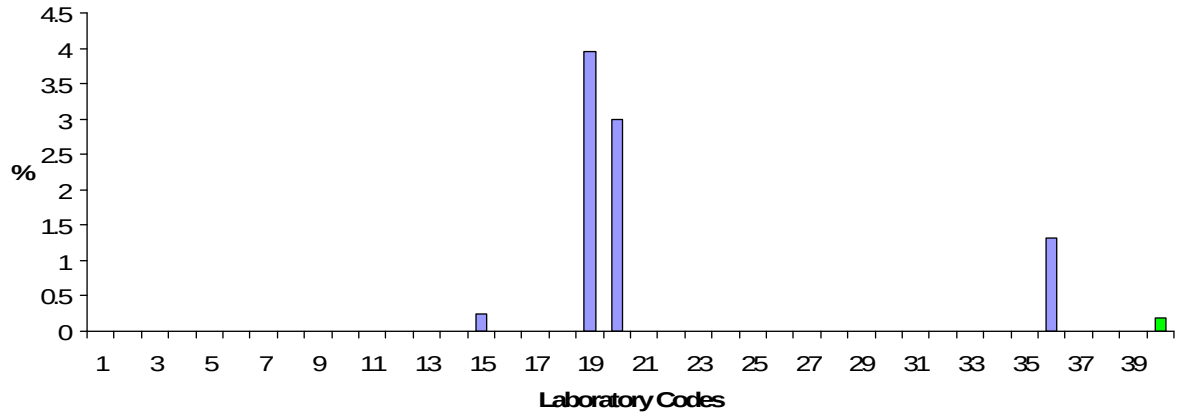


Fig. 36: Sum of the relative abundances of *Fragilaria* cf. *leptostauron, Fragilaria leptostauron* var. *dubia* and var. *leptostauron* and *Fragilaria martyi* from sample D 1.1. Blue bars: participants, green bars: auditors.

Overall, the here discussed taxa from the *F. brevistriata*-group and similar taxa together only contributed low relative abundances in sample D 1.1 (average of auditors 4.5 %, Fig. 32). Thus, the presence or absence of these taxa in the counting results may not necessarily be due to identification mistakes. For example, the variability among slides may explain some of the discrepancies. Still, the results of some laboratories suggest identification problems with these taxa when they are compared to the results of the auditors. For example, laboratory 3 and laboratory 15 identified above average relative abundances of *F. construens* (Fig. 34), laboratories 3, 19, 20, 24 and 28 did not detect any *F. pinnata* (Fig. 35) and laboratories 19 and 20 identified relatively high abundances of *F. leptostauron* (Fig. 36).

Fragilaria brevistriata can usually be easily identified due to its very peripheral striae (Plate 9, Tab. 16). More problematic is the differentiation of *Fragilaria construens*, *F. pinnata* and *F. leptostauron*. In valve view, *Fragilaria construens* and *F. leptostauron* can be easily distinguished from one another by their width, striae density and striae orientation.

According to Hofmann et al. (2011), especially valve width and striae density can be used to separate *Fragilaria pinnata* and *F. leptostauron*. However, large valves with coarse striae density of *F. pinnata* var. *pinnata* may be difficult to identify, as their valve ranges overlap with *F. leptostauron* var. *dubia* and *F. leptostauron* var. *martyi* (asymmetric). In contrast, *Fragilaria parasitica* is easily identifiable due to their typical lanceolate shape with protracted ends (in valve view) (Plate 9, Tab. 16).

To complete the picture: *Fragilaria pseudoconstruens* has a shape that is most similar to *F. construens* f. *construens*. However, they can be distinguished by the prominently dotted striae of *Fragilaria pseudoconstruens*, i.e. the visible areolae in the light microscope. Similarly, *Fragilaria construens* f. *subsalina* has punctate striae in the light microscope and consequently differs from similarly shaped *F. brevistriata* and *F. construens* f. *venter*.

The main problem with identifying most taxa of this group is the girdle view, in which these valves are often present. Here, the only measures of valve length and striae density often do not suffice for a definite identification. According to Hofmann et al. (2011), *Fragilaria brevistriata* and *F. construens* f. *venter* can be distinguished in girdle view by the length of the striae on the mantle area (for *F. brevistriata* shorter compared to *F. construens* f. *venter*). The distinct distension of the middle valve part of *Fragilaria leptostauron* var. *leptostauron* and *Fragilaria construens* f. *construens* is also visible in girdle view. However, often the valves in girdle view can only be allocated according to their relative abundance in valve view among possible candidates.

We recommend to identify the here discussed small fragilarioid diatoms according to the taxonomy in Hofmann et al. (2013) when applying the German method, due to the ongoing controversial discussions and still insufficient clarification of the taxonomy. The training set and counting data of the German method are based on the old taxonomy (Krammer & Lange-Bertalot 1986-2004) that is also largely used by Hofmann et al. (2013) for these diatoms. Thus, using other taxonomic concepts (see top of the text) is unfeasible, when using the German Phylib-method, because the other concepts are not comparable or applicable to the taxonomy that the German method is based on.

Tab. 16: Measurements of selected taxa from the small fragilarioid diatoms that were potentially mistaken for *Fragilaria brevistriata* and *F. pinnata* that occurred in sample D 1.1. Sources: Hofmann et al. (2011), Krammer & Lange-Bertalot (1986-2004).

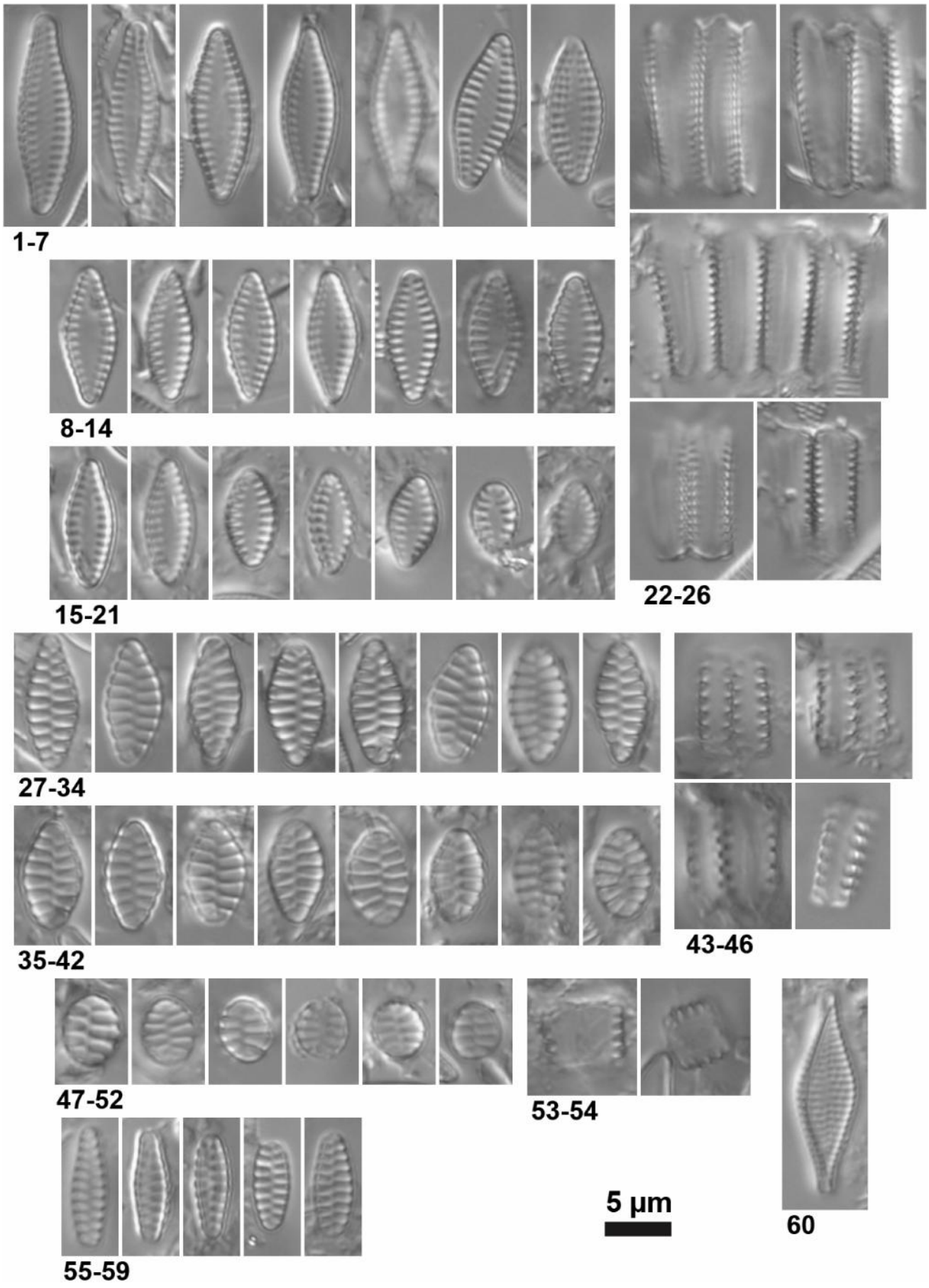
Taxon	Length (µm)	Width (µm)	Striae in 10 µm	Comment
F. brevistriata	5-30 (more?)	3-7	12-17	striae extremely peripheral
F. construens f. *construens*	15-27	5-7	13-14	striae parallel, shape
F. construens f. *venter*	4-9	3-6	19-21	striae parallel, shape
F. elliptica	3-10 (more?)	2.8-6	11-16 (more?)	punctate striae
F. lapponica	10-30	3-6	6-10	striae extremely peripheral
F. leptostauron	(6?) 15-36	(3?) 10-23	5-9 (11)	shape
F. leptostauron var. *dubia*	(6?) 15-36	10-23	5-9	shape
F. leptostauron var. *leptostauron*	(6?) 15-36	10-23	5-9	shape
F. martyi	(6?) 15-36	10-23	5-9	shape
F. parasitica (var. *parasitica*)	10-25	3-5	16-20	shape
F. pinnata	3-35(60?)	2-8 (more?)	(5)6-12	striae mainly parallel
F. pinnata var. *pinnata*	3-35	2-8	8-12	striae mainly parallel
F. pseudoconstruens	3-22	3-7	15-18	punctate striae!
F. construens f. *subsalina*	12-24	4-4.5	12-14	punctate striae!

We recommend to document the small fragilarioid diatoms with pictures to enable a subsequent allocation to the new taxonomy and names once the taxonomic discussion is settled. However, it remains uncertain, if the German Phylib-method can implement new taxonomic models in the near future by recounting the training set samples according to the newest taxonomy.

The identification of valves in girdle band views will remain a problem. Especially the small fragilarioid diatoms in slides often lay in girdle view. We recommend to measure them and identify these valves as much as possible. If this is impossible, the girdle band views should be allocated proportionally to the appropriate taxa present in valve view. The girdle views should also be documented photographically. Please note that the German method asks for counting objects and not valves. Objects may be a frustule or a valve (for details see Schaumburg et al. (2011), page 25). Each frustule within a chain of girdle bands is one object (Hofmann, personal communication). Valves at the end of the chains are also one object (Hofmann, personal communication).

Plate 9 (next page): Small fragilarioid diatoms from the intercalibration exercise sample D 1.1 (Lake Geneva, Switzerland). The depicted valves are named according to Hofmann et al. (2013): ***Staurosira brevistriata* (Grunow) Grunow** or alternatively *Fragilaria brevistriata* Grunow (1-26); ***Staurosira mutabilis* (W. Smith) Grunow** or alternatively *Fragilaria pinnata* Ehrenberg (27-54); ***Staurosira* cf. *mutabilis* (W. Smith) Grunow** or alternatively *Fragilaria* cf. *pinnata* Ehrenberg (55-59); ***Fragilaria parasitica* var. *parasitica* (W. Smith) Grunow** (60). Figures 22-26; 43-46 and 53-54 are girdle band views, with figures 22 and 23, as well as 25 and 26 depicting the same valves in different foci. The depicted taxa were found during a detailed screening of sample D 1.1 and are the only taxa of small fragilarioid diatoms that could be detected, i.e. no other, similar taxa were found during the detailed screening. For this search, a total of 5,000 diatom valves were looked at in sample D 1.1. It is possible, that participants found other very rare single finds in sample D 1.1 that are not shown here. However, they are statistically irrelevant.

Measurements of depicted valves:
1-26: length: 5.5-15.5 µm, width: 3.5-4.8 µm, striae in 10 µm: 14-15
27-42: length: 7.0-10.5 µm, width: ~4.5 µm, striae in 10 µm: ~10
47-52: length: 4.5-5.5 µm, width: 4.0-4.2 µm, striae in 10 µm: 10-12
55-59: length: 6.8-9.1 µm, width: 2.5-3.0 µm, striae in 10 µm: 12-13 (14)


1-7
8-14
15-21
22-26
27-34
35-42
43-46
47-52
53-54
55-59
60
5 µm

3.2.8 *Fragilaria capucina* and similar taxa in samples D 1.1 and D 2

Several taxa from the formerly called *Fragilaria capucina*-complex occurred in samples D 1.1 and D 2 (Plates 10 and 11), with a higher diversity and also abundance of these taxa in sample D 2 compared to sample D 1.1. The results suggest that many participants seem to have problems with identifying these *Fragilaria*-taxa and also that identification literature of various age was used. Both led to a number of names for these *Fragilaria*-taxa (see Fig. 37 and Fig. 41) that exceeds the real diversity within this group in both samples. Participants assigned 13 names to the here discussed *Fragilaria*-species in sample D 1.1 and 19 names to the *Fragilaria*-taxa in sample D 2.

Fragilaria capucina-complex in sample D 1.1

All participants of the intercalibration exercise determined taxa of the former *Fragilaria capucina*-complex or similar taxa (0.4-6.7 %, mean: 2.5 %) in sample D 1.1 (Fig. 37). The three auditors identified 1.4-2.4 % (mean: 1.9 %) of this group in good agreement with one another (Fig. 37).

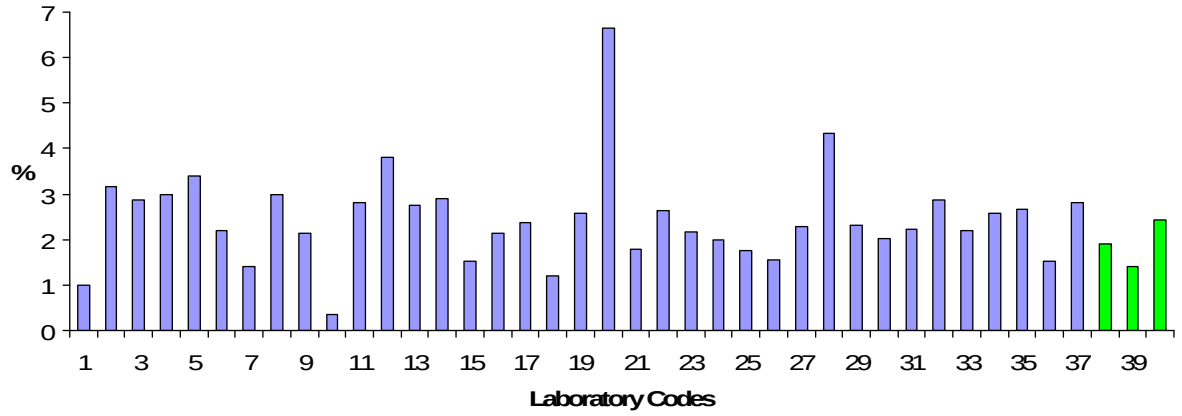


Fig. 37: Sum of the relative abundance of ***Fragilaria* spec.**, ***F. amphicephaloides***, ***F. austriaca***, ***F. capucina*** (including cf.), ***F. capucina* var. *capucina***, ***F. capucina* var. *vaucheriae***, ***F. famelica***, ***F. famelica*** var. ***famelica***, ***F. gracilis***, ***F. pararumpens***, ***F. perminuta***, ***F. rumpens*** (including cf.) and ***F. vaucheriae*** from sample D 1.1. Blue: participants, green: auditors.

Of 37 participants 23 detected *Fragilaria perminuta* in sample D 1.1 (0.2-3.2; mean: 2.0) (Fig. 38). All three auditors identified *F. perminuta* in sample D 1.1 (0.2-1.9 %; mean: 1.2) (Fig. 38). The absence of *F. perminuta* in sample D 1.1 in the results of the participants may

be due to an identification mistake or due to the variability among slides (see Chapter 2). As the relative abundance of this species was relatively low, both seem possible.

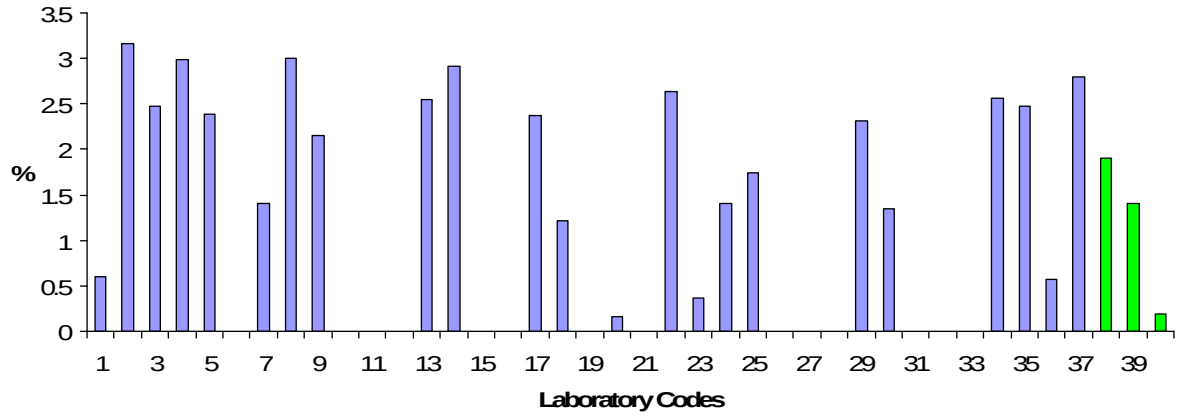


Fig. 38: Relative abundances of ***Fragilaria perminuta*** from sample D 1.1. Blue: participants, green: auditors.

Fragilaria capucina or *F. capucina* var. *capucina* were detected by one auditor (0.6 %) and by seven participants (0.4-4.0 %; mean: 1.6 %) (Fig. 39). Interestingly, laboratories with relatively high abundances of *F. capucina* (>1 %) did not detect any (laboratories 19 and 26) or only relatively low abundances (laboratories 20 and 23) of *F. perminuta*. Thus, it seems possible that the species were mistaken for one another or that they were misidentified.

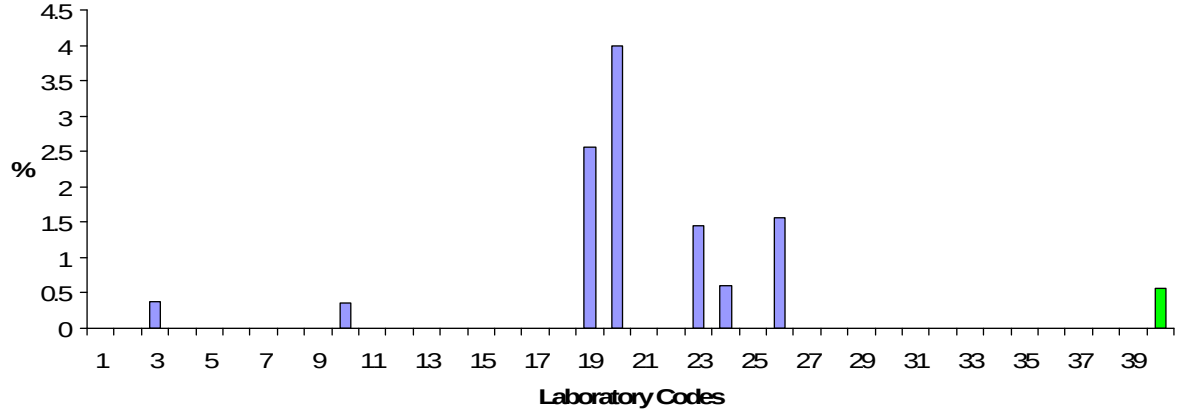


Fig. 39: Relative abundances of ***Fragilaria capucina*** (including cf.) and ***F. capucina* var. *capucina*** from sample D 1.1. Blue: participants, green: auditors.

Fragilaria capucina var. *vaucheriae* or *F. vaucheriae* was identified with 0.2 % to 2.8 % by 12 of the 37 participants (Fig. 40). The three auditors did not detect any *F. vaucheriae* in

sample D 1.1. Of the 12 laboratories that identified *F. capucina* var. *vaucheriae* or *F. vaucheriae* in sample D 1.1, especially laboratories with relatively high abundances (>1 %) of these two names, did not detect any *F. perminuta* (laboratories 6, 11, 15, 16, 21, 27 and 33). Thus, it seems likely that *F. perminuta* was mistaken for *F. capucina* var. *vaucheriae* or *F. vaucheriae*.

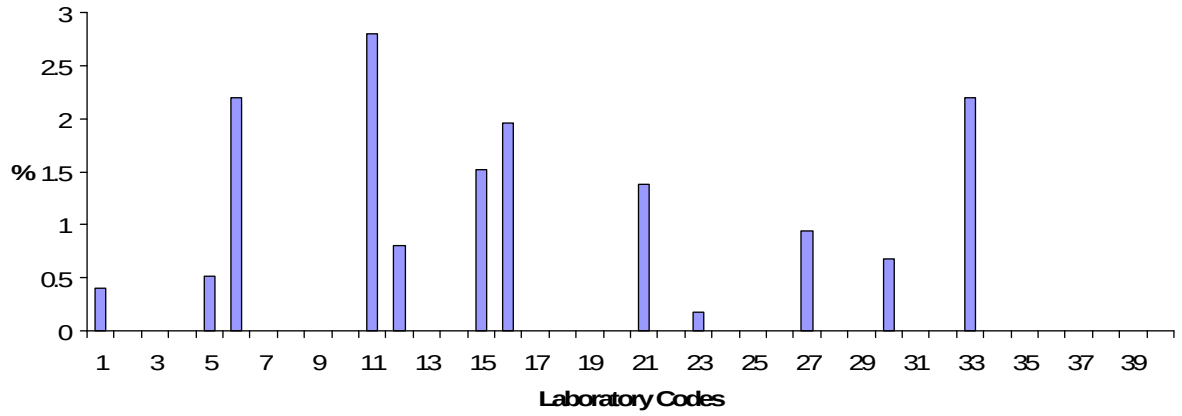


Fig. 40: Relative abundances of ***Fragilaria vaucheriae*** or ***F. capucina* var. *vaucheriae*** from sample D 1.1. Blue: participants, no. 38-40: auditors (none).

One auditor (1.5 %) and four laboratories (0.2-0.5 %) identified *Fragilaria* spec. in sample D 1.1 (not shown). *Fragilaria pararumpens* was not detected by any auditor, but by three participants with 0.9-2.9 % (not shown). Two of these laboratories did not detect *F. perminuta* in sample D 1.1. *Fragilaria rumpens* or *F.* cf. *rumpens* were also not detected by any auditor, but by four participants with 0.2-4.3 % (not shown). Two of these laboratories did not detect any *F. perminuta* in sample D 1.1. *Fragilaria austriaca* (2.5 %) and *Fragilaria gracilis* (2.8 %) were only detected once, misidentifications seem likely (not shown).

The results from sample D 1.1 suggest that there were probably considerable identification problems with respect to the former *Fragilaria capucina*-complex. The only taxon from this group, where regular occurrence can be assumed, was *F. perminuta* (see also Plate 10). Thus, many denominations (see Fig. 37) probably occurred due to mistaking *F. perminuta* for other taxa.

Fragilaria capucina-complex in sample D 2

The former *Fragilaria capucina*-complex in sample D 2 was more divers compared to sample D 1.1 (Plate 11). The auditors identified similar relative abundances from the *F. capucina*-aggregate with 10.5-12.1 % (mean: 11.3 %) (Fig. 41). The participants determined 4.4-17.3 % (mean: 9.6 %) from this group (Fig. 41).

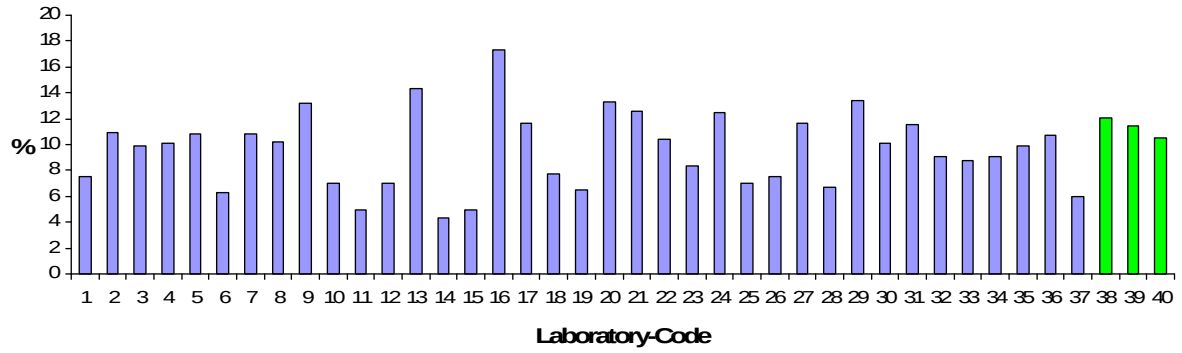


Fig. 41: Sum of the relative abundances of ***Fragilaria* spec.**, ***F. amphicephaloides***, ***F. austriaca*** (including cf.), ***F. capitellata***, ***F. capucina*** (including cf.), ***F. capucina* var. *austriaca***, ***F. capucina* var. *capucina***, ***F. capucina* var. *gracilis***, ***F. capucina* var. *rumpens***, ***F. capucina* var. *vaucheriae***, ***F. delicatissima***, ***F. gracilis*** (including cf.), ***F. pararumpens***, ***F. pectinalis***, ***F. perminuta***, ***F. radians***, ***F. recapitellata***, ***F. rumpens*** (including cf.) and ***F. vaucheriae*** in sample D 2. Blue: participants, green: auditors.

Of the here discussed taxa, the most abundant one was probably *F. vaucheriae* in sample D 2 (Plate 11). For example, all three auditors determined this taxon (named *F. capucina* var. *vaucheriae*) with 2.8-9.5 % (mean: 6.9) in sample D 2 (Fig. 42). Of the 37 intercalibration-participants 28 identified *F. vaucheriae* or *F. capucina* var. *vaucheriae* with 0.6-12.5 % (mean: 7.0 %) (Fig. 42). Nine participants did not identify this taxon.

Despite the obvious difficulties with identifying the here discussed *Fragilaria*-species, only 11 of the 37 participants indicated any ambiguity with the identification (by using cf. or spec.) (0.2-5.2 %) (Fig. 43). Two auditors identified a part of the objects as *Fragilaria* spec. (1.0 and 6.2 %) (Fig. 43).

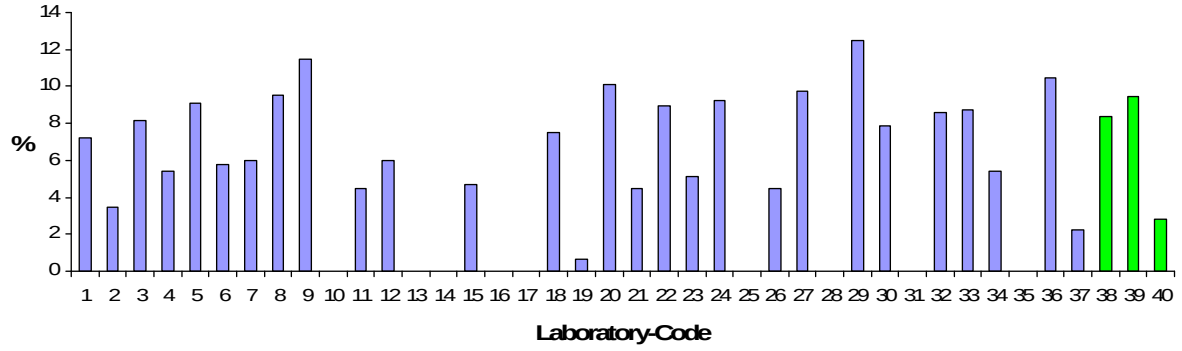


Fig. 42: Relative abundances of ***Fragilaria vaucheriae*** and ***F. capucina* var. *vaucheriae*** from sample D 2. Blue: participants, green: auditors.

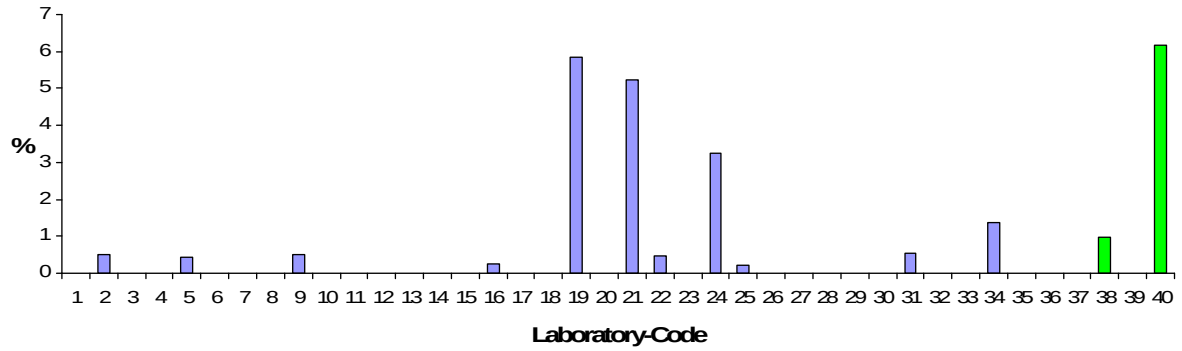


Fig. 43: Sum of the relative abundances of **ambiguous objects from the *Fragilaria capucina*-complex** (*Fragilaria* spec., *F.* cf. *austriaca*, *F.* cf. *capucina*, *F.* cf. *gracilis*, *F.* cf. *radians* and *F.* cf. *rumpens*) from sample D 2. Blue: participants, green: auditors.

One auditor determined *Fragilaria austriaca* with 0.2 % (Fig. 44). Almost a third of the participants also identified *F. austriaca* in sample D 2 with 0.2-1.8 % (Fig. 44).

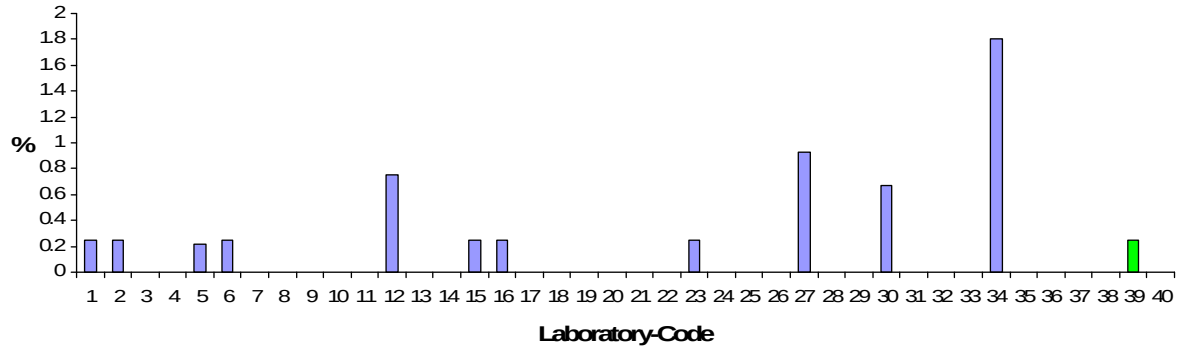


Fig. 44: Relative abundances of ***Fragilaria austriaca*** and ***F. capucina* var. *austriaca*** from sample D 2. Blue: participants, green: auditors.

One auditor determined *Fragilaria capucina* (named *F. capucina* var. *capucina*) with 1.6 % in sample D 2 (Fig. 45). 12 of the 37 participants also found *F. capucina* or *F. capucina* var. *capucina* with distinctly varying relative abundances (0.3-12.6 %; mean: 4.9 %) (Fig. 45).

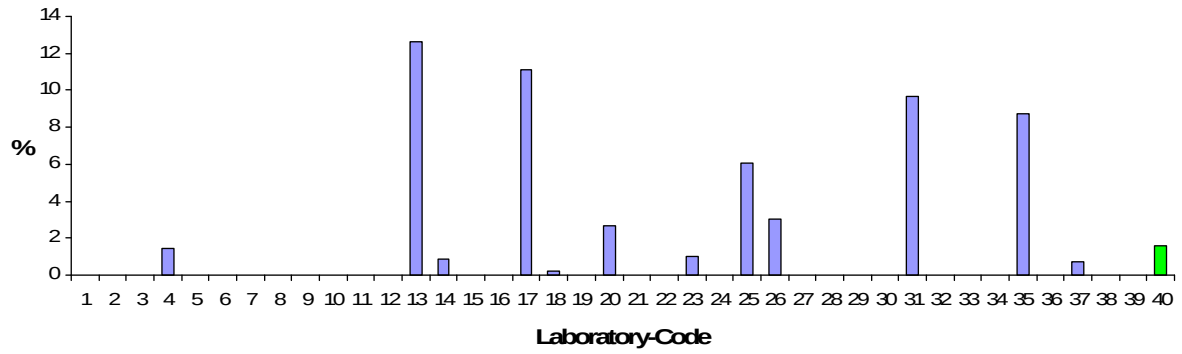


Fig. 45: Relative abundances of ***Fragilaria capucina*** and ***F. capucina* var. *capucina*** from sample D 2. Blue: participants, green: auditors.

Two auditors determined *Fragilaria gracilis* in sample D 2 (1.5 % and 1.2 %, respectively) (Fig. 46). 15 of the 37 participants also identified *F. gracilis* or *F. capucina* var. *gracilis* (0.2-1.5 %; mean: 0.8 %) (Fig. 46).

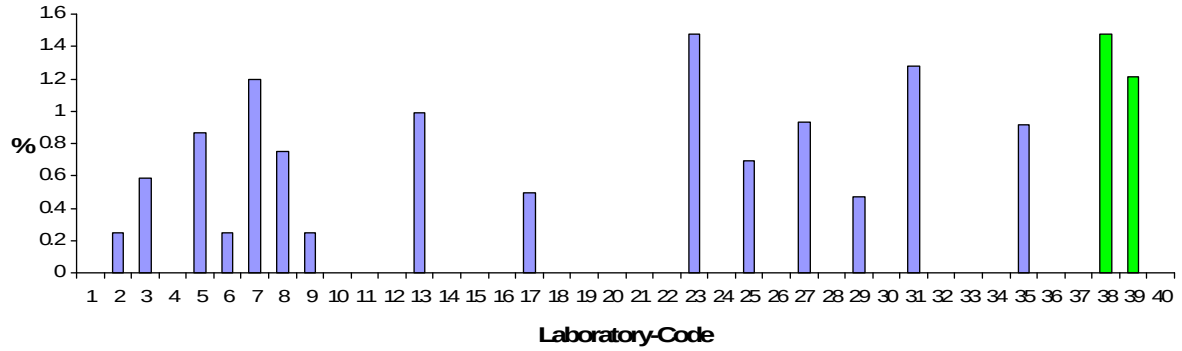


Fig. 46: Relative abundances of ***Fragilaria gracilis*** and ***F. capucina* var. *gracilis*** from sample D 2. Blue: participants, green: auditors.

Apart from the above mentioned names, several participants of the intercalibration exercise also used additional names for the taxa from the *F. capucina*-aggregate in sample D 2, namely *Fragilaria perminuta*, *F. rumpens* or *F. capucina* var. *rumpens*, *F. pectinalis*, *F. delicatissima* and *F. amphicephaloides* (not shown). These five taxa were not detected by any of the auditors in sample D 2. *F. perminuta* was determined by five participants (laboratories 2, 3, 7, 14 and 20) with 0.5-2.8 % (mean: 1.4 %). *F. rumpens* or *F. capucina* var. *rumpens* was

identified by 14 laboratories (laboratories 2, 5, 7, 9, 11, 12, 14, 16, 21, 22, 28, 30 and 37) (0.2-6.7 %; mean: 1.3 %). *F. pectinalis* was only identified by laboratory 16 with 16.1 % and *F. delicatissima* (4.1 %) as well as *F. amphicephaloides* (3.0 %) only by laboratory 10.

The results of the participants in samples D 1.1 and D 2, and to a lesser extend partly the results of the auditors, demonstrate the taxonomic problems with identifying species of the former *Fragilaria capucina*-complex. For example, taxa that were detected by all three auditors were not determined or found by a considerable percentage of the participants, such as *F. perminuta* in sample D 1.1 and *Fragilaria vaucheriae* in sample D 2. Also, taxa that were not found by any of the auditors, were determined by several participants with more or less high relative abundances, such as *F. vaucheriae* in sample D 1.1 and *F. perminuta* or *F. rumpens* in sample D 2. Still, ambiguities were hardly labelled with "spec." or "cf", despite these obvious difficulties.

Many taxa of the *Fragilaria capucina*-aggregate *sensu lato* are very similar. Transitional forms between some of the here discussed taxa exist and identification traits of many taxa overlap (see Tab. 17). Also, in many cases several basic and detailed taxonomic questions are still unanswered. Thus, for an unambiguous determination of the species within the *Fragilaria capucina*-aggregate *sensu lato* it is imperative to measure all possible parameters (length, width, striae density) for several individuals and to carefully compare these with the pictures and the details given in the species descriptions in the identification literature. Hofmann et al. (2013) is suitable as identification literature for most of the here discussed taxa (see below).

Of the taxa listed in Tab. 17 with a width up to 3 µm (*F. amphicephaloides*, *F. gracilis*, *F. delicatissima* and *F. pararumpens*), *F. gracilis* can be identified based on the high striae density of about 20 in 10 µm and *F. pararumpens* based on the distinctive valve view: the short central part is more or less widened, the shape is lanceolate, gradually narrowing from the middle to the capitate ends (Hofmann et al, 2013). *F. delicatissima* (14-16 striae in 10 µm) and *F. amphicephaloides* (10-14 striae) can usually be distinguished from one another based on their striae density. Additionally, *F. amphicephaloides* has valve ends that are more strongly capitated compared to *F.delicatissima*.

Of the species with a width >3.5 µm (*F. capucina* var. *capucina*, *F. rumpens*, *F. vaucheriae*, *F. radians*, *F. mesolepta* and *F. pectinalis*), *F. mesolepta* can usually (see below) be easily distinguished by the constriction in the middle part of the valve. *F. radians*

usually differs by their low striae density of 9-11 striae in 10 µm from the other taxa. *F. capucina* var. *capucina* and *F. rumpens* can be distinguished from the other taxa by their shape (in valve view), if the striae density does not allow a differentiation. Valves of *F. radians* narrow gradually from the middle to the capitate ends (Hofmann et al. 2013). In contrast, the sides of the valves of *F. vaucheriae* are almost parallel from the middle almost to the ends, where they narrow distinctly ("like shoulders"). *F. capucina* var. *capucina* differs from *F. rumpens* by the striae density. Of the taxa with a valve width >3.5 µm, the most difficult is probably the differentiation of *F. vaucheriae* and *F. pectinalis*. Here, the entire combination of traits always need to be considered. In contrast to the other here discussed taxa *F. famelica* has (usually visible) dotted striae. *F. famelica* is taxonomically not part of the *F. capucina*-aggregate, but is listed here because it could be confused with some of these species.

The remaining species *F. austriaca, F. perminuta, F. recapitellata, F. acidoclinata* (and *F. famelica*) have a valve width of 3-4 µm or 3-5 µm (Tab. 17). Thus, they cannot clearly be allocated to either of the previous two groups. Within the *F. capucina*-aggregate *F. perminuta* can be distinguished relatively well by the one-sided central area, where the valve margin is usually slightly widened outward (Hofmann et al. 2013). Often, a blurry patch can be seen near the central area. *F. austriaca* can also be distinguished relatively well by their lanceolate shape and capitate ends. *F. recapitellata* can be similar to *F. vaucheriae*. However, *F. recapitellata* (14-18 striae in 10 µm) has a higher striae density and is often more capitate than *F. vaucheriae* (9-14 striae). The striae of *F. acidoclinata* are coarsely dotted and the valves have a characteristic shape with relatively long protracted and capitately rounded ends.

We think that a documentation with pictures is particularly important for the here discussed taxa, to ensure the comparability of counting results among diatomists and to enable a later revision of counting results. According to Hofmann et al. (2013) the autecology should be used as an additional trait for species identification in some cases (see e.g. details about *F. austriaca* in Hofmann et al. 2013). We disagree, as the diatomist often does not know the ecology of the water body or as the limnological data of the sampling sites are often missing, especially when implementing the EU-Water Framework Directive. Additionally, it would be a circular argument to infer the water quality based on species that were identified based on the water quality. When in doubt, we recommend using „cf.“ in addition to the photographic documentation, to avoid pretending unambiguous naming. In the following we present an overview of the most important identification traits of the here discussed species in Tab. 17 and in notes.

Tab. 17: Measureable dimensions and some traits of taxa of the former *Fragilaria capucina*-complex and similar taxa. All names were either used by the participants or auditors for objects from samples D 1.1 and D 2 (exception: additionally listed are *F. mesolepta* and *F. acidoclinata*). Details according to Hofmann et al. (2013), Krammer & Lange-Bertalot (1986-2004), Tuji (2007)* and Tuji & Williams (2008). For more information see text. Apart from *F. pectinalis* all taxa listed here are depicted in Hofmann et al. (2013).

Taxon	Length (µm)	Width (µm)	Striae/10µm	Comment
F. acidoclinata	35-60	3-4	11-13	shape, dotted
F. amphicephaloides	40-75	2-3	10-14	shape, central area
F. austriaca	20-60	3-4	12-15	shape
F. capucina var. *capucina*	<25-75	3.5–4.5	12-17	
F. delicatissima	30->100	2.5-3	14-16	
F. famelica	10-70	2.5-4	11-16	striae dotted
F. gracilis	<10-60	2-3 (-3.6*)	~20	
F. mesolepta	~20-60	3.5–4.5	15-18	shape
F. pararumpens	25-50	2.5-3	16-18	shape, central area
F. pectinalis	28-37	3.5-4	14-15	central area
F. perminuta	7-40	3-4	17-21	shape, central area
F. radians	35-55	3.5-4.5	9-11	shape, central area
F. recapitellata	11-38	3-5	14-18	shape, central area
F. rumpens	~20-65	3.5-4	18-20	
F. vaucheriae	<10-50	4-5	9-14	shape

***Fragilaria acidoclinata* Lange-Bertalot & Hofmann**

- in Krammer & Lange-Bertalot (1986-2004) not listed
- synonyms: unknown
    - valves linear to narrow linear-lanceolate
    - ends more or less protracted and rounded capitated
    - distinctly coarsely dotted, axial area lanceolate. Central area more or less rectangular

***F. amphicephaloides* Lange-Bertalot**

- in Krammer & Lange-Bertalot (1986-2004) named *Fragilaria capucina amphicephala*-Sippe
- synonyms: *Fragilaria capucina* var. *amphicephala* (Kützing) Lange-Bertalot, *F. capucina* subsp. *amphicephala* (Kützing) Lange-Bertalot, *Synedra amphicephala* Kützing, *F. amphicephala* Ehrenberg
    - valves linear, ends more or less capitately protruded
    - axial area moderately narrow to somewhat widened
    - striae in the valve middle weakened by a vague central area

***F. austriaca* (Grunow) Lange-Bertalot**

- in Krammer & Lange-Bertalot (1986-2004) named *Fragilaria capucina austriaca*-Sippe
- synonym: *Fragilaria capucina* var. *austriaca* (Grunow) Lange-Bertalot
    - valves narrow lanceolate to linear-lanceolate
    - ends more or less capitately protruded
    - axial area (usually) small linear, central area variable

F. capucina* Desmazieres var. *capucina

- in Krammer & Lange-Bertalot (1986-2004) named *Fragilaria capucina*-Sippe *sensu stricto*
    - valves variable from lanceolate to linear
    - striae are often disrupted or weakened by a more or less distinct central area
    - striae are not visibly dotted

***F. delicatissima* (W. Smith) Lange-Bertalot**

- in Krammer & Lange-Bertalot (1986-2004) also named *F. delicatissima*
- basionym: *Synedra delicatissima* W. Smith
    - named *Ulnaria delicatissima* by Aboal & Silva (2004)
    - valves narrow linear to narrow lanceolate, ends long protruded and capitate
    - valves narrow gradually from the middle to the ends

***F. famelica* (Kützing) Lange-Bertalot**

- in Krammer & Lange-Bertalot (1986-2004) named *F. famelica*-Sippe sensu stricto
- synonyms: *Synedra famelica* Kützing, *S. minuscula* Grunow, *Nitzschia famelica* (Kützing) Rabenhorst, *N. palea* f. *famelica* (Kützing) Rabenhorst, *N. palea* var. *famelica* (Kützing) Peragallo
    - valves linear-elliptical to linear-lanceolate, ends obtusely rounded to slightly protracted
    - striae dotted in light microscope (usually just visible)
    - axial area very narrow, central area variable, often irregular or absent

***F. gracilis* Østrup**

- in Krammer & Lange-Bertalot (1986-2004) named *F. capucina gracilis*-Sippe
- synonym: *Fragilaria capucina* var. *gracilis* (Østrup) Hustedt
    - valves linear to linear-lanceolate
    - ends obtusely rounded, not or only slightly protracted
    - striae often weakened or disrupted by a more or less distinct central area
    - areolae of striae not visible

***F. mesolepta* Rabenhorst**

- in Krammer & Lange-Bertalot (1986-2004) named *F. capucina mesolepta*-Sippe sensu lato
- synonyms: *Fragilaria capucina* var. *mesolepta* (Rabenhorst) Rabenhorst, *F. virescens* var. *mesolepta* (Rabenhorst) Schönfelt, *F. virescens* f. *mesolepta* (Rabenhorst) Cleve-Euler, *F. capucina* f. *mesolepta* (Rabenhorst) Hustedt, *Staurosira mesolepta* (Rabenhorst) Cleve & Möller, *S. capucina* var. *mesolepta* (Rabenhorst) Comère
  - in Hofmann et al. (2013) and Krammer & Lange-Bertalot (1986-2004) three "Sippen":
    - *mesolepta*-Sippe, in Krammer & Lange-Bertalot Plate 110, Fig. 14-16
    - *subconstricta*-Sippe, in Krammer & Lange-Bertalot Plate 110, Fig. 17-18
    - *tenuistriata*-Sippe, Krammer & Lange-Bertalot Plate 110, Fig. 19-21
    - according to Tuji & Williams (2008) these are three separate species

  mesolepta-Sippe
    - valves almost linear, in the middle more or less strongly constricted
    - ends slightly capitately protruded

  subconstricta-Sippe and *tenuistriata*-Sippe:
    - valves almost straight, in the middle only slightly or not at all constricted
    - ends widely to distinctly obtuse rounded

***F. pararumpens* Lange-Bertalot, Hofmann, Werum**

- in Krammer & Lange-Bertalot (1986-2004) listed with various names
- in Krammer & Lange-Bertalot (1986-2004) in Plate 110, Fig. 22; Plate 112, 10-11
- synonyms: *Fragilaria familiaris* Krasske
  - valves relatively narrow lanceolate
  - valves gradually narrowing from the middle to the ends
  - valves in a small area more or less slightly widened in the middle
  - ends capitate, dots on striae not visible
  - axial area narrow to very narrow

  Central area relatively large, almost square hyaline or with „ghost-striae“

***F. pectinalis* (O.F. Müller) Lyngbye**

- in Hofmann et al. (2013) and Krammer & Lange-Bertalot (1986-2004) not included
- depicted and described in Tuji & Williams (2006 and 2008)
- synonyms: *Conferva pectinalis* O.F. Müller, *Synedra capitellata* var. *cymbelloides* Grunow, *S. capitellata* f. *striis-distantioribus* Grunow, *S. gloiophila* Grunow
  - valves mainly linear to linear -lanceolate
  - ends usually more or less weakly protracted to slightly capitate
  - central area one-sided

***F. perminuta* (Grunow) Lange-Bertalot**

- in Krammer & Lange-Bertalot (1986-2004) named *F. capucina perminuta*-Sippe
- synonyms: *Fragilaria perminuta*-Sippe Krammer & Lange-Bertalot
    - valves variable, linear-elliptical (linear-lanceolate) to almost linear in large cells
    - ends more or less capitately protracted, central area one-sided, often slightly widened

***F. radians* (Kützing) Williams & Round**

- in Krammer & Lange-Bertalot (1986-2004) named *F. capucina radians*-Sippe
- synonyms: *Synedra radians* Kützing and many more
    - valves almost linear, ends capitately protracted
    - valves often slightly widened around the central area
    - no dots visible on the striae
    - central area square to apical square with blurred „ghost-striae“

***F. recapitellata* Lange-Bertalot & Metzeltin**

- in Krammer & Lange-Bertalot (1986-2004) named *F. capucina capitellata*-Sippe
- synonyms: *Synedra capitellata* Grunow, *S. vaucheriae* var. *capitellata* (Grunow) Hustedt, *Fragilaria intermedia* var. *capitellata* (Grunow) Cleve-Euler, *F. capitellata* (Grunow) Petersen, *F. vaucheriae* var. *capitellata* (Grunow) Ross, *F. capucina capitellata*-Sippen Krammer & Lange-Bertalot
    - valves with decreasing size from linear to lanceolate
    - ends more or less capitate, central area one-sided distinct to blurry

***F. rumpens* (Kützing) Carlson**

- in Krammer & Lange-Bertalot (1986-2004) named *F. capucina rumpens*-Sippen
- synonyms: *Fragilaria capucina* subsp. *rumpens* (Kützing) Lange-Bertalot, *F. capucina* var. *rumpens* (Kützing) Lange-Bertalot, *Synedra rumpens* Kützing
    - valves linear to linear-lanceolate, ends not or only slightly protracted, obtuse rounded

***F. vaucheriae* (Kützing) Petersen**

- in Krammer & Lange-Bertalot (1986-2004) named *F. capucina vaucheriae*-Sippen s.t.
- synonyms: *Exilaria vaucheriae* Kützing, *Fragilaria vaucheriae* var. *parvula* (Kützing) Cleve-Euler, *F. capucina* var. *vaucheriae* (Kützing) Lange-Bertalot, *Synedra vaucheriae* (Kützing) Kützing, *Ctenophora vaucheriae* (Kützing) Schönfeldt, *Ceratoneis vaucheriae* (Kützing) Kobayasi, *C. vaucheriae* (Kützing) Kobayashi
    - valves variable; linear-elliptical (small valves) to linear (large valves)
    - central area one-sided, not always pronounced

Plate 10 (next page): ***Fragilaria perminuta*** (**1-18**) and ***F.* cf. *perminuta*** (**19-21**) from sample D 1.1 (Lake Geneva, Switzerland). Valves 19-20 are slightly too narrow (2.7-2.8 µm wide), all other traits correspond with the definition of *F. perminuta*. Other taxa of the *F. capucina*-aggregate than the ones depicted here could not be found during a detailed screening of sample D 1.1 (1,500 diatom valves were looked at and all valves of the *F. capucina*-aggregate or similar taxa were identified). For more information see text.

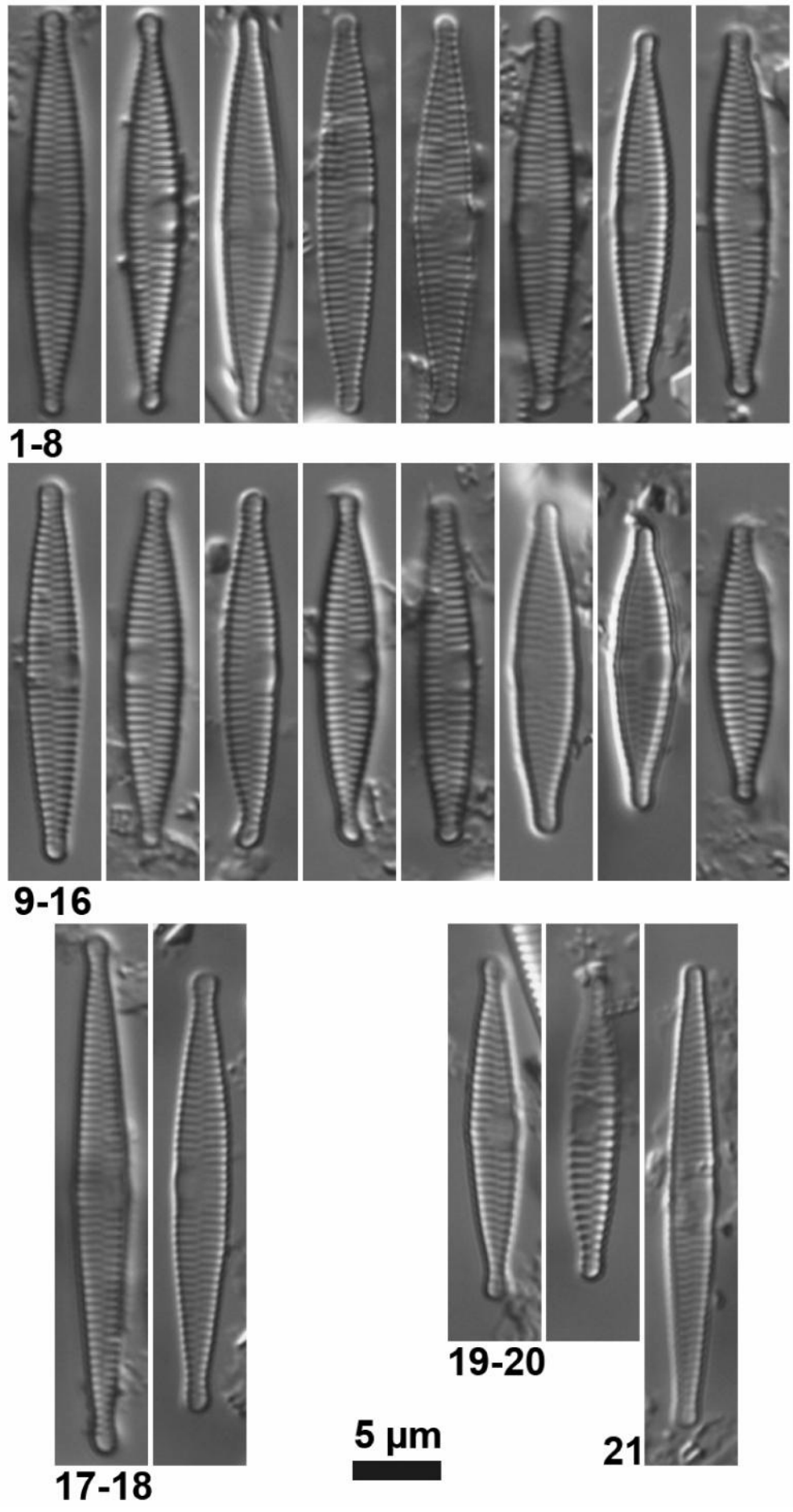
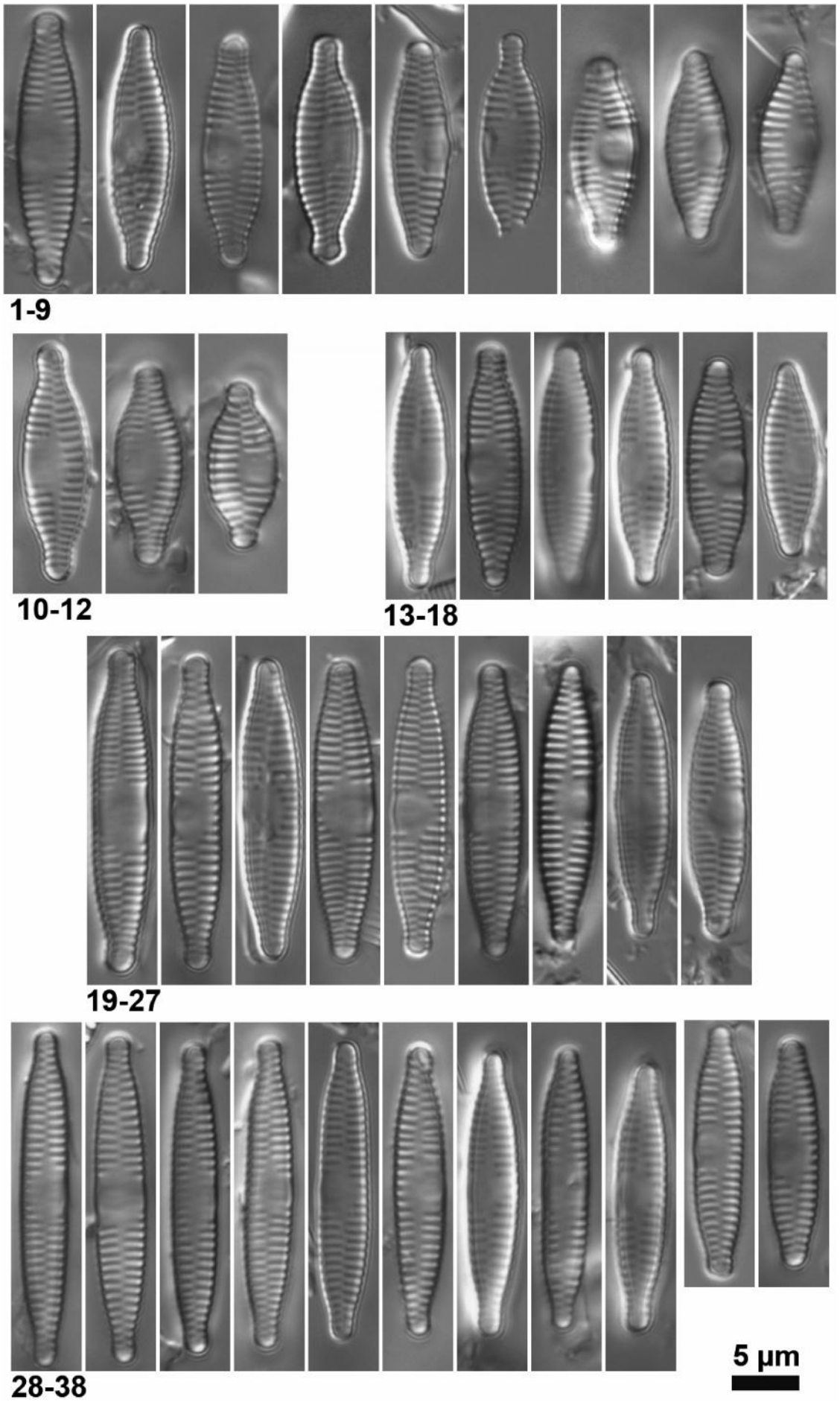

1-8
9-16
17-18
19-20
21
5 µm

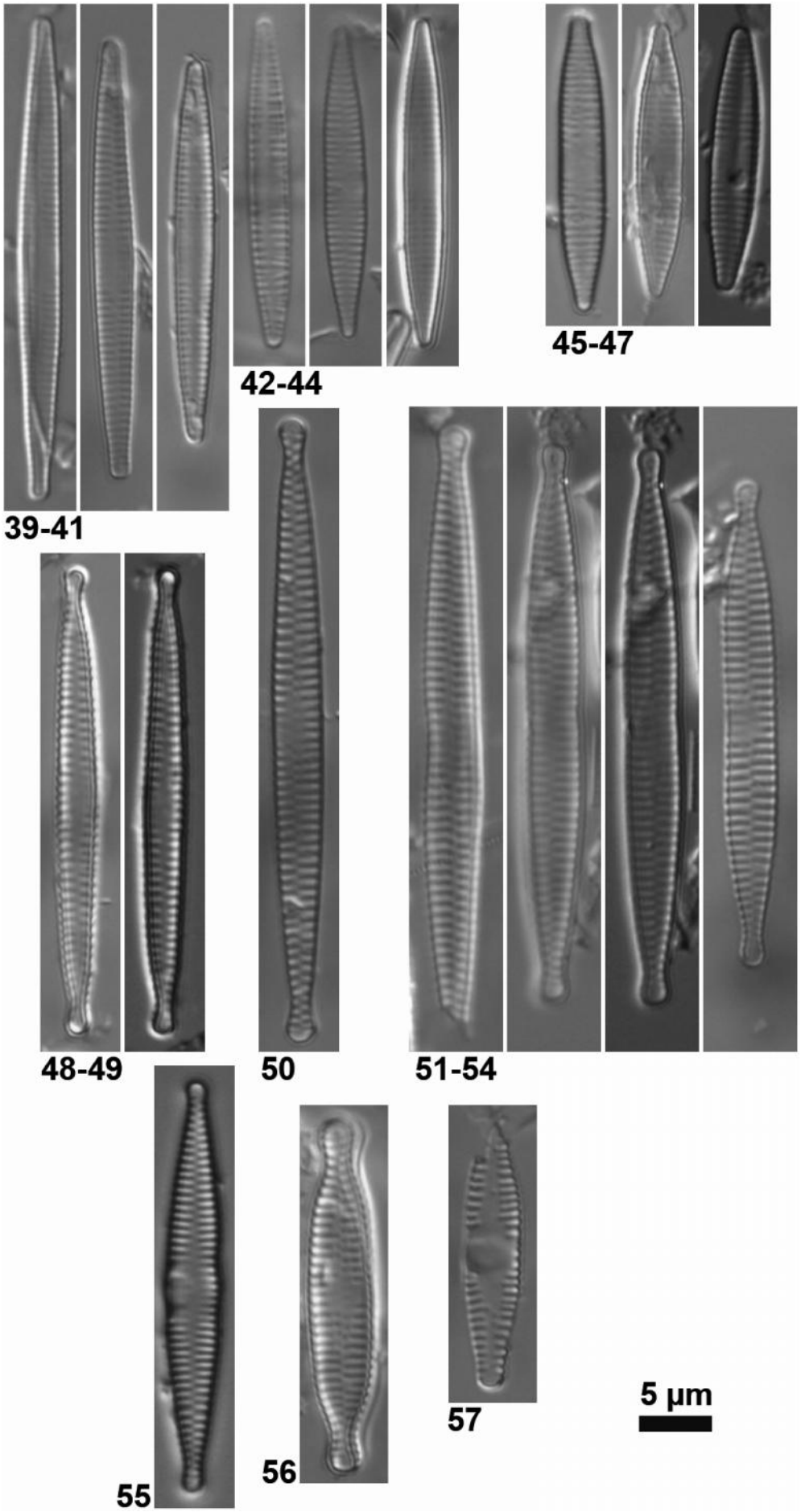
Plate 11 (the following two plates): ***Fragilaria recapitellata*** (**1-9**), ***F.* cf. *recapitellata*** (**10-12**), ***Fragilaria*-valves that are difficult to unambiguously identify** (*F. capucina* var. *capucina* or *F. vaucheriae*) (**13-38**), ***F. gracilis*** (**39-44**), ***F.* cf. *gracilis*** (**45-47**), ***F.* cf. *austriaca*** (**48-49**), ***F. austriaca*** (**50**), ***Fragilaria* spec. 1** (**51-54**), ***F.* cf. *pararumpens*** (**55**), ***Fragilaria* spec. 2** (**56**) and ***Fragilaria* spec. 3** (**57**) from sample D 2 (River Drau, Austria). Other taxa of the *F. capucina*-aggregate than the ones depicted here could not be found during a detailed screening of sample D 2 (1,500 diatom valves were looked at and all valves of the *F. capucina*-aggregate or similar taxa were identified). For more information see text.

The valves named ***F.* cf. *recapitellata* (10-12)** are slightly too wide for *F. recapitellata* (5.1-5.3 µm instead of 3-5 µm). All other traits correspond to the species description, suggesting that the valve width range of *F. recapitellata* may actually be larger than given. Thus, the depicted valves are probably *F. recapitellata*.

The ***Fragilaria*-valves labelled "difficult to identify"** (**13-38**) correspond to a mixture of the measurable dimensions of *F. capucina* var. *capucina* (3.5-4.5 µm wide, 12-17 striae in 10 µm) and *F. vaucheriae* (4-5 µm wide, 9-14 striae), as they where 14.6-25.1 µm long, 3.4-4.5 µm wide and had 14.5-16.5 striae in 10 µm (exception valve 25 with 13.5 striae in 10 µm), whereby the minimum length of *F. capucina* var. *capucina* is not clearly identified in the literature. However, the shape of these valves are more similar to the description of *F. vaucheriae*, for which the striae are too dense. Valves 13-27 are ~4.0-4.5 µm wide and the valves 28-38 are 3.4-3.9 µm wide. Apart from valve 25 none of the here depicted valves (13-38) can only be allocated ambiguously to either *F. vaucheriae* or *F. capucina* var. *capucina*. Ultimately, these valves need to be labelled with "cf." or "spec.". Only valve 25 (length: 25.8 µm; width 4.2 µm; 13.5 striae in 10 µm) corresponds in all traits with the description of *F. vaucheriae* and could consequently be named as such.

Valves labelled ***F.* cf. *gracilis*** (**45-47**) are slightly too wide according to the details given in Hofmann et al. (2013) (3.1-3.4 µm instead of 2-3 mm). However, Tuji (2007) pointed out that *F. gracilis* is sometimes wider than 3.0 µm (e.g. 3.6 µm). Consequently, valves 45-47 are probably *F. gracilis*. The valves named ***F.* cf. *austriaca*** (**48-49**) are too narrow (2.8-2.9 µm instead of 3-4 µm) and striae are too dense (16-16.5 instead of 12-15 striae in 10 µm) for *F. austriaca*. The valves labelled ***Fragilaria* spec. 1** (**51-54**) could be named *F.* cf. *mesolepta*, as the valves are similar to *F. mesolepta*. However, shape (in fact with a constriction in the middle, however, valves hardly linear) and striae density (14-15 instead of 15-18 in 10 µm) slightly deviate from the species description of *F. mesolepta*. ***F.* cf. *pararumpens*** (**55**) is too wide (3.9 instead of 2.5-3 µm) and striae are too dense (19 instead of 16-18 in 10 µm). ***Fragilaria* spec. 2** (**56**) (length: 24.3 µm; width 4.4 µm; 16 striae in 10 µm) and ***Fragilaria* spec. 3** (**57**) (length: 18.7 µm; width 3.9 µm; 18 striae in 10 µm) could not be allocated to any of the here discussed taxa.


1-9
10-12
13-18
19-27
28-38
5 µm


39-41
42-44
45-47
48-49
50
51-54
55
56
57
5 µm

3.2.9 *Gomphonema olivaceolacuum* in sample D 1.1

In sample D 1.1 substantial problems became apparent with identifying *Gomphonema olivaceolacuum* (Lange-Bertalot & Reichardt) Lange-Bertalot & Reichardt, which was confused with *Gomphonema olivaceum* (Hornemann) Brébisson by several participants. The sum of both taxa (*G. olivaceolacuum* and *G. olivaceum*) were similar between most participants (n= 36; 2.7-17.6 %, average 6.8 %) and the three auditors (4.7-7.1 %, average 5.6 %) (Fig. 47). Laboratory 10 did not identify any of the two species. Laboratories 19 and 36 identified above average abundances of this group (17.6 % and 17.2 %, respectively) (Fig. 47).

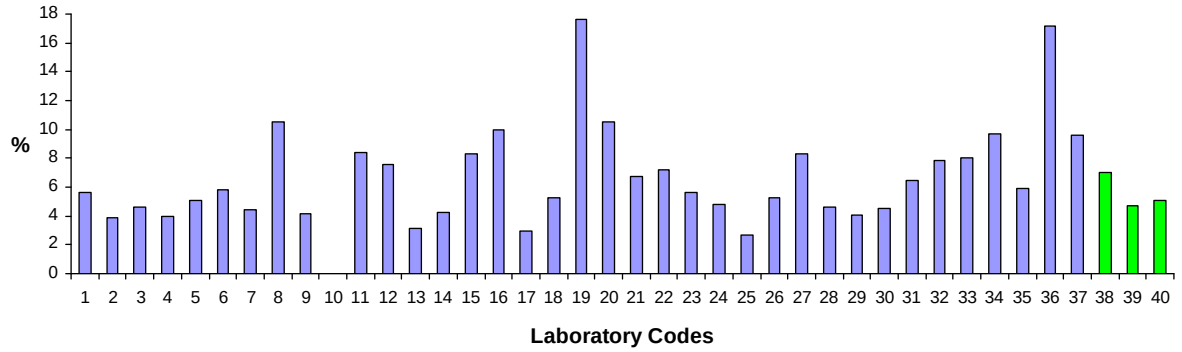


Fig. 47: Sum of the relative abundances of *Gomphonema olivaceolacuum* (including cf.) and *G. olivaceum* (including cf. and varieties *balticum*, *calcareum*, *olivaceolacuum* and *olivaceum*) in sample D 1.1 as identified by the participants (blue bars) and auditors (green bars).

The three auditors identified *Gomphonema olivaceolacuum* in similar abundances (4.7-6.9 %, average 5.5 %). In contrast, only 22 of the 37 participants identified this taxon with certainty (2.7-16.4 %, average 6.8 %) (Fig. 48). Three laboratories (13, 23, 24) identified this taxon as *G.* cf. *olivaceolacuum*. Laboratories 2, 3, 7, 10, 15, 19, 20, 22, 28, 29, 32 and 35 did not identify any *G. olivaceolacuum* in sample D 1.1 (Fig. 48). Laboratory 7 counted this taxon as *Gomphonema olivaceum* var. *olivaceolacuum* Lange-Bertalot & Reichardt.

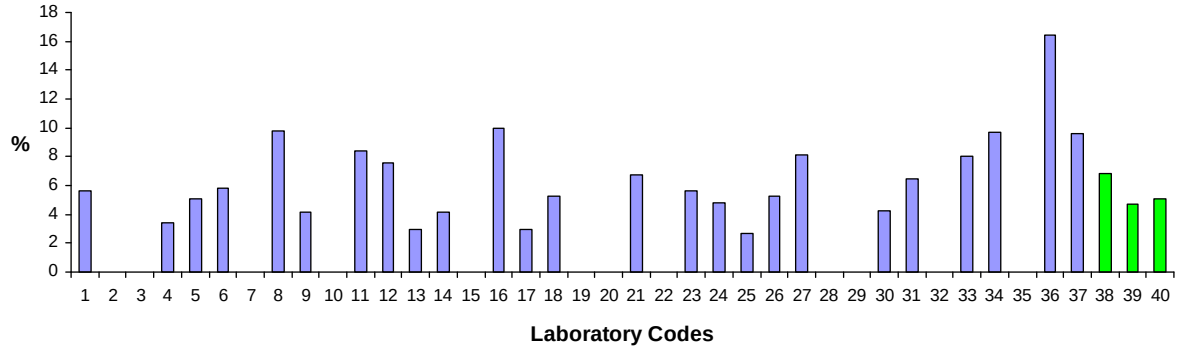


Fig. 48: Relative abundances of ***Gomphonema olivaceolacuum*** (including cf.) in sample D 1.1 as identified by the participants (blue bars) and auditors (green bars).

Eleven of the 12 laboratories that did not detect any *G. olivaceolacuum* in sample D 1.1 (laboratories 2, 3, 7, 15, 19, 20, 22, 28, 29, 32 and 35) identified solely *G. olivaceum* in abundances similar to the abundances of *G. olivaceolacuum* of the other laboratories and auditors (Fig. 49). Thus, it can be assumed that *G. olivaceolacuum* was mistaken for *G. olivaceum*. However, *G. olivaceum* was also present in sample D. 1.1, but in very low abundances (e.g. detected by one auditor with 0.2 %) (Fig. 49). Additionally, the use of the varieties *olivaceum*, *balticum*, *calcareum* and *olivaceolacuum* indicate that several participants did not use or ignored the mandatory identification literature according to the current instructions (e.g. page 27 in Schaumburg et al. 2011), which were also listed in the letter accompanying the slides.

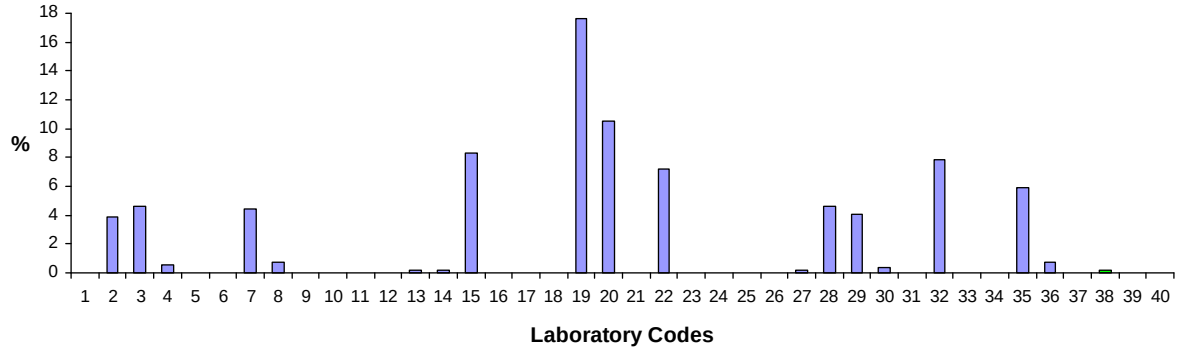


Fig. 49: Relative abundances of ***Gomphonema olivaceum*** (including cf. and varieties *balticum*, *calcareum*, *olivaceolacuum* (just laboratory 7) and *olivaceum*) from sample D 1.1 as identified by the participants (blue bars) and auditors (green bar).

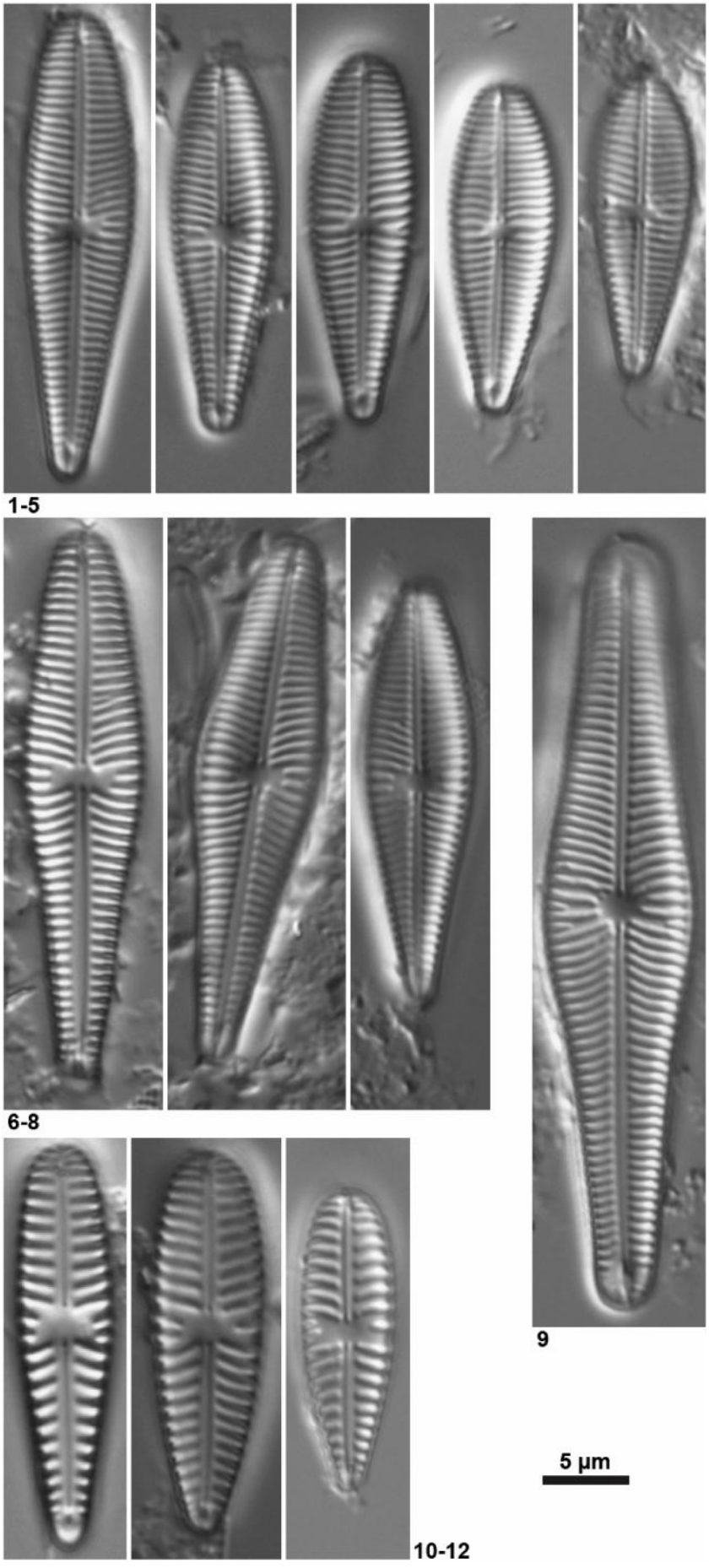
G. olivaceolacuum and *G. olivaceum* can not be confused, if valves are careful identified by using the entire set of characteristics of each species and by using the current identification literature (Hofmann et al. 2011). *G. olivaceum* is smaller (12-42 µm long; 5.5-9 µm wide) than *G. olivaceolacuum* (20-50 µm long; 7.5-13 µm wide). However, there is some overlap in size. Yet, the species can be well distinguished by their different striae density (*G. olivaceum* 8-12 striae in 10 µm; *G. olivaceolacuum* 12-18 in 10 µm). Additionally the two species differ in shape (different width ratio of head- and foot-pole).

Theoretically it is possible to confuse *G. olivaceolacuum* with *G. olivaceoides* (see Hofmann et al. 2011, page 310). However, this species is distinctly smaller than *G. olivaceolacuum* and possesses four stigmata around the central area that are absent in *G. olivaceolacuum*. These stigmata can probably only be overlooked, if the quality of the technical equipment is insufficient.

G. calcareum and *G. balticum* are also very similar to *G. olivaceolacuum* and more similar than aforementioned taxa. One participant of this intercalibration exercise confused these taxa, however using the names *G. olivaceum* var. *balticum* and var. *calcareum* (old taxonomy). The differentiation of these taxa is currently still problematic according to Hofmann et al. (2011). A picture of *G. calcareum* is given on Plate 95 (Fig. 15 and 16, Hofmann et al. 2011, p. 792). However, no description is given for this taxon. *G. balticum* is depicted in Krammer & Lange-Bertalot (1986-2004) on page 772 (Fig. 165: 8) and labelled as *G. olivaceum* var. *balticum*. For further information about the taxonomic problems of the *G. olivaceum*-complex see Lange-Bertalot (1993), Krammer & Lange-Bertalot (1986-2004) and Hofmann et al. (2011).

Finally, we note that a differentiation between *G. olivaceolacuum* and *G. olivaceum* is impossible, when exclusively using old identification literature. For example, the picture on Plate 95, Figure 19 (page 792) in Hofmann et al. (2011) is identical to Fig. 165: 1 (page 772) in Krammer & Lange-Bertalot (1986-2004; Band 2/1). However, they are labelled *G. olivaceolacuum* (Hofmann et al. 2011) and *G. olivaceum* var. *olivaceum* (Krammer & Lange-Bertalot 1986-2004; Volume 2/1), respectively. Additionally, *G. olivaceolacuum* fits into the broad morphological range of *G. olivaceum* described in Krammer & Lange-Bertalot (1986-2004) (see page 374, description of taxon No. 24; Band 2/1). Thus, *G. olivaceolacuum* is going to be named *G. olivaceum* var. *olivaceum*, if just Krammer & Lange-Bertalot (1986-2004) is used for identification. This emphasizes the need to use the given literature.

Plate 12 (next page): Light microscopic pictures of *Gomphonema olivaceolacuum* (1-5); *Gomphonema* cf. *olivaceolacuum* (6-8), *Gomphonema* spec. (9) and *Gomphonema olivaceum* (10-12). Valves 1-9 are from sample D 1.1 (Lake Geneva, Switzerland). Valves 10-12 are given for comparison from sample D 11 (Lake Krossinsee, northern Germany). The shape of valves 6-8 differs slightly compared to the shape of valves 1-5 and the valves *G. olivaceolacuum* depicted in Hofmann et al. (2011), especially in the upper half. However, Hofmann et al. (2011) state that overall the shape of *G. olivaceolacuum* varies only very little. Valves are described to always be club-shaped with a broadly rounded head and a comparably very small rounded foot. The greatest width of the valve is supposed to always be distinctly above the middle (Hofmann et al. 2011). Thus, valves 6-8 were labelled with a cf. The shape of valve 9 differs even more than for valves 6-8. Also, valve 9 is too large for a *G. olivaceolacuum* (Hofmann et al. 2011). It is possible that *G. olivaceolacuum* is morphologically more variable than listed in the literature and that valves 6-9 indeed are *G. olivaceolacuum*. However, more taxonomic examinations are necessary to resolve this uncertainty.


1-5
6-8
9
10-12
5 µm

3.2.10 *Gomphonema pumilum* and similar taxa in samples D 11 and D 1.1

In sample D 11 and D 1.1 various small *Gomphonema*-taxa occurred that were part of the *Gomphonema pumilum* (Grunow) Reichardt & Lange-Bertalot – aggregate or more or less similar such as *Gomphonema minutum* (Agardh) Agardh. Reichardt (1997) already showed that the differentiation of the species from the *G. pumilum* aggregate is particularly difficult (see e.g. Plate XII Reichardt 1997) and that the taxonomy is not yet sufficiently clarified, as he examined this group intensively. These known difficulties for differentiating these species and additional problems are also reflected in the results of this intercalibration exercise (see below).

Counting results for sample D 11

In sample D 11 the auditors determined 4.9-7.7 % (mean: 6.2 %) of species from the *Gomphonema pumilum*-aggregate and similar species (Fig. 50). The participants used the following names for the same species: *Gomphonema* spec., *G. elegantissimum* (Reichardt & Lange-Bertalot) Reichardt & Lange-Bertalot, *G. micropumilum* Reichardt, *G. minusculum* Krasske, *G. minutum*, *G. pumilum*, *G. pumilum* var. *elegans* Reichardt & Lange-Bertalot (synonymous for *G. elegantissimum*), *G. pumilum* var. *pumilum* Reichardt & Lange-Bertalot and *G. pumilum* var. *rigidum* Reichardt & Lange-Bertalot. 34 of the 37 participants identified these taxa with 0.5-7.5 % (mean: 4.0 %) (Fig. 50). Three participants did not find any of these taxa (laboratories 19, 28, 36) and six laboratories (10, 14, 15, 17, 26, 32) counted very low abundances (<2 %; Fig. 50).

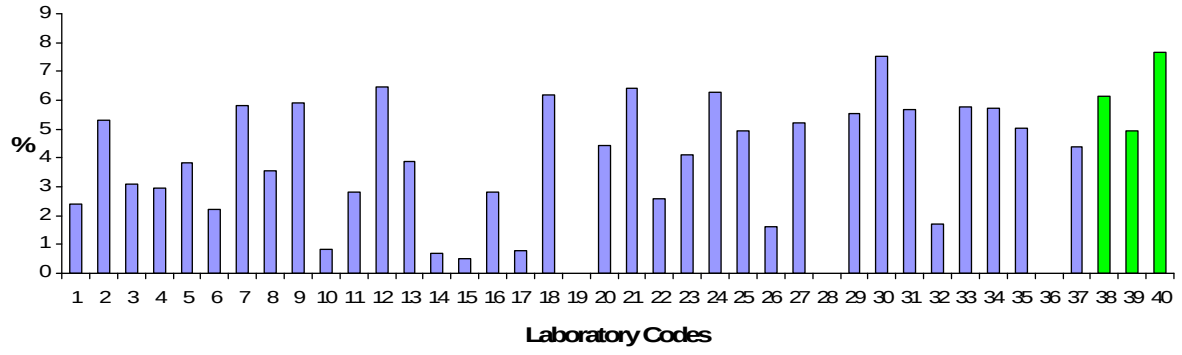


Fig. 50: Sum of the relative abundances of ***Gomphonema* spec.**, ***G. elegantissimum*** (including cf.), ***G. micropumilum***, ***G. minusculum***, ***G. minutum***, ***G. pumilum*** (including cf.) and ***G. pumilum* var. *elegans***, **var. *pumilum*** and **var. *rigidum*** (var. *rigidum* including cf.) from sample D 11. Blue: participants of the intercalibration exercise, green: auditors.

All three auditors identified *G. pumilum* with 0.4-5.2 % (mean: 2.3 %) in sample D 11 (Fig. 51), whereby the varieties *pumilum* and *rigidum* were not further differentiated. Of the 37 participants of the intercalibration exercise, only 27 participants determined *G. pumilum* (including the varieties *pumilum* and *rigidum*) with 0.2-5.3 % (mean: 2.8 %) (Fig. 51).

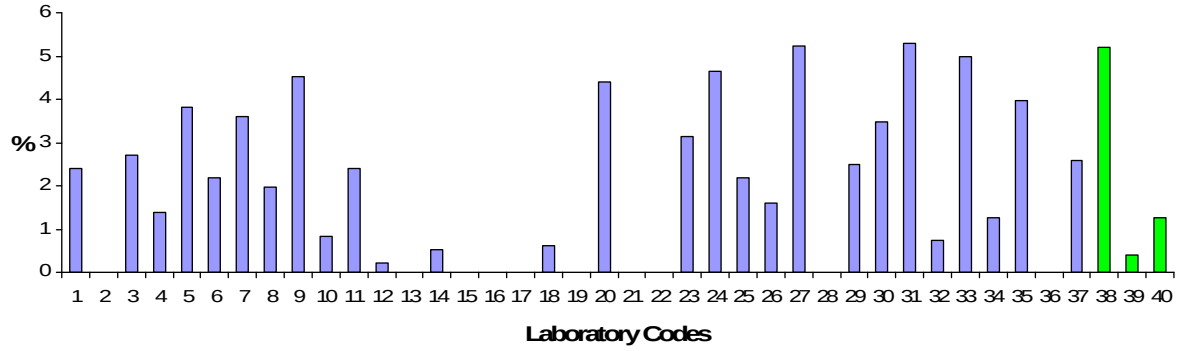


Fig. 51: Sum of the relative abundances of ***Gomphonema pumilum***, ***G. pumilum* var. *pumilum*** and ***G. pumilum* var. *rigidum*** from sample D 11. Blue: participants of the intercalibration exercise, green: auditors.

15 of the 37 participants identified *Gomphonema elegantissimum*, *G. pumilum* var. *elegans*, *G. micropumilum* and *G. minusculum* with 0.2-2.6 % (mean: 1.2 %) (Fig. 52). The auditors did not detect any of these taxa in sample D 11 (Fig. 52).

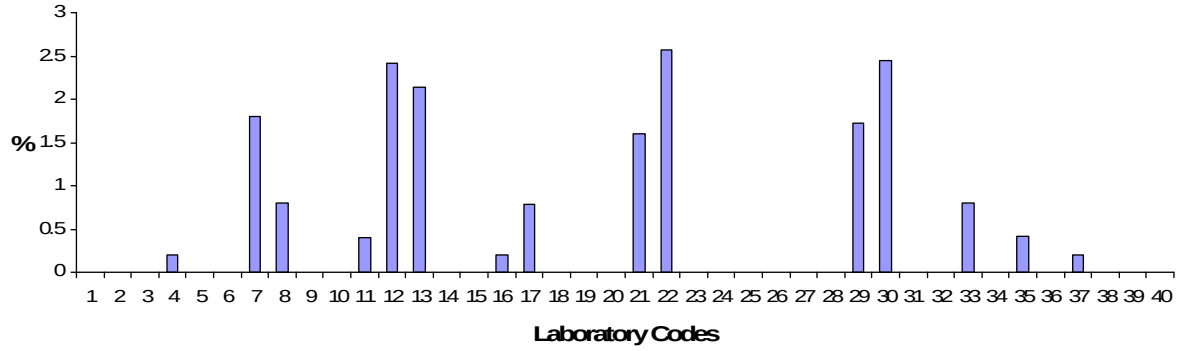


Fig. 52: Sum of the relative abundance of ***Gomphonema elegantissimum***, ***G. pumilum* var. *elegans***, ***G. micropumilum*** and ***G. minusculum*** from sample D 11. Blue: participants of the intercalibration exercise, green: auditors (no. 38-40).

In sample D 11 *G. minutum* also occurred. All three auditors identified this taxon with 0.4-0.9 % (mean: 0.6 %) (Fig. 53). 17 of the 37 participants also determined *G. minutum* with 0.2-1.8 % (mean: 0.9 %) (Fig. 53).

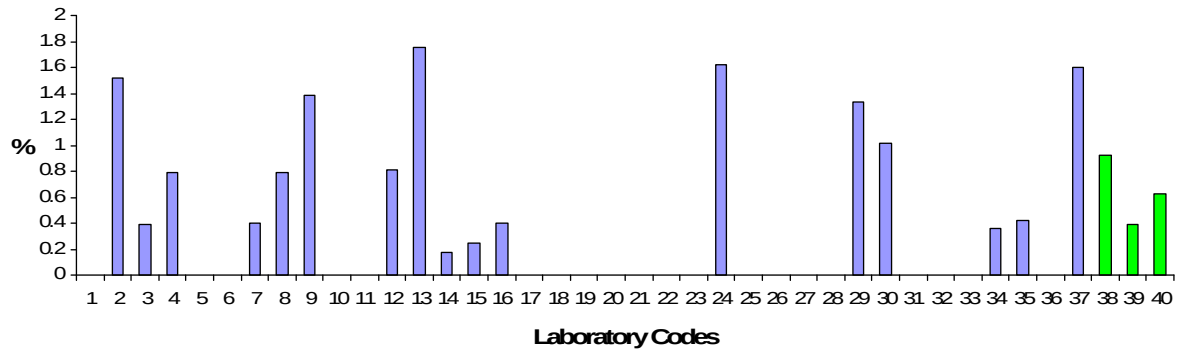


Fig. 53: Relative abundances **of *Gomphonema minutum*** in sample D 11. Blue: participants of the intercalibration exercise, green: auditors.

With the known difficulties with identifying species from the *G. pumilum*-aggregate and varieties (see e.g. Reichardt 1997, Hofmann et al. 2013) we expect that a certain percentage of these taxa in sample D 11 is identified ambiguously. Correspondingly, two of the three auditors determined *Gomphonema* spec. with 3.9 % and 5.8 % (Fig. 54). Only 14 of the 37 participants indicated any ambiguous identifications (labelling with „spec." or „cf.") for the here discussed *Gomphonema*-taxa (0.2-5.6 %) (Fig. 54). The remaining 23 participants listed the here discussed *Gomphonema*-taxa without doubt, i.e. they only listed unambiguous species.

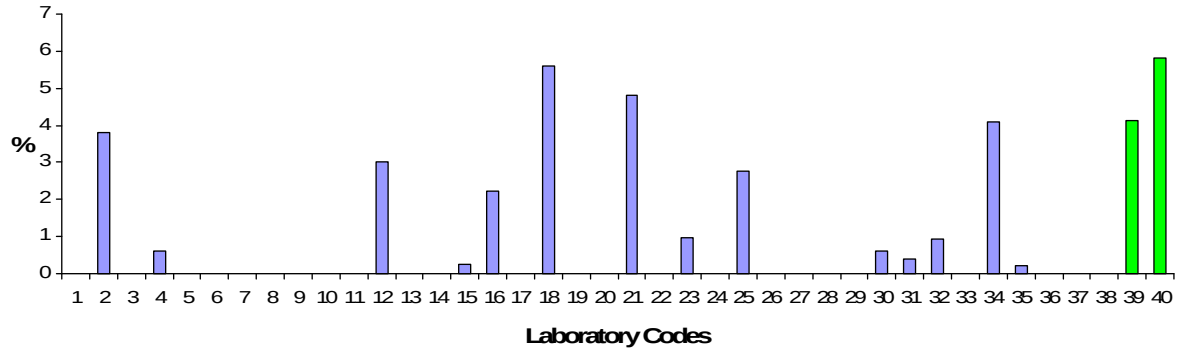


Fig. 54: Sum of the relative abundance of ***Gomphonema* spec**. and all *Gomphonema*-taxa from sample D 11 that are listed in Fig. 50 that were labelled with a „cf.", i.e. ***G.* cf. *elegantissimum***, ***G.* cf. *pumilum*** and ***G.* cf. *pumilum* var. *rigidum***. Blue: participants of the intercalibration exercise, green: auditors.

Counting results for sample D 1.1

In sample D 1.1 the auditors determined 20.4-24.1 % (mean: 22.0 %) of taxa from the *Gomphonema pumilum*-aggregate and similar species (Fig. 55). The participants used the following names for these taxa: *Gomphonema* spec., *G. angustivalva* Reichardt, *G. elegantissimum*, *G. lacus-vulcani* Reichardt & Lange-Bertalot, *G. micropumilum*, *G. minusculum*, *G. minutum*, *G. pseudotenellum* Lange-Bertalot, *G. pumilum*, *G. pumilum* var. *pumilum* and *G. pumilum* var. *rigidum*. 36 of the 37 participants also identified taxa from this group (0.5-23.9 %; mean: 15.9 %) (Fig. 55). Laboratory 36 did not find any of these taxa. Two laboratories (15, 19) counted only very low abundances (<2 %) from this group (Fig. 55).

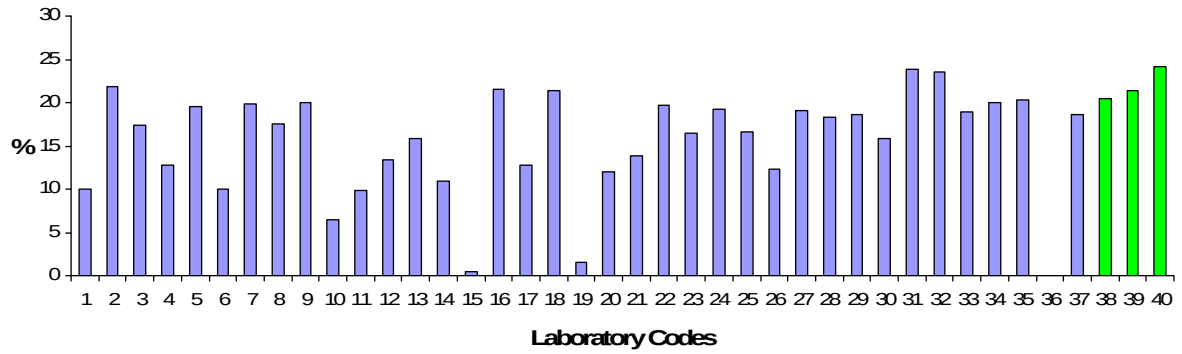


Fig. 55: Sum of the relative abundances of ***Gomphonema* spec**, ***G. angustivalva***, ***G. elegantissimum***, ***G. lacus-vulcani***, ***G. micropumilum***, ***G. minusculum***, ***G. minutum***, ***G. pseudotenellum***, ***G. pumilum***, ***G. pumilum* var. *pumilum*** and **var. *rigidum*** from sample D 1.1. Blue: participants of the intercalibration exercise, green: auditors.

In sample D 1.1 a scientifically not yet described small *Gomphonema*-species dominated the assemblage (for more details see below). Consequently, in the following we focus only on how many participants correctly used „*Gomphonema* spec." or „cf." to account for this *Gomphonema*-species. One auditor identified *Gomphonema* spec. with 15.7 % in sample D 1.1 (Fig. 56). 16 of the 37 participants determined to 0.2-21.4 % (mean: 12.6 %) ambiguous taxa of this group (Fig. 56).

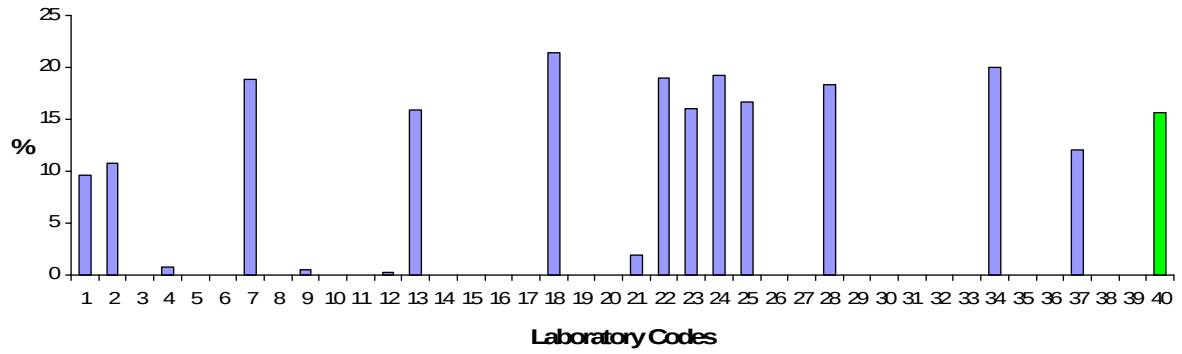


Fig. 56: Sum of the relative abundances of ***Gomphonema* spec**. and all *Gomphonema*-taxa from sample D 1.1 that are listed in Fig. 55 that were labelled with a „cf.“, i.e. ***G.* cf. *angustivalva***, **G. cf. *micropumilum***, ***G.* cf. *pumilum***, ***G.* cf. *pumilum* var. *pumilum*** and ***G.* cf. *pumilum* var. *rigidum***. Blue: participants of the intercalibration exercise, green: auditors.

In contrast to the ambiguous taxa depicted in Fig. 56, the three auditors identified 8.4-21.4 % (mean: 16.8 %) of unambiguous taxa of the here discussed *Gomphonema* species (Fig. 57). These valves were exclusively identified as *G. pumilum* without any further differentiation of the varieties. 30 of the 37 participants indicated unambiguous *Gomphonema* species with 0.4-23.9 % (mean: 12.3 %) (Fig. 57). These species were identified as *G. angustivalva*, *G. elegantissimum*, *G. lacus-vulcani*, *G. micropumilum*, *G. minusculum*, *G. minutum*, *G. pseudotenellum*, *G. pumilum*, *G. pumilum* var. *pumilum* and var. *rigidum*.

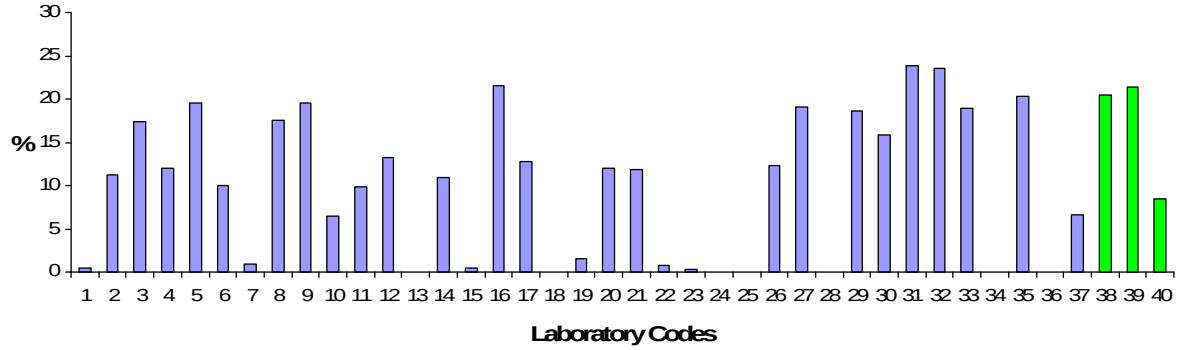


Fig. 57: Sum of the relative abundances of **all unambiguously identified small *Gomphonema* taxa** from sample D 1.1 (*G. angustivalva*, *G. elegantissimum*, *G. lacus-vulcani*, *G. micropumilum*, *G. minusculum*, *G. minutum*, *G. pseudotenellum*, *G. pumilum*, *G. pumilum* var. *pumilum* and var. *rigidum*). Blue: participants of the intercalibration exercise, green: auditors.

Several problems became apparent when analysing above results. For one, they suggest that there were probably considerable problems with identifying and differentiating *G. pumilum*

and similar species, such as *G. elegantissimum*, *G. micropumilum* and *G. minusculum* and possibly also *G. minutum* (see results of sample D 11). Additionally, the results of sample D 11 suggest that there are problems with differentiating the varieties of *G. pumilum* (not shown). However, partly, this may simply reflect the recommendation in Hofmann et al. (2011) to refrain from differentiating these varieties.

Similar to other already discussed taxonomic problems (see e.g. Chapter 3.2.1 and Chapter 3.2.5), here too the results indicate that various identification literature was used, leading to the use of different names (e.g. *G. elegantissimum* and *G. pumilum* var. *elegans*) (see results of sample D 11). Consequently, a comparison of the counting results among diatomists was hampered.

A great problem indicated by the here presented results, especially by the example of the small *Gomphonema*-species in sample D 1.1, are the different details about how ambiguous the identification was (usage of „spec." and „cf." for ambiguous taxa). 30 of 37 participants indicated that they identified all here discussed *Gomphonema*-species unambiguously in sample D 1.1, despite a large percentage of valves of a not yet described species that was somewhat similar to the *G. pumilum*-aggregate (see below). Chapter 5 details the general problems with respect to ambiguous taxa when applying the German Phylib-method for identifying the ecological status of lakes and rivers (Schaumburg et al. 2006, 2011).

The identification of the here discussed species of the *G. pumilum*- aggregate and similar species will remain difficult, because many traits overlap among many of the already described species (see e.g. Tab. 18), because transitional forms between taxa are common (Reichardt 1997), because the taxonomy is not entirely resolved (Reichardt 1997, Hofmann et al. 2013) and because the entire species-aggregate is characterised by a relatively high morphologic variability. Next to the measurable dimensions, the following light-microscopic traits are particularly important for the here discussed species: shape, raphe structure, size and shape of the stigma, striae orientation, size and width of the axial area and size as well as shape of the central area, all of which need to be considered for the species identification.

Tab. 18: Measureable dimensions of taxa from the *Gomphonema pumilum*-aggregate and similar species. Listed names were used by the participants or auditors for objects from samples D 11 or D 1.1. Details according to Hofmann et al. (2013), Krammer & Lange-Bertalot (1986-2004), Reichardt & Lange-Bertalot (1991) and Reichardt (1997). For more information see text.

Taxon	Length [µm]	Width [µm]	Striae in 10 µm
G. angustivalva	11-24	2.7-3.7	15-18
G. elegantissimum	10-35	3.6-5.4	11-15
G. lacus-vulcani	6.5-20	3.1-4.0	20-23
G. micropumilum	10-22	3.5-4.4	14-18
G. minusculum	14-32.7	2.8-4.6	12-16
G. minutum	10-35	4-8	8-18
G. pseudotenellum	14-28	2.5-4	14-18.5
G. pumilum var. *pumilum*	12-36 (38)	3.5-5.5	11.5-14
G. pumilum var. *rigidum*	12-36	(3) 3.5-5.3	11.5-14
G. spec (this study, see below)	7.1-15.2	2.7-3.8	17-24

G. angustivalva (figures e.g. in Reichardt 1997 and Hofmann et al. 2013)

- valves hardly club-shaped to almost entirely naviculoid, linear to linear-lanceolate
- ends fairly bluntly rounded, wide end (head-pole) often (not always) slightly protracted
- raphe slightly undulated to straight, filiform or hardly visible lateral
- axial area narrow, central area laterally rectangularly widened
- stigma distinctly offset from the striae, close to the central node
- striae slightly radial

G. elegantissimum (figures e.g. in Hofmann et al. 2013)

- shape lanceolate
- ends bluntly rounded, wide end ("head-pole") moderately wider compared to small end ("foot-pole")
- foot pole may vary and is up to moderately pointy
- raphe distinctly undulated, central pores look bend sideways
- axial area variable from moderately narrow to moderately wide lanceolate
- central area variably transversely widened
- stigma distinctly offset from striae, close to the central node
- striae radial, slightly curved
- sometimes areolae visible

G. lacus-vulcani (figures in Reichardt 1997)

- valves hardly asymmetrical, shape lanceolate to oval-lanceolate
- "head pole" rounded bluntly to pointy, sometimes slightly protracted
- raphe straight, filiform
- axial area narrow, central area slightly rectangularly widened
- stigma distinctly offset from striae, close to central node
- striae slightly radial to parallel

G. micropumilum (figures in Reichardt 1997)

- valves stout club-shaped
- ends curved bluntly cuneiform
- "head-pole" at apex usually distinctly flattened, sometimes hardly visible protracted
- "foot-pole" rounded narrowly, raphe straight, almost filiform
- axial area narrow linear, central area laterally widened
- stigma distinctly offset from striae (isolated), close to central node
- striae weakly to moderately radial
- areolae hardly visible

G. minusculum (figures e.g. in Reichardt 1997 and Hofmann et al. 2013)

- shape narrow linear-lanceolate, in the middle widened, i.e. greatest width in the middle
- ends bluntly rounded, often protracted, "foot-pole" slightly narrower rounded compared to "head-pole"
- "head-pole" sometimes slightly capitately widened
- raphe straight to hardly visibly curved, filiform or very slightly lateral
- axial area narrow linear to very slightly lanceolately widened
- central area laterally squared (even shortening of middle striae)
- stigma distinctly offset from striae, close to central node
- striae parallel to slightly radial

G. minutum (figures e.g. in Hofmann et al. 2013)

- valves oval club-shaped to lanceolate
- "head-pole" broadly rounded, "foot-pole" distinctly smaller than "head-pole"
- raphe slightly curved
- axial area very narrow, central area usually very small and one-sided
- stigma relatively large and distinct, close to valve centre
- striae radial, looking thick in light microscope (areolae in double rows)
- areolae not visible, in girdle view there is a distinct dot next to the marginal striae

G. pseudotenellum (figures e.g. in Hofmann et al. 2013)

- valves hardly club-shaped, narrow lanceolate, almost naviculoid
- ends more or less pointy, not protracted
- raphe distinctly curved, in smaller valves only „slightly undulated“
- axial area narrow to very narrow, central area more or less rectangularly widened
- stigma close to central node
- striae slightly radial

G. pumilum* var. *pumilum (figures e.g. in Reichardt 1997 and Hofmann et al. 2013)

- valves more lanceolate and less club-shaped compared to var. *rigidum*
- ends more or less bluntly rounded, "head-pole" only slightly wider than "foot-pole"
- raphe fairly straight, filiform or very slightly lateral
- axial area broadly lanceolate, central area hardly to moderately distinct
- stigma distinctly offset from striae, close to central node
- striae very slightly radial to almost parallel, hardly bent

G. pumilum var. rigidum (figures e.g. in Reichardt 1997 and Hofmann et al. 2013)

- shape more linear-lanceolate compared to nominate variety
- ends rounded bluntly, "head-pole" moderately wider compared to "foot-pole"
- raphe fairly straight, filiform or very slightly lateral
- axial area narrow, central area very distinctly and abruptly offset
- stigma distinctly offset from striae, close to central node
- striae very slightly radial to almost parallel, hardly bent

Above details of morphologic traits of each species are given according to Hofmann et al. (2013), Krammer & Lange-Bertalot (1986-2004), Reichardt & Lange-Bertalot (1991) and Reichardt (1997). Next to the detailed taxa above, there are other similar species, which potentially need to be considered during the identification, such as *G. procerum* Reichardt & Lange-Bertalot, *G. cuneolus* Reichardt, *G. designatum* Reichardt and other species listed e.g. in Reichardt (1997).

Among the here discussed *Gomphonema*-species there was a very small taxon in sample D 1.1 (Plate 13: 1-39). During an intensive screening of sample D 1.1, where overall 1,500 diatom valves were looked at, *Gomphonema olivaceolacuum* occurred with ~6 %, *G. tergestinum* with ~1 % and also one single valve of *G. angustivalva* and *G. minutum* were found (Plate 13). *Gomphonema pumilum* or other similar species were not found in sample

D 1.1 during this screening. All other valves, that occurred with the highest percentages from the *Gomphonema*-taxa in sample D 1.1 (~15-20 %) were the above mentioned small *Gomphonema*-species (Plate 13).

The valves of this *Gomphonema*-species are 7.1-15.2 µm long, 2.7-3.8 µm wide and have 17-24 striae in 10 µm (based on measurements of 150 valves from sample D 1.1). The valves are rather elliptical-lanceolate, only very slightly to not visibly club-shaped, i.e. mainly naviculoid. The ends are more or less bluntly rounded and not protracted. The "head-end" is only slightly wider compared to the "foot-end" to almost identical ends. Despite the small valve size, the raphe is distinctly visible, filiform straight to very weakly curved. The axial area is narrow near the ends of the valve and gradually widens towards the middle. The width of the axial area and the location of where the axial area starts to widen is very variable. In some few valves the axial area already starts to widen relatively close to the valve ends. In most other valves the axial area starts to widen more or less close to the central area. The central area is relatively large, elliptically elongated and never abruptly set apart from the axial area (as is typical e.g. for *G. pumilum* var. *rigidum*). Instead, the central area is a distinct widening of the axial area, somewhat similar to *G. pumilum* var. *pumilum*, except that the transition from axial area to central area is more pronounced compared to *G. pumilum* var. *pumilum*. A distinctly visible stigma is located near the valve centre and clearly offset from the middle striae. The striae are parallel to slightly radial and straight to slightly bend.

The characteristics of this taxon coincide with none of the here discussed taxa or the taxa named in Fig. 57 and differs especially to the description of *G. pumilum* (this name was used the most often for taxa from sample D 1.1). Probably, this species has not yet been scientifically described (Erwin Reichardt, personal communication March 2012). A species description of this taxon is currently in preparation (May 2014).

Recommendations for routine counts:

Similar to identifying many other diatom species, particularly here too, it is essential to use the entire combination of traits that define species of the *G. pumilum* – aggregate. We additionally recommend to identify species and varieties with as much detail as possible, even though Hofmann et al. (2013) suggest not to separate the varieties of *G. pumilum* for now. The German Phylib-method does not yet differentiate the varieties and consequently, the separation or lumping currently does not affect the ecological assessment. However, with further assessments of all of these taxa, we expect different ecological preferences that could subsequently refine the ecological water assessment.

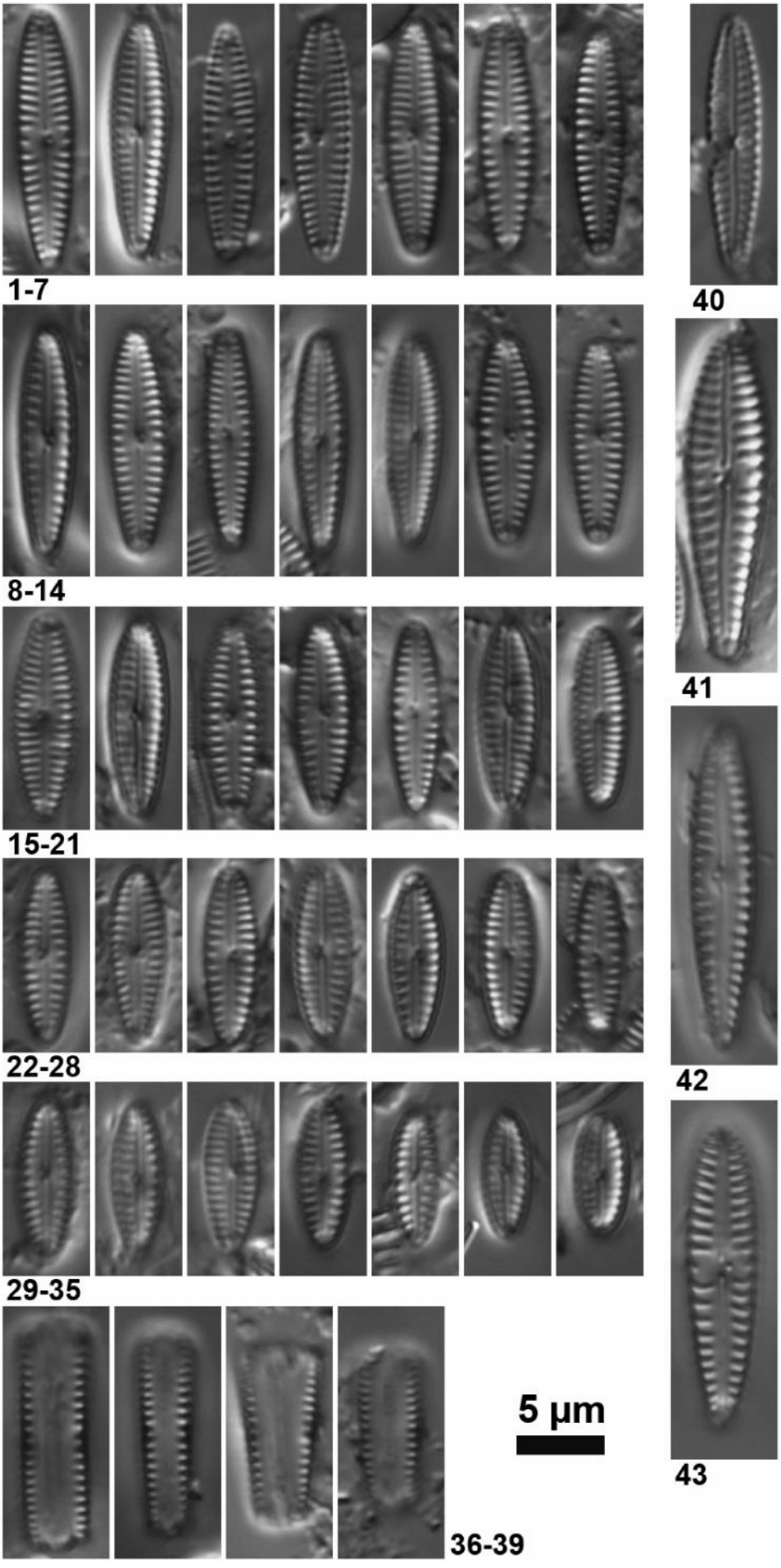
Some of the here discussed species are described and/or depicted in Hofmann et al. (2013). However, Hofmann et al. (2013) is not entirely sufficient for a by and large complete identification of taxa of the *G. pumilum*-aggregate, because several taxa are not listed in Hofmann et al. (2013) that occur in various eco-regions and that also need to be considered. Thus, additional literature should be used (such as Reichardt 1997).

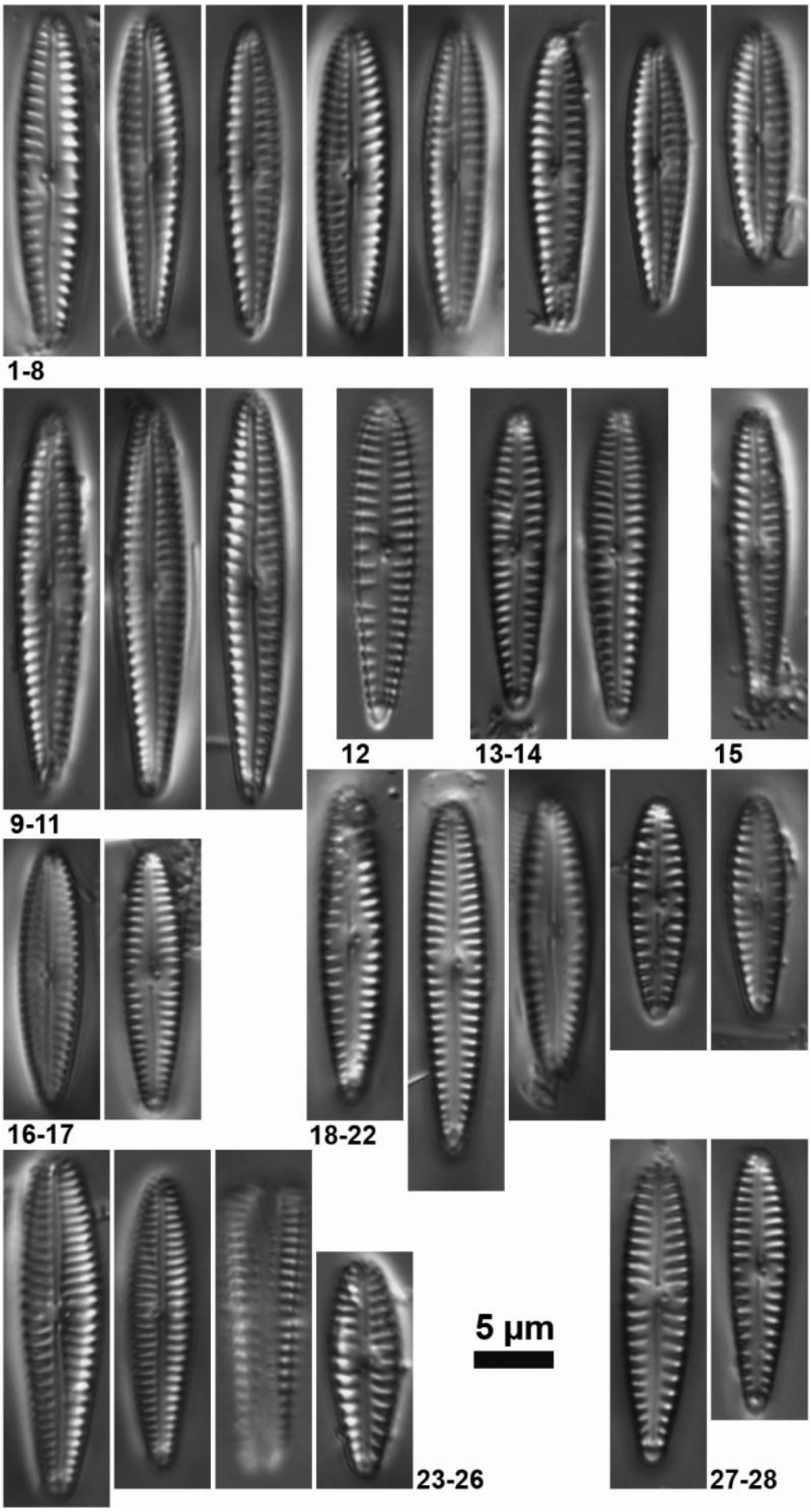
Another problem are the girdle band views, a position that the here discussed taxa are often present on the slides. In these cases, an identification is often not possible. We recommend to allocate these girdle view valves into groups (using length, shape, striae density and areolae, if present). For using the German Phylib-method and software, these groups can be allocated according to the percentage of the valve views of the appropriate *Gomphonema*-species. Here too, a brief description and pictures of the girdle view groups are essential.

As valves of the here discussed *Gomphonema*-taxa can often not be allocated to one of the described species despite careful identification and as there are many taxonomically problematic cases that are not sufficiently solved, we strongly recommend, especially for ambiguous identifications, to use „cf.“. Here too, the valves should be photographed and be briefly described.

Plate 13 (page 117): *Gomphonema* spec. (1-39; 36-39 girdle views) and single finds of *G. angustivalva* (40) and *G. minutum* (41) from sample D 1.1 (Lake Geneva, Switzerland). *G. pumilum* var. *pumilum* (42) and *G. pumilum* var. *rigidum* (43) are from other lakes of the north-German lowlands and are depicted here for comparison. For more information see text.

Plate 14 (page 118): Taxa of the *Gomphonema pumilum*-aggregate and similar species from sample D 11 (Lake Krossinsee, northern Germany). *G. elegantissimum* (1-11), *G.* cf. *elegantissimum* (12) (striae not bend), *G.* cf. *elegantissimum* or *G.* cf. *minusculum* (13-14) (shape?), *G. minusculum* (15), *Gomphonema* spec. (16), *Gomphonema* cf. *pumilum* (17) (striae too dense), *G. pumilum* var. *pumilum* (18-22) (traits fit more or less with species description, partly transitional forms to *G. pumilum* var. *rigidum*: e.g. 19), *G. minutum* (23-26) and *G. pumilum* var. *rigidum* (27-28) (typical forms). All taxa of the *G. pumilum*-complex and similar species were photographed during an intensive screening of sample D 11, where altogether 1,500 diatom valves were looked at. During this screening, no other than the shown taxa were found.


1-7
40
8-14
41
15-21
42
22-28
43
29-35
5 µm
36-39


1-8
9-11
12
13-14
15
16-17
18-22
23-26
27-28
5 µm

3.2.11 *Mayamaea atomus* var. *permitis* in sample D 12

The results of sample D 12 from the intercalibration exercise suggest that there are taxonomic problems with identifying *Mayamaea atomus* (Kützing) Lange-Bertalot. The problems result from difficulties by identifying the varieties of *Mayamaea atomus* and also by detecting *Mayamaea atomus* in the first place. It seems the latter issue is the bigger problem. The reasons for overlooking this taxon are not clear with the data at hand. Theoretically, it is possible that the technical equipment was insufficient (aperture, magnification) or that participants lacked experience with diatom identification. However, we can exclude these reasons for the results of this exercise based on the additionally given information by the participants. The objective type, aperture, experience with counting diatoms, optical illumination technique, regional origin of samples commonly counted by the participants or number of samples counted per year did not significantly influence the statistical distance (see Chapter 4.4) of the participant results to the auditor results (mixed-effect model according to Pinheiro & Bates (2000), $p<0.05$).

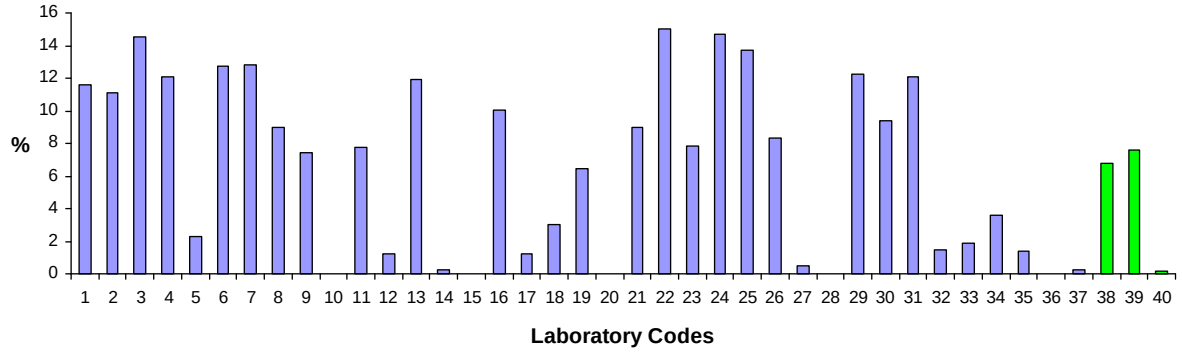


Fig. 58: Sum of the relative abundances of *Mayamaea atomus* (including cf.), *Mayamaea atomus* var. *atomus*, var. *alcimonica* and var. *permitis* (including cf.) in sample D 12 as identified by the participants (blue bars) and the auditors (green bars).

Two auditors counted 6.8 % and 7.6 %, respectively, of the *Mayamaea atomus*-group in sample D 12, one auditor just 0.2 % (Fig. 58). Five participants (laboratories 10, 15, 20, 28, 35) did not detect any *M. atomus* (including varieties), other participants only in abundances of less than one percent (laboratories 14, 27, 37) (Fig. 58), the maxima abundances were 15.0 % and 14.5 %. Most participants and the auditors had mainly identified *M. atomus* var. *permitis* (Hustedt) Lange-Bertalot of this taxa group, few participants and one auditor (with 0.5 %) also detected *M. atomus* var. *atomus*. Four participants did not distinguish between the varieties of *M. atomus*, possibly due to difficulties allocating the varieties.

Tab. 19: Selected traits for differentiating the varieties of *Mayamaea atomus* listed for sample D 12 and *M. agrestis*. Source: Hofmann et al. (2011) and *Lange-Bertalot (2001). Length and width of *M. atomus* var. *alcimonica* are derived from measurements of figures 14-20 on Plate 104 according to the given scale in Lange-Bertalot (2001, page 444).

taxon	length (µm)	width (µm)	striae/10µm	comment
M. atomus var. *atomus*	8.5-13	4-5.5	19-22	in girdle view not distinguishable
M. atomus var. *permitis*	6-9	3-4	(25)30-36	
**M. atomus* var. *alcimonica*	9.3-11.4	4.0-4.7	24-26	
**M. agrestis*	9-11	(2.5)3-3.8	24-28	

Among others the width and the striae density can be used to distinguish the varieties *atomus*, *permitis* and *alcimonica* (see Tab. 19), as these traits differ more or less distinctly among the three varieties. These three taxa may be present in German inland waters (Reichardt 1984, Lange-Bertalot 2001, Hofmann et al. 2013) and should be distinguished, as they have different ecological preferences (Reichardt 1984, Lange-Bertalot 2001; see also Chapter 3.3). Even more difficult than differentiating these three varieties of *M. atomus* is the distinction between *M. atomus* var. *alcimonica* and *M. agrestis*. Both taxa share many traits that are visible with a light microscope (Lange-Bertalot 2001) and can probably not be separated unambiguously in individual cases.

Several other taxa that are similarly thinly silicified as *M. atomus* may be confused with this taxa group, such as *Mayamaea fossalis* (Krasske) Lange-Bertalot and *Craticula molestiformis* (Hustedt) Mayama. For example, next to *M. atomus* there were also the thinly silicified *Adlafia minuscula* var. *minuscula* (Grunow) Lange-Bertalot, *Fistulifera pelliculosa* (Brébisson) Lange-Bertalot and *F. saprophila* (Lange-Bertalot & Bonik) Lange-Bertalot in sample D 12. Thus, we recommend to use an aperture of 1.4 and a high quality oil-immersion objective to unambiguously identify taxa such as *M. atomus* and their varieties.

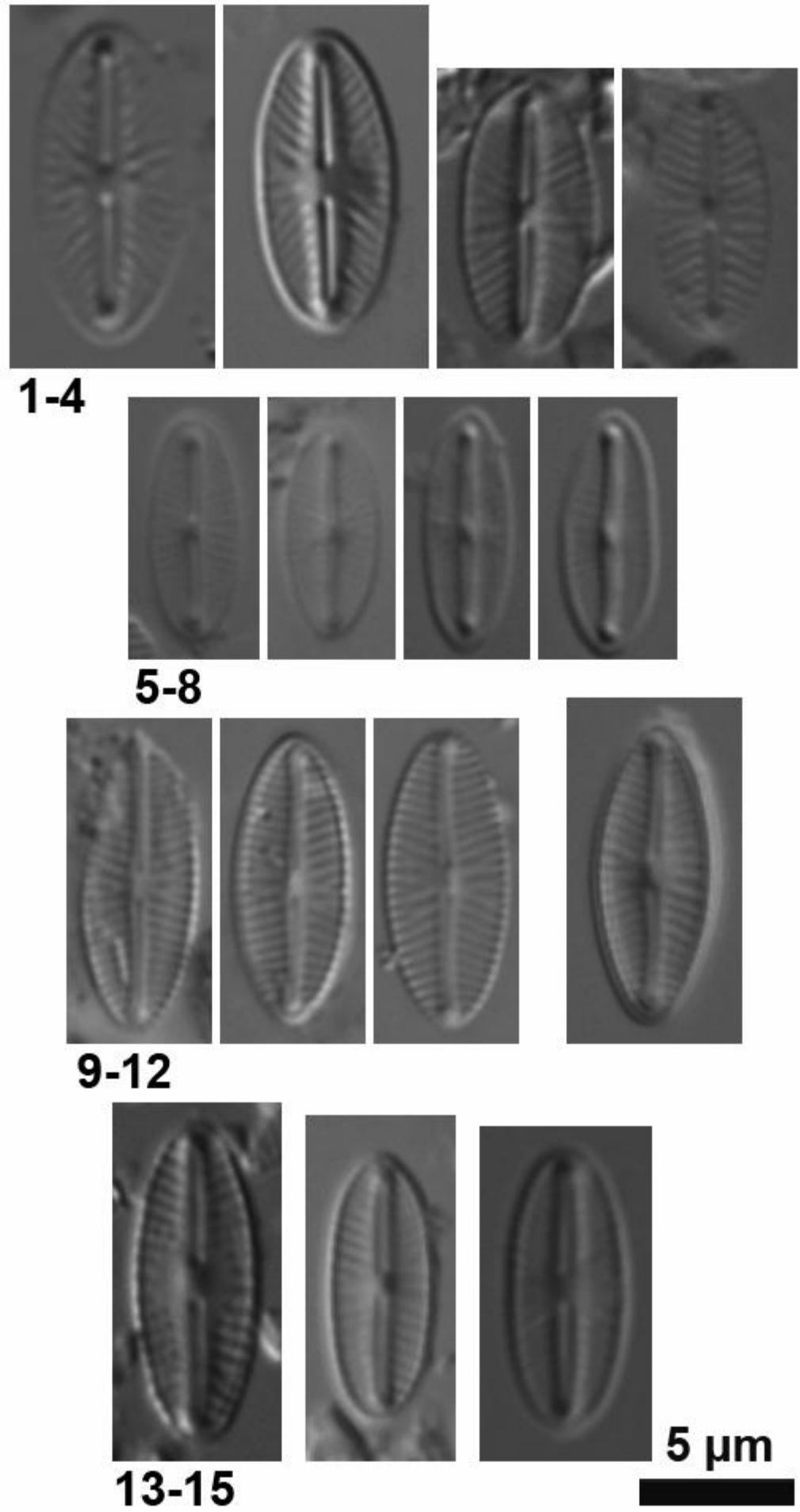
Plate 15 (next page): Light microscopic pictures of *Mayamaea atomus* var. *atomus* (1-4), *M. atomus* var. *permitis* (5-8), *M. atomus* var. *alcimonica* (9-11), *M.* cf. *agrestis* (12), *M. fossalis* var. *fossalis* (13), *M. atomus* cf. var. *permitis* (14) and *Mayamaea* spec. (15). Valves 5-8 are from sample D 12 (River Klepelshagener Bach, North German Lowlands). All other valves are from elsewhere and just depicted for comparison (1-2 and 15 are from biological soil crusts from the North German Lowlands, 3-4 and 12-14 from running waters of the North-German Lowlands, Fig. 9-12 (Maria Kahlert) from Swedish running waters, see http://www.norbaf.net/courses/suggestions_final.pdf).

The valve in Fig. 4 is 8.3 µm long, 4.0 µm wide and has 20-24 striae in 10 µm and is thus slightly too short and the striae are sometimes too dense when compared to the ranges given for *M. atomus* var. *atomus* in Lange-Bertalot (2001). As these deviations are minimal, we did not use „cf." and are confident that this is unambiguously *M. atomus* var. *atomus*.

The valve in Fig. 12 was labelled *Mayamaea* cf. *agrestis* (9.9 µm long, 3.9 µm wide, 24 striae in 10 µm), due to the intermediate width between *M. agrestis* and *M. atomus* var. *alcimonica* (see Tab. 19). The size is within the range of both taxa. However, the shape and distinct sternum is more similar to *M. agrestis* than to *M. atomus* var. *alcimonica*.

The valve in Fig. 14 is 3.3 µm wide and 8.5 µm long, which corresponds with *M. atomus* var. *permitis*. However, with 24 striae in 10 µm the striae density is too low and thus this valve was labelled with a „cf.".

The valve *Mayamaea* spec. (Fig. 15) is 9.3 µm long, 3.8 µm wide and has ~25 striae in 10 µm and fits the measurable dimensions of *M. atomus* var. *alcimonica* or *M. agrestis*. However, the valve shape and butterfly-shaped, very big central area do not fit for these taxa. For more information see text.


1-4
5-8
9-12
13-15
5 µm

3.2.12 *Navicula cryptotenella* and *N. cryptotenelloides* in samples D 11 and D 1.1

In **sample D 11** the genus *Navicula* Bory de Saint-Vincent was dominated by *Navicula cryptotenella* Lange-Bertalot, while *Navicula antonii* Lange-Bertalot occurred subdominant and *Navicula reichardtiana* Lange-Bertalot and *Navicula tripunctata* (O.F. Müller) Bory de Saint-Vincent (both not further discussed here) occurred regularly but with less than 2 % according to the results of the auditors. All other *Navicula*-taxa were present with less than 1 % based on the results of the auditors and were statistically not relevant in sample D 11.

The greatest taxonomic problems within this group (Fig. 59) occurred with identifying *N. cryptotenella* and *N. antonii* in sample D 11 (Figs. 60 and 62). Occasionally, *N. cryptotenella* was confused with *Navicula menisculus* Schumann (Fig. 63). *N. cryptotenelloides* Lange-Bertalot was only present in very low abundances in sample D 11. Thus, it remains unclear if a participant did not detect any *N. cryptotenelloides* due to taxonomic problems or due to the absence of the taxon in that sample (Fig. 61).

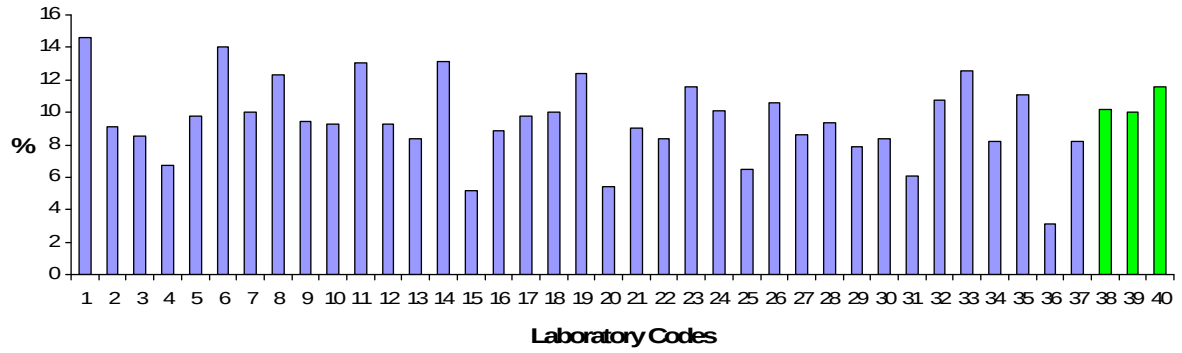


Fig. 59: Sum of the relative abundances of ***Navicula cryptotenella, N. cryptotenelloides, N. antonii*** and ***N. menisculus*** including all taxa with cf. in sample D 11, as identified by the participants (blue bars) and auditors (green bars).

The three auditors identified *N. cryptotenella* with 5.9 %, 8.8 % and 10.0 % (average 8.3 %) (Fig. 60), while 33 of the 37 participants detected *N. cryptotenella* with 1.4-8.9 % (average: 5.4 %) in sample D 11 (Fig. 60).

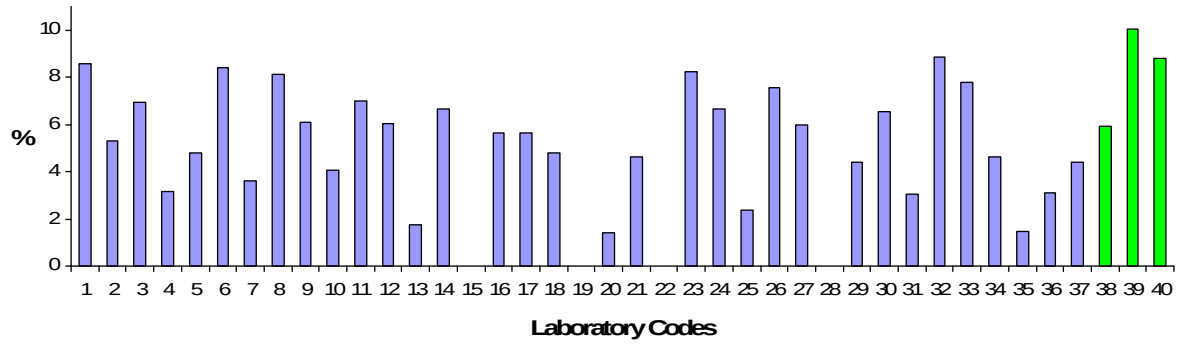


Fig. 60: Relative abundances of ***Navicula cryptotenella*** in sample D 11, as identified by the participants (blue bars) and auditors (green bars).

Navicula cryptotenelloides was detected in two auditor samples with 0.4 % and 0.6 % (average: 0.5 %) and in 24 of 37 participant samples with 0.2-3.1 % (average: 0.9 %) in sample D 11 (Fig. 61).

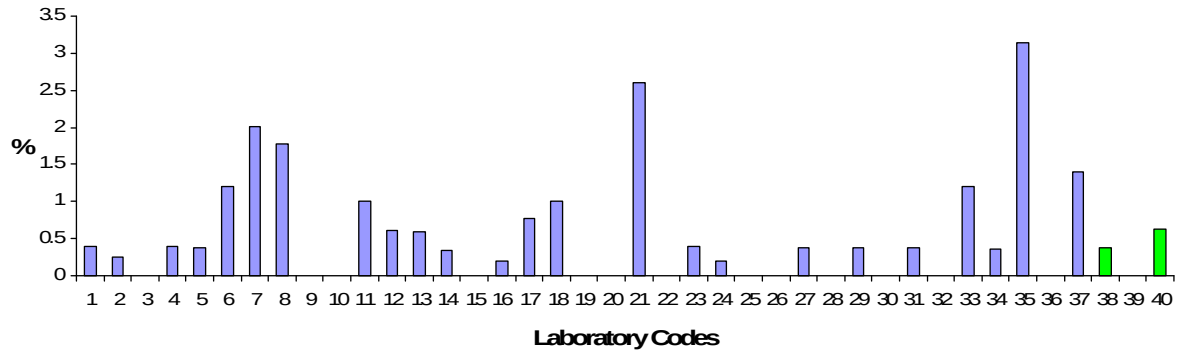


Fig. 61: Relative abundances of ***Navicula cryptotenelloides*** in sample D 11, as identified by the participants (blue bars) and auditors (green bars).

Navicula antonii was identified with 3.9 % and 0.8 % (average: 2.3 %) by two of the three auditors and with 1.2-9.3 % (average: 3.5 %) by 34 of 37 participants in sample D 11 (Fig. 62).

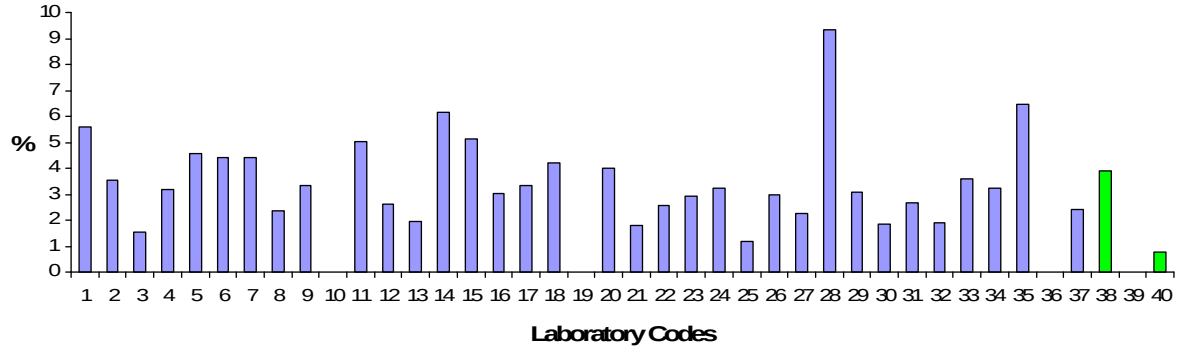


Fig. 62: Relative abundances of ***Navicula antonii*** in sample D 11, as identified by the participants (blue bars) and auditors (green bars).

Especially the results of the participants 10, 13, 15, 19, 22, 25, 28 and 36 expose taxonomic problems. Of these, laboratories 15, 19, 22 and 28 did not detect any *N. cryptotenella* in sample D 11. Laboratories 10, 19 and 36 did not detect any *N. antonii* and laboratories 10 and 19 counted *N. menisculus* in very high abundances in sample D 11 (Fig. 63). Additionally, laboratories 13, 19, 22 and 25 detected a considerable percentage of the here discussed taxa with ambiguity (labelled with a cf.) (Fig. 64).

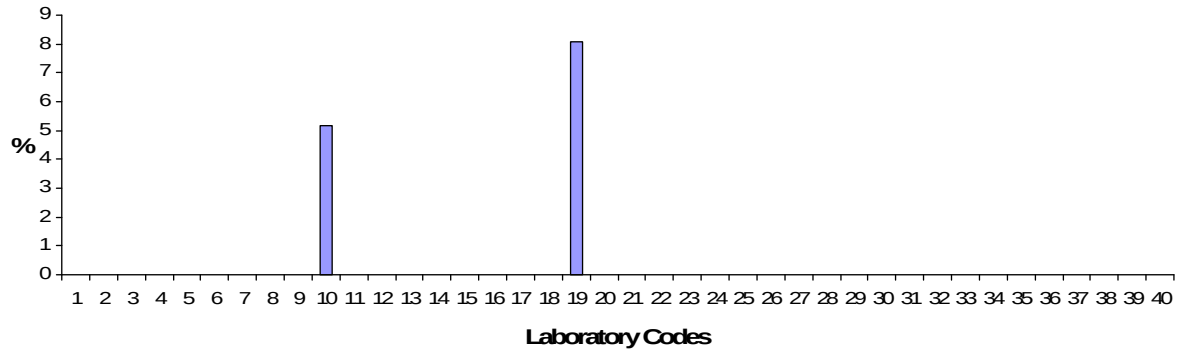


Fig. 63: Relative abundances of ***Navicula menisculus*** in sample D 11, as identified by the participants (blue bars) and auditors (no. 38-40).

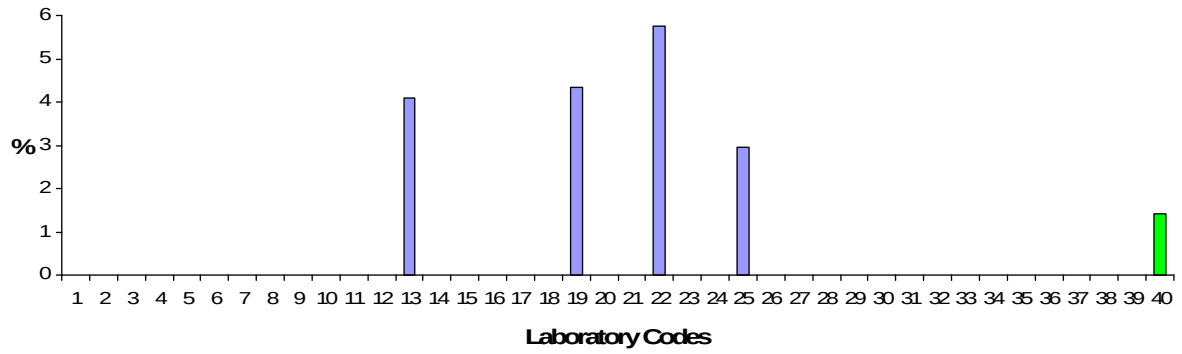


Fig. 64: Sum of the relative abundances of ***Navicula* cf. *antonii*, *N.* cf. *cryptotenella*** and ***N.* cf. *cryptotenelloides*** in sample D 11, as identified by the participants (blue bars) and auditors (green bar).

Similar to the participants, the results of the auditors were not uniform and suggest taxonomic difficulties within the here discussed *Navicula*-taxa. For example, auditor 39 exclusively identified *N. cryptotenella,* while auditors 38 and 40 additionally detected *N. antonii* and *N. cryptotenelloides*. Also, just auditor 40 named some counted objects as *N.* cf. *cryptotenelloides* (1.4 %; Fig. 64).

In **sample D 1.1** the auditors identified *N. cryptotenelloides* as the dominating taxon of the genus *Navicula*. All other *Navicula*-taxa occurred with distinctly less than 1 % and were thus statistically not relevant in sample D 1.1. In contrast to sample D 11 the main problem was differentiating *N. cryptotenella* and *N. cryptotenelloides*. Both taxa occurred in sample D 1.1 (Fig. 65). However, *N. cryptotenella* only occurred in low abundances (<1 %). Thus, not detecting any *N. cryptotenella* does not suggest an identification error or counting mistake.

35 of 37 participants detected *N. cryptotenelloides* (+ cf.) and/or *N. cryptotenella* (+ cf.) in sample D 1.1 (1.4-11.8 %, average: 7.0 %) (Fig. 65). Laboratories 28 and 36 did not identify any *N. cryptotenelloides* or *N. cryptotenella*. In contrast to single taxa abundances the sum of *N. cryptotenelloides* (+ cf.) and/or *N. cryptotenella* (+ cf.) were uniform among auditors (average: 7.0 %) (Fig. 65).

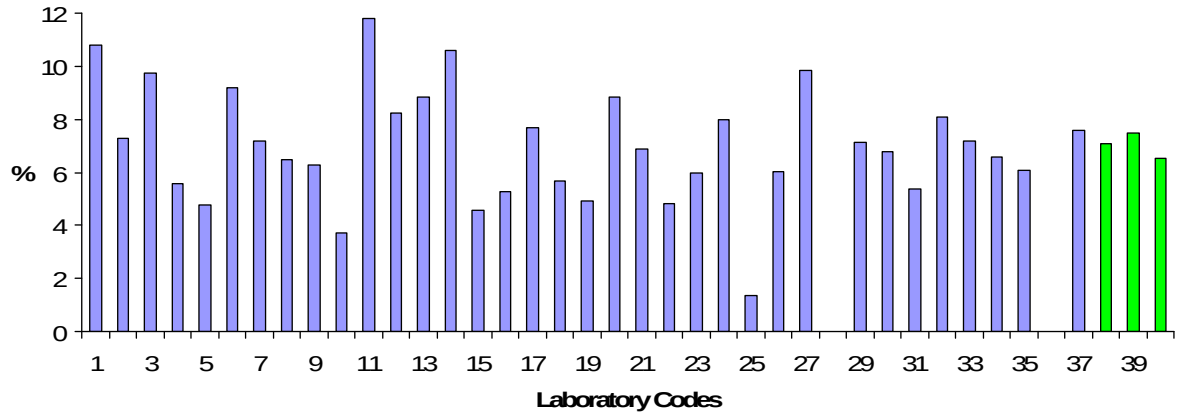


Fig. 65: Sum of the relative abundances of ***Navicula cryptotenella*** and ***N. cryptotenelloides*** including cf. in **sample D 1.1**, as identified by the participants (blue bars) and auditors (green bars).

In sample D 1.1 *N. cryptotenelloides* was detected by 31 of 37 participants (1.4-11.0 %, average: 6.4 %) (Fig. 66) and by two of three auditors (average: 7.3 %) (Fig. 66).

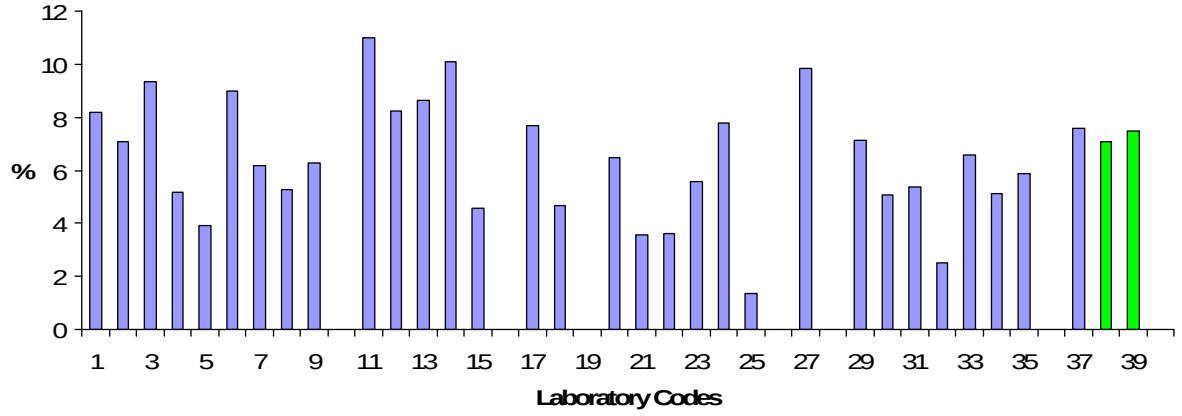


Fig. 66: Relative abundances of ***Navicula cryptotenelloides*** in sample D 1.1, as identified by the participants (blue bars) and auditors (green bars).

Navicula cryptotenella was not detected by any auditor in sample D 1.1 (Fig. 67). In contrast, 25 of the 37 participants identified *N. cryptotenella* (0.2-6.1 %, average: 1.6 %) (Fig. 67).

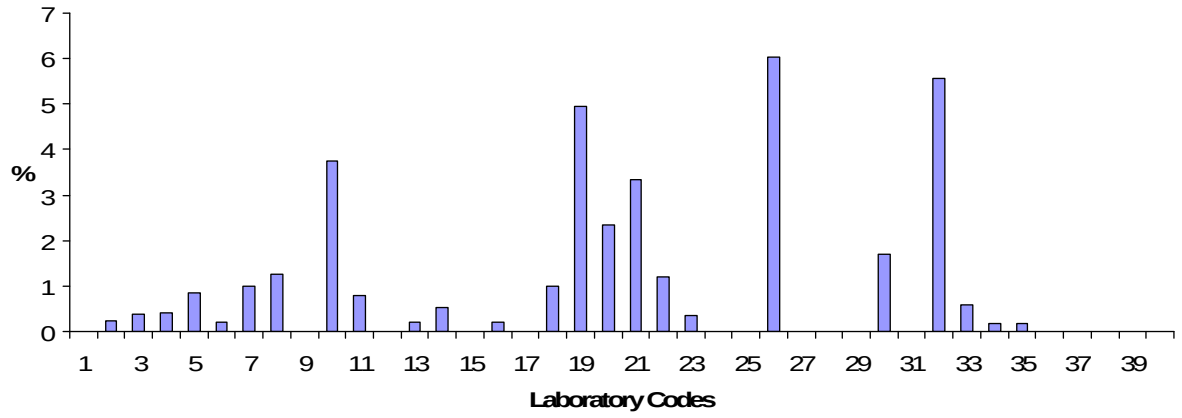


Fig. 67: Relative abundances of ***Navicula cryptotenella*** in sample D 1.1, as identified by the participants (blue bars) and auditors (no. 38-40).

One auditor (no. 40) counted solely *N*. cf. *cryptotenella* instead of *N*. *cryptotenella* in sample D 1.1 (Fig. 68). Similarly, four participating laboratories counted *N*. cf. *cryptotenella* (laboratories 1 and 24) or *N*. cf. *cryptotenelloides* (laboratories 16 and 34) in sample D 1.1 (Fig. 65).

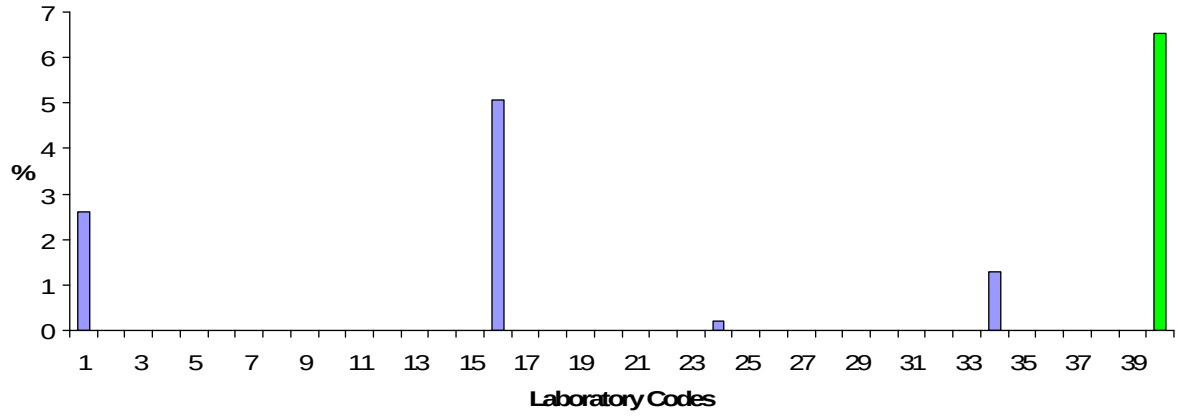


Fig. 68: Sum of the relative abundances of ***Navicula* cf. *cryptotenella*** and ***N*. cf. *cryptotenelloides*** in sample D 1.1, as identified by the participants (blue bars) and auditors (green bars).

The results of participants 10, 16, 19, 26, 28 and 36 particularly reveal the taxonomic problems. Laboratories 28 and 36 did not detect either *N. cryptotenelloides* or *N. cryptotenella*. Laboratory 28 counted 7.2 % *N. antonii* instead of the here discussed taxa. Laboratory 36 identified 13.4 % *Navicula gregaria* Donkin (not detected by the auditors in sample D 1.1), but no other *Navicula*-taxon that we think can possibly be mistaken for *N. cryptotenelloides*.

Laboratories 10, 16, 19 and 26 did not detect any *N. cryptotenelloides* in sample D 1.1. Laboratories 10, 19 and 26 solely identified *N. cryptotenella*. Laboratory 16 counted low abundances of *N. cryptotenella* and mainly *N.* cf. *cryptotenelloides*. Similarly, the results of auditor no. 40, who solely identified *N.* cf. *cryptotenella* in sample D 1.1, emphasize the taxonomic problems within the *N. cryptotenella* and *N. cryptotenelloides*-group.

Overall, the counting results of samples D 11 and D 1.1 suggest in practice difficulties when identifying *N. cryptotenella* and *N. cryptotenelloides* and similar species, such as *N. antonii*. Mainly, the problems probably stem from several overlapping measurable dimensions such as length, width and striae density, and a generally fairly similar habitus among the here discussed species (Tab. 20).

Tab. 20: Selected characteristics for differentiating the *Navicula*-taxa identified in samples D 11 and D 1.1 and similar species. Measurements according to Lange-Bertalot (2001) and Hofmann et al. (2011).

species	length (µm)	width (µm)	striae (n/10 µm)	areolae (n/10 µm)
N. cryptotenella	12-40	5-7	14-16	38
N. cryptotenelloides	9-18	3.7-4.2	16-18	42-44
N. antonii	11-30	6-7.5	10.5-15	28-32
N. menisculus	32-50	11-12.5	8.5-9.5	24-25
N. upsaliensis	18-47	9.5-12	9-11.5	25-27

Next to the measurable criteria (Tab. 20) other details may help to an extend during the identification, especially when differentiating *N. cryptotenella* from *N. cryptotenelloides*. The raphe is filiform (*N. cryptotenella*) or filiform to hardly visible (*N. cryptotenelloides*), the central area is irregular and small (*N. cryptotenella*) or very small to not visible (*N. cryptotenelloides*) and the lineolae are hardly visible (*N. cryptotenella*) or never visible (*N. cryptotenelloides*) when using a light microscope. Additionally the sternum is raised in *N. cryptotenella* and never raised in *N. cryptotenelloides*. However, this trait is almost only visible using an electron microscope and thus not really useful during a routine count.

Apart from the discussed similarities, there is an additional problem that led to distinct difficulties with differentiating *N. cryptotenella* and *N. cryptotenelloides*. According to Lange-Bertalot (1993, 2001) the two taxa can always and certainly be distinguished by their

width, as *N. cryptotenelloides* is 3.7-4.2 µm wide and *N. cryptotenella* is 5.0-7.0 µm wide. Overlapping valve width (4.2-5.0 µm) is not supposed to occur. In contrast, valves with a valve width of 4.2-5.0 µm occurred regularly in the here examined samples. For elucidating this problem of overlapping valve width 38 valves of the *N. cryptotenella* / N. *cryptotenelloides*-group were measured and photographed in each of the D 1.1 and D 11 samples (Fig. 69; Plate 17).

Of the 38 measured valves in **sample D 1.1**

- 23 had a valve with of 3.6-4.2 µm, corresponding to *N. cryptotenelloides*. Interestingly, all 23 valves had a striae density of 18.5-21.0 in 10 µm, which consistently exceeds 16-18 striae in 10 µm, the given range for *N. cryptotenelloides* (Lange-Bertalot 2001, Hofmann et al. 2011). Still, we assume that these valves are probably *N. cryptotenelloides*, as already small differences in the method for measuring striae density can lead to different results of measured striae density (see Chapters 3.2.14 and 6);
- one valve had a width of 5.5 µm and 17 striae in 10 µm. Even though the striae density is slightly too high according to Lange-Bertalot (2001) and Hofmann et al. (2011), this valve is probably *N. cryptotenella* (see arguments above);
- the remaining 14 valves (36.8 %) had a width between 4.2-5.0 µm, contrasting the details given in Lange-Bertalot (1993 and 2001), i.e. the width were in the range between *N. cryptotenella* and *N. cryptotenelloides* (Fig. 69). These 14 valves had a striae density between ~17.3 and 21.0 in 10 µm and were thus more similar to *N. cryptotenelloides* than to *N. cryptotenella*. However, it remains disputable, which taxon these valves belong to. Thus, we suggest to label these valves with a cf. for now.

Of the 38 measured valves in **sample D 11**

- two valves had a width of 3.7 and 4.0 µm and a striae density of ~18 and 20 in 10 µm, respectively. Correspondingly, these valves were probably *N. cryptotenelloides* (see discussion above).
- 13 valves had a width >5.0 µm, matching *N. cryptotenella*. Of these 13 valves 10 valves had ~14-16 striae in 10 µm and are thus probably *N. cryptotenella*. Three of these 13 valves had ~17-18 striae in 10 µm and are therefore not unambiguously *N. cryptotenella*.
- the remaining 23 valves (60.5 %) had a width between 4.2-4.9 µm, i.e. in a range between the width of *N. cryptotenella* and *N. cryptotenelloides*. Similarly, the striae density ranged between 15.5 and 19.9 in 10 µm. Thus, it remains unclear if the valves belong to

N. cryptotenella or to *N. cryptotenelloides* and should probably also best be labelled with a cf.

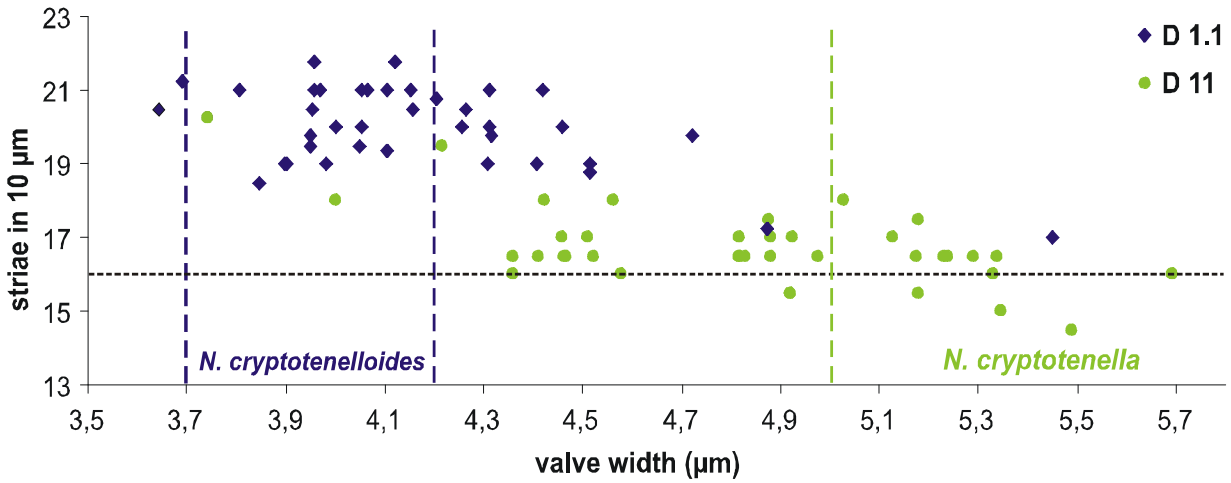


Fig. 69: Measured striae density and valve width from valves of the *N. cryptotenella* and *N. cryptotenelloides*–complex from 38 valves from sample D 1.1 (blue diamonds) and 38 valves from sample D 11 (green circles). Dashed lines denote the valve width of *N. cryptotenelloides* (3.7-4.2 µm) and *N. cryptotenella* (5.0-7.0 µm) and the differentiating striae density of 16 striae in 10 µm according to Lange-Bertalot (2011). For more information see text.

The participants (and auditors) chose different approaches to dealing with these ambiguous taxa description of the *N. cryptotenella*–complex according to counting results (see above) and discussions during the workshop of the intercalibration exercise.

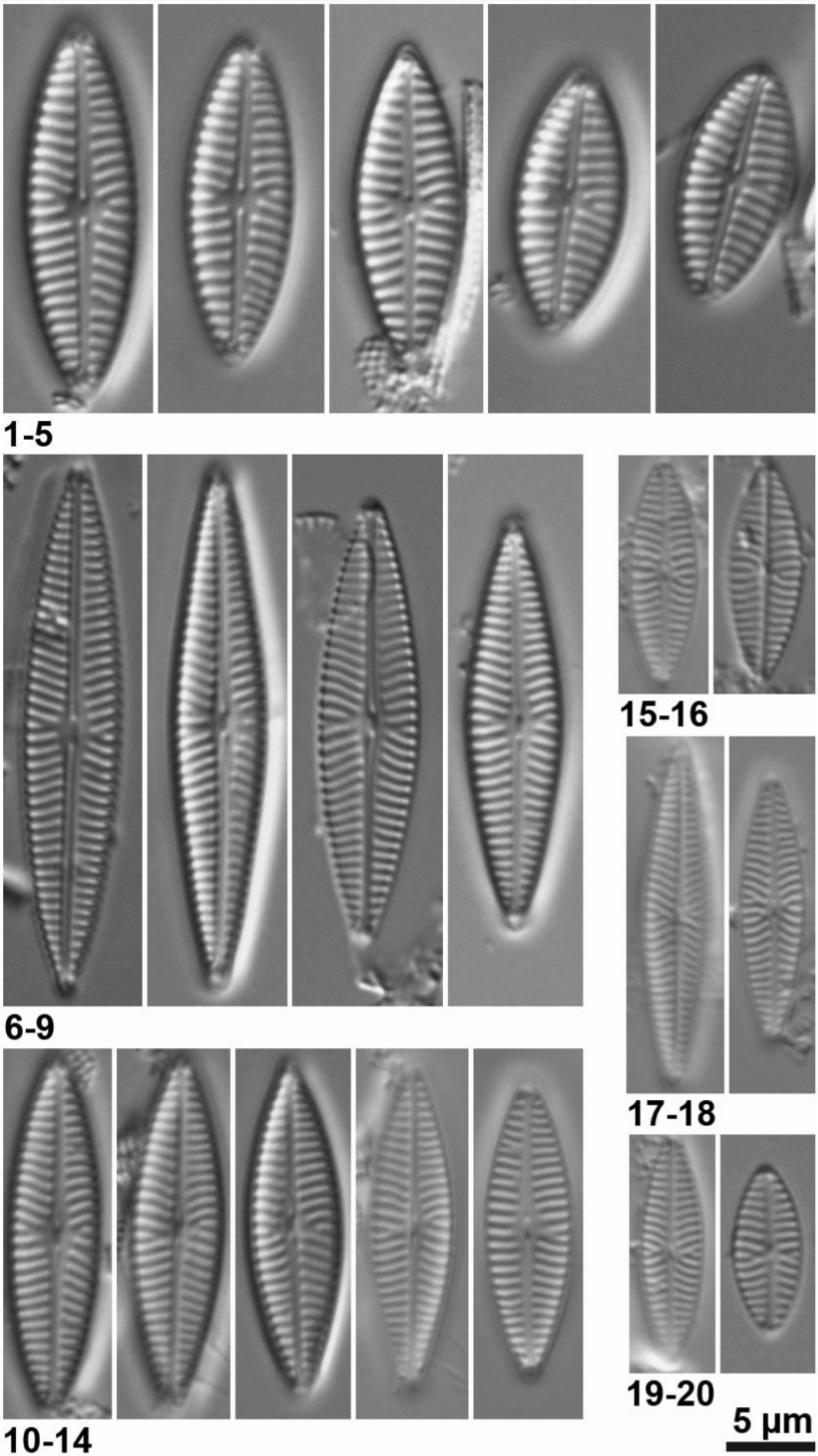
Some participants measured and determined (as good as possible) the first few valves of this *Navicula*-complex and allocated all additional valves of this complex to this determined name during the remaining count. We consider this approach as problematic, because only one name is given, but potentially both taxa (*N. cryptotenella* and *N. cryptotenelloides*) may be present in any given sample.

Other participants measured and determined every encountered valve. Here, three approaches were used for valves with a valve width between 4.2 and 5.0 µm. Some participants labelled these valves with a "cf.". Other participants refrained from using "cf." and deliberately allocated the valves to either *N. cryptotenella* or *N. cryptotenelloides*, depending on which species width was closer. They argued that they wanted to avoid an unreliable assessment, as the German method assumes water quality evaluations to be unreliable in both lakes and running waters, if the sum of diatom objects that could not be determined (sp., spp.) and/or could not unambiguously be determined (cf.) exceeds 5 % (Schaumburg et al. 2006, 2011) (for more details see Chapters 3.3 and 5).

A third approach was used by another participant, who calculated the average striae density of all valves with a valve width between 4.2 and 5.0 µm. If the striae density average was greater than 16 these valves were allocated to *N. cryptotenelloides* and to *N. cryptotenella* if the average was smaller than 16. The participant argued that a species identification is more reliable when based on the entire population than on single valves. We generally agree. However, during a routine count of composite samples with many different diatom taxa, we cannot assume that there is always only one taxon of a species-complex present and thus that there is only the population of one taxon present. Therefore, using this approach two species may be counted as just one species, especially very similar species and species with overlapping traits such as the here discussed *N. cryptotenella* and *N. cryptotenelloides*.

Recommendations:
It is essential to consider the entire combinations of traits, when identifying the here discussed taxa. For *N. cryptotenella* and *N. cryptotenelloides* especially valve width and striae density are important. Valves with a width between these two taxa (4.2 to 5.0 µm) should be labelled with a „cf.“, documented with a picture and the approach should be briefly explained in the results. Thus, a later review and possible revision of the results is possible without an entire recount of the sample. The approach remains relevant until the taxonomy of the *N. cryptotenella*–complex is revised, particularly as Lange-Bertalot (2001) also considers *N. cryptotenella* as a heterogeneous taxon.

Plate 16 (next page): *Navicula antonii* (1-5), *N. cryptotenella* (6-14) and *N. cryptotenelloides* (15-20) from the intercalibration exercise samples D 11 (Lake Krossinsee, northern Germany) and D 1.1 (Lake Geneva, Switzerland). These valves all conform with all relevant identification traits given by Lange-Bertalot (2001) and Hofmann et al. (2011). Valves 1-14 and 20 are from sample D 11, valves 15-19 from sample D 1.1.


1-5
6-9
10-14
15-16
17-18
19-20
5 µm

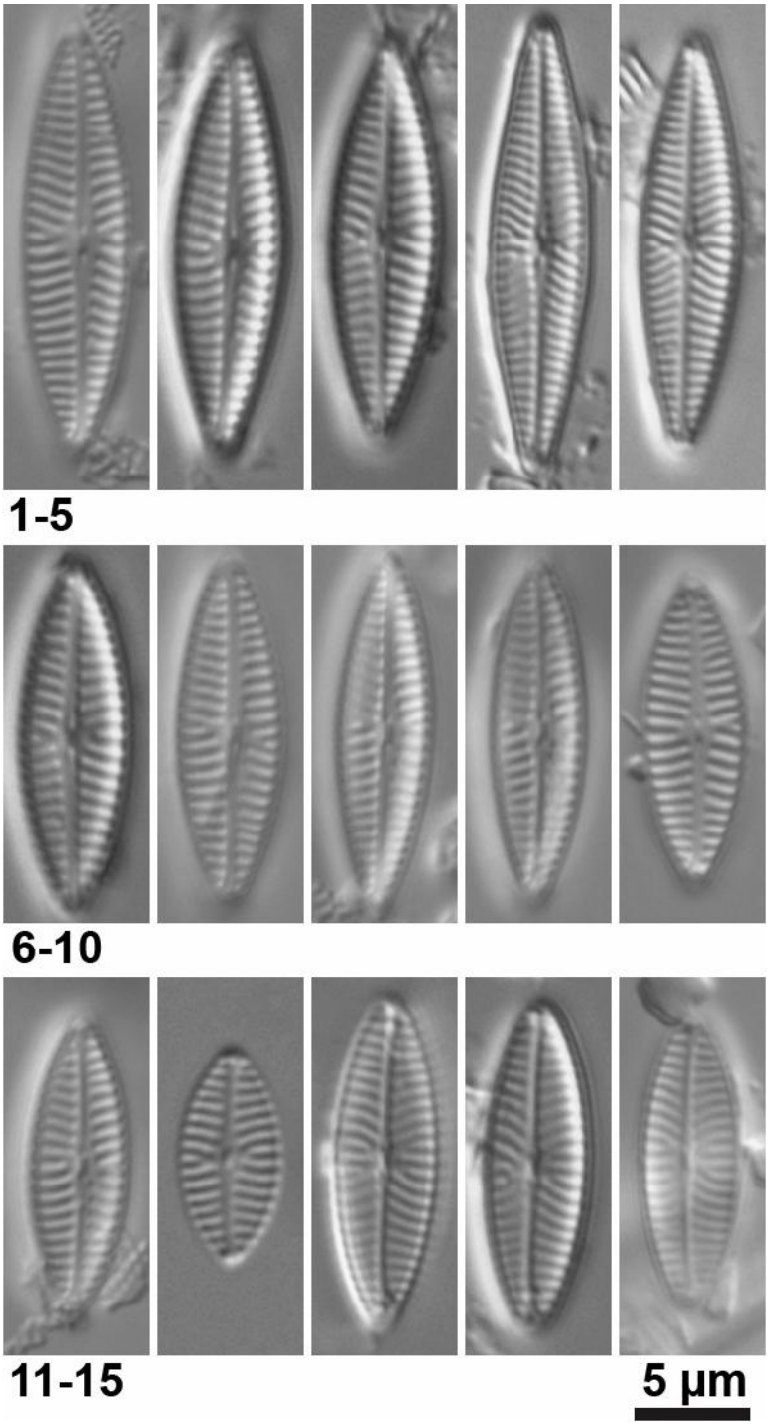


Plate 17: Ambiguously determined valves (cfs.) of the *Navicula cryptotenella* and *N. cryptotenelloides*–complex with a valve width of 4.2 to 5.0 µm from the intercalibration exercise samples D 11 (Lake Krossinsee, northern Germany; valves 1-3 and 6-12) and sample D 1.1 (Lake Geneva, Switzerland; valves 4-5 and 13-15). For more information see text.

3.2.13 *Navicula lundii* and *N. veneta* in sample D 12

During this diatom intercalibration exercise *Navicula veneta* Kützing and *N. lundii* Reichardt were only found in sample D 12. 34 of 37 participants identified valves from the *N. lundii*, *N. veneta*, *N. exilis* Kützing and *N. cryptocephala* Kützing–complex in sample D 12 (0.5-6.5 %; average: 3.8 %) (Fig. 70). Three of the thirty-seven laboratories did not find any species of this group (laboratories 15, 22, 28). All auditors reported *N. veneta* (1.5-2.2 %; average 1.7%) and *N. cryptocephala* (var. *cryptocephala*) (0.6-2.2 %; average 1.2%). Two auditors reported *N. lundii* (1.2-2.4; average 1.8 %). *N. exilis* was not detected by the auditors in sample D 12.

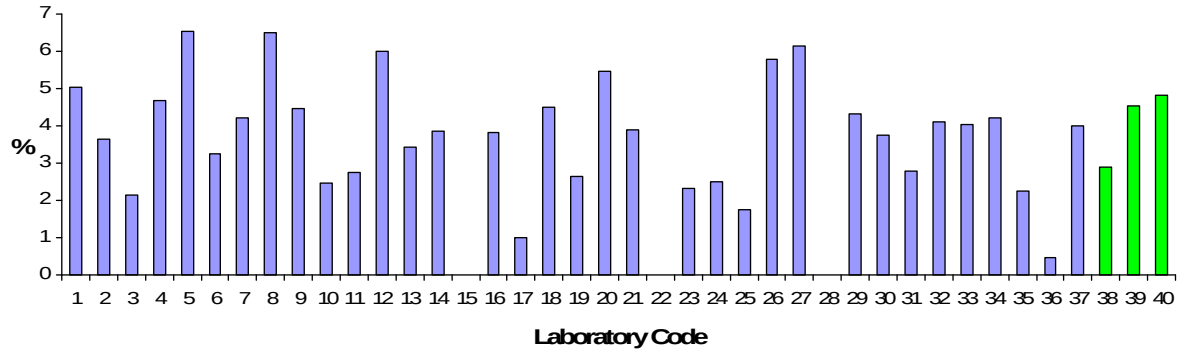


Fig. 70: Sum of the relative abundance of *Navicula cryptocephala* (var. *cryptocephala*), *N. exilis*, *N. lundii* (including cf.) and *N. veneta* (including cf.) from sample D 12 from the participants (blue bars) and the auditors (green bars).

Thirty of the thirty-seven participants reported *N. veneta* and one participant reported *N.* cf. *veneta* in addition to *N. veneta* (Fig. 71). The relative abundance was between 0.5 and 5.4 % with an average of 2.1 %. Thirty of the thirty-seven participant reported *N. cryptocephala* (var. *cryptocephala*) (Fig. 72). The relative abundance of this species was between 0.5 and 5.0 % with an average of 1.7 %.

Nine laboratories reported *N. lundii* or *N.* cf. *lundii* (1.0-2.7 %; average 1.5 %) (Fig. 72), four laboratories reported *N. exilis* (0.5-0.7 %; average 0.7 %) (not shown). Four participants reported only *N. cryptocephala* (laboratories 10, 17, 19, 36).

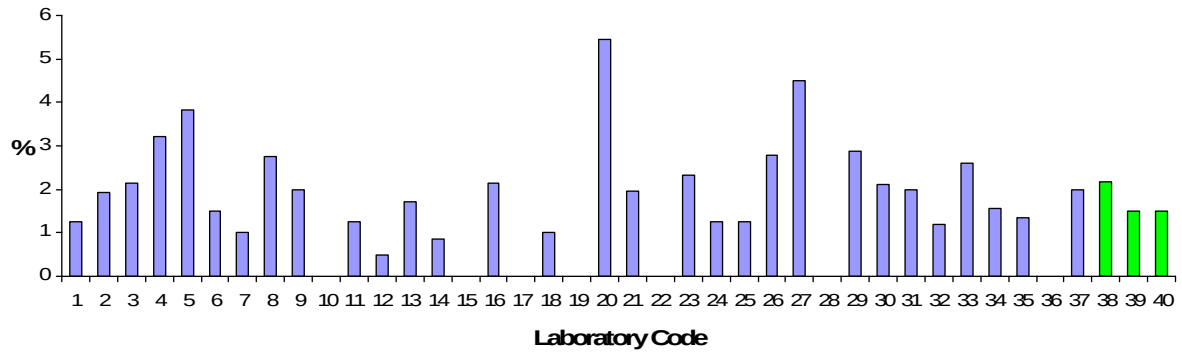


Fig. 71: Relative abundance of ***Navicula veneta*** (including cf.) from sample D 12 as counted by each participant (blue bars) and auditor (green bars).

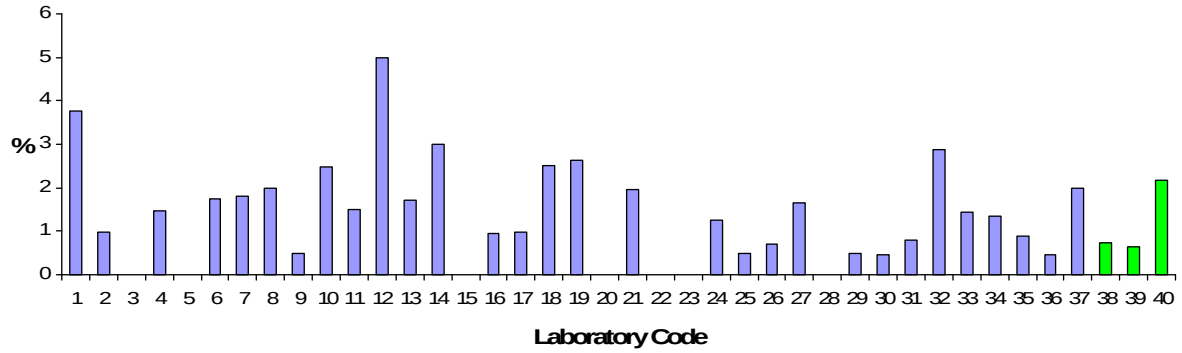


Fig. 72: Relative abundance of ***Navicula cryptocephala*** (var. *cryptocephala*) from sample D 12 as counted by each participant (blue bars) and auditor (green bars).

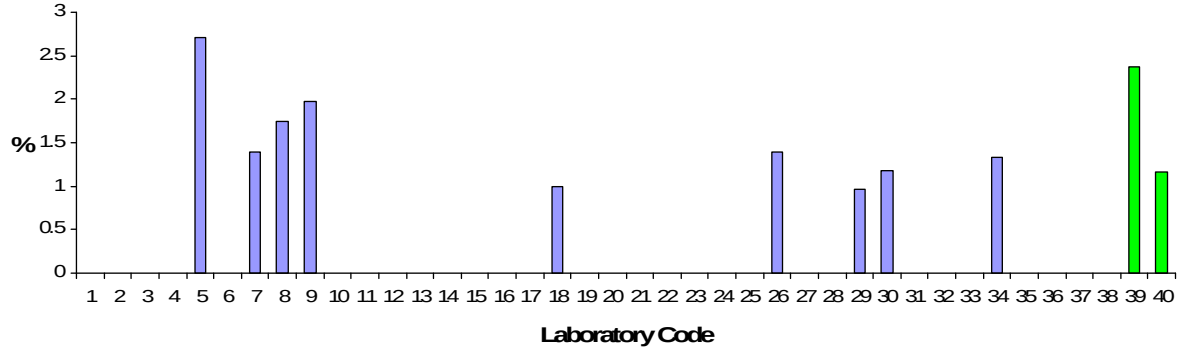


Fig. 73: Relative abundance of ***Navicula lundii*** (including cf.) from sample D 12 as counted by each participant (blue bars) and auditor (green bars).

Formerly, *Navicula veneta*, *N. lundii* and *N. exilis* were considered to be varieties of *N. cryptocephala* (see e.g. Krammer & Lange-Bertalot 1986-2004) and are not easy to distinguish. Especially *N. lundii* and *N. exilis* are very similar species and it is possible that *N. exilis* and *N. lundii* are conspecific, i.e. that they belong to the same species (Lange-Bertalot 2001). In order to distinguish these species the entire character spectrum must be compared with one another and with that of other taxa of similar morphology (see below).

Tab. 21: Measurable dimensions for differentiating *Navicula lundii*, *N. veneta*, *N. exilis* and *N. cryptocephala* according to Lange-Bertalot (2001) and Hofmann et al. (2013). Length and width is given in µm, striae and puncta in n/10 µm.

Taxon	Length	Width	Striae	Puncta
N. veneta	13-30	4.4-6	13.5-15	~35
N. lundii	13-35	4-6.3	14-15	---
N. exilis	20-45	6-8	13-15	~40
N. cryptocephala	20-40	5-7	14-17	~40

Navicula veneta is linear-lanceolate to rhombic-lanceolate with protracted, usually slightly wedge-shaped ends. The raphe is filiform. The central area is rather small and almost symmetrical transversely widened to a rectangle. The striae of *N. veneta* are weakly radiate, convergent at the ends.

Navicula lundi is lanceolate with an often asymmetrical and cymbelloid appearance. The ends are wedge-shaped, obtusely rounded and sometimes very weakly protracted. The raphe is filiform to very weakly lateral. The central area is of average width and round to transversely rectangular. The striae of *N. lundii* are radiate, slightly curved and parallel to convergent at the ends.

Navicula exilis is lanceolate as well but the ends are shortly wedge-shaped to longer and gradually narrowed and acutely to obtusely rounded. The raphe is filiform and rarely very weakly lateral. The central area is comparatively large transversely elliptical to rectangular and clearly asymmetrical. The striae are radiate and convergent at the ends.

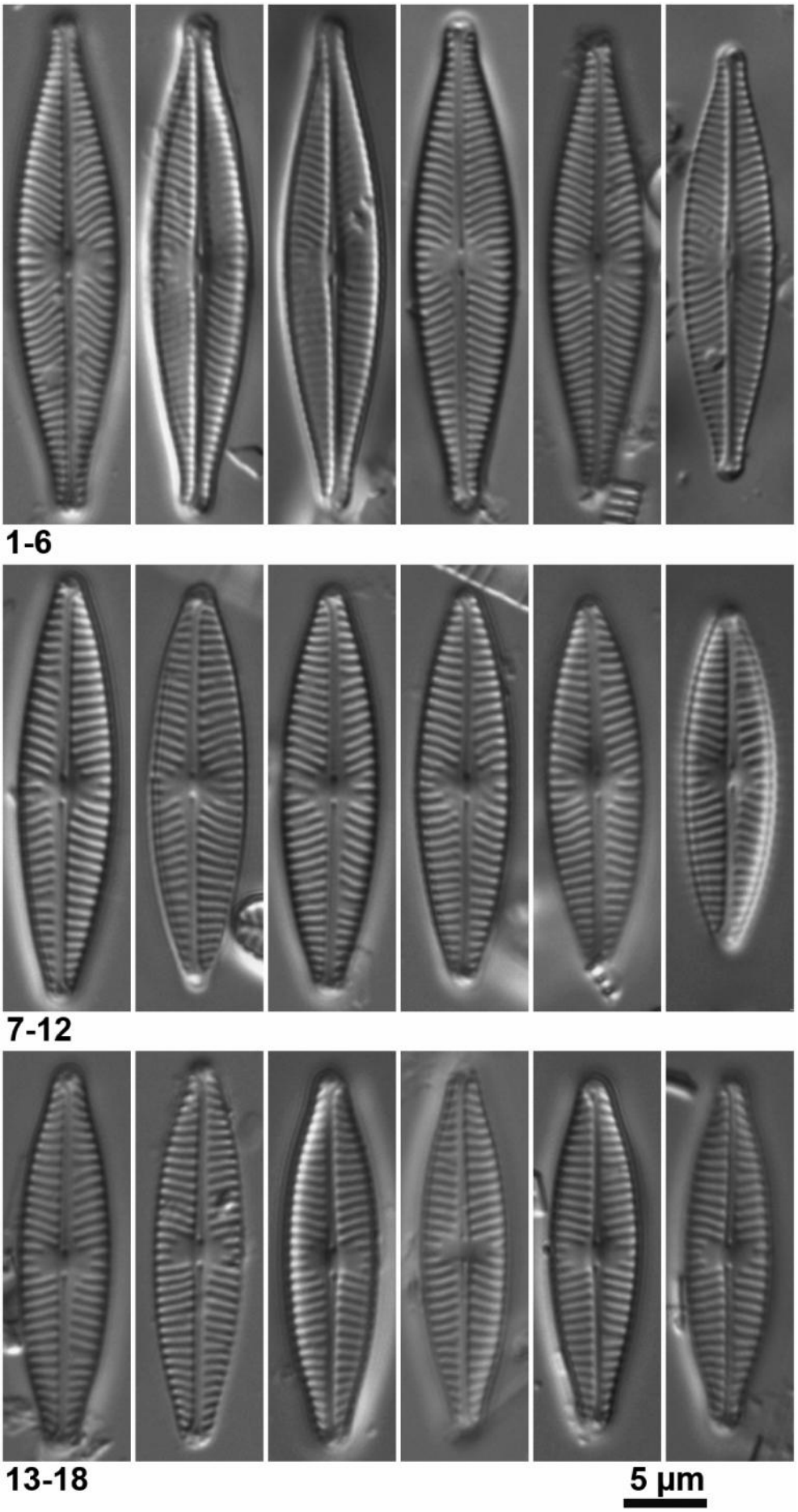
Navicula cryptocephala is lanceolate or narrowly lanceolate. The ends are gradually narrowing or weakly rostrate, obtusely rounded or subcapitate. The raphe is filiform. The central area is small to moderately large, roundish to transversely elliptical and a little asymmetrical. The striae are strongly radiate and weakly convergent at the ends.

Navicula exilis and *N. lundii* are easy to distinguish from *N. cryptocephala* and *N. veneta*. *N. cryptocephala* has more tapered ends and strongly radiate striae, *N. veneta* has linear-lanceolate valves, weakly radiate striae and the puncta are often visible when using the light microscope. It is more difficult to distinguish *N. exilis* from *N. lundii*. The characters of both species are almost the same (except for the valve width, see Tab. 21). The ecology of both species is supposed to be distinctly different (Lange-Bertalot 2001). *N. lundii* is a soil diatom and is found in periodically wet habitats with an average to moderately high electrolyte concentration. *N. exilis* occurs in low conductivity, oligotrophic to weakly eutrophic and oligosaprobic waters (Lange-Bertalot 2001). In contrast, *N. lundii* is also regularly present in running waters of the North-German lowlands, such as sample D 12, suggesting that the ecological preferences of this species needs further investigation and that ecological details from the literature should not be used for species identification.

It is possible to confuse the abundant taxa of sample D 12 (*N. lundii*, *N. veneta* and *N. cryptocephala*) with other related or similar taxa, e.g. *N. hofmanniae* Lange-Bertalot, *N. cryptofallax* Lange-Bertalot & Hofmann, *N. leistikowii* Lange-Bertalot and *N. aquaedurae* Lange-Bertalot. Their size-range and other characters overlap and thus these taxa should be taken into account. However, it should be possible to keep the confusion to a feasible minimum, if all characteristics are considered.

Recommendations:
It is essential to consider the entire combination of traits when identifying species from this *Navicula*-complex. All (new) species from the former *Navicula cryptocephala*-group should be distinguished. Note a “cf.” and take a picture, if the identity of the valve is ambiguous and explain where the valve deviates from the species description. In case of cf-deletion for the PHYLIB software to avoid more than 5 % ambiguous taxa, note the procedure and consider the deletion when assessing the PYHLIB-results.

Plate 18 (next page): Light microscopic images of *Navicula cryptocephala* (1-6), *N. lundii* (7-12) and *N. veneta* (13-18) from sample D 12 (River Klepelshagener Bach, Northern Germany). For further details see the text.


1-6
7-12
13-18
5 µm

3.2.14 *Navicula reichardtiana* and *N. caterva* in sample D 1.1

Navicula reichardtiana was identified in most samples from both the participants and auditors (Tab. 22). *Navicula caterva* was identified from a few participants, mainly in sample D 1.1. The auditors did not detect *N. caterva* in any of the samples of the intercalibration exercise (Tab. 22).

Tab 22: Number of participants and auditors that have identified *Navicula reichardtiana* and *N. caterva* definitively or with cf. in the four samples.

	sample	participants (n=37)		auditors (n=3)	
		definitively	cf.	definitively	cf.
N. reichardtiana	D 11	19	2	2	-
N. reichardtiana	D 1.1	25	1	2	1
N. reichardtiana	D 2	13	-	2	-
N. reichardtiana	D 12	6	-	-	-
N. caterva	D 11	1	1	-	-
N. caterva	D 1.1	5	-	-	-
N. caterva	D 2	1	-	-	-
N. caterva	D 12	1	-	-	-

The relative abundances of *N. reichardtiana* and *N. caterva* were usually relatively low (together <2.5% per sample and participant, Fig. 74). Thus, it is not possible to evaluate the absence of these taxa as a counting or identification error. However, the present results indicate problems of identification and possibly also of taxonomy. In the following these problems will be discussed in detail using the example of sample D 1.1.

Overall, this precise differentiation is important, as it may affect the water quality assessment. *N. caterva* has no indicator value for all running water types and most lake types, while *N. reichardtiana* has indicator values for most water body types (see Chapter 3.3).

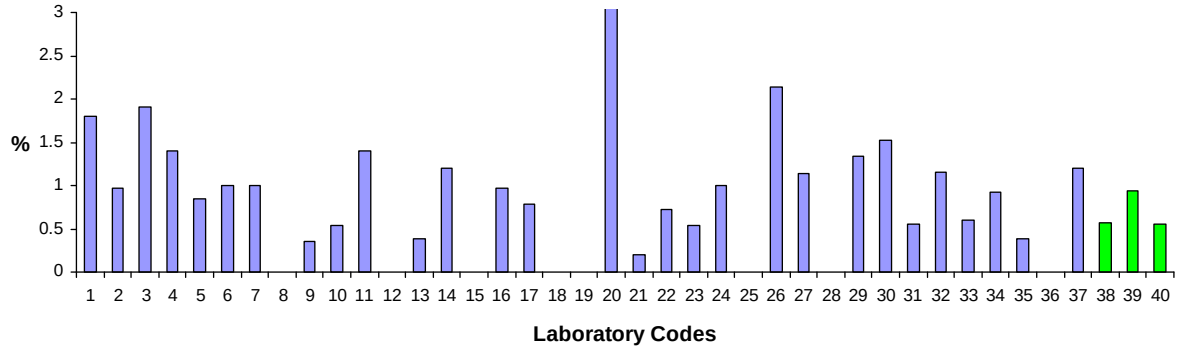


Fig. 74: Sum of the relative abundance of ***Navicula reichardtiana*** (**var. *reichardtiana***), ***N.* cf. *reichardtiana*** and ***N. caterva*** detected by each participant (blue) and auditor (green) in sample D 1.1. Laboratory 20 counted 9.5 % (not fully shown).

29 of 37 participating laboratories (0.2-9.5 %, mean: 1.3 %) and all three auditors (0.6-0.9 %, mean: 0.7 %) identified taxa of the *N. reichardtiana*- and *N. caterva*-complex in sample D 1.1 (Fig. 74). 25 participants (0.2-2.1 %, mean: 1.0 %) and two auditors (0.6-0.9 %, mean: 0.8 %) identified *N. reichardtiana* (var. *reichardtiana*) with certainty (Fig. 75). One participant (1.2 %) and one auditor (0.6 %) identified *N.* cf. *reichardtiana* (Fig. 76).

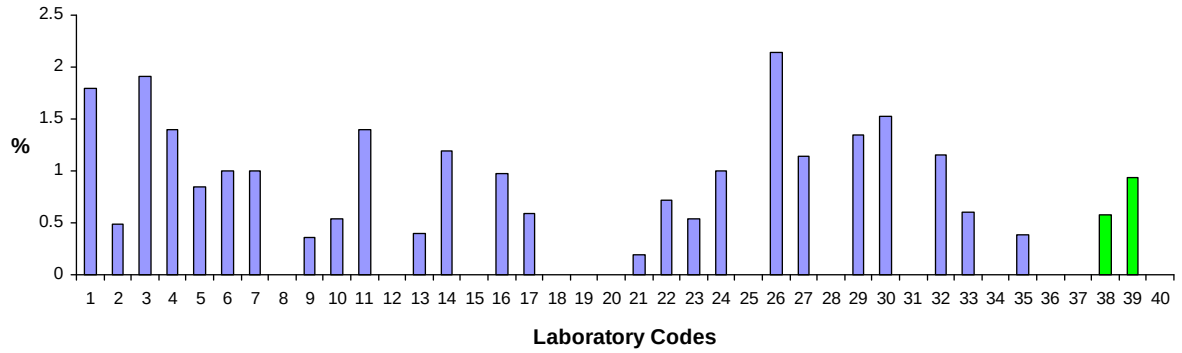


Fig. 75: Relative abundance of ***Navicula reichardtiana*** (**var. *reichardtiana***) detected by each participant (blue) and auditor (green) in sample D 1.1.

N. caterva was identified with certainty by five participants (0.2-9.5 %, mean: 2.3 %) in sample D 1.1 (Fig. 77). The auditors did not identify any *N. caterva*. One laboratory (no. 20) identified above-average abundances (9.5 %) of *N. caterva*. Furthermore, seven participants identified *N. associata* Lange-Bertalot and one participant *N. moskalii* Metzeltin, Witkowski & Lange-Bertalot in sample D 1.1 (not shown in figures).

Fig. 76: Relative abundance of ***Navicula* cf. *reichardtiana*** detected by each participant (blue bars) and auditor (green bars) in sample D 1.1.

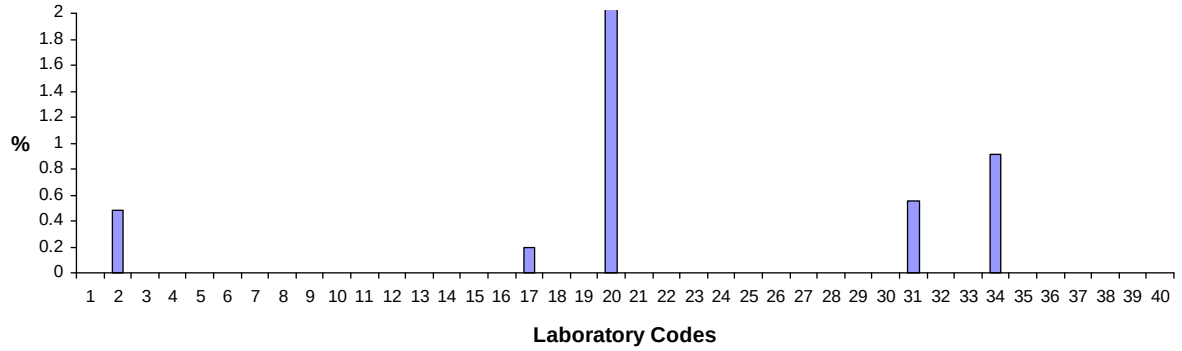


Fig. 77: Relative abundance of ***Navicula caterva*** detected by each participant (blue bars) and auditor (no. 38-40) in sample D 1.1. Laboratory 20 counted 9.5 % (not fully shown).

In summary, two auditors and most participants identified *N. reichardtiana* (var. *reichardtiana*) with certainty and only five participants and no auditor identified *N. caterva*. Thus, we could easily assume that *N. caterva* was misidentified. However, this can not be conclusively verified within the context of this intercalibration exercise, as additional taxonomic examinations would be necessary. Still, we can demonstrate (see below), why some participants identified *N. caterva* instead of *N. reichardtiana*, why some participants (laboratories no. 17 and 2) identified both taxa in one sample and why one auditor identified all objects of this taxonomic complex as *N.* cf. *reichardtiana*.

N. reichardtiana and *N. caterva* are mainly distinguished by their striae density (Lange-Bertalot 2001, Hofmann et al. 2011). Accordingly, *N. reichardtiana* has 14-16 striae/10 µm and *N. caterva* (16)18-21 striae in 10 µm. Additionally, the striae orientation is supposed to change abruptly in *N. reichardtiana* and gradually in *N. caterva*. Other criteria of differentiation overlap, i.e. the width and the size of the central area, which is small to very

small in *N. caterva* and small in *N. reichardtiana*. Also, the areolae-density of the striae differs between the two species. However, this trait is usually not sufficiently visible with the light microscope (Lange-Bertalot 2001, Hofmann et al. 2011, Tab. 23).

Tab. 23: Measureable dimensions of *Navicula reichardtiana*, *N. caterva* and similar taxa according to Lange-Bertalot (2001) and Hofmann et al. (2011).

taxon	length (µm)	width (µm)	striae (n/10 µm)	areolae (n/10 µm)
N. caterva	10.4-17	4.2-5.5	(16)18-21	40
N. reichardtiana	12-22(26)	5-6	14-16	33-36
N. moskalii	24-27	6.8-8	11.5-15	30
N. associata	16-20	6-6.6	12-15	28-30

At a first glace *N. reichardtiana* and *N. caterva* should be easily separated on the basis of above traits, especially on the basis of striae density and characteristic change of striae orientation towards the poles. However, the evaluation of the intercalibration exercise demonstrated that problems occur when these two species are looked at in more detail. In the following these problems will be revealed exemplarily by showing the different approaches to identifying *N. reichardtiana* and *N. caterva* by two participants. Both participants, anonymously called participant A and B, identified both *N. reichardtiana* and *N. caterva* in the sample and agree on the names of several frustules. However, participants A and B disagree on the names of a few frustules due to a different measuring approach and different interpretation of the identification traits (Fig. 78).

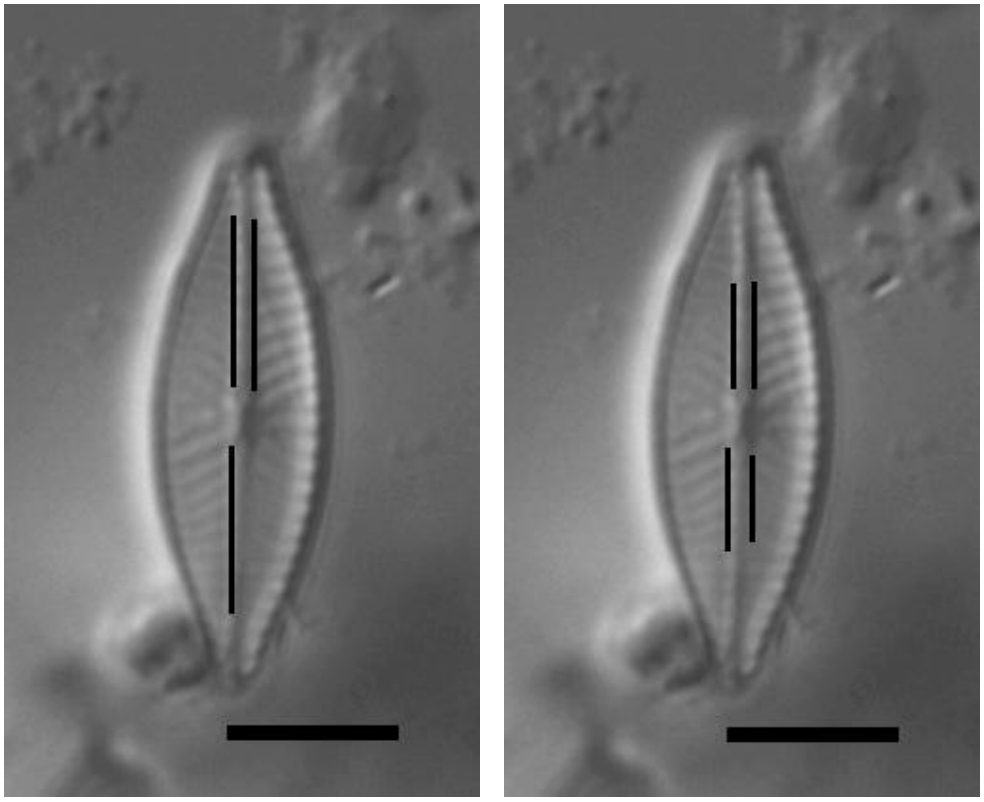


Fig. 78: Example of the different striae-counting approach (vertical lines) from participant A (left) and B (right) on a valve from the *Navicula reichardtiana-caterva*-group from sample D 1.1. Scale (horizontal line): 5 µm. For more details see text.

Both participants measure the striae density along the margin of the axial area, beginning at the end of the central area. Participant A counts the striae along a 5 µm scale, while participant B uses a shorter scale (Fig. 78). Both then extrapolate to striae density in 10 µm (Tab. 24). If possible, striae density should be measured along a 10 µm scale. However, if this is not possible (as is the case here), striae densities should be measured in 5 µm or a shorter interval (see Chapter 6). The most important criterion for the scale-length is that striae density is not supposed to change substantially in the area measured (see Chapter 6). Participant A argues that striae density varies, but not substantially, in the area used by participant A (Fig. 78), i.e. that the striae get only slightly denser towards the poles. Participant B is more stringent and tries to use a relatively short interval without any change in striae density (Fig. 78). These different interpretations of striae counting methods and consequently varying measuring methods only lead to small differences in the extrapolated number of striae in 10 µm (Tab. 24). However, these small differences are crucial for taxonomic identification (see Plate 19).

Tab. 24: Results of measurements of participant A and B of the valve shown in Fig. 78 of the *Navicula reichardtiana-caterva*-group from sample D 1.1. Participant A measures directly in µm, while participant B measures in pixel and then converts into µm. In this given example 0.0549 pixel convert to 1 µm. For more explanations see text.

participant	length (µm)	width (µm)	striae/µm (pixel)				striae/10µm
			top left	top right	bottom left	bottom right	
A	15.93	4.78	8.25/5	8/5	8/5	-	16.17
B	16.36	4.78	5/3.3 (60)	5/3.4 (62)	5/3.3 (60)	4/2.6 (47)	15.14

Interpretation and species identification by participant A:
Participant A argues that the width of the valve in the given example (Fig. 78) fits the range of *N. caterva* and is too small for *N. reichardtiana*. The striae density (16.17 in 10 µm) only slightly exceeds the range for *N. reichardtiana* and fits the range of *N. caterva*. Participant A denotes the central area as small, which characterises both species. The striae orientation changes only once abruptly (bottom right in Fig. 78) and three times gradually, which participant A thinks fits better to *N. caterva* than to *N. reichardtiana* (see also the following remarks about the discrepancies between the text that describes the species and the figures that depict the species in Lange-Bertalot 2001 and Hofmann et al. 2011). In summary participant A identifies the valve in Fig. 78 as *N. caterva*.

Interpretation and species identification by participant B:
Participant B argues that the width of the valve in the given example (Fig. 78) only so slightly exceeds the range of *N. reichardtiana* that this valve may still be identified as *N. reichardtiana*. Striae density (15.14 in 10 µm) fits the range of *N. reichardtiana* and is outside the range of *N. caterva*. Similar to participant A, participant B denotes the central area as small, which characterises both species. The striae orientation changes abruptly once (bottom right, see Fig. 78), which distinctly characterises *N. reichardtiana* according to participant B. In summary participant B argues that the valve in Fig. 78 more resembles *N. reichardtiana* than *N. caterva*. However, as the width is slightly outside the range of *N. reichardtiana* and the striae orientation changes only gradually on three sides, participant B identifies the valve in Fig. 78 as *N.* cf. *reichardtiana*.

Both participants carefully considered the whole combination of characters to identify the valve in Fig. 78. However, they differ in their identification of the valve in Fig. 78 due to

different approaches and interpretation, especially of the traits "striae density" and "changes of striae orientation towards the poles". It is difficult or even impossible to decide, which participant is correct, as there are some ambiguities that cannot be resolved. It is not documented in sufficient detail, in which way the describing authors counted the striae density. Thus, no decision can be made, which measuring approach is more appropriate. Ultimately, striae density should be counted in the same manner as the describing authors. However, this is often not known or documented.

The text of Lange-Bertalot (2001) and Hofmann et al. (2011) describe that the striae orientation towards the poles of *N. reichardtiana* changes abruptly and of *N. caterva* gradually. However, the pictures in Lange-Bertalot (2001) and Hofmann et al. (2011) do not always correspond to this description. For example, Hofmann et al. (2011) depict valves of *N. caterva* with abruptly changing striae orientation towards the poles (Plate 31, Figure 38 in Hofmann et al. (2011), striae top left and bottom left). The same valve is also depicted in Lange-Bertalot (2001) (Fig. 7, Plate 33 in Lange-Bertalot 2001). Similarly, valves of *N. reichardtiana* are depicted with gradually changing striae orientation towards the poles in Hofmann et al (2011) (Plate 31, Figure 29 and 30, on both: striae top right) and in Lange-Bertalot (2001) (Plate 13 Fig. 26, striae top right and bottom left). Thus, for identifying *N. reichardtiana* and *N. caterva* the questions arises what to do, if the describing text does not correspond to the describing pictures in the identification literature. Currently, there is a rather wide range of possibilities to interpret the trait "striae orientation towards the poles", which explains the different approaches of participants A and B. Again, it would be key to know how the describing authors interpreted this trait.

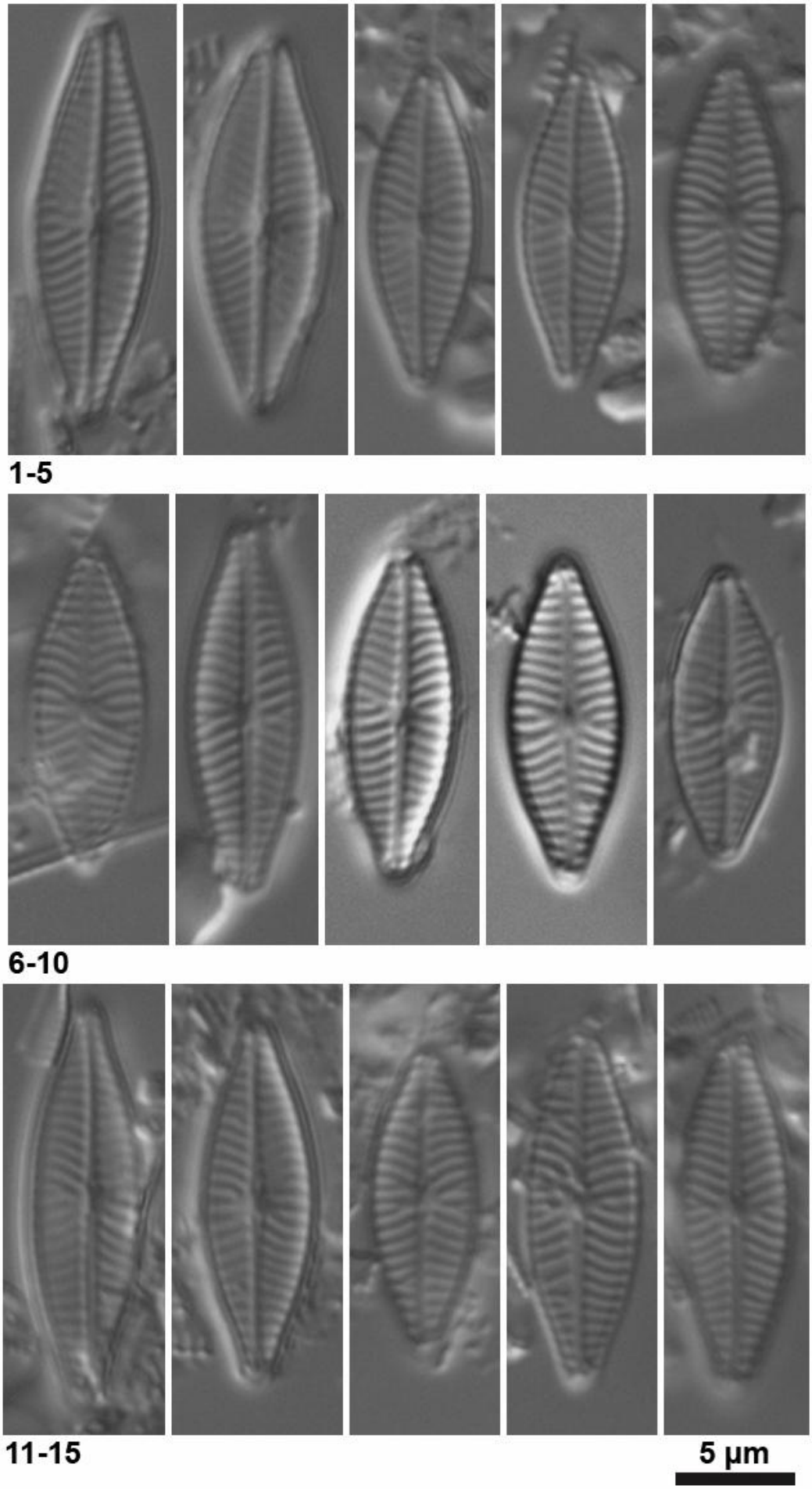
Recommendations:

For the identification of *N. caterva* and *N. reichardtiana* it is necessary and essential to carefully consider the whole combination of their characteristic traits. Particular traits are the different striae density and the change of striae orientation towards the ends (abrupt or gradual). Other, yet overlapping, distinguishing traits are the different width and different size of the central area. If the combination of characters are not definitively attributable to one of the two species, the object should be counted with cf., documented with a picture and the approach to identification should be noted to enable a validation and possibly a revision in hindsight.

Tab. 25: Naming of valves from the *Navicula reichardtiana* and *N. caterva* group of Plate 19 by participant A and B. For more information see text and Plate 19.

Valves in Plate 19	participant A	participant B
1	*reichardtiana*	*reichardtiana*
2	*reichardtiana*	*reichardtiana*
3	*caterva*	*caterva*
4	*caterva*	cf. *reichardtiana*
5	cf. *reichardtiana*	cf. *reichardtiana*
6	cf. *reichardtiana*	cf. *reichardtiana*
7	*reichardtiana*	cf. *reichardtiana*
8	cf. *caterva*	cf. *reichardtiana*
9	cf. *caterva*	cf. *reichardtiana*
10	cf. *caterva*	cf. *reichardtiana*
11	cf. *caterva*	cf. *reichardtiana*
12	cf. *caterva*	cf. *reichardtiana*
13	cf. *caterva*	cf. *reichardtiana*
14	cf. *caterva*	*reichardtiana*
15	cf. *caterva*	*caterva*

Plate 19 (next page): Light microscope pictures of valves from the *Navicula reichardtiana* and *N. caterva* group (valves 1-15). Valve 9 is from sample D 11, the remaining valves are all from sample D 1.1. Both valves of one frustule are valves 3 + 4; 5 + 6 and 14 + 15. For more explanations see text and Tab. 25.


1-5
6-10
11-15
5 µm

3.2.15 *Nitzschia lacuum* and *N. fonticola* in samples D 11 and D 1.1

Counting results of sample D 11

According to the results of the auditors the genus *Nitzschia* Hassall contributed 4.5 % (mean) to sample D 11 (mean of participants 4.0 %). The most abundant *Nitzschia* was *Nitzschia fonticola* (Grunow) Grunow in sample D 11 (Plate 20, Fig. 80). During a screening of sample D 11, where 900 diatom objects were looked at, the following taxa were also found: *Nitzschia* cf. *fonticola*, *N. amphibia* Grunow, *N. dissipata* ssp. *dissipata* Grunow, *N. intermedia* Hantzsch, *N. gracilis* Hantzsch and *N. paleacea* (Grunow) Grunow (Plate 20). The latter taxa had relative abundances of less than 0.8 %. Thus, we focus on *N. fonticola* in the following and on *Nitzschia*-taxa which names were used by participants and that were more or less similar to *N. fonticola* and may be confused with *N. fonticola* (Fig. 79).

Next to the taxa names listed in Fig. 79 the participants also used the following 17 names for *Nitzschia*-species in sample D 11: *Nitzschia acicularis* (Kützing) W. Smith, N. *dissipata* Grunow, *N. dissipata* ssp. *dissipata*, *N. dissipata* var. *media* (Hantzsch) Grunow, *N. graciliformis* Lange-Bertalot & Simonsen, *N. gracilis*, *N. intermedia*, *N. palea* (Kützing) W. Smith, *N. palea* var. *debilis* (Kützing) Grunow, *N. palea* var. *palea* (Kützing) W. Smith, *N. palea* var. *tenuirostris* Lange-Bertalot, *N. paleacea*, *N. paleaeformis* Hustedt, *N. recta* var. *recta* Hantzsch, *N. sociabilis* Hustedt, *N. subacicularis* Hustedt and *N. tubicola* Grunow. We assume that these 17 taxa cannot be confused with *N. fonticola*. Consequently, they are not further discussed here (however, some are depicted on Plate 20).

Interestingly, the participants used a total of 37 different names for *Nitzschia*-species in sample D 11 compared to ~6-7 *Nitzschia*-taxa that were found during the screening. This fact already suggest that problems occurred with identifying these *Nitzschia*-species, even though it is possible that a few other *Nitzschia*-taxa than the 6-7 species identified during the screening were also present in sample D 11.

Of the 37 participants 36 identified *N. fonticola* or taxa that may potentially be confused with *N. fonticola* (0.7-5.6 %, mean: 2.9 %; Fig. 79). The auditors counted 2.2-4.5 % of these *Nitzschia*-taxa (mean: 3.4 %) (Fig. 79).

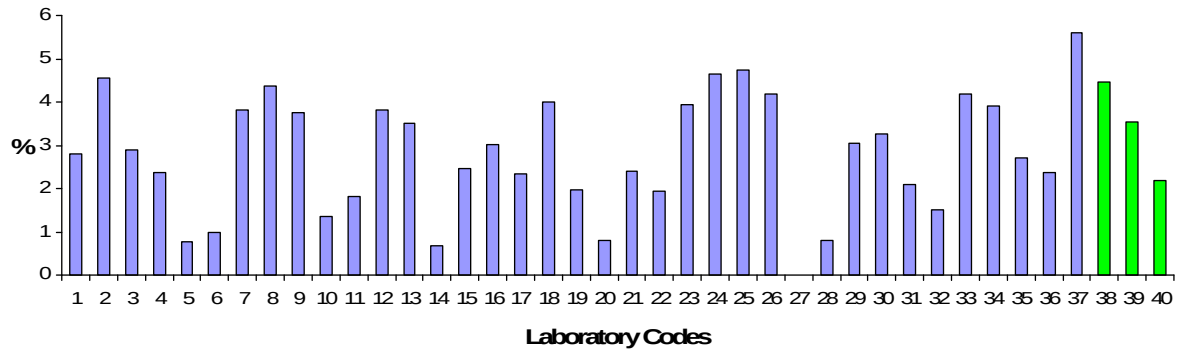


Fig. 79: Sum of the relative abundances of *Nitzschia* spec., *N. abbreviata* Hustedt, *N. amphibia*, *N. angustatula* Lange-Bertalot, *N. archibaldii* Lange-Bertalot, *N. bulnheimiana* (Rabenhorst) H.L. Smith, *N. desertorum* Hustedt, *N. fonticola* (var. *fonticola*), *N. fossilis* (Grunow) Grunow, *N. frustulum* (var. *frustulum*) Grunow, *N. frustulum* var. *inconspicua* (Grunow) Grunow, *N. hantzschiana* Rabenhorst, *N. incognita* Legler & Krasske, *N. lacuum* Lange-Bertalot, *N. liebetruthii* Rabenhorst, *N. pusilla* Grunow, *N. radicula* Hustedt, *N. solita* Hustedt and *N. supralitorea* Lange-Bertalot from sample D 11 (including these taxa with cf.). Blue bars: results of participants, green bars: results of the auditors. For more information see text.

Of the 37 participants 27 participants determined *N. fonticola* (including cf.) with 0.2-3.4 % (mean: 1.8 %) (Fig. 80). The three auditors identified *N. fonticola* with 0.9-3.2 % (mean: 2.0 %) (Fig. 80).

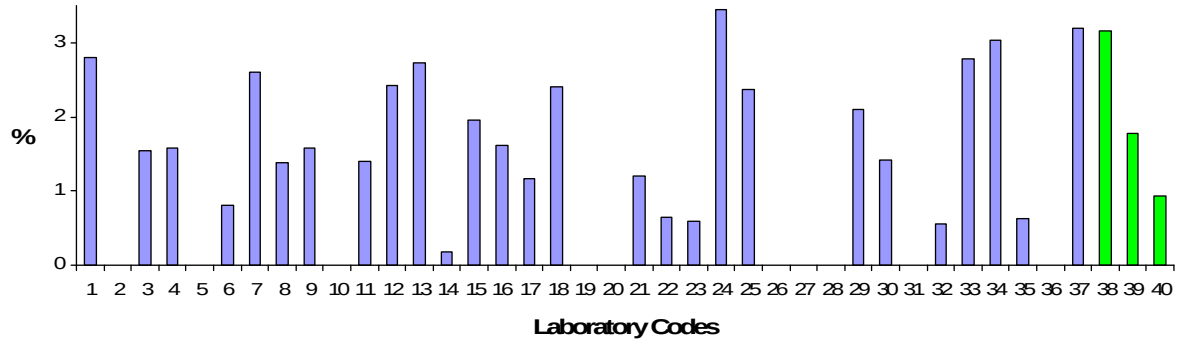


Fig. 80: Sum of the relative abundances of ***Nitzschia fonticola*** (var. *fonticola*) (including "cf.") in sample D 11. Blue bars: results of participants, green bars: results of the auditors.

15 of 37 participants indicated ambiguity for the *Nitzschia*-taxa listed in Fig. 79 by using „cf." or „spec.", with relative abundances of 0.2-3.4 % (mean: 1.1 %) (Fig. 81). Similarly, two auditors indicated ambiguous *Nitzschia*-taxa with 0.8-1.0 % (mean: 0.8 %) (Fig. 81).

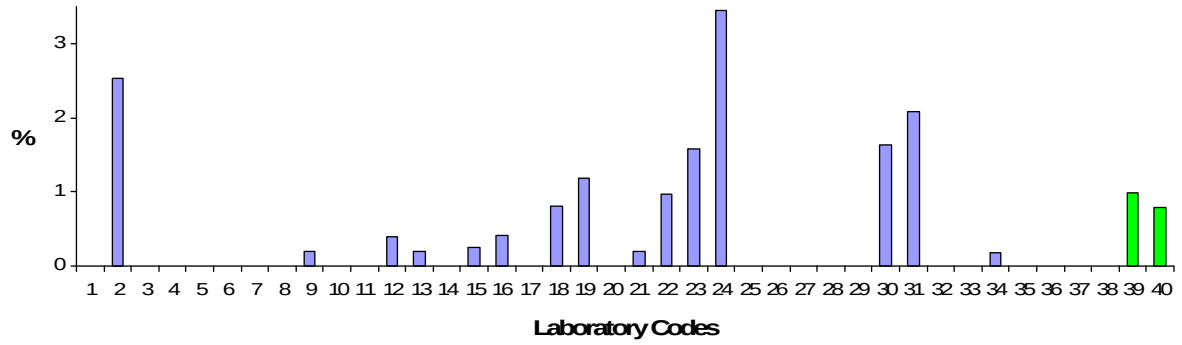


Fig. 81: Sum of the relative abundances of all **ambiguous *Nitzschia*-taxa** (*Nitzschia* spec. and all taxa labelled „cf.“ that are listed in Fig. 79) in sample D 11. Blue bars: results of participants, green bars: results of the auditors.

Counting results of sample D 1.1

The genus *Nitzschia* contributed 6.0 % (mean) to sample D 1.1 according to the auditors (mean participants: 6.8 %). The most abundant species of the genus *Nitzschia* in sample D 1.1 were *N. lacuum* and *N.* cf. *fonticola* (Plate 21, Figs. 83 and 84). During a screening of sample D 1.1, where 1,000 diatom objects were looked at, the following taxa were also found: *N. oligotraphenta* (Lange-Bertalot) Lange-Bertalot, *N.* cf. *oligotraphenta*, *N. dissipata*, *N.* cf. *dissipata*, *N. fonticola*, *N. alpinobacillum* Lange-Bertalot and *Nitzschia* spec. (Plate 21). The greatest problems with the *Nitzschia*-taxa in sample D 1.1 occurred with *N. lacuum* and *N. fonticola* and taxa that were more or less similar with *N. lacuum* or *N. fonticola* and could have been mistaken for them (Fig. 82).

Next to the taxa listed in Fig. 82, the participants additionally used the following 14 names: *N. angustata* (W. Smith) Grunow, *N. brunoi* Lange-Bertalot, *N. constricta* (Kützing) Ralfs, *N. dissipata*, *N. dissipata* ssp. *dissipata*, *N. dissipata* ssp. *oligotraphenta* Lange-Bertalot, *N. dissipata* var. *media*, *N. gracilis*, *N. oligotraphenta*, *N. paleacea*, *N. pura* Hustedt, *N. recta* Hantzsch, *N. recta* var. *recta* Hantzsch and *N. sociabilis*. We assume that these 14 taxa cannot be mistaken for *N. lacuum* or *N. fonticola* and consequently they are not discussed any further (however, some are depicted on Plate 21).

In sample D 1.1 all 37 participants identified *N. lacuum*, *N. fonticola* or taxa that may potentially be confused with *N. lacuum* or *N. fonticola* with 1.5-8.9 % (mean: 5.2 %). The auditors counted 2.9-5.8 % of these *Nitzschia*-taxa (mean: 4.6 %) (Fig. 82).

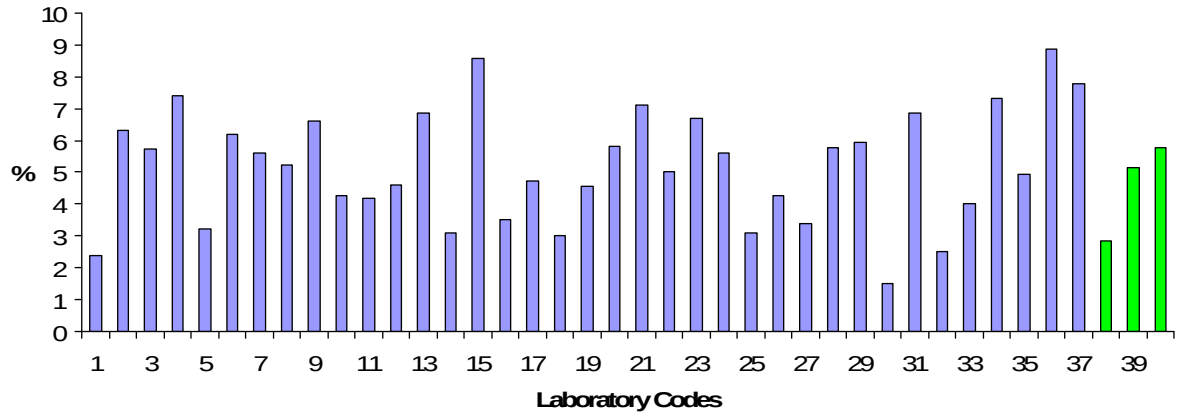


Fig. 82: Sum of the relative abundances of *Nitzschia* spec., *N. abbreviata*, *N. alpinobacillum*, *N. amphibia*, *N. angustatula*, *N. angustiforaminata* Lange-Bertalot, *N. bryophila* (Hustedt) Hustedt, *N. dealpina* Lange-Bertalot & Hofmann, *N. denticula* Grunow, *N. fonticola*, *N. fonticola* var. *fonticola*, *N. frustulum*, *N. frustulum* var. *frustulum*, *N. frustulum* var. *inconspicua*, *N. lacuum*, *N. liebetruthii*, *N. palea*, *N. palea* var. *palea*, *N. palea* var. *tenuirostris*, *N. pusilla* and *N. supralitorea* from sample D 1.1 (including all of these taxa that were labelled with cf.). Blue bars: results of participants, green bars: results of the auditors. For more information see text.

Nitzschia lacuum was detected with 1.4-6.9 % (mean: 4.0 %) by 32 of the 37 participants (Fig. 83). Two of the three auditors identified *N. lacuum* with 2.5 % and 4.7 % (mean: 3.6 %) (Fig. 83).

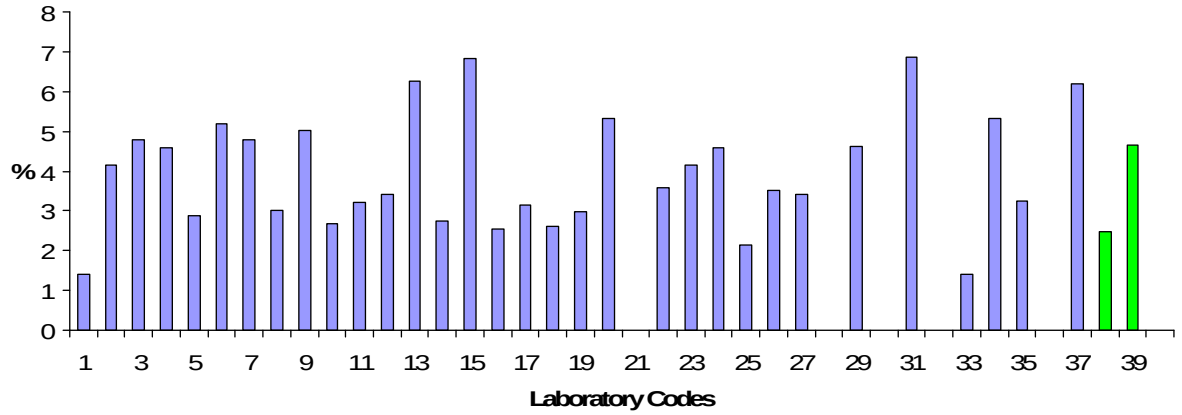


Fig. 83: Relative abundance of ***Nitzschia lacuum*** (including cf.) from sample D 1.1. Blue bars: results of participants, green bars: results of the auditors.

Nitzschia fonticola was detected with 0.2-2.3 % (mean: 0.8 %) by 22 participants (Fig. 84). Two auditors identified *N. fonticola* with 0.4 % and 0.2 % (mean: 0.3 %) (Fig. 84).

Seven participants and the auditor that did not detect any *N. lacuum* or *N. fonticola*, identified *N. palea* in sample D 1.1 (participants: 0.6-5.8 %, mean: 1.6 %, auditor 5.6 %) (Fig 85). Of course it is possible that single finds of *N. palea* were present in the sample. However, during the intensive screening of 1,000 diatom objects, where all *Nitzschia*-taxa were photographed (Plate 21), *N. palea* could not be found.

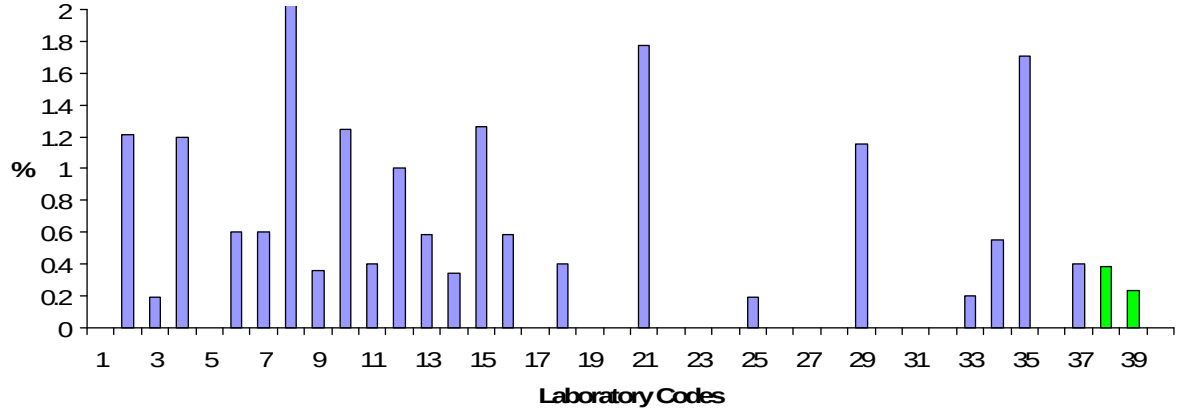


Fig. 84: Relative abundances of ***Nitzschia fonticola*** (including cf.) from sample D 1.1. Blue bars: results of participants, green bars: results of the auditors.

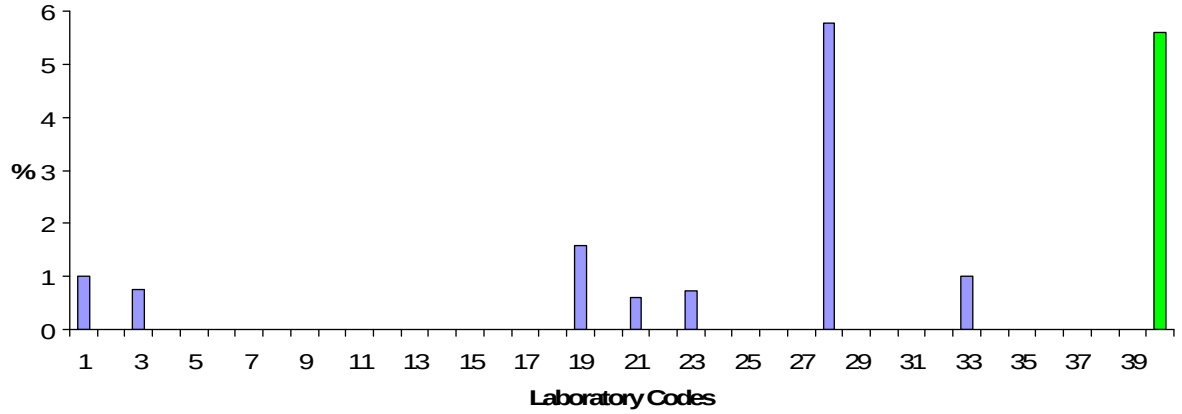


Fig. 85: Relative abundances of ***Nitzschia palea*** (including cf.) from sample D 1.1. Blue bars: results of participants, green bars: results of the auditors. For more information see text.

14 of the 37 participants labelled 0.4-6.9 % (mean: 1.8 %) as ambiguous by using „cf.“ or „spec.“ for *Nitzschia*-taxa that are listed in Fig. 82 (Fig. 86). The three auditors did not identify any ambiguous *Nitzschia*-taxa.

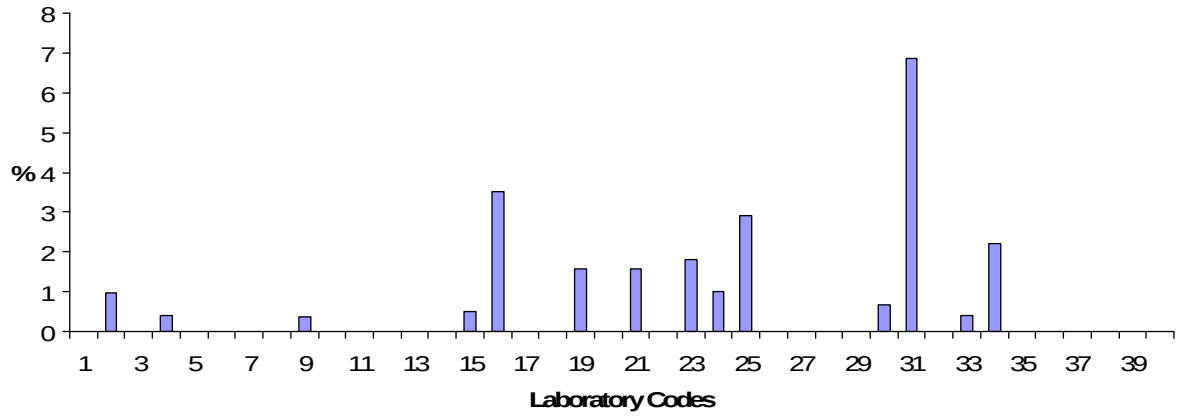


Fig. 86: Sum of the relative abundances of all **ambiguous *Nitzschia*-taxa** (*Nitzschia* spec. and all taxa from Fig. 82 that were labelled „cf.“) from sample D 1.1. Blue bars: results of participants, no. 38-40: results of the auditors.

The results suggests that the main identification problems within the genus *Nitzschia* were with *Nitzschia fonticola* in sample D 11 and with *N. lacuum* as well as possibly *N. fonticola* in sample D 1.1. Thus, in the following we only discuss these two species and other more or less similar taxa that were named in the results by the participants in samples D 11 and D 1.1 (see Tab 26 and 27).

Nitzschia fonticola and *N. lacuum* can hardly be confused with one another. *N. fonticola* has a central node (gap), i.e. the distance between the two middle fibulae is greater compared to the distance between the remaining fibulae. In *N. lacuum* the central node (gap) is absent. Additionally, they differ among others in striae density and shape of the ends (see plates 20 and 21). The measurable dimensions of these two taxa overlap, however only very slightly and most valves can also be well distinguished by length, width and/or fibulae density. In contrast, the differentiation of *N. fonticola* from a number of other taxa with a central node can be tricky and of *N. lacuum* from other taxa without a central node. For example, in the results from samples D 11 and D 1.1 nine *Nitzschia*-taxa with gap were given next to *N. fonticola* and 13 *Nitzschia*-taxa without a gap next to *N. lacuum* (see Tab 26 and 27). The measurable dimensions and the most important criteria for differentiating *N. fonticola* and *N. lacuum* from similar species are given in Tabs. 26 and 27, respectively.

Tab. 26: *Nitzschia*-taxa **with a gap** between the two middle fibulae compared to the distance of the remaining fibulae (i.e. central node present) that were listed in the results for samples D 11 and D 1.1 (except: *N. costei* that is additionally listed) and that were *N. fonticola* or more or less similar to *N. fonticola* and may be confused with *N. fonticola*. Please note that there are other *Nitzschia*-taxa that can also be confused with *N. fonticola* that are not listed here, but that need to be considered when identifying *N. fonticola*. Details from Hofmann et al. (2013), Krammer & Lange-Bertalot (1986-2004) and Tudesque et al. (2008). Sh = shape; E = ends; F: fibulae; P = points; S = striae.

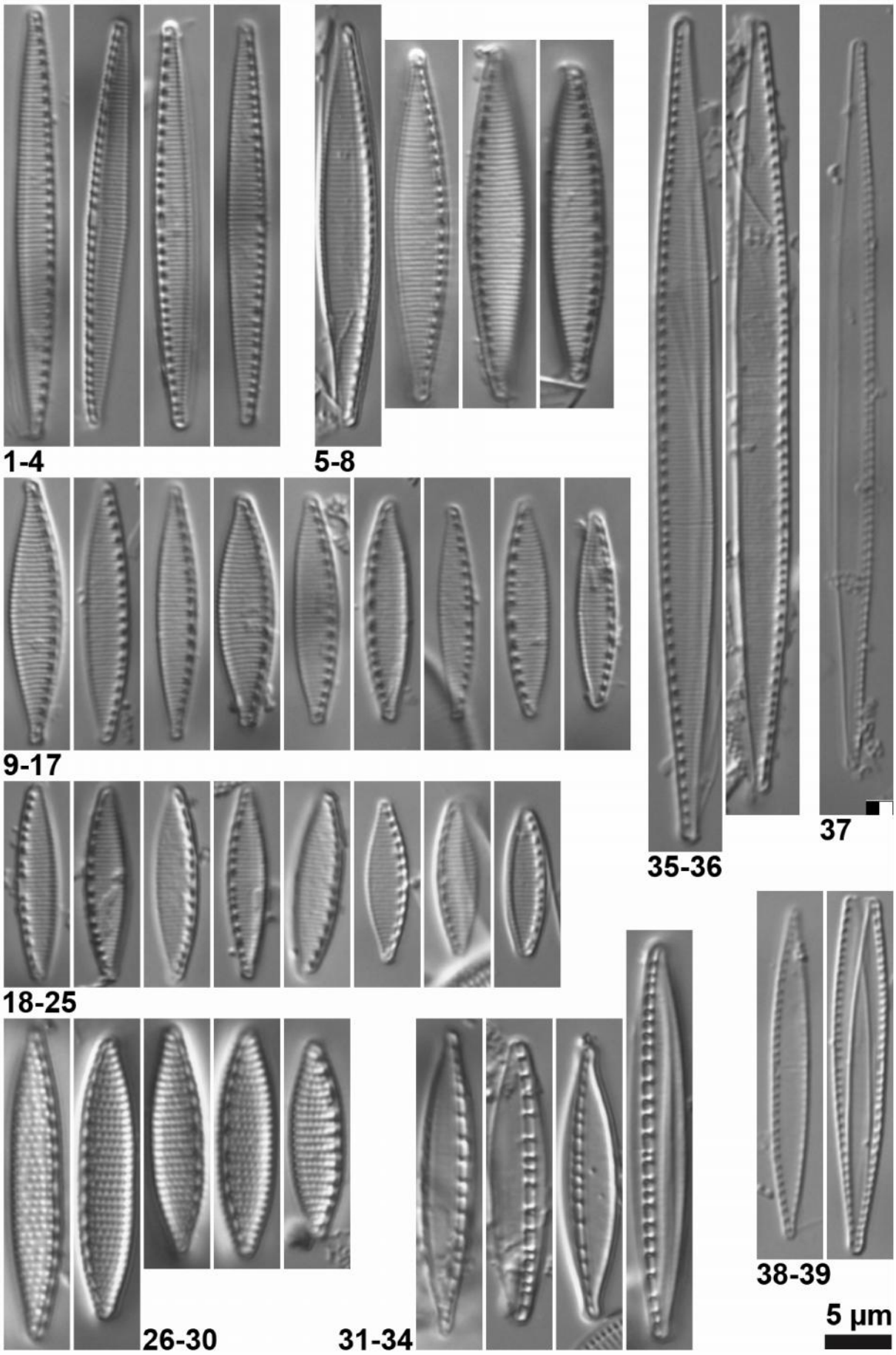
Taxon	Length [µm]	Width [µm]	Fibulae /10µm	Striae /10µm	Comments
N. abbreviata	3-22	2.5-3.5	8-13	23-30	Sh: elliptical, linear elliptical, E: widely rounded, never wedge-shaped, F: look thick
N. amphibia	6-50	4-6	7-9	13-18	Sh: very variable, E: often pointy, F: elongated like a tooth root, S: often coarsely dotted
N. bulnheimiana	12-60	4-4.7	8-13	19-22	Sh: lanceolate to linear, E: ~pointy, P: usually visible
N. costei	8-45	2.5-4.5	(7)9-12(13)	23-27	Sh: linear- lanceolate to lanceolate, E: sub-rostrate, not to hardly capitate
N. fonticola	7-46	2.5-5.5	(8)9-14	24-33	Sh: always lanceolate, E: rostrate, sub-capitate, slightly capitate, but more so compared to *N. costei*
N. fossilis	30-85	3.5-5	7-9	18-21	Sh: lanceolate to linear, E: slightly capitate, S: often delicately dotted
N. frustulum var. *frustulum*	5-60	2-4.5	10-16	19-30	Sh: lanceolate, linear-lanceolate, linear, E: ~pointy rounded
N. frustulum var. *inconspicua*	3-22	2.5-3.5	8-13	23-32	Sh: linear-lanceolate, E: ~pointed rounded, sometimes slightly protracted
N. hantzschiana	8-50	3-5	7-13	20-26	Sh: lanceolate to linear, E: rather blunt, wedge-shaped, slightly capitate, P: visible
N. incognita	~20-70	2-3	10-15	28-30	Sh: narrow lanceolate, in the middle linear, E: pointy rounded to slightly capitate
N. radicula	33->70	2.5-3	10-13	28-30	Sh: narrow lanceolate, E: pointy rounded to slightly capitate, F: stubby

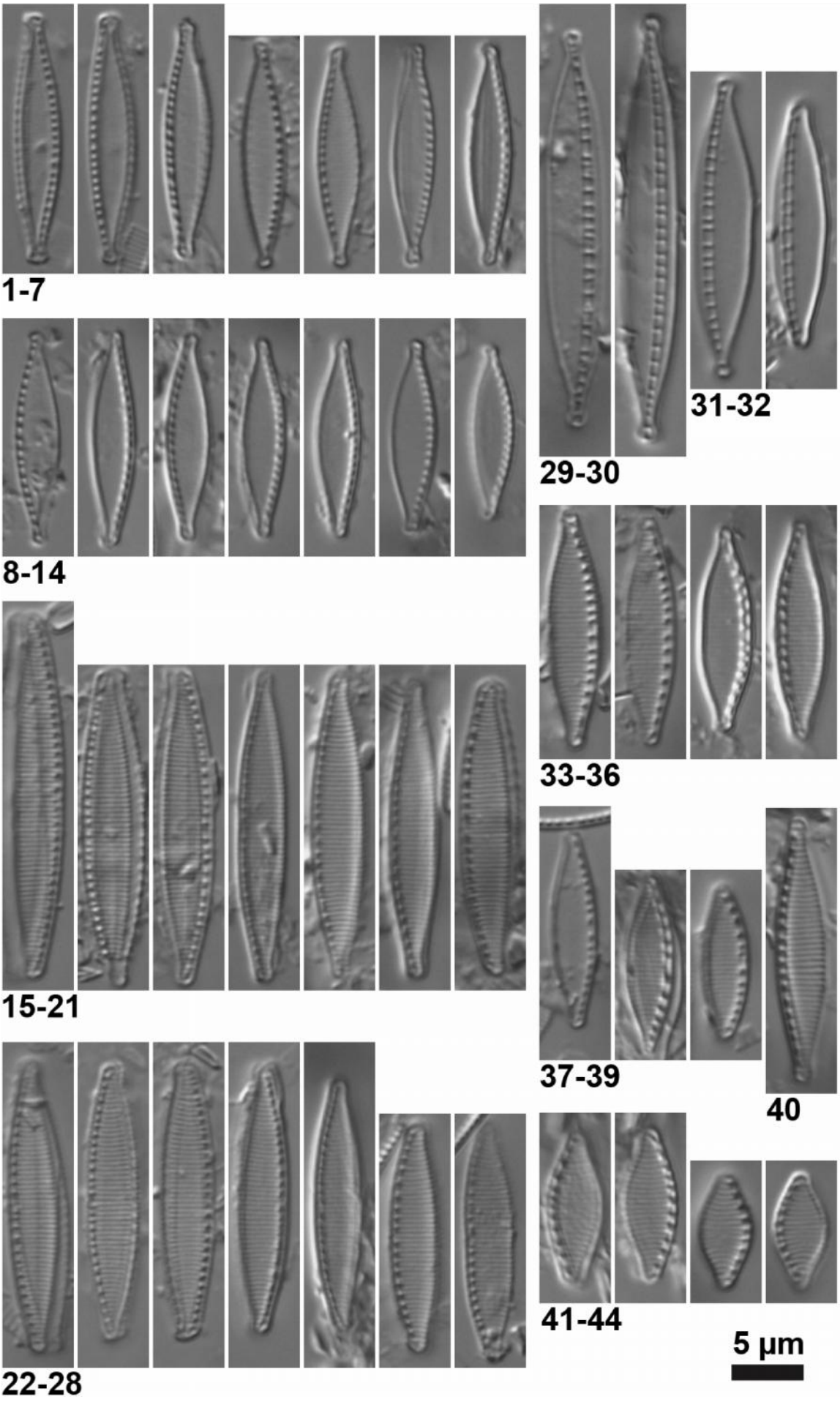
Tab. 27: *Nitzschia*-taxa **without a gap** between the two middle fibulae compared to the distance between the remaining fibulae (central node absent) that were named in the results for samples D 11 and D 1.1 and that were *N. lacuum* or more or less similar to *N. lacuum* and that may be confused with *N. lacuum*. Please note that next to the here listed *Nitzschia*-taxa, other taxa exist that can be confused with *N. lacuum*. Details from Hofmann et al. (2013) and Krammer & Lange-Bertalot (1986-2004). Sh = shape; E = ends; F: fibulae; P = points (areolae); S = striae.

Taxon	Length [µm]	Width [µm]	Fibulae /10 µm	Striae /10µm	Comments
N. alpinobacillum	14-24	3-4	9-11	25-27	Sh: lanceolate, E: capitate, P: not visible
N. angustatula	13-24	~4	16-20	16-20	Sh: lanceolate, linear-lanceolate, E: pointy to ~capitate, F: not visible
N. angustiforaminata	8-24	3-4	10-12	21-25	Sh: elliptical to linear, E: pointy, S: dotted
N. archibaldii	15-40	2-3	14-19	46-55	Sh: narrow lanceolate, E: pointy, sometimes slightly capitate
N. bryophila	15-26.5	4-5	(8)9-10(12)	30-32	Sh: lanceolate, linear-lanceolate, E: short rostrate to slightly capitate
N. dealpina	8-13	3-4	12-14	26-28	Sh: wide-lanceolate, E: shortly protracted, pointy, S: distinctly dotted
N. denticula	10-60	3-8	5-8	(13)14-18(20)	Sh: variable E: pointy, blunt F: transverse walls across entire valve width
N. desertorum	17-20	4-5	10-15	25-26	Sh: elliptical-lanceolate, E: short rostrate, pointy, S: distinctly dotted
N. lacuum	10-20	2-3	13-18	35-40	Sh: strictly lanceolate, E: capitate to pointy, S: not always visible
N. liebetruthii	14-32	2.8-3.2	12-14	23-25	Sh: lanceolate, linear-lanceolate, E: pointy rounded
N. palea-Aggregat	15-70	2.5-5	9-17	28-40	Sh: variable, E: wedge-shaped, pointy, never capitate, S: usually not visible
N. pusilla	8-33	2.5-5	14-20(24)	40-55	Sh: elliptical, usually linear-lanceolate, linear, E: blunt to wide
N. solita	18-50	4-6	11-16	24-28	Sh: wide to narrow lanceolate, E: rostrate pointed, slightly capitate, S: delicately dotted
N. supralitorea	10-25	2.5-4	10-14	25-34	Sh: lanceolate to linear-lanceolate, E: ~protracted, rarely slightly capitate

Plate 20 (page 158): *Nitzschia*-taxa from sample D 11 (Lake Krossinsee, Northern Germany) that were found during a screening of the sample for a total of 900 valves. All *Nitzschia*-taxa were photographed. Other taxa than the ones presented here were not found in sample D 11 during the screening. ***Nitzschia* cf. *fonticola*** (1-4) (shape and the relation of width to length differ distinctly from *N. fonticola*), ***N. fonticola*** (5-25), ***N. amphibia*** (26-30), ***N. dissipata* ssp. *dissipata*** (31-34), ***N. intermedia*** (35-36), ***N. gracilis*** (37) and ***N. paleacea*** (38-39).

Plate 21 (page 159): *Nitzschia*-taxa from sample D 1.1 (Lake Geneva, Switzerland) that were found during a screening of the sample for a total of 1000 valves. All *Nitzschia*-taxa were photographed. Other taxa than the ones presented here were not found in sample D 1.1 during the screening. ***N. lacuum*** (1-14), ***N.* cf. *fonticola*** (15-28) (shape differs, rather similar to *N. costei*), ***N.* cf. *oligotraphenta*** (29) (valve too short with 21 µm instead of 30-45 µm length), ***N. oligotraphenta*** (30), ***N.* cf. *dissipata*** (31) (*N. dissipata* does not have capitate ends, all other traits agree with the description of *N. dissipata*), ***N. dissipata*** (32), ***N. fonticola*** (33-39), ***N. alpinobacillum*** (40), ***Nitzschia* spec.** (41-44) (these valves are 6.3-11.0 µm long, 3.7-4.0 µm wide, with 12-14 fibulae/10 µm and 30 striae/10 µm).


1-4
5-8
9-17
18-25
26-30
31-34
35-36
37
38-39
5 µm


1-7
8-14
15-21
22-28
29-30
31-32
33-36
37-39
40
41-44
5 µm

3.3 Effects of counting result variances on the ecological assessment with Phylib

The German implementation of the EU-Water Framework Directive assesses the water quality using benthic diatoms with the Diatom-Index (DI) for lakes and with the Diatom Indicted Ecological Status$_{\text{running waters}}$ (DIÖZ$_{\text{Fließgewässer}}$) for running waters (Schaumburg et al. 2006–2012). The results signify one of five possible ecological status classes (ES) with one representing the "very good ecological status" or "high ecological status" (ES 1), two the "good" (ES 2), three the "moderate" (ES 3), four the "poor" (ES 4) and five the "bad" ecological status (ES 5) (REFCOND 2003).

The indices were calculated from the ungrouped (compare Chapter 3.1) relative abundance data of the diatom species submitted by the participants and auditors using the Phylib-Software 4.1. Prior to analysis, the data were manipulated according to the Phylib-Software. For example, redundant entries had to be pooled to one entry, e.g. several "*Pennales"* entries in one sample of one participant were summarized to one "*Pennales*" entry. None of these data changes affected the outcome of the indices or water quality evaluation.

The diatom-indices are assumed to be unreliable in both lakes and running waters, if the sum of diatom objects that could not be determined (sp., spp.) and/or could not unambiguously be determined (cf.) exceeds 5 %, if the sum of diatom objects that represent planktonic taxa exceed 5 % (counting error as they should be excluded from the count) or if the sum of diatom objects that represent aerophilic taxa exceeds 5 % (Schaumburg et al. 2006, 2007, 2011a, 2012). Planktonic or aerophilic taxa abundances never exceeded 5 % during this calibration exercise and are consequently not further discussed or presented here.

3.3.1 The lakes

The Diatom-Index (DI) indicates the ecological status for the two lake samples **Lake Krossinsee (German Lake-Type D 11)** and **Lake Geneva (German Lake Type D 1.1)**. The DI is calculated as the average of the module „Trophic-Index“ (TI) and the module „Quotient of Reference Species“ (RAQ). In addition to above criteria of reliability (Schaumburg et al. 2011a), the DI is assumed to be reliable

- if the TI is based on at least 60 % of the counted objects (only valid for Lake-Type D 11),
- if the TI is based on at least 11 taxa (only valid for Lake-Type D 1.1) and
- if the RAQ is based on at least 10 taxa (Lake-Type D 11) or 12 taxa (Lake-Type D 1.1).

Lake Krossinsee, sample D 11

The results of 18 of the 37 participants received no comments from the Phylib-Software. Thus, these 18 results generated a "reliable assessment". In contrast, the assessment was deemed unreliable for the following 19 participants and three auditors.

- Three laboratories (laboratory codes 26, 29, 36) and one auditor (39), because the sum of indicative taxa was less then 60 % **(<60 %)**.
- Three laboratories (10, 15, 21), because the number of RAQ-taxa was less then 10 (**<10 RAQ**).
- Two laboratories (18 and 34) and one auditor (42), because the sum of diatom objects that could not be determined (sp., spp.) and/or could not unambiguously be determined (cf., aff.) exceeded 5 % (**>5 % cfs**).
- Seven laboratories (2, 12, 13, 16, 24, 30, 31), because <60 % and >5 % cfs.
- Two laboratories (22 and 25) and one auditor (41), because <60 %, >5 % cfs and <10 RAQs.
- Two laboratories (19 and 28), because <10 RAQs and >5 % cfs.

Overall 13 laboratories and two auditors had too many spec/cfs according to the Phylib-Software, as the participants indicated 0-12 % cfs (average 2 %, n = 37) and had 0-15 % specs (average 2 %, n = 37). The auditors had 0-6 % cfs and 0-12 % specs (n = 3).

Seven participants and one auditor had an insufficient number of RAQ-species for a reliable assessment. Of these, less then 410 objects were counted instead of the required 500 objects (laboratories 10, 15, 22 and 28) and/or the participant did not search for rare taxa after the count as was mandatory (laboratories 10, 15, 19, 21, 22, 25 and 28). Thus, not following the mandatory instructions by not counting a sufficient number of objects or by not searching for rare taxa may lead to an unreliable assessment. In contrast to these seven participants with the RAQ-error message, the auditor followed the German instruction-protocol (Schaumburg et al. 2011a).

The module „Quotient of Reference Species“ (RAQ) ranged from 0.20-0.25 (average 0.22) for the auditors (n = 3) and from 0 to 0.25 (0.12) for the participants (n = 37) with a standard deviation (stdev.) of 0.03 and 0.07, respectively (Fig. 87). The module „Trophic-Index“ (TI) ranged from 0.36 to 0.44 (0.40) for the auditors and from 0.20 to 0.47 (0.35) for the participants with a stdev. of 0.04 and 0.06, respectively (Fig. 87). Thus, the TI assessed the water quality with a better ecological status then the RAQ. The average of the RAQ and

TI modules, i.e. the Diatom-Index (DI), ranged from 0.29 to 0.35 (0.31) for the auditors and from 0.12 to 0.34 (0.23) for the participants with a standard deviation of 0.03 and 0.05, respectively (Fig. 87). Thus, Lake Krossinsee (sample D 11) is assessed as having a poor water quality (ecological status 4), except by one auditor and one participant, who assess a moderate water quality (ecological status 3) (Fig. 87).

Overall, the differing counting results of sample D 11 did effect the water quality assessment. The taxonomy of six laboratories (10, 15, 19, 20, 23 and 28) differed the most from the results of the auditors and had to be marked as "unsuccessful" on the certificates for sample D 11 (red circles in the DCA of Chapter 3.1.1). These six laboratories and laboratories with a taxonomy that differed significantly from two of the three auditors (7, 12, 21, 22, 30, 31, 35, 36, 37; green circles in DCA of Chapter 3.1.1) also deviated the most in the water quality assessment from the auditors. For example, the greatest difference of the participants-DI to the average DI of the auditors was 0.13-0.20 (n = 6) and belonged either to a laboratory that was outside of all three (red circle in DCA) or of two auditor (green circle in DCA) confidence intervals when comparing the taxonomic differences (Fig. 87 and Chapter 3.1.1). The DI of the remaining laboratories differed 0.01 to 0.12 from the average DI of the auditors (n = 31). Interestingly, the DI of laboratory 35 (green circle) was the most similar to the average DI of the auditors (Fig. 87), as it differed only 0.01 units. Thus, not all taxonomic differences affect the DI and some taxonomic differences have a greater impact on the water quality assessment than others, as not all taxa have indicator values (see below and Tab. 28). The assessment of the ecological status (3 or 4) was hardly affected, which is surprising, as the average DI of the auditors (0.31) was very close to the ecological status classes limits between three and four (0.33) (Fig. 87).

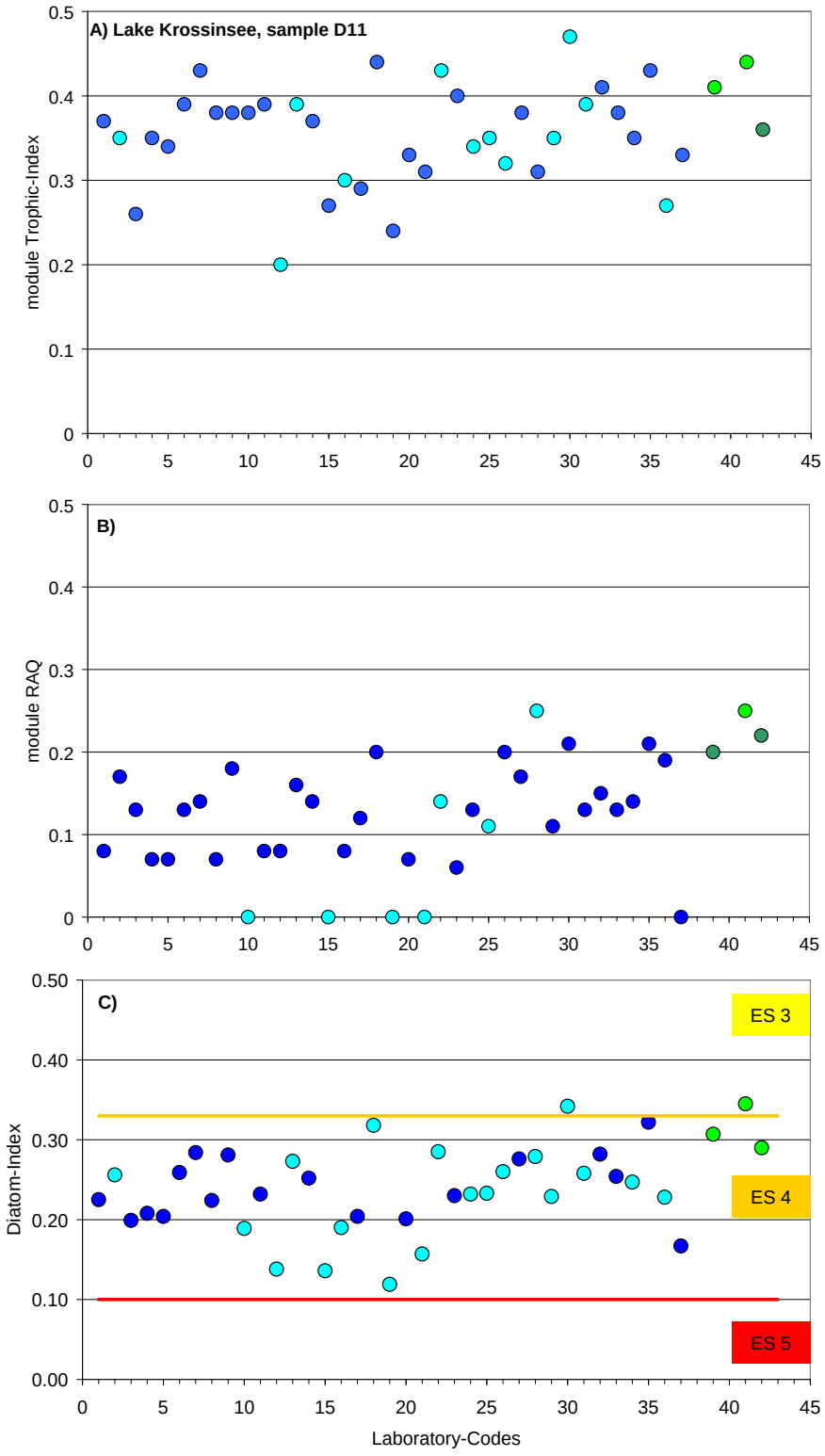


Fig. 87: A) Module Trophic-Index, B) module Quotient of Reference Species (RAQ) and C) Diatom-Index calculated from the identified diatom assemblages from the participants (blue circles) and auditors (green circles) in **Lake Krossinsee (sample D 11)**. Coloured horizontal lines = boundary between moderate (ES 3), poor (ES 4) and bad ecological status (ES 5) for Lake-Type D 11. Unreliable estimates, i.e. A) <60 %, B) <10 RAQ and C) additionally >5 % cfs = light blue and light green circles; reliable estimates dark blue and dark green circles (for more information see text).

Lake Geneva, sample D 1.1

The results of 16 of the 37 participants and two of the three auditors received no comments from the Phylib-Software. Thus, these 18 results generated a "reliable assessment". In contrast, the assessment was deemed unreliable for the following 21 participants and one auditor.

- Four laboratories (6, 11, 21, 26), because the number of RAQ-taxa was less then 12 (**<12 RAQ**).
- Seven laboratories (2, 7, 13, 16, 25, 31, 34) and one auditor (42), because the sum of diatom objects that could not be determined (sp., spp.) and/or could not unambiguously be determined (cf., aff.) exceeded 5 % (**>5 % cfs**).
- Two laboratories (23, 37), because <12 RAQ and >5 % cfs.
- Two laboratories (15, 36), because <12 RAQ and because the number of taxa with a trophic indicator value was less then 11 **(**<**11 Tr**).
- Six laboratories (1, 18, 19, 22, 24, 28), because <12 RAQ, >5 % cfs and <11 Tr.

Overall 15 laboratories and one auditor had too many spec/cfs according to the Phylib-Software, as the participants indicated 0-50 % ambiguous taxa (cfs, specs or pennates) (average 12 %, n = 37). The auditors had 0-25 % ambiguous taxa (n = 3). Here, a taxon similar to *Gomphonema pumilum* was responsible for the high relative abundances of *Gomphonema* spec. in most of these counts (see Chapter 3.2.10 for details). See Chapter 3.1.2 for an overview of other additionally problematic taxa.

The module „Quotient of Reference Species“ (RAQ) ranged from 0.17-0.29 (average 0.23) for the auditors (n = 3) and from 0.06 to 0.50 (0.25) for the participants (n = 37) with a standard deviation (stdev.) of 0.06 and 0.10, respectively (Fig. 88). The module „Trophic-Index“ (TI) ranged from 0.15 to 0.19 (0.17) for the auditors and from 0.13 to 0.36 (0.21) for the participants with a stdev. of 0.02 and 0.05, respectively (Fig. 88). Thus, differing taxonomy affected the RAQ assessment more strongly than the TI in sample D 1.1 (Fig. 88). The average of the RAQ and TI modules, i.e. the Diatom-Index (DI), ranged from 0.16 to 0.24 (0.20) for the auditors and from 0.11 to 0.41 (0.23) for the participants with a stdev. of 0.04 and 0.06, respectively (Fig. 88). Thus, Lake Geneva (sample D 1.1) is assessed as having a poor water quality (ecological status 4) by all three auditors and 32 participants and as a

moderate water quality (ecological status 3) by five participants (laboratories 3, 13, 26, 28, 36) (Fig. 88).

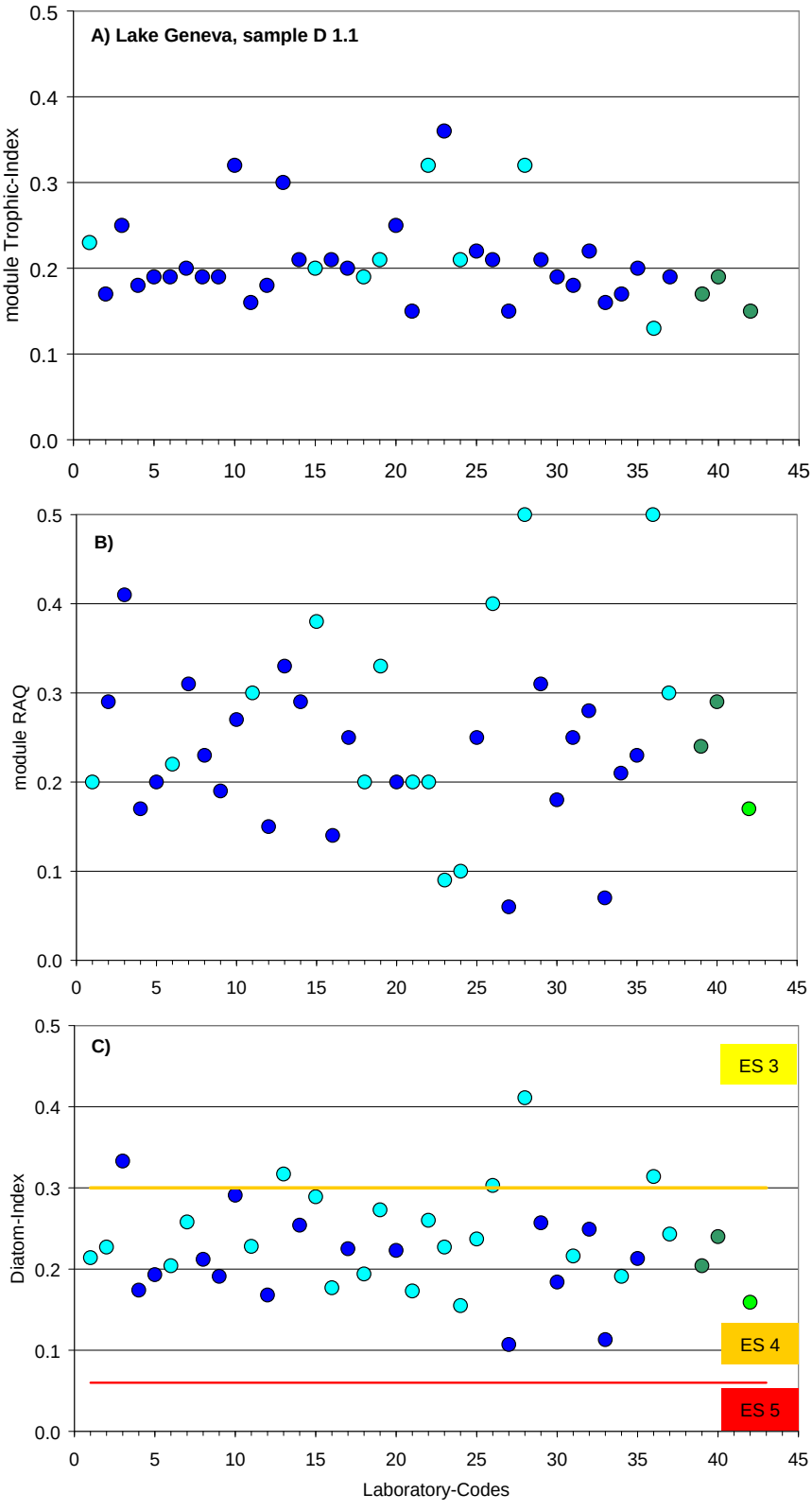


Fig. 88: A) Module Trophic-Index, B) module Quotient of Reference Species (RAQ) and C) Diatom-Index calculated from the identified diatom assemblages from the participants (blue circles) and auditors (green circles) in **Lake Geneva (sample D 1.1)**. Coloured horizontal lines = boundary between moderate (ES 3), poor (ES 4) and bad ecological status (ES 5) for Lake-Type D 1.1. Unreliable estimates, i.e. A) <11 Tr, B) <12 RAQ and C) additionally >5 % cfs = light blue and light green circles; reliable estimates dark blue and dark green circles (for more information see text).

The Bray-Curtis distance of the participants to the auditors was only weakly correlated with the difference of the participants DI to the auditors DI ($R^2 = 0.08$). Again, not all taxa have indicator values. Also, all ambiguous taxa or taxa that were identified only to the genus level, do not contribute to the ecological assessment, independent of the name used. Another reason for the weak correlation is that the postulated taxonomic resolution is not (yet) reflected in the resolution of the indicator values in the Phylib-Software. For example, *Gomphonema pumilum, G. pumilum* var. *pumilum, G. pumilum* var. *rigidum, G. pumilum* var. *elegans* and *G. elegantissimum* all share identical indicator values (see below, Tab. 28 and Chapter 7). Overall, the assessment of the ecological status (3 or 4) was only mildly affected by the different taxonomy, as the DI of the auditors were close to the middle of the ecological status class 4 (Fig. 88).

3.3.2 The rivers

The Diatom Indicted Ecological Status$_{\text{running waters}}$ (**DIÖZ**) indicates the ecological status for the two river samples **River Klepelshagener Bach** (German Running Water Type **D 12**) and **River Drau** (German Running Water Type **D 2**).The DIÖZ is derived from the Diatom Index$_{\text{Fließgewässer}}$ (**DI$_{\text{FG}}$** or Diatom Index$_{\text{running waters}}$), which is calculated as the average of the module „Trophic-Index“ (**TI**) and the module „Species Composition and Abundance“ (**SCA**).

River Klepelshagener Bach, sample D 12

Only the results of three laboratories (18, 28 and 31) were unreliable according to both Schaumburg et al. (2012) and the Phylib-Software, as the sum of diatom objects that could not be determined (sp., spp.) and/or could not unambiguously be determined (cf., aff.) exceeded 5 %.

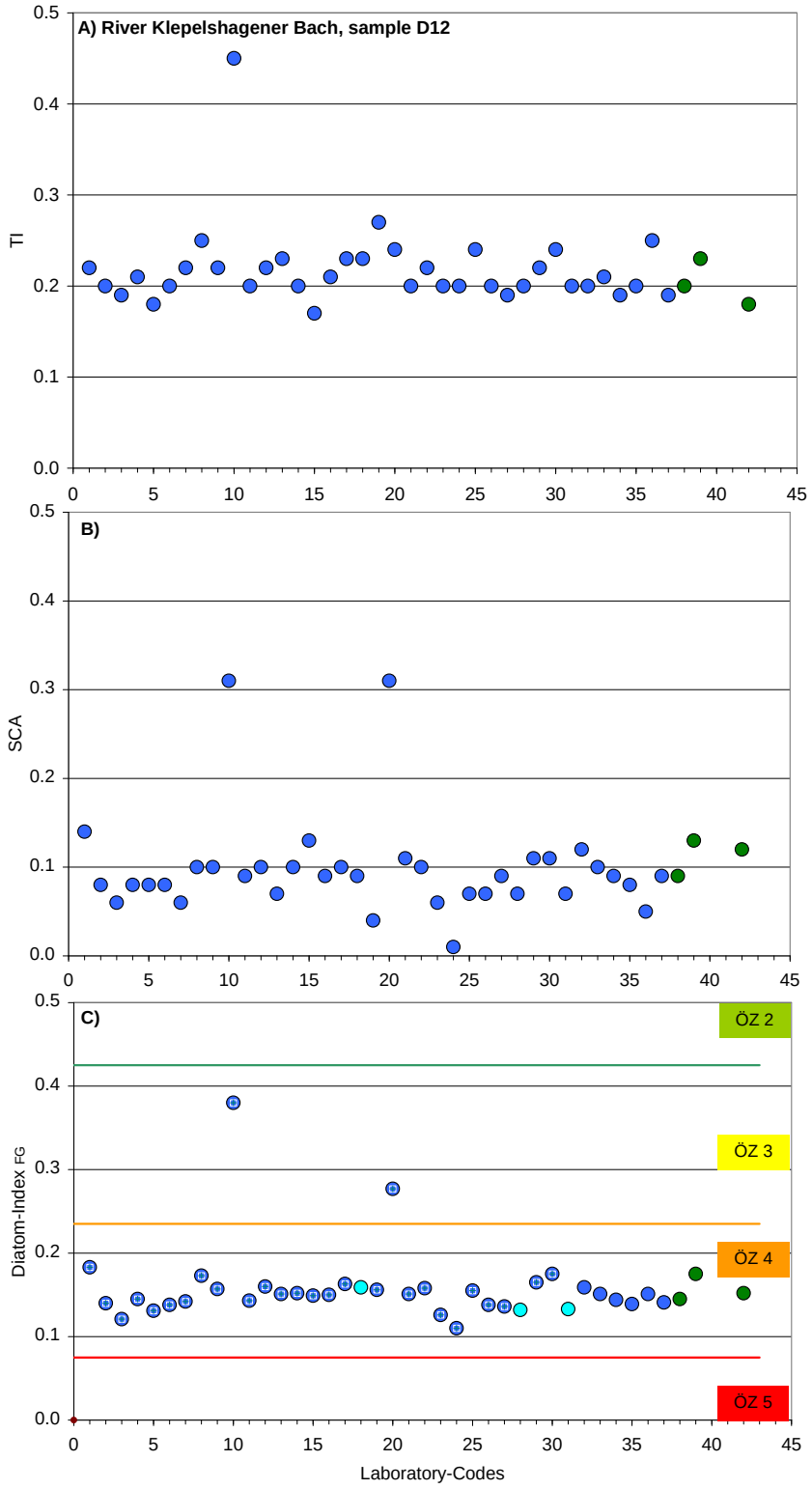


Fig. 89: A) Module „Trophic-Index“ (TI), B) module „Species Composition and Abundance“ (SCA) and C) Diatom-Index$_{\text{running waters}}$ (Diatom-Index$_{\text{FG}}$) calculated from the identified diatom assemblages from the participants (blue circles) and auditors (green circles) in **River Klepelshagener Bach (sample D 12)**. Coloured horizontal lines = boundary between high (ES 1), good (ES 2), moderate (ES 3) and poor ecological status (ES 4) for River-Type D 12. Unreliable estimates (>5 % cfs) = light blue circles; reliable estimates dark blue and dark green circles (for more information see text).

The Trophic-Index (**TI**) derived from the results of the participants ranged from 0.17-0.45 (average 0.22; standard deviation (stdev.) 0.04) and from the results of the auditors from 0.18-0.23 (average 0.20; stdev 0.03) (Fig. 89). The „Species Composition and Abundance“ (**SCA)** of the participants ranged from 0.01-0.31 (average 0.10; stdev. 0.06) and of the auditors from 0.09-0.13 (average 0.11; stdev. 0.02) (Fig. 89). The average of TI and SCA, i.e. the $\mathbf{DI_{FG}}$ of the participants ranged from 0.11-0.38 (average 0.16; stdev. 0.05) and from 0.15-0.18 (average 0.16; stdev. 0.02) for the auditors, which translates into a poor **DIÖZ** (ES 4) for all three auditors and all participants, except laboratories 10 and 20, which determined a moderate DIÖZ (ES 3) (Fig. 89).

The two laboratories (10 and 20) with a different ecological assessment of sample D 12 were also outside the confidence intervals of all three auditors with respect to the Bray-Curtis distances (see Chapter 3.1.3). For example, laboratory 10 determined an assemblage that was dominated by *Achnanthes lanceolata*–Sippen with 13 % (auditors: 27-44 % *Planothidium lanceolatum*), 13 % *Sellaphora pupula* and 8 % *Sellaphora stroemii* (auditors: 1-4 % *Sellaphora joubaudii*, 5-7 % *Sellaphora seminulum*). The taxon *Achnanthes lanceolata*–Sippen has no indicator values, while *Planothidium lanceolatum* has a TI of 3.3 (weight 3.0). The TI of *S. stroemii* identified by laboratory 10 is 1.2 (weight 2.0), while *S. joubaudii* and *S. seminulum* (auditor species) have a TI of 3.6 (weight 5.0) and 3.2 (weight 2.0), respectively (Tab. 28). Therefore, the different taxonomy distinctly affected the ecological assessment.

The main difference between the results of laboratory 20 and the auditors that affected the assessment was *Achnanthidium minutissimum* var. *minutissimum* (reference species and low trophic index with 1.2; Tab. 28). The auditors determined this species with 3-5 %, while laboratory 20 identified it with 22 %. Consequently, TI, SCA and the water quality assessment was higher and better, respectively, with the results from laboratory 20 compared to the auditors. Here, different taxonomy only indirectly affected the assessment, as the relevant species really occurred in the sample, but with distinctly different abundances.

River Drau, sample D 2

The results of five laboratories (2, 12, 18, 21, 22) and one auditor (42) were unreliable according to both Schaumburg et al. (2012) and the Phylib-Software, as the sum of diatom objects that could not be determined (sp., spp.) and/or could not unambiguously be determined (cf., aff.) exceeded 5 %. The remaining 34 counting results were deemed reliable.

The Trophic-Index (**TI**) derived from the results of the participants ranged from 0.50-0.70 (average 0.62; standard deviation (stdev.) 0.05) and from the results of the auditors from 0.65-0.72 (average 0.68; stdev 0.04) (Fig. 90). The **SCA** of the participants ranged from 0.19-0.89 (average 0.59; stdev. 0.18) and of the auditors from 0.71-0.75 (average 0.73; stdev. 0.02) (Fig. 90). The average of TI and SCA, i.e. the **DI_{FG}** of the participants ranged from 0.34-0.79 (average 0.61; stdev. 0.11) and from 0.69-0.72 (average 0.70; stdev. 0.02) for the auditors, which translates into a high **DIÖZ** (ES 1) for laboratory 25, a good DIÖZ (ES2) for all three auditors and 26 laboratories and into a moderate DIÖZ (ES3) for 10 laboratories (5,7,17,19, 21, 23, 28, 31, 35, 37) (Fig. 90). Thus, the different taxonomy affected the ecological assessment of the River Drau, especially the module „Species Composition and Abundance“.

For the River Drau, the three auditors had fairly similar results (see Chapter 3.1.4 for more details) and consequently a very consistent assessment of the water quality (low auditor standard deviations for TI, SCA and DI_{FG} and three times ES2). In contrast, 27 % (10 laboratories) of the participants obtained a "reliable" water quality assessment that differed from the results of the auditors (either ES1 or ES3) (Fig. 90). The differences are mainly based on deviating SCA-values, i.e. the percentage of general reference species and type specific reference species, both of which contributed a total of 71-75 % to the auditor assemblages (which corresponds to a SCA of 0.71-0.75). Here, the reference species were mainly *Achnanthidium minutissimum* var. *minutissimum*, *Achnanthidium pyrenaicum*, *Diatoma ehrenbergii* and *Diatoma mesodon* that together contributed 61-69 % to the auditor assemblages (see Chapter 3.1.4). The samples of five of the participant results (laboratories 19, 28, 31, 35, 37) that assessed another ecological status compared to the auditors also had Bray-Curtis-distances outside the confidence- interval of two or all three auditors (Tab. 8, red- or green-rimmed samples in Fig. 3 in Chapter 3.1.4). Similarly, the result of laboratory 21 assessed an ES3, but was deemed unreliable, and was also outside the confidence-intervals of all three auditors. Thus, in these six cases the different taxonomy affected the ecological assessment. E.g. laboratory 28 identified *Achnanthidium minutissimum* (DV-no. 26060) with 50 %, which is not considered to have any indicator values in the Phylib-Software (which makes sense, as this is a lumping group). In the accompanying letter with the slides it was explicitly pointed out to the participants that in the DV-list 2011 the nominate variety is NOT characterised by omitting the variety. Thus, we have to assume that the participant was not sure, which variety was present in the sample. Also, laboratory 28 did not find any *Achnanthidium pyrenaicum*. Consequently, the different taxonomy affected the ecological water assessment.

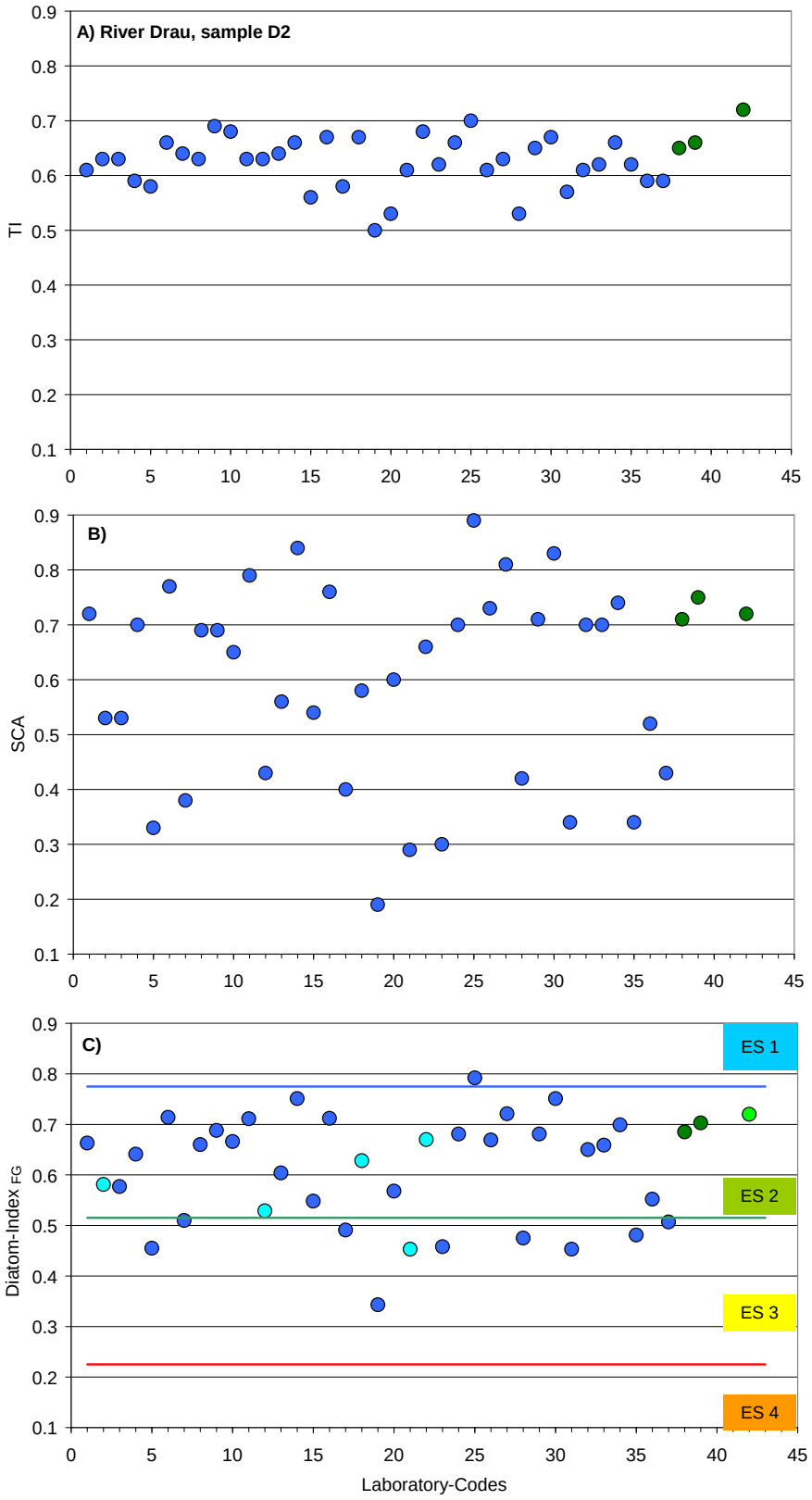


Fig. 90: A) Module „Trophic-Index“ (TI), B) module „Species Composition and Abundance“ (SCA) and C) Diatom-Index$_{\text{running waters}}$ (Diatom-Index$_{\text{FG}}$) calculated from the identified diatom assemblages from the participants (blue circles) and auditors (green circles) in **River Drau (sample D 2)**. Coloured

horizontal lines = boundary between high (ES 1), good (ES 2), moderate (ES 3) and poor ecological status (ES 4) for River-Type D 2. Unreliable estimates (>5 % cfs) = light blue and light green circles; reliable estimates dark blue and dark green circles (for more information see text).

Tab. 28: Indicator values of problematic taxa as identified by this intercalibration exercise according to the newest Phylib-Software (version 4.1, 02.10.2012). Chap = see given Chapter; saprobic (S) and trophic (T) value with corresponding weighting (g), RA = is the species a general reference species or a type specific reference species for running water type D2 or D12? Given is either "no" (-), "yes" (for both types D2 and D12) or "12" (yes, just for type D12). Gray columns = running water indices, white columns = lake indices. Trophic-index (Ti) North (N) or South (S), A = type specific reference species, C = types specific degradation indicator for lake types D1.1 and 11.

Chap.	DV-no.	Taxon	S	g	T	g	RA	TiN	TiS	gS	A	C
3.2.1	26005	Achnanthidium pyrenaicum	1.4	3	1.3	1	yes	-	-	-	-	-
	26006	Achnanthidium subatomus	-	-	-	-	yes	-	-	-	-	-
	36012	Achnanthidium minutissimum	-	-	-	-	-	-	-	-	-	-
	26060	Achnanthidium minutissimum var. minutissimum	1.7	1	1.2	1	yes	-	-	-	11	-
	26063	Achnanthidium minutissimum var. jackii	-	-	1.2	3	-	-	-	-	-	-
	26024	Achnanthidium eutrophilum	-	-	-	-	-	-	-	-	-	-
	26088	Achnanthidium straubianum	-	-	-	-	-	0.0	-	-	11	-
	6271	Achnanthes petersenii	1.0	5	0.6	1	yes	0.66	2.0	2	yes	-
	6272	Achnanthes pusilla	1.0	5	0.6	3	yes	0.75	1.5	3	yes	-
3.2.2	6171	Amphora inariensis	1.2	4	2.1	1	yes	0.98	2.5	1	-	-
	-	Amphora minutissima	*Not comprised in PHYLIB.*									
	36245	Amphora indistincta	-	-	-	-	-	-	-	-	-	-
	6983	Amphora pediculus	2.1	2	2.8	2	yes	2.89	-	-	-	-
	-	Amphora subatomus	*Not comprised in PHYLIB.*									
3.2.3	36025	Cocconeis placentula	1.8	2	2.6	2	yes	3.45	-	-	-	-
	6726	Cocconeis placentula var. euglypta	-	-	2.3	2	yes	-	-	-	-	-
	6728	Cocconeis placentula var. lineata	-	-	2.3	2	yes	2.93	-	-	-	-
	6021	Cocconeis placentula var. placentula	1.8	2	2.6	2	yes	3.45	-	-	-	-
	16179	Cocconeis placentula var. tenuistriata	-	-	-	-	-	-	-	-	-	-
3.2.4	26301	Encyonema lange-bertalotii	-	-	-	-	-	-	-	-	-	-
	26318	Encyonema ventricosum	-	-	-	-	12	-	-	-	-	-
	16992	Encyonema reichardtii	1.5	4	2.7	3	12	3.97	4.4	3	-	yes
	26208	Encyonema minutum	1.6	2	2.0	1	yes	0.7	2.0	2	1.1	-
	36062	Encyonema silesiacum	-	-	-	-	12	-	-	-	-	-
3.2.5	26207	Encyonopsis microcephala	1.2	4	1.2	1	yes	1.02	-	-	11	-
	16619	Encyonopsis minuta	1.2	4	1.2	1	yes	1.02	-	-	11	-
	26329	Encyonopsis subminuta	1.2	4	1.2	1	yes	1.02	-	-	11	-
	-	Encyonopsis tavirana	*Not comprised in PHYLIB.*									
	26326	Encyonopsis krammeri	1.2	4	1.2	1	yes	1.02	-	-	11	-
	26321	Encyonopsis alpina	1.2	4	1.2	1	yes	1.02	-	-	11	-
3.3	36214	Sellaphora pupula	-	-	-	-	-	-	-	-	-	-
	16614	Sellaphora pupula var. pupula	2.4	2	3.7	5	-	3.01	-	-	-	-
	26633	Sellaphora stroemii	1.0	5	1.2	2	yes	0.72	1.8	2	yes	-
3.2.6	26568	Eolimna minima	-	-	2.9	2	-	-	-	-	-	-
	26624	Sellaphora seminulum	3.2	2	3.2	2	-	5.7	-	-	-	yes
	36265	Sellaphora joubaudii	1.8	3	3.6	5	-	3.04	4.0	2	-	-
	6556	Navicula utermoehlii	1.4	4	1.8	2	yes	2.43	4.0	1	-	1.1
	16587	Naviculadicta raederae	-	-	-	-	-	-	-	-	-	-
	16589	Naviculadicta schaumburgii	-	-	-	-	-	-	-	-	11	-

Tab. 28 continued, II of III												
Chap.	**DV-no.**	**Taxon**	**S**	**g**	**T**	**g**	**RA**	**TiN**	**TiS**	**gS**	**A**	**C**
3.2.7	36079	Fragilaria brevistriata	1.3	4	3.0	1	12	2.81	-	-	-	-
	6388	Fragilaria brevistriata var. brevistriata	1.3	4	3.0	1	12	2.81	-	-	-	-
	6034	Fragilaria construens f. construens	1.4	3	2.3	2	12	-	-	-	-	-
	6828	Fragilaria construens f. venter	-	-	2.3	2	12	-	-	-	-	-
	6400	Fragilaria elliptica	-	-	-	-	-	-	-	-	-	-
	6403	Fragilaria lapponica	-	-	-	-	-	2.5	-	-	11	-
	16593	Fragilaria leptostauron	-	-	-	-	-	-	-	-	-	-
	6774	Fragilaria leptostauron var. dubia	-	-	-	-	12	4.18	-	-	-	-
	6076	Fragilaria leptostauron var. leptostauron	-	-	2.0	1	12	-	-	-	-	-
	16669	Fragilaria martyi	-	-	-	-	-	3.98	-	-	-	-
	6237	Fragilaria parasitica var. parasitica	2.2	3	2.3	3	-	3.28	4.0	2	-	-
	36086	Fragilaria pinnata	1.4	3	2.2	1	12	2.57	-	-	-	-
	6078	Fragilaria pinnata var. pinnata	1.4	3	2.2	1	12	2.57	-	-	-	-
	6407	Fragilaria pseudoconstruens	-	-	-	-	yes	-	-	-	-	-
	16790	Fragilaria construens f. subsalina	-	-	-	-	-	-	-	-	-	-
3.2.8	36274	Fragilaria amphicephaloides	1.0	5	0.9	2	yes	0.51	1.6	2	yes	-
	26372	Fragilaria austriaca	1.0	5	0.5	4	yes	0.98	2.5	1	yes	-
	16570	Fragilaria capucina	-	-	-	-	-	-	-	-	-	-
	6033	Fragilaria capucina var. capucina	-	-	1.8	2	12	3.79	4.5	3	-	1.1
	6186	Fragilaria capucina var. vaucheriae	2.5	2	1.8	1	-	5.33	5.0	3	-	yes
	16996	Fragilaria vaucheriae	2.5	2	1.8	1	-	5.33	5.0	3	-	yes
	6399	Fragilaria delicatissima	1.0	5	1.4	2	yes	0.9	2.0	2	yes	-
	36082	Fragilaria famelica	-	-	-	-	-	-	-	-	-	-
	16995	Fragilaria gracilis	1.3	4	1.1	2	yes	-	-	-	-	-
	36266	Fragilaria pararumpens	-	-	-	-	-	-	-	-	-	-
	26385	Fragilaria pectinalis	-	-	-	-	-	-	-	-	-	-
	26374	Fragilaria perminuta	1.5	3	2.1	4	-	-	4.2	2	-	1.1
	36259	F. radians	-	-	2.0	2	12	0.38	-	-	11	-
	36260	Fragilaria recapitellata	-	-	-	-	-	-	-	-	-	-
	26375	Fragilaria rumpens	1.6	3	1.0	2	yes	4.12	-	-	-	-
3.2.9	26422	Gomphonema olivaceolacuum	1.9	4	1.9	3	-	4.23	4.5	3	-	1.1
	36093	Gomphonema olivaceum	-	-	-	-	-	-	-	-	-	-
	16254	Gomphonema olivaceum var. balticum	-	-	-	-	-	-	-	-	-	-
	16255	Gomphonema olivaceum var. calcareum	-	-	1.8	3	-	-	-	-	-	-
	6432	Gomphonema olivaceum var. olivaceolacuum	1.9	4	1.9	3	-	4.23	4.5	3	-	1.1
	6867	Gomphonema olivaceum var. olivaceum	2.1	4	2.9	1	12	4.3	4.1	2	-	yes
	36275	Gomphonema olivaceoides	1.5	3	1.5	2	yes	0.98	2.5	1	yes	-
	-	Gomphonema calcareum	*Not comprised in PHYLIB.*									
	-	Gomphonema balticum	*Not comprised in PHYLIB.*									

Tab. 28 continued, III of III												
Chap.	**DV-no.**	**Taxon**	**S**	**g**	**T**	**g**	**RA**	**TiN**	**TiS**	**gS**	**A**	**C**
3.2.10	26404	Gomphonema angustivalva	-	-	-	-	-	-	-	-	-	-
	36095	Gomphonema pumilum	1.6	3	1.1	1	yes	2.75	4.3	2	-	1.1
	6437	Gomphonema pumilum var. pumilum	1.6	3	1.1	1	yes	2.75	4.3	2	-	1.1
	26430	Gomphonema pumilum var. rigidum	1.6	3	1.1	1	yes	2.75	4.3	2	-	1.1
	26429	Gomphonema pumilum var. elegans	1.6	3	1.1	1	yes	2.75	4.3	2	-	1.1
	36276	Gomphonema elegantissimum	1.6	3	1.1	1	yes	2.75	4.3	2	-	1.1
	16661	Gomphonema lacus-vulcani	-	-	-	-	yes	-	-	-	-	-
	26420	Gomphonema micropumilum	-	-	-	-	-	-	-	-	-	-
	16559	Gomphonema minusculum	-	-	-	-	-	-	-	-	-	-
	6912	Gomphonema minutum	2.0	5	2.2	1	-	4.23	4.5	3	-	yes
	6911	Gomphonema pseudotenellum	-	-	-	-	-	0.66	2.0	2	-	-
3.2.11	26469	Mayamaea atomus var. atomus	3.4	2	2.8	3	-	4.74	-	-	-	yes
	26472	Mayamaea atomus var. permitis	3.4	2	3.1	4	-	5.74	-	-	-	yes
	26470	Mayamaea atomus var. alcimonica	-	-	-	-	-	-	-	-	-	-
	26463	Mayamaea agrestis	-	-	-	-	-	-	-	-	-	-
3.2.12	6889	Navicula cryptotenella	1.5	2	2.3	1	yes	-	-	-	-	-
	16307	Navicula cryptotenelloides	-	-	-	-	-	1.37	-	-	-	11
	16653	Navicula antonii	2.2	2	2.1	2	-	3.04	4	2	-	yes
	36125	Navicula menisculus	-	-	-	-	-	-	-	-	-	-
	16897	Navicula upsaliensis	-	-	2.9	2	-	4	-	-	-	11
3.2.13	6511	Navicula lundii	-	-	-	-	yes	-	-	-	-	-
	6890	Navicla veneta	3.3	2	3.5	5	-	-	-	-	-	yes
	6917	Navicula exilis	1.1	4	2.0	1	yes	0.66	2.0	2	-	-
	36114	Navicula cryptocephala	2.5	2	3.5	4	-	3.0	4.9	3	-	11
3.2.14	6221	Navicula reichardtiana var. reichardtiana	2.1	4	2.3	1	-	3.51	4.3	2	-	1.1
	16596	Navicula caterva	-	-	-	-	-	-	-	-	-	5, 7
	16584	Navicula moskalii	-	-	-	-	-	-	-	-	-	-
	26612	Navicula associata	-	-	2.3	1	-	-	4.3	2	-	-
3.2.15	36154	Nitzschia fonticola	2.1	4	-	-	-	3.72	4.5	3	-	yes
	6025	Nitzschia fonticola var. fonticola	2.1	4	-	-	-	3.72	4.5	3	-	yes
	36155	Nitzschia frustulum	-	-	-	-	-	-	-	-	-	-
	6597	Nitzschia lacuum	1.2	4	1.2	1	yes	1.27	-	-	-	-

3.3.3 Comparison of results with 2007 and 2012 taxonomy

For all four samples three auditors and 13 participants also submitted their results according to the old taxonomy, i.e. mainly based on Krammer & Lange-Bertalot (1986-2004) according to the older German instruction protocol (Schaumburg et al. 2006, 2007) instead of Hofmann et al. (2011 or 2013) as is mandatory according to the new instruction protocol (Schaumburg et al. 2011a, 2012).

The results of the German Phylib-indices reference species, trophic state and diatom index, on which the ecological assessment is based on, are not always identical between the old (2007) and new (2011) taxonomy from the *same* participant or auditor (Fig. 91). For example, one auditor identified 7 % *Amphora indistincta* and 1% *Amphora pediculus* according to the new literature (see also Chapter 3.2.2) and 9 % *Amphora pediculus* according to the old literature in sample D 11. *Amphora indistincta* was not yet described in the older identification literature. *Amphora indistincta* has no indicator values yet, while *Amphora pediculus* contributed with a TI of 2.89 (Tab. 28) to the ecological assessment. Thus, the training set of the German Phylib indices needs to be recounted based on the new taxonomy to fully use the potential that diatoms have as bioindicators and to increase the precision of the tool. Relatively newly described species need to be included with indicator values, especially the ones that are fairly common, such as *Amphora indistincta*, *Amphora minutissima* or *Navicula lacuum*.

Also, this result emphasises the need of using cf., if a taxon does not fully meet the species description (see Chapter 5). A counting result with *Amphora pediculus* cannot be allocated to *Amphora indistincta* in hindsight, if a cf. is not used and the taxon is not documented with a picture and description. The information is lost, the assessment biased towards the ecology of *Amphora pediculus* (see also Hofmann et al. 2011a, p. 97).

Another example of distinctly differing water quality assessment according to the new and old taxonomy (Fig. 91) is due to *Achnanthidium eutrophilum*. Laboratory 3 identified 29 % *Achnanthidium minutissimum* var. *minutissimum* (indicating a relatively good water quality, see Tab. 28) and 12 % *Achnanthidium eutrophilum* (no indicator values) according to the new taxonomy and 42 % *Achnanthes minutissima*-Sippen (no indicator values) according to the old taxonomy in sample D 2. Similar to *Amphora indistincta*, *Achnanthidium eutrophilum* is fairly common (see e.g. page 80 in Hofmann et al. 2011). This taxon used to be part of the *Achnanthes minutissima*-aggregate but was not further differentiated according to the old literature. It was labelled *Achnanthes minutissima* var. "Sippe mit rhombisch-lanzettlichen Schalen" (taxon with rhombic–lanceolate valves) (Plate 32, figures 57-61, page 312 in Krammer & Lange-Bertalot 2004). Thus, the new taxonomy differentiates the diatom taxa in more detail, potentially increasing the precision of the water quality assessment, as *A. eutrophilum* indicates eutrophic to polytrophic waters (Hofmann et al. 2011). However, *A. eutrophilum* has no indicator values yet. Again, this suggests that a recount of the training set according to the new taxonomy is essential to fully use the potential that diatoms have as bioindicators.

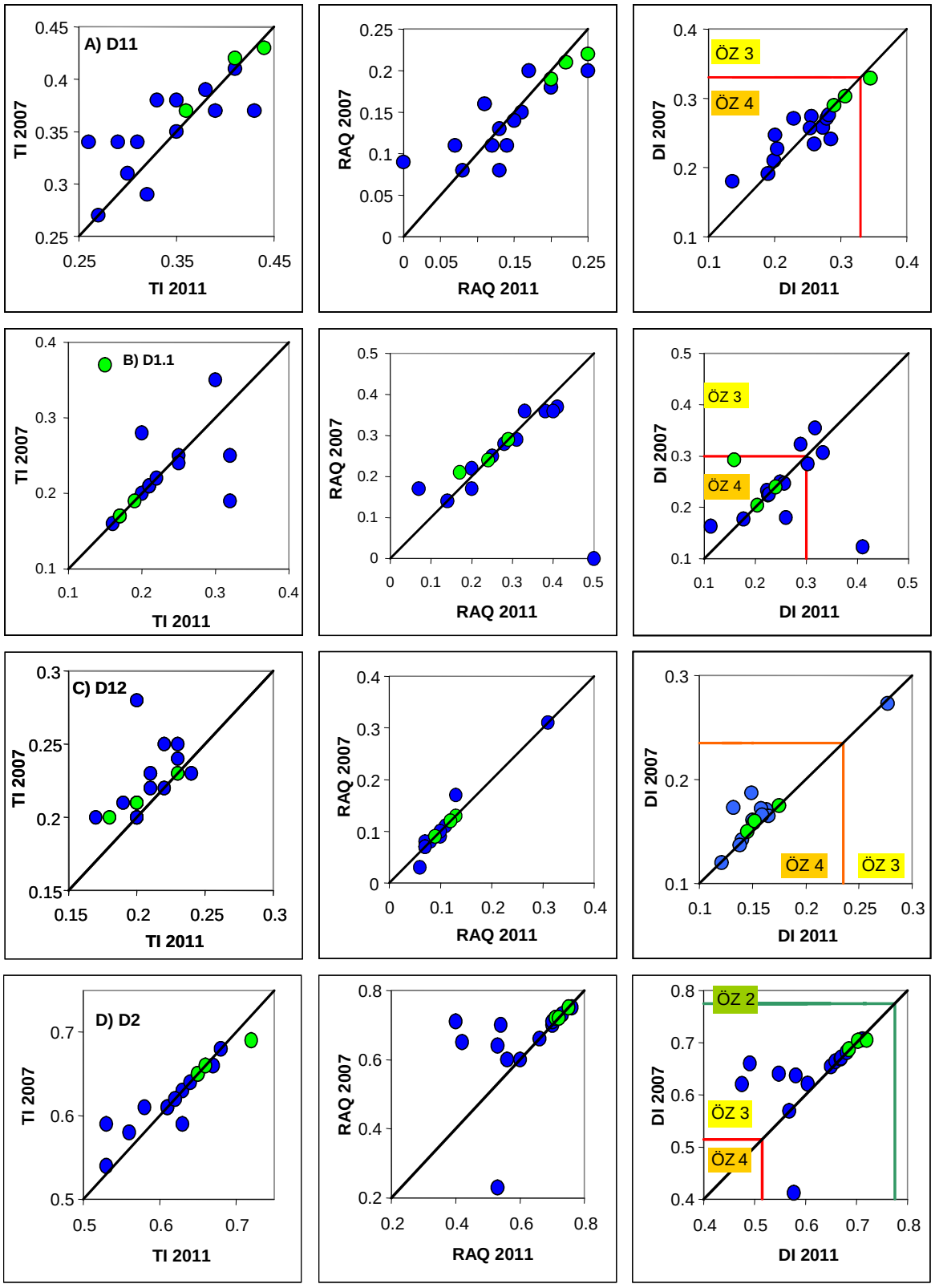


Fig. 91: Phylib-indices according to the old (2007) versus new (2011) taxonomy from samples A) Lake Krossinsee (sample D 11), B) Lake Geneva (sample D 1.1), C) River Klepelshagener Bach (sample D 12) and D) River Drau (sample D 2). TI = Trophic-Index for both lakes and rivers (A-D), RAQ = Quotient of Reference Species for lakes (A-B) and Species Composition and Abundance for

rivers (C-D), DI = Diatom-Index (A-D), ÖZ = ecological status class (ökologische Zustandsklasse); Solid bold line: 1:1 line. Circles on the 1:1 line indicate identical results with both taxonomies. Blue circles = participants, green circles = auditors. For more information see text.

4. Quality assurances of diatom counts in Europe

Today, there is no common EU standard about the Quality Assurance/Quality Control (QA/QC) of diatom counts in the different EU countries, even if there are now European Standards under way, which hopefully will harmonize efforts and should be tested in the future. Instead, every country has different attempts to ensure QA/QC of diatom counts used for biomonitoring. These attempts are quite different (Tab. 29) despite the fact that it has been shown that only QA/QC including a harmonization of diatom identification among countries enables the comparison of diatom data across countries (Kelly et al. 2012; Kahlert et al. 2009). In fact, studies using data from different countries are usually forced to pool species counts into genera to ensure a comparison of counts of the same taxa (for example Vyverman et al. 2007).

The following compilation of diatom count quality control in Europe is as complete as possible, all countries had the possibility to answer an enquiry, and 15 countries did. The compilation is probably not complete, as many experts noticed that diatom work in each country is going on at different levels, and different authorities are responsible for different monitoring, or research, and it is not an easy task to get an overview. Furthermore, using diatoms for monitoring is in many countries an expanding task; so many answers from 2012 might be outdated when this report is published. Still, this is the first overview about this question and gives the possibility to compare the efforts of the different countries.

Diatom counts are done at many different levels, from local authorities to regional and national monitoring in each country, with additional counts for investigate applied research projects by water authorities as well as co-operations with research institutions, and last but not least pure research projects done at universities. The number of counts per country and year differs greatly, from “several thousand” in the UK, Germany and Spain to approximately a hundred samples per year in Estonia. However, most countries report at least several hundred samples per year for routine monitoring. The number of samples counted for various research projects varies a lot between years. In 14 out of the 15 countries answering the

questionnaire, diatom counts according to the EU standards (EN 14407 and EN 13946) are included in the monitoring programs, only in the Czech Republic all periphyton is assessed, and in this case diatoms are not counted but assessed according to a semi-quantitative scale. However, also in the Czech Republic diatoms are counted according to the EU standards in three international monitoring projects.

There are great differences between countries on who is counting the diatom samples. Usually, it is a mixture of people working at a central authority such as the national water authority, regional water authorities, plus consultants and researchers including sometimes graduate students. Some countries have the ambition to keep the counts central at one national authority, as for example Ireland, whereas other countries as for example Spain only have consultants including researchers doing this work.

All countries have the ambition to ensure a quality control of the diatom counts, but the methods are again very different. In most countries, there is a central water authority officially responsible for the quality assurance (QA) of diatom counts. In some countries, the responsible authority requires the use of accredited laboratories for diatom counts in monitoring programs, but the content of these official diatom accreditations is very shifting: Rather seldom the identification and counting of the diatoms is actually part of the QA, instead, laboratories are expected to participate in ring-tests, harmonization exercises or intercalibrations, if available. The requirement for the diatom accreditation in The Netherlands is rather an exception naming the number of ring-tests to be done (once per year). Often, only the sampling, laboratory handling, and sometimes the calculation of diatom indices are included in an accreditation, which leaves the largest source of variation beside. Usually, not all laboratories in a country are accredited via lab QA schemes, and it is up to the authority in question if they want to use an accredited laboratory, and if they require additional tasks. For example, because the official accreditation procedure in Sweden does not include the QA of diatom identification and counting, many water authorities now require the proof that a laboratory passed the Nordic-Baltic Network for Benthic Algae in Freshwater (NORBAF) intercalibration tests. There is a consensus among the authorities and diatomists that the current accreditation schemes are not adapted to biological analyses, and need to be developed to be a useful tool to ensure the quality control of diatom counts. All countries are in some stage of development of these QA, and there will probably be more formal rules in the future.

Most countries and also most diatomists have understood the importance to harmonize their diatom counts to ensure high quality, and also understood the importance of practical

harmonization efforts. However, these efforts are very different again, ranging from a very formal test like in Italy where even a time restriction is used, to tests including a mixture of more informal meetings coupled to formal certificates with a threshold to have passed, to the UK ring-test which has the official focus on reflective learning instead of being an examination. Small countries with few experts usually recommend the participation in neighbouring countries' ring-tests, but many countries have their own national ring-tests. Examples for ring-tests including several countries are the ring-tests of the UK, Germany and The Netherlands, which are officially nationally, but include people from neighbouring countries (Ireland uses to participate in the UK test, Wallonia in the test of The Netherlands). Another example is the NORBAF intercalibration, where experts from Sweden, Finland and the Baltic countries use to participate.

The ring-tests are often organized by a water authority, but also by private consultants and universities. Most ring-tests have few participants, usually around five. The UK ring-test has though many more (70), and also the German (37), the Italian (32) and the NORBAF test (20) have more participants. In many countries, tests are done on annual basis, NORBAF and the Czech test biannually. The UK test is continuously sending one slide approximately every second month. Some countries report that they have done one test so far, or that tests are done infrequently. Usually, tests are organized by a national water authority, however, the UK test is organized by a consultant, and the NORBAF test by a co-operation of a university and a consultant.

Also the practical process is different among the ring-tests. The number of samples to be counted for one test ranges from one to five. In most cases, the samples are sent out either as oxidized material and the participants must prepare the slide themselves, or as readymade slides. In two cases the preparation of a raw sample was part of the intercalibration, and in only one of these cases also the sampling was part of the test. Then, the practical part of the counting also follows very different protocols. Only in Italy the calculation of diatom indices is part of the test. Sometimes very strict protocols even with time restriction for different parts are to be followed like in the case of Italy. In most cases though, experts have a more or less unlimited time to count the sample according to their normal laboratory procedures in their own laboratory, and send the results to a central place for evaluation. In some countries, samples are not counted, but only the taxonomical problems of each sample are notified and discussed in a meeting. Workshops discussing the test results are however not part of the exercise in the UK and Italy.

In some way the test must ensure the QA of the counting, again this is done in different ways in the exercises. Only in the tests of NORBAF, Germany and Hungary, a similarity threshold with the auditor(s) must be met to pass the test. Usually, there are more informal rules about when a diatomist is counted as “passed” by comparison with an expert. For example, in the UK test, analysts need to have passed a series of tests to be "accredited". The target in these tests is to achieve a value that lies not far from the mean value of all participants’ results. However, this German ring-test suggests that the mean value of all participants may not necessarily reflect the correct taxonomy. For example, only 17 of the 37 participating laboratories identified *Achnanthidium pyrenaicum* and *Achnanthidium minutissimum* var. *minutissimum* in abundances that are in good agreement with the abundances of the auditors in sample D 2 (see Chapter 3.2.1). If the target is missed by a defined error value, participants get a “warning” or in severe cases a recommendation for “action”. However, warning/action implicates that the laboratory in question is recommended to handle the problem in some way, but it is up to the single laboratory on how (Kelly 2013). Also in other exercises, focus is rather on reflective learning and helping the participants to understand mistakes and improve their counting and identification. In these cases certificates are handed out only occasionally, which implies more a confirmation about the participation, not about having passed a threshold. There was information that the rules about how an analyst counts as “accredited” are not or not well formulated, and only unsatisfactorily communicated. Not all countries replied on what exactly is required to achieve an accredited status for a diatom expert.

Of course, the choice of the auditor or expert (or groups of experts) is very important in such an exercise. Diatom taxonomy is not straight forward, taxonomy is re-evaluated continuously, and there are many problematic groups where an agreement must be made to achieve harmonized results. Opinions of different experts about these questions will probably vary widely, and it is a sensitive question. At the same time, most experts are at the same time counting samples for different projects themselves, and often receive funding from water authorities, which means that they need to evaluate themselves as well. UK found the solution to choose the most experienced experts in UK and take turns on the responsibility for different slides, and additionally have several experts counting one slide to get the natural-, and expert-variability in a slide. The Nordic countries in NORBAF, having much less analysts, chose to agree on the two auditors for the intercalibration. In other countries, the expert is picked by the responsible authority in question. Other exercises were done mostly as a common discussion, with no explicit expert.

In summary, QA of diatom identification and counting still is in its infancy, and is not at all harmonized among EU countries. The risk is that countries with low formal requirements for a diatom analyst will employ the cheapest one, with the large risk to get the most unqualified one, which in turn will lead to bad quality data. A second large problem is that a country which gets data from different analysts, i.e. almost all countries, might get much unharmonized data which are impossible to compare in a national dataset, and this is certainly already the case when trying to compare data from different EU countries. It is necessary to have agreed EU standards on diatom identification and counting QA, which are followed preferably by all laboratories, both public and private ones, but at least in all cases where samples are counted for routine monitoring and investigative research projects.

Tab. 29: Different attempts to ensure Quality Assurance/Quality Control (QA/QC) of diatom counts used for bio-monitoring in various EU countries. The data are based on two enquiries from 2012/2014. Note that many answers might be outdated when this report is published. NORBAF = Nordic-Baltic Network for Benthic Algae in Freshwater. The numbers in the first column correspond to the following data or questions:

1 Country

2 What diatom samples are being counted - national monitoring programs (NM) and/or regional monitoring programs (RM)?

3 How many samples are being counted per year approximately in national monitoring programs (NM) and regional monitoring programs (RM)?

4 Who is actually doing the diatom analyses - authorities (A), Consultants (C), Researchers (R) or a mixture and which mixture?

5 Are there any ring tests or similar to ensure diatom identification quality?

6 If yes (question 5), how often?

7 Who is organizing ring-tests or workshops: authorities (A), Consultants (C), Researchers (R) or a mixture and which mixture?

8 How many diatomists attend the ring-tests or workshops?

9 Are diatom samples to be counted?

10 Is diatom sampling a part of the ring-test or workshop?

11 Is sample oxidation a part of the ring-test or workshop?

12 Is preparation of permanent slides a part of the ring-test or workshop?

13 Number of samples?

14 Is there a workshop?

15 Are there certificates?

16 How is the workshop followed up (i.e. is there any written outcome)?

17 How much is the participation?

18 Is there a quality protocol, i.e. a demand that a diatom expert must meet to be a confirmed expert?

19 Are there auditors in your ring-tests?

Tab. 29

1	UK	Germany	Netherlands	Flanders	Wallonia	SE (NORBAF)	Hungary	Estonia	FIN (NORBAF)	Czech R.	France	Italy	Portugal	Ireland	Spain	Austria
2	NM, RM	NM, RM	NM, RM	NM, RM	NM	NM, RM	NM, RM	NM	NM, RM	NM	NM	NM, RM	RM	NM, RM	NM, RM	NM
3	>1000	>>>1000	900	380	150	~200	~500	100	450	>360	~2000	>800	~400 *15	250	>>>1000	>200
4	A, C	A, C, R	A, C, R	A	A, C	A, C, R	A, R	R	C, R	A, R	A, C, R	A, R	A, C, R	A	C, R	C
5	yes	yes	yes	no *1	yes	yes	yes	no *2	yes *14	no	yes *3	yes *4	no	*5	no	yes
6	annually *10	every 2nd year	annually *13		annually	every 2nd year	every 4th year		every 2nd year		annually	occasionally		annually		every 3rd year
7	C	A, R	A	A *1	A	C, R	A		C, R		A, R	A				A
8	50-60	30-40	10			15-20	7		15-20		15	32				5-6
9	yes	yes	yes		yes	yes	yes		yes		yes	yes				yes
10	no	no	no			no	no		no		yes	no				no
11	no	no	no			no	yes		no		yes	no				no
12	no	no	yes			yes	yes		yes		yes	No				no
13	5	2-4	1			3-5	2		3-5		1 to 3	1				5-7
14	no *11	yes	no *6		yes	yes	yes		yes		no	yes				no
15	no	yes	no			yes	yes		yes		no	yes				no
16	report	report *7	report			report *7	report		report *7		report	report				*16
17	£300	300 euro	550 euro			300 euro *8	100 euro		300 euro *8			free				
18	yes*12	yes	yes	no	yes	yes	yes	no	yes	no	yes	yes		no	no	yes
19	yes *9	yes	no	no		yes	yes		yes	no	yes					yes

Explanatory notes (Tab. 29):

*1 Flanders Government is planning to organise a ring-test in future. Some analysts from Flanders join ring-tests in other (neighbouring) countries

*2 we try to participate in international ring-tests

*3 annual ring-tests for regional authorities. In a near future, those ring-tests will also concern consultants

*4 in 2011 there has been a national intercalibration process between agencies through ISS (Superior Institute of Sanity) ISPRA circuit, about the counting and application of the protocol ICMi (Intercalibration Common Metric Index)

*5 some analysts from Ireland join ring-tests in other countries (Great Britain, Germany and Sweden)

*6 planned for the future

*7 sometimes scientific articles

*8 exclusive the workshop

*9 a pool of six “experts” and nine “mentors”

*10 five slides per year spread over the year, roughly every two months

*11 but there is a parallel series of training workshops which often dovetail with the ring-tests

*12 the ring-test is part of a broader accreditation scheme for Agency staff

*13 changed to biannually (2014)

*14 taking part in NORBAF test

*15 this preview was based on the number of available monitoring sites defined during the implementation of the WFD by the national water Institute

*16 internal information, not published

5. How to deal with taxonomic ambiguities (cf., aff., spec.)

One main problem affecting the quality of diatom counting results that became apparent in this intercalibration exercise is the question about how to deal with taxonomic ambiguities, i.e. with taxa that do not fit the species description to one hundred percent, such as the small *Amphora*-species (see Chapter 3.2.2), the varieties of *Cocconeis placentula* (Chapter 3.2.3) or various *Navicula*-taxa (see Chapters 3.2.12 and 3.2.14).

There are three options to signify taxonomic ambiguities. If the taxon can only be determined to a genus level, „spec.“ is used for "species indeterminata". If the taxon looks similar to a described species (and is probably closely related to it), but differs to this species in a way that suggests that the named taxon is probably another (unknown) species, „aff.“ is used for "species affinis". Finally, „cf.“ (confer; compare) is used, if the taxon differs slightly from the given species in some traits, but is probably identical to the species in question. Omitting one of these three addendums for taxa with differing traits suggests or pretends an unambiguous species identification and consequently hampers or hinders a comparison of such diatom counting results among diatomists.

Usually, the addendums „spec.“, „aff.“ and „cf.“ in diatom counting results are no problem and rather document a careful approach during identification. However, the German method (Schaumburg et al. 2011, 2012) deems diatom counting results unreliable in both lakes and running waters, if the sum of diatom objects that could not be determined (sp., spp.) and/or could not unambiguously be determined (cf.) exceeds 5 %. As a consequence, some diatomists (according to several participants of this intercalibration exercise) commonly lump ambiguous taxa with the most likely taxa for the ecological assessment when using the German method to avoid using "spec.", "cf." or "aff.", but do not sufficiently and comprehensible document these diatom taxa. This approach allows a seemingly reliable ecological assessment of the sample. Ultimately, this issue is also a psychological problem. A contractor may increase (or think to increase) the chances of future assignments, if he or she always delivers "good, clean and reliable results" and who seemingly identifies all or almost all diatoms in a sample with certainty. Thus, theoretically there is the danger that a low number of ambiguous taxa becomes an allegedly quality attribute, especially for the German method.

Generally, ambiguous diatom identifications may be due to various reasons. Often, the causes are not a lack of knowledge or lack of experience. Instead, ambiguous identifications usually stem from ambiguous species descriptions, greater morphological ranges of diatom

taxa than given in the species description (see e.g. Chapters 3.2.2; 3.2.3 and 3.2.12) or the occurrence of species that have not yet been scientifically described (see Chapter 3.2.10) as demonstrated by the results of this intercalibration exercise. We highly recommend using „spec.“, „aff.“ or „cf.“ in case of ambiguous diatoms to clearly identify which taxa occurred in a sample. Additionally, it is necessary to briefly describe the ambiguity and to take pictures of the corresponding diatom valves. Only then a comparability of diatom counting results and also later corrections/amendments can be ensured. For example, *Navicula lacuum* has only been described in 2009, although it is a common species in some areas (Hofmann et al. 2011). Some diatomists called this species *Navicula* cf. *seibigiana*, others *Navicula* cf. *wiesneri.* Others lumped this taxon e.g. with *Navicula seibigiana* prior to 2009 to avoid the use of *cf.* Only in the first two cases, a re-evaluation of the samples is possible, if the species has been documented. In the latter case, the information is lost, the ecological assessment remains distorted, water quality changes (or stability) over time remains obscured and the comparison among results from different diatomists is hampered or hindered.

In some cases, it may makes sense to lump ambiguous taxa with unambiguous taxa (or manipulate the data in some other way), when using the German method for implementing the EU-Water Framework Directive (Schaumburg et al. 2011, 2012) to avoid an unreliable assessment. However, this should only be done for calculating the ecological status of a water body with the German method and only if the lumping is detailed, reasoned and comprehensibly explained.

Overall, we strongly recommend to abolish the above mentioned 5 %-limit and so improve the German method by avoiding meaningless or counterproductive measures for avoiding unreliable assessments. Instead, it would make a lot more sense to use other measures for quality control for diatom results that really reflect the reliability of a water quality assessment result. For example, the reliability could be met, if a minimum number or relative abundance of indicative taxa is present in a sample (Werner & Dreßler 2007). This suggestion (a minimum percentage of indicative taxa) has now been implemented for the German assessments of lakes (Schaumburg et al. 2011) as an additional criterion of quality control. However, the 5 %-limit of ambiguous diatoms has not been abolished. Consequently, many sample deliver an "unreliable" assessment result despite, for example, a high number and relative abundance of indicative taxa that would make an assessment result very reliable.

6. Measurement of striae-density

One important measure for differentiating diatom-taxa is the striae-density (see e.g. Chapters 3.2.5 and 3.2.14). During the workshop we recognised that it is not always clear where exactly and how to measure striae density. Similarly, the results confirm the affects and problems on taxa identification, when measuring striae density differently. For example, striae-density helps to distinguish *Navicula reichardtiana* and *N. caterva*. Here, different means to measure striae-density may lead to a different decision, whether or not the object is *N. reichardtiana* or *N. caterva* (Chapter 3.2.14). Partly, this problem stems from the sparse description in the identification literature about how to measure striae density (however, see e.g. Krammer 1997, page 22, in German) and also partly from contradictory statements by different authors (Krammer 1997). Thus, we contacted Dr. Kurt Krammer, Prof. Dr. Dr. h.c. Horst Lange-Bertalot and Erwin Reichardt for detailed instructions. Still, one has to keep in mind that not necessarily all taxonomists who described a taxon have followed exactly this method. Thus, a certain source of error remains.

The striae-density should be measured close to the axial area, beginning adjacent to the central node or –if present- central area (Krammer 1997 page 22 and pers. comm.; Lange-Bertalot, pers. comm. 2012, Reichardt, pers. comm. 2012, Reichardt 1984) (Fig. 92). If an axial area is missing (e.g. Eunotia or Nitzschia) striae density should be measured close to the median area (Lange-Bertalot, pers. comm. 2012) (Fig. 92). Stop counting striae, if they begin to get denser, i.e. measure in the area where Str-density is (relatively) constant (Reichardt, pers. comm. 2012, see also comment about striae measurements for *Gomphonema rhombicum* in Reichardt 2007). Thus, the given striae-density for the "middle portion (dorsal)", e.g. for *Cymbopleura* (Krammer 2003), does not really refer to measurements in the middle of the valve but to striae counts as shown in Fig. 92 on an *Encyonema*-valve (Lange-Bertalot, pers. comm. 2012).

Ideally, the scale should be 10 µm long. For small taxa a shorter scale must be used (Krammer and Lange-Bertalot, pers. comm. 2012) and a quarter or half a striae must then be taken into account (Lange-Bertalot, pers. comm. 2012). However, there does not seem to be a consensus, if the scale should be as long as possible or better consistently 5 µm or 2 µm long, even though this might affect the striae-density count (see Chapter 3.2.14). Thus, even with these detailed instructions, some uncertainties and errors remain.

Usually it is sufficient to note that striae are getting more dense towards the ends for distinguishing taxa, i.e. striae-density at the ends is hardly measured when using light microscopy (Reichardt, pers. comm. 2012). If the striae-density differs between the dorsal and ventral side of the valve, both densities should be noted (Krammer, pers. comm. 2012), if this trait is given as an important criterion for species differentiation, as e.g. for many *Amphora*-taxa (Levkov 2009).

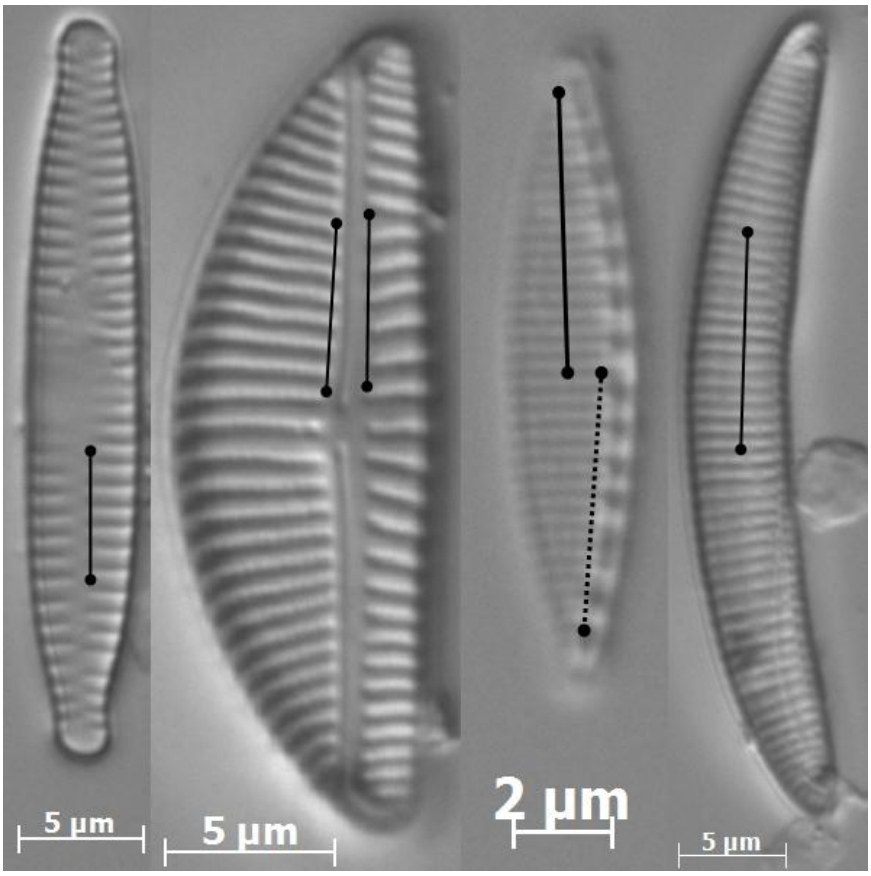


Fig. 92: Where to measure striae-density (solid line) or fibulae-density (dotted line) according to Lange-Bertalot (pers. comm. 2012). From left to right: *Fragilaria capucina*, *Encyonema silesiacum*, *Nitzschia fonticola*, *Eunotia bilunaris*. Pictures taken from various samples (not part of the intercalibration exercise).

7. Recommendations

Recommendations for applied diatomists

Overall, this intercalibration exercise revealed that it is essential for the applied diatomist to carefully consider all traits of a species when identifying diatoms. This measure alone would prevent some taxonomic problems occurring in this intercalibration exercise. Similarly, it is necessary to really use the postulated identification literature to obtain comparable diatom counting results among diatomists. For example, the *Achnanthes minutissima*-aggregate based on Krammer & Lange-Bertalot (1986-2004) comprises far more taxa compared to the *Achnanthidium minutissima*-aggregate based on Hofmann et al. (2011 or 2013). Consequently, both denominations do not equate each other.

When diatoms occur that are difficult to identify or only ambiguously identifiable, we recommend to concisely explaining the naming, to document the respective valves with pictures (next to the general photographic documentation of the most abundant taxa) and to detail the used literature for each case. For example, it makes a considerable difference, if Hofmann et al. (2011 or 2013) is exclusively used when identifying taxa of the genus *Encyonopsis* or if Krammer (1997) is additionally used, because Krammer (1997) incorporates distinctly more species of the genus *Encyonopsis* compared to Hofmann et al. (2011 or 2013).

Additionally, we generally recommend labelling ambiguously identified diatoms with “spec.”, “aff.” or “cf.” in the counting results to allow a later comparison of diatom counting results among different diatomists.

To ensure a permanently high quality standard for diatom counts and consequently the comparability of diatom counting results, we recommend to regularly participate in intercalibration exercises or taxonomic workshops. This recommendation is particularly addressed to the applied diatomist, who is implementing the European Water Framework Directive or working on other bio-monitoring projects. Alternatively, there is the possibility that the authorities that assign diatom contracts conduct quality control via a third party.

Finally, we recommend ignoring any details about the ecology, distribution and occurrence of species in the identification literature for the diatom identification, except maybe on rare occasions. Often these details have not been sufficiently verified (see e.g. Chapter 3.2.2) and the applied diatomist uses the taxonomy to infer water quality. Thus, they need to be carful about a circular argument.

Recommendations for the German Method

Abolish the 5% limit of ambiguous taxa (spec., cf. and aff.) that now indicate an unreliable water quality assessment in the German method (Schaumburg et al. 2011, 2012; current Phylib-Software). Instead, use other (useful) criteria to indicate the reliability of water quality assessment results, such as the amount of indicative taxa in a sample (see Chapters 3.3 and 5), as is already implemented for lakes (Schaumburg et al. 2011). However, the 5 %-limit is still and simultaneously in place (Schaumburg et al. 2011). Ideally, the percentage of diatom objects or number of diatom taxa that have indicator values are given in the Export-file of the Phylib-Software. This would enable the limnologist or water manager to put more emphasis on water quality assessments that are based on a higher percentage of counted objects (e.g. more weight on results from 90% indicative taxa than from 60% indicative taxa). Consequently, this would help interpreting diatom counting results and their changes over time or space, i.e. when comparing diatom data with other indicators or when comparing diatom results among each other.

The number of indicative taxa will greatly improve (increase and be more precise!)(see e.g. Chapter 3.3) once the **training set** is **recounted** based on the new taxonomy, i.e. Hofmann et al. (2011 or 2013) and supplementary identification books as specified in Schaumburg et al. (2011 and 2012) rather then mainly on Krammer & Lange-Bertalot (1986-2004). Benthic diatoms are well established, robust bio-indicators (e.g. Smol & Stoermer 2010). We can make an even stronger case, i.e. reduce error and uncertainties, if we fully use the potential at hand by recounting the training set.

We recommend **including the following species to the list of planktonic pennates** (e.g. Table 15, page 49, in Schaumburg et al. 2012):

- *Diatoma tenuis* Agardh
- *Nitzschia draveillensis* Coste & Ricard
- *Nitzschia graciliformis* Lange-Bertalot & Simonsen.

These three taxa are considered to be planktonic according to the mandatory main identification literature (Hofmann et al. 2011, pages 172, 463 and 456, respectively) and other experienced diatomists. For example, *Diatoma tenuis* and *Nitzschia graciliformis* contribute to the assessment tool for planktonic diatom remains in the profundal by Dr. I. Schönfelder ("Bewertungsmodul für planktische Diatomeen-Reste im Profundal" = DI-PROF; Mischke & Nixdorf 2008, page 38). Alternatively, a clear statement that these taxa are to be included despite the fact that they are planktonic, because they are incorporated in the Phylib-Software,

would avoid some confusion. In any case, these taxa should be addressed to avoid different handling of the issue by different diatomists, i.e. the contradiction between the instruction protocol and the mandatory main identification literature.

We also recommend to **count valves instead of objects** (the method used should consequently also be used for the recommended recounts of the training set). Counting objects (valves or frustules each count 'one') instead of valves introduces a greater error than counting valves instead of objects. The reason given for counting objects is that for some representatives of the Naviculaceae it is supposed to often be impossible to tell from a valve view whether one is looking at single valves or entire frustules (Schaumburg et al. 2011, 2012). We think this only applies to very few taxa, such as *Fistulifera saprophila*, as the aperture of most used microscopes are currently very good (22 % of the participants used an aperture of 1.25, 51 % used 1.3 and 27 % as well as the auditors used an aperture of 1.4 in this intercalibration exercise). Additionally, the main problem with identifying these thinly silicified species were more related to the fact, if they were found at all (see Chapter 3.2.11). In contrast, the laboratory method (type of acid, centrifugation, duration of treatment) will strongly determine, if frustules will separate into two valves or not. However, the laboratory method is not specified in the instruction protocol (which makes sense, as some samples need a more vigorous treatment than others depending on substrate etc.). Thus, the error or differences among diatomists will be greater when counting objects instead of valves.

Recommendations for taxonomists

When describing new diatom-taxa and when compiling identification books the taxonomist should keep in mind that his or her work will also be used by the applied limnologist and not just by fellow scientists. Accordingly, ambiguous documentations should be avoided (see e.g. Chapters 3.2.3; 3.2.12 and 3.2.13), i.e. all traits necessary for identification should be depicted in detail and without ambiguity and according to unambiguous text descriptions.

For an assessment of the possible morphological variability of a taxon during diatom identifications, the taxonomist needs to provide basic data with the species description, i.e. on how many valves are the given morphometric ranges of the species description based on, from how many samples and/ or populations and from how many different inland waters (sites). Similarly, data about the ecology of each taxon should be part of the basic data (if available). Precise ecological data are more useful than general statements for a reliable identification.

Additional misconceptions between taxonomists and applied limnologists or diatomists can be resolved, if the taxonomist describes the method that was used to generate the measured ranges, especially of striae density. These recommendations have often already been followed, but this intercalibration exercise demonstrates that there is room and need for improvement (see e.g. Chapters 3.2.12 and 3.2.13).

Overall, the listed recommendations will benefit the applied limnologist by facilitating diatom identifications and consequently by leading to an improved application of diatoms as bio-indicators for inland-water quality assessments. Also, the intercalibration exercise revealed the need for fundamental diatom research, as the taxonomist can help to further improve the use of diatoms as bio-indicators by further investigating species taxonomy and also ecology.

8. Summary

The first German intercalibration exercise for benthic diatoms was conducted in 2011 and 2012 to compare diatom counting results of the same samples from different diatomists to identify taxonomic problems that are relevant to comply with the European Water Framework Directive for running waters and lakes. Also, this intercalibration exercise was carried out to promote the taxonomic comparability of counting results and consequently to improve the application of the German implementation (Schaumburg et al. 2006, 2011) of the European Water Framework Directive.

A total of 37 participants from ten countries (Belgium, the Czech Republic, France, Germany, Ireland, Italy, the Netherlands, Slovakia, Spain and Sweden) and three auditors with many years of experience with identifying benthic diatoms took part in the intercalibration exercise. All 37 participants and the three auditors counted benthic diatoms in the following four samples: **Lake Krossinsee**, Northern Germany, lowland lake, calcareous, polymictic, German Lake Type D 11, **Lake Geneva**, Switzerland, Alps/Alpine foothills lake, calcareous, dimictic, German Lake Type D 1.1, **River Klepelshagener Bach**, Northern Germany, lowland river, calcareous, German River Type D 12 and **River Drau**, Austria, Alps/Alpine foothills river, siliceous, German River Type D 2.

The statistical analysis of the four samples identified the similarity of counting results per sample between participants and auditors using both Bray-Curtis dissimilarity distance and a multivariate graph (Detrendet Correspondence Analysis=DCA). These are two independent methods that were used to confirm and ensure the assessment of the counting results of each laboratory. In the Lake Geneva sample (D 1.1) the average Bray-Curtis-distance of the counting results among auditors was great (exceptionally high standard deviation). Thus, the 95 %-confidence interval almost covered the entire possible range from zero to one. Consequently, this sample cannot be evaluated statistically, i.e. the results of the auditors cannot be used to assess the quality of the results of the participating laboratories. The remaining three samples (Lake Krossinsee D 11, River Klepelshagener Bach D 12 and River Drau D 2) could be evaluated statistically and were used to assess the quality of the results of the participating laboratories. Accordingly, the evaluation given on the certificates were based on the similarity comparisons for these three samples between auditors and participant.

The results from nine of the 37 laboratories differed significantly from the results of the auditors for at least one of the three evaluable samples. Among others based on these

deviations this intercalibration exercise identified considerable taxonomic problems with 12 diatom-genera. At least 15 problems occurred when looking at the results in detail of the following taxa or groups: (1) *Achnanthidium pyrenaicum* and *A. minutissimum* in sample D 2; (2) various small *Amphora*-species in sample D 11; (3) *Cocconeis placentula*-aggregate and similar taxa in sample D 11; (4) *Encyonema silesiacum* and similar taxa in sample D 2; (5) *Encyonopsis subminuta* and similar taxa in sample D 1.1; (6) *Eolimna minima* and similar taxa in sample D 12; (7) *Fragilaria* (*Staurosira*) *brevistriata* and similar taxa in sample D 1.1; (8) *Fragilaria capucina* and similar taxa in samples D 1.1 and D 2; (9) *Gomphonema olivaceolacuum* in sample D 1.1; (10) *Gomphonema pumilum* and similar taxa in samples D 11 and D 1.1; (11) *Mayamaea atomus* var. *permitis* in sample D 12; (12) *Navicula cryptotenella* and *N. cryptotenelloides* in samples D 11 and D 1.1; (13) *Navicula lundii* and *N. veneta* in sample D 12; (14) *Navicula reichardtiana* and *N. caterva* in sample D 1.1 and (15) *Nitzschia fonticola* and *N. lacuum* in samples D 11 and D 1.1.

Despite these taxonomic problems, the present results demonstrate that the objective type or aperture, work experience with counting diatoms, optical illumination technique, regional origin of samples commonly counted by the participants or number of samples usually counted per year by the participants did not significantly influence the statistical distance of the participants results to the results of the auditors (mixed-effect model, $p<0.05$). Thus, these parameters had no distinct effect on the given counting results.

The taxonomic problems that emerged occurred due to both insufficient use of given taxonomic details by the participants of this intercalibration exercise and ambiguous species descriptions and documentation in the current identification literature. Next to simple identification mistakes of single diatom taxa, the following five major issues were identified by this intercalibration exercise for benthic diatoms as reasons for deviating counting results: (1) Partly, the use of different identification literature led to different species names of the same taxa, which hampered or hindered the comparability of the results in some cases. (2) Some diatom valves of the intercalibration exercise had morphological measurements outside the given species ranges to a certain degree in one or more traits. Such valves were present in all four samples and occurred frequently and could consequently only be identified ambiguously. (3) Participants and auditors also allocated different names to the same taxa due to species descriptions with insufficient details or partly due to a mismatch of pictures and species description in the identification literature. (4) In several cases taxa were reported with more certainty than actually present, i.e. despite morphologic deviations from the defined ranges in the identification literature the valves were often not labelled with a "cf." or "spec".

(5) Partly, insufficient knowledge about the ecology of some species with misleading recommendations based on this ecology in the identification literature led to different naming of some diatoms.

The taxonomic differences among participants and auditors for the same samples affected the water quality assessment with the German PYHLIB-method, further emphasizing the need for documenting ambiguous species and the need of quality assurances (QA) of diatom counts. A comparison of the QA of diatom counts in Europe demonstrates that this national intercalibration exercise promotes practical taxonomic harmonization efforts. Among others, this exercise shows that the use of auditors is beneficial, as the mean value of all participants may not necessarily reflect the correct taxonomy. Similarly, the workshop conducted following the exercise helped to pinpoint and solve some taxonomic problems. Among others, the workshop identified the need to clarify where to measure striae-density, which was consequently also included in this report.

Ultimately, we generally recommend to the applied diatomist, i.e. during routine monitoring work with diatoms, to carefully consider all traits defining a species and to use the mandatory identification literature. Also, ambiguous diatom valves should be photographed, concisely lyrically documented and be labelled with „spec.“, „cf.“ or „aff.“ in the counting results. We also recommend to further investigate the taxonomy and ecology of some of the here discussed common diatom taxa in future scientific investigations and to generally provide more details and basic information in the identification literature for each of the presented diatom taxa. Only thus, the use of diatoms as bio-indicators can be improved, whereas both parties (the applied limnologist and the taxonomist or scientist) need to combine their efforts for a better cooperation.

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