

## Applied use of taxonomy: lessons learned from the first German intercalibration exercise for benthic diatoms

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**Abstract** – The first German intercalibration exercise for benthic diatoms was conducted to improve the application of the implementation of the European Water Framework Directive for running waters and lakes in Germany. The intercalibration exercise revealed several taxonomic problems. Among others, considerable problems occurred with identifying and differentiating species of the following four groups: (I) *Amphora indistincta* Levkov and *A. pediculus* (Kützing) Grunow, (II) *Cocconeis placentula* var. *euglypta* Ehrenberg and *C. placentula* var. *lineata* (Ehrenberg) Van Heurck, (III) *Navicula cryptotenella* Lange-Bertalot and *N. cryptotenelloides* Lange-Bertalot and (IV) *N. reichardtiana* Lange-Bertalot and *N. caterva* Hohn & Hellermann. The taxonomic problems that emerged occurred due to both insufficient use of given taxonomic details (by limnologists) and ambiguous species descriptions and documentation (by taxonomists). Thus, we recommend to the applied limnologist to use the mandatory identification literature and to document any ambiguous valves during routine counts. Also, it would be desirable to further investigate certain species by taxonomists and, in general, to provide more basic data with species descriptions or in identification manuals. These measures will improve the use of diatoms as bioindicators and consequently benefit both applied limnologists and taxonomists.

**Key words:** *Amphora*, benthic diatoms, *Cocconeis*, intercalibration exercise, *Navicula*, quality control, taxonomy

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## Introduction

Benthic diatoms are well-established, robust bioindicators (SMOL and STOERMER 2010) that are widely used, e.g. in paleolimnology (HÜBENER et al. 2009, DREBLER et al. 2011) or when implementing the European Water Framework Directive (EU-WFD 2000) (PRYGIEL 2002, WERNER and DRESSLER 2007, KELLY 2013). In various European countries intercalibration exercises and taxonomic workshops are conducted:

- to ensure the comparability of diatom counting results among diatomists when implementing the Water Framework Directive,
- to facilitate a uniform approach for dealing with taxonomic problems, and
- to perform the basic quality control for diatom counting results (KELLY 2001, PRYGIEL et al. 2002, KAHLERT et al. 2012).

In Germany, the first intercalibration exercise for benthic diatoms was conducted in 2011 and 2012 to improve the application of the German instruction protocol that assesses the water quality of lakes and rivers using diatoms according to the European Water Framework Directive (SCHAUMBURG et al. 2006, 2007, 2011). The 37 participants of this German intercalibration exercise came from Germany, Belgium, the Czech Republic, France, Ireland, Italy, the Netherlands, Slovakia, Spain and Sweden and counted benthic diatom samples from two rivers and two lakes according to the German method (SCHAUMBURG et al. 2006, 2007, 2011). The counting results of three auditors of these four samples were used as references.

The auditor results were a statistically reliable reference for three of the four samples and provided the diatom assemblage to which the participant results were compared to and assessed with. Nine of the 37 participant results deviated significantly to the results of all three auditors in at least one sample. These significant differences identified taxonomic problems within twelve genera.

The aim of this study was a detailed description and analysis of four identified taxonomic problems to recommend measures that may improve the applied use of diatoms as bioindicators. These four problems apparent from the first German intercalibration exercise were the identification and differentiation of

- (I) some small *Amphora*-taxa (mainly *A. indistincta* Levkov and *A. pediculus* (Kützing) Grunow);
- (II) the varieties of *Cocconeis placentula* Ehrenberg (mainly *C. placentula* var. *euglypta* Ehrenberg and *C. placentula* var. *lineata* (Ehrenberg) Van Heurck);
- (III) *Navicula cryptotenella* Lange-Bertalot and *N. cryptotenelloides* Lange-Bertalot and
- (IV) *Navicula reichardtiana* Lange-Bertalot and *Navicula caterva* Hohn & Hellermann.

## Material and methods

The first German intercalibration exercise was based on four samples of benthic diatoms:

1. Lake Krossinsee, Northern Germany, lowland lake, calcareous, polymictic
2. Lake Geneva, Switzerland, Alps/Alpine foothills lake, calcareous, dimictic
3. River Klepelshagener Bach, Northern Germany, lowland river, calcareous
4. River Drau, Austria, Alps/Alpine foothills river, siliceous

The taxonomic problems discussed here occurred only in sample Lake Krossinsee (Northern Germany) and in sample Lake Geneva (Switzerland). Thus, we will only address the two lake samples in the following. Lake Krossinsee (52°36'31.5"N; 13°69'46.5"E) was sampled on 12<sup>th</sup> July 2010 from various stones and various stalks (below water surface) of *Phragmites australis* (Cavanilles) Trinius ex Steudel and *Typha latifolia* L. Lake Geneva (46°22'31.3"N; 6°15'18.0"E) was sampled on 7<sup>th</sup> August 2011 from various stones. The selected and here presented taxonomic problems concern the differentiation and identification of the following taxa:

1. *Amphora indistincta* and *A. pediculus*
2. *Cocconeis placentula* var. *euglypta* and var. *lineata*
3. *Navicula cryptotenella* and *N. cryptotenelloides*
4. *Navicula reichardtiana* and *N. caterva*

Diatom samples were taken according to the German instructions for implementing the European Water Framework Directive (SCHAUMBURG et al. 2006, 2007, 2011). Only naturally prevalent substrates were sampled (stones and macrophytes in Lake Krossinsee and stones in Lake Geneva) in at least 20 cm water depth. Periphyton was scratched from at least 15 stones and plants per site into a 500 mL bottle and alcohol was added. Diatom samples were oxidised and prepared with HCl, H<sub>2</sub>O<sub>2</sub>, H<sub>2</sub>SO<sub>4</sub>, KMnO<sub>4</sub> and C<sub>2</sub>H<sub>2</sub>O<sub>4</sub> following modified KALBE and WERNER (1974). Dried slurries containing the diatoms were then mounted with Naphrax® (refraction index 1.73) onto slides. Each slide from the two samples was prepared by one person and then sent to the participants. The slides were labelled with the diatom water body type, i.e. participants knew the region and lake-type. However, participants did not know which lake 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 here discussed lake samples were counted by two auditors and the running water samples (which are not part of this paper) 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 SCHAUMBURG et al. (2006, 2007) (both in English) and additionally the new and relevant changes of instructions given in SCHAUMBURG et al. (2011), which were translated and sent to the participants in the letter accompanying the samples. The standard identification literature was HOFMANN et al. (2011). Additionally, identification had to be based on the supplementary books KRAMMER and LANGE-BERTALOT (1986–91, 2004), LANGE-BERTALOT (1993, 2001), LANGE-BERTALOT and MOSER (1994), LANGE-BERTALOT and METZELTIN (1996), KRAMMER (1997a, 1997b, 2000, 2002, 2003), REICHARDT (1999), WITKOWSKI et al. (2000) and LEVKOV (2009).

Participants and auditors entered the diatom counting results via the EQAT-webpage ([www.planktonforum.eu](http://www.planktonforum.eu)) into an 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.

Diatom photos were taken with a ProgRes® SpeedXTcore3 (Jenoptik) camera 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).

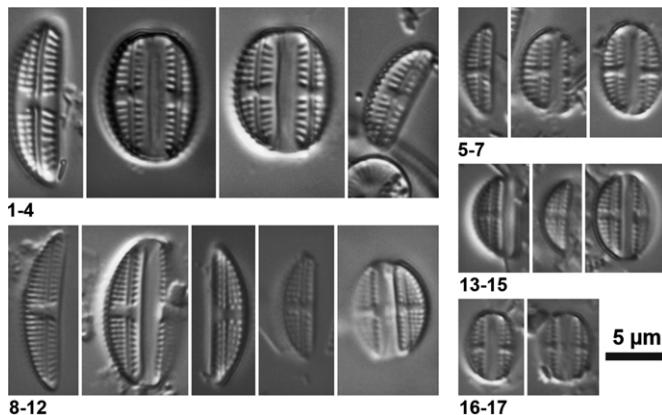
## Results

### Small *Amphora*-species in Lake Krossinsee

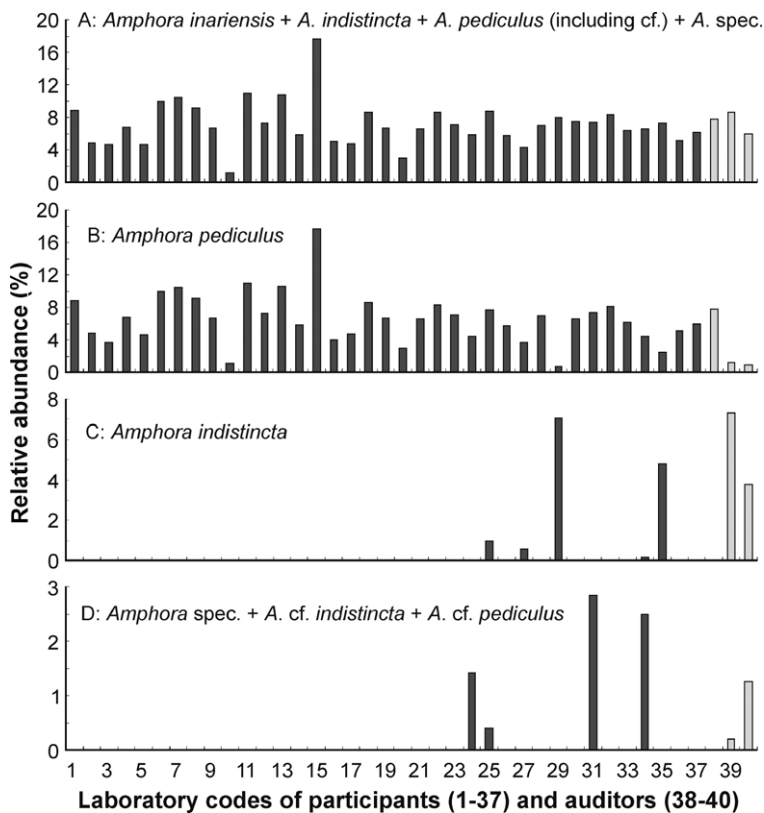
The most abundant *Amphora* species in the sample from Lake Krossinsee were *A. indistincta* (Pl. 1: 1–4) and *A. pediculus* (Pl. 1: 8–12; Fig. 1). Additionally, valves of *A. cf. indistincta* (Pl. 1: 5–7), *A. cf. pediculus* (Pl. 1: 13–15) and *Amphora* spec. (Pl. 1: 16–17) occurred in low abundances in this sample. Rare *Amphora*-taxa (< 0.2%) were *Amphora copulata* (Kützing) Schoeman & Archibald, *A. cf. copulata*, *Amphora eximia* Carter, *Amphora inariensis* Krammer, *Amphora cf. subatomus* Levkov, *Amphora minutissima* W. Smith, *A. cf. minutissima* and *Amphora lange-bertalotii* Levkov & Metzeltin, which are not further discussed here.

The valves 1–4 depicted in Pl. 1 perfectly fit the ranges given for *A. indistincta* and 8–12 (Pl. 1) for *A. pediculus* (see LEVKOV 2009 or HOFMANN et al. 2013). Thus, they are named without problems. The valves 5–7 (Pl. 1) were labelled *A. cf. indistincta*, because their habita morphologically corresponded to *A. indistincta*, but some traits differed from the species description in LEVKOV (2009). The valve widths were too small (1.9–2.9 µm instead of 3–4 µm) and dorsal striae density was too high (24–25 instead of 18–22 striae in 10 µm).

The valves 13–15 (Pl. 1) were labelled *A. cf. pediculus*, because they mainly correspond to *A. pediculus* as even the dorsal areolae are visible using light microscopy despite their small size. However, the valves are too small (5.8–6.9 µm long instead of 7–15 µm and 1.9–2.3 µm wide instead of 2.5–4.0 µm) and the striae are too dense (e.g. dorally 25 and more striae in 10 µm instead of 18–24 in 10 µm).



**Pl. 1.** Light microscopic pictures of *Amphora indistincta* (1–4), *A. cf. indistincta* (5–7), *A. pediculus* (8–12), *A. cf. pediculus* (13–15) and *Amphora* spec. (16–17) from Lake Krossinsee, Germany.



**Fig. 1.** Relative abundance of small *Amphora*-species from Lake Krossinsee, Germany, based on the results of the first German intercalibration exercise for benthic diatoms. A: Sum of *A. inariensis*, *A. indistincta*, *A. cf. indistincta*, *A. pediculus*, *A. cf. pediculus* and *Amphora spec.* B: *A. pediculus*, C: *A. indistincta*, D: *Amphora spec.*, *A. cf. indistincta* and *A. cf. pediculus*. Dark grey bars: participants; light grey bars: auditors.

The valves 16–17 (Pl. 1) distinctly deviate from the species descriptions of *A. indistincta* and *A. pediculus*. Thus, it is even more difficult to allocate an appropriate name to these valves, which were thus labelled *Amphora spec.*

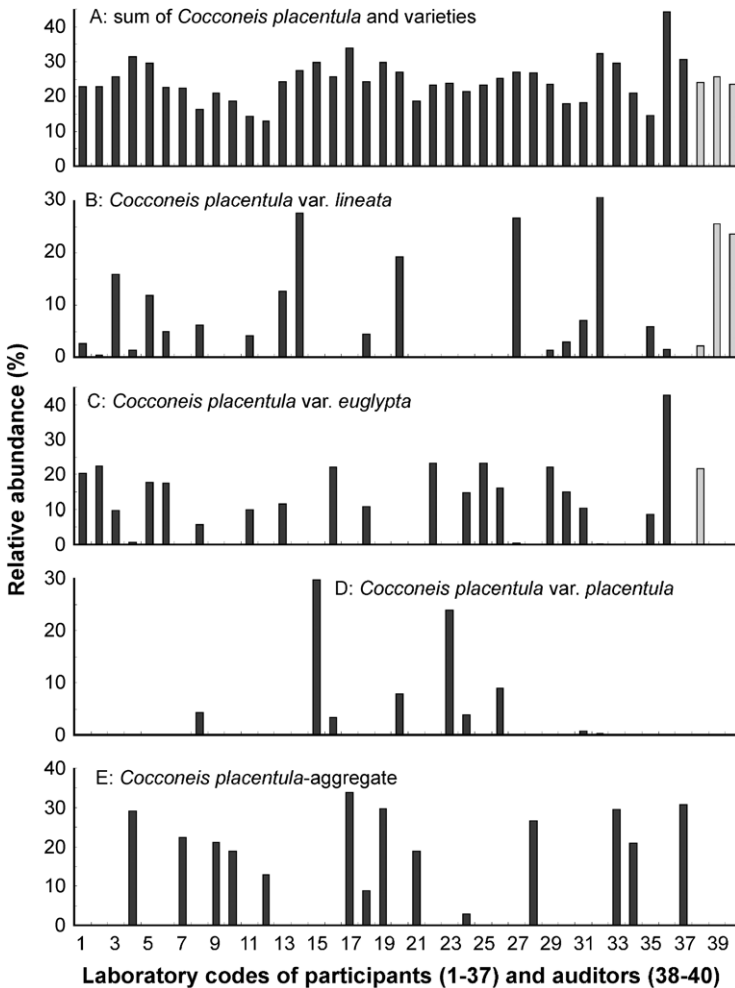
Overall, the sum of small *Amphora*-species were more or less similar among participants (1.1–17.6%, average 7.2%) and auditors (6.0–8.7%, average 7.5%) in the sample of Lake Krossinsee (Fig. 1A). Just the results of laboratory 10 (1.1%) and laboratory 15 (17.6%) deviated distinctly from the rest. However, 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 *A. pediculus* (Fig. 1B). 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* (Fig. 1C). Of the 37 participants only five detected *A. indistincta* in sample Lake Krossinsee (Fig. 1C).

Despite the occurrence of some small *Amphora*-valves in Lake Krossinsee (see Pl. 1: 5–7 and 13–17) that do not fit any *Amphora*-taxa description in HOFMANN et al. (2011) or

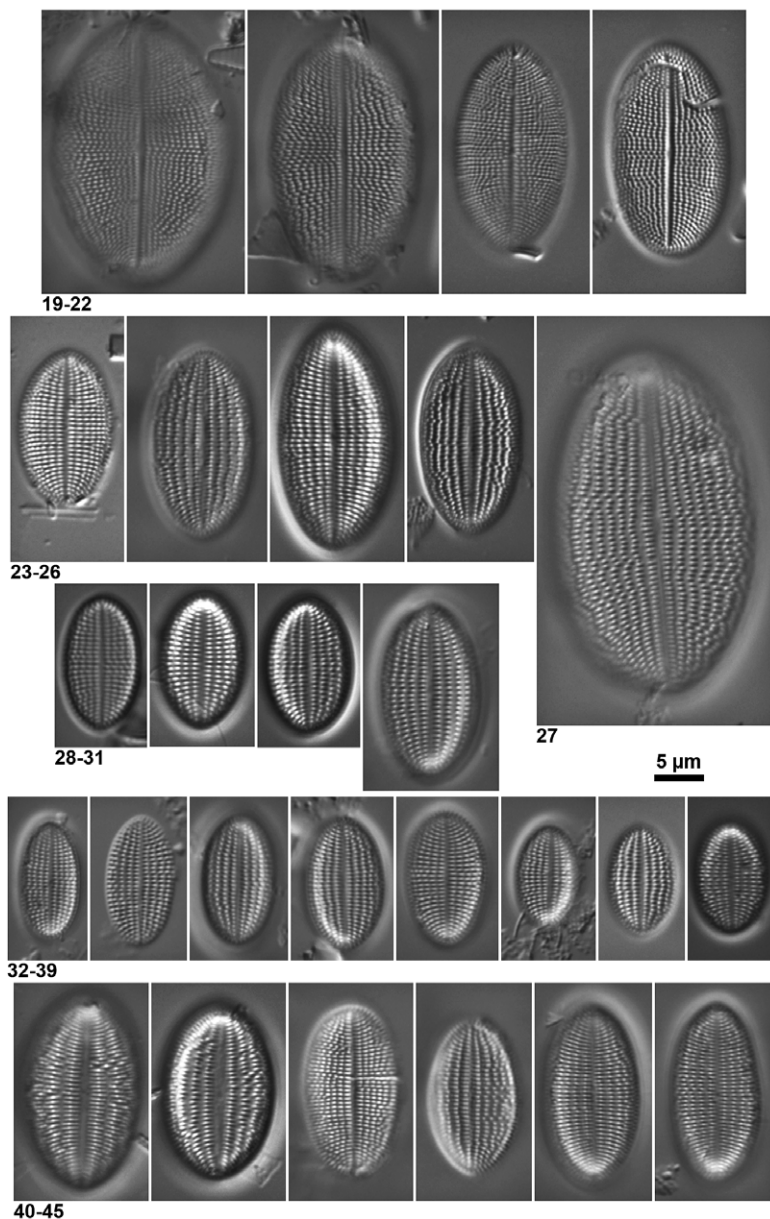
LEVKOV (2009) only four laboratories and two auditors called some valves *Amphora* spec., *A. cf. pediculus* or *A. cf. indistincta* (Fig. 1D), i.e. they were uncertain about the identity of some valves from this taxonomic group.

***Cocconeis placentula* in Lake Krossinsee**

*Cocconeis placentula* was one of the dominant taxa in the Lake Krossinsee sample (Fig. 2). One main problem was the differentiation of *C. placentula* var. *placentula* (Pl. 2: 19–22), *C. placentula* var. *lineata* (Plate 2: 23–27) and *C. placentula* var. *euglypta*



**Fig. 2.** Relative abundance of *Cocconeis placentula* Ehrenberg from Lake Krossinsee, Germany, based on the results of the first German intercalibration exercise for benthic diatoms. A: Sum of *C. placentula* var. *lineata*, *C. placentula* var. *euglypta*, *C. placentula* var. *placentula* and *C. placentula*-aggregate, B: *C. placentula* var. *lineata*, C: *C. placentula* var. *euglypta*, D: *C. placentula* var. *placentula* and E: *C. placentula*-aggregate, dark grey bars: participants; light grey bars: auditors.



**Pl. 2.** Light microscopic pictures of the rapheless valves of *Cocconeis placentula*-varieties from the intercalibration exercise sample Lake Krossinsee, Germany, labelled according to the morphological concept of KRAMMER and LANGE-BERTALOT (2004). *C. placentula* var. *placentula* (19–22), *C. placentula* var. *lineata* (23–27), *C. placentula* var. *euglypta* (28–31) and rapheless valves of *C. placentula* with a striae density of  $\sim 24\text{--}26$  in  $10\ \mu\text{m}$  (32–45). These latter valves are *C. placentula* var. *placentula* based on their striae density according to KRAMMER and LANGE-BERTALOT (2004). However, the arrangement and shape of the areolae better fit with *C. placentula* var. *euglypta* (32–39) or *C. placentula* var. *lineata* (40–45) according to the concept of KRAMMER and LANGE-BERTALOT (2004).

(Pl. 2: 28–31) according to the results of this intercalibration exercise. The remaining *Cocconeis*-taxa in Lake Krossinsee (*C. placentula* var. *tenuistriata* Geitler, *C. neothumensis* Krammer, *C. pseudolineata* (Geitler) Lange-Bertalot and *C. pediculus* Ehrenberg) were either only present in low abundances (< 1.0%) or were taxonomically not problematic according to the results of this intercalibration exercise. Thus, they are not presented or discussed below.

Various morphological concepts exist for differentiating the varieties of *C. placentula* (see e.g. HUSTEDT 1930, GEITLER 1982, KRAMMER and LANGE-BERTALOT 2004, JAHN et al. 2009, ROMERO and JAHN 2013), which are currently in practical use simultaneously. The German instruction protocol (SCHAUMBURG et al. 2006, 2007, 2011) stipulates using HOFMANN et al. (2011) as standard identification literature for counting diatoms. HOFMANN et al. (2011) refer to KRAMMER and LANGE-BERTALOT (2004) for differentiating the varieties of *C. placentula*. Thus, we identified the varieties according to KRAMMER and LANGE-BERTALOT (2004).

All 37 participants identified *C. placentula* including the varieties *euglypta*, *lineata* or *placentula* or *C. placentula*-aggregate (12.9–44.3%, average 24.4%) (Fig. 2A). 19 participants detected *C. placentula* var. *lineata* (0.5–31.8%, average 9.9%) (Fig. 2B) and 22 participants identified *C. placentula* var. *euglypta* (0.2–42.8%, average 14.8%) (Fig. 2C). Eleven participants identified *C. placentula* var. *placentula* (0.18–29.7%, average 7.6%) (Fig. 2D) of which two (laboratories 15 and 23) did not detect any other varieties of *C. placentula*. 14 participants detected *C. placentula*-aggregate (2.8–33.9%, average 21.9%) (Fig. 2E).

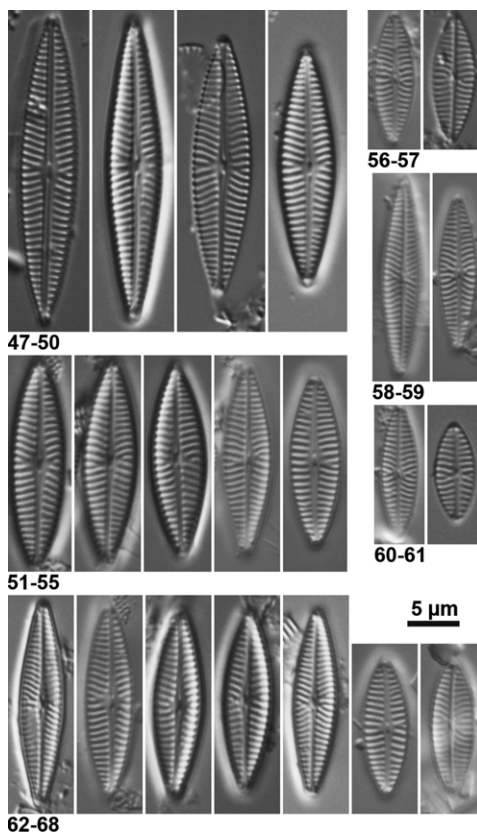
The results of the auditors suggest that *C. placentula* was dominated by the varieties *euglypta* and/or *lineata* in sample Lake Krossinsee. Two auditors exclusively identified *C. placentula* var. *lineata* (25.5% and 23.5%, respectively) (Fig. 2B). The third auditor detected 21.7% *C. placentula* var. *euglypta* (Fig. 2C) and 2.2% *C. placentula* var. *lineata* (Fig. 2B).

### ***Navicula cryptotenella* and *N. cryptotenelloides* in Lake Krossinsee and Lake Geneva**

In the sample from Lake Krossinsee the genus *Navicula* Bory de Saint-Vincent was dominated by *Navicula cryptotenella*, while *N. cryptotenelloides* occurred with less than 0.2% (see Pl. 3: 47–68). In contrast, in the sample from Lake Geneva the auditors identified *N. cryptotenelloides* as the dominant taxon within the genus *Navicula*, while *N. cryptotenella* was very rare (< 1%) but present as well (Pl. 3: 47–68). In both samples other *Navicula*-taxa occurred too, such as *N. antonii* Lange-Bertalot, *N. reichardtiana* and *N. tri-punctata* (O.F. Müller) Bory de Saint-Vincent. However, they will not be further discussed here.

In the Lake Krossinsee sample the three auditors identified *N. cryptotenella* with 5.9–10.0% (average 8.3%) (Fig. 3B), while 33 of the 37 participants detected *N. cryptotenella* with 1.4–8.9% (average: 5.4%) (Fig. 3B). *N. 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%) (Fig. 3C). Especially the results of the participants 15, 19, 22 and 28 expose taxonomic problems. These laboratories did not detect any *N. cryptotenella* in sample Lake Krossinsee (Fig. 3B). Laboratories 15 and 28 only identified *N. antonii* and were the only participants that did not find any evidence of *N. cryptotenella* or *N. crypto-*

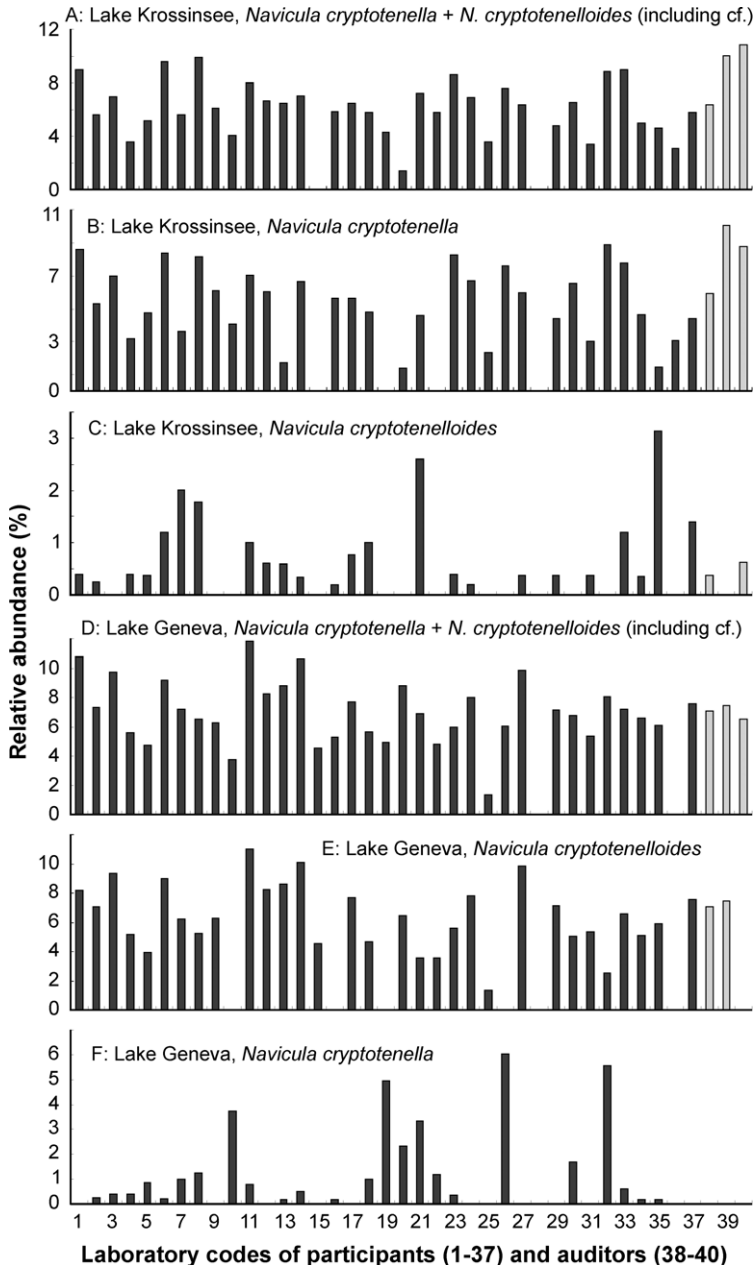




**Pl. 3.** Light microscopic pictures of *Navicula cryptotenella* (47–55), *N. cryptotenelloides* (56–61) and valves that can not be identified with certainty with a valve width between 4.2 and 5.0  $\mu\text{m}$  (62–68) from the Lake Krossinsee sample, Germany (47–55 and 61, 63–65 and 67), and Lake Geneva sample, Switzerland (56–60, 62, 66 and 68).

*tenelloides* (see Fig. 3A). Additionally, laboratories 13, 19, 22 and 25 detected a considerable percentage of the taxa discussed here with ambiguity (labelled with cf.). Similarly to the participants, the results of the auditors suggest taxonomic difficulties of these *Navicula*-taxa. For example, auditor 40 named some counted objects as *N. cf. cryptotenelloides* (1.4%).

In the Lake Geneva sample 35 of 37 participants detected *N. cryptotenelloides* (including cf.) and/or *N. cryptotenella* (including cf.) (1.4–11.8%, average: 7.0%) (Fig. 3D). Laboratories 28 and 36 did not identify any *N. cryptotenelloides* or *N. cryptotenella* (Fig. 3D). In contrast to single taxa abundances (Figs. 3E–F) the sum of *N. cryptotenelloides* and/or *N. cryptotenella* were uniform among auditors (average: 7.0%) (Fig. 3D). *N. cryptotenelloides* was detected by 31 of 37 participants (1.4–11.0%, average: 6.4%) and by two of three auditors (average: 7.3%) (Fig. 3E). *N. cryptotenella* was not detected by any auditor in this sample (Fig. 3F). In contrast, 25 of the 37 participants identified *N. cryptotenella* (0.2–6.1%, average: 1.6%) (Fig. 3F). One auditor (no. 40) counted solely *N. cf. cryptotenella*. Similarly, four participating laboratories counted *N. cf. cryptotenella* (laboratories

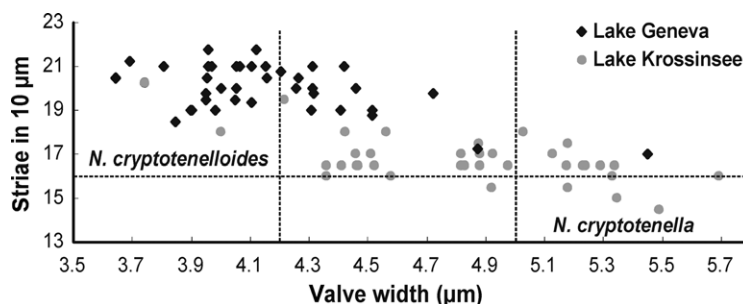


**Fig. 3.** Relative abundance of *Navicula cryptotenella* and *N. cryptotenelloides* from samples Lake Krossinsee, Germany (A–C), and Lake Geneva, Switzerland (D–F), based on the results of the first German intercalibration exercise for benthic diatoms. A: Sum of *Navicula cryptotenella* and *N. cryptotenelloides* (including cf.), B: *N. cryptotenella*, C: *N. cryptotenelloides*, D: Sum of *N. cryptotenella* and *N. cryptotenelloides* (including cf.), E: *N. cryptotenelloides*, F: *N. cryptotenella*, dark grey bars: participants; light grey bars: auditors.

1 and 24) or *N. cf. cryptotenelloides* (laboratories 16 and 34). The results of participants 10, 16, 19, 26, 28 and 36 particularly reveal taxonomic problems. Laboratories 28 and 36 did not detect either *N. cryptotenelloides* or *N. cryptotenella* (Fig. 3D). Laboratories 10, 16, 19 and 26 did not detect any *N. cryptotenelloides* (Fig. 3E). Laboratories 10, 19 and 26 solely identified *N. cryptotenella* (Figs. 3E–F). Laboratory 16 counted mainly *N. cf. cryptotenelloides*. Similarly, the results of auditor no. 40, who solely identified *N. cf. cryptotenella*, emphasize the taxonomic problems within the *N. cryptotenella* and *N. cryptotenelloides*-group.

Besides general problems with identifying *N. cryptotenella* and *N. cryptotenelloides*, 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 the given width for *N. cryptotenelloides* is 3.7–4.2  $\mu\text{m}$  and for *N. cryptotenella* is 5.0–7.0  $\mu\text{m}$ . Overlapping valve width (4.2–5.0  $\mu\text{m}$ ) is not supposed to occur (LANGE-BERTALOT 1993, 2001). In contrast, valves with a valve width of 4.2–5.0  $\mu\text{m}$  occurred regularly in the samples examined here. For elucidating this problem of overlapping valve width 38 valves of the *N. cryptotenella* and *N. cryptotenelloides*-group were measured and photographed in each of the Lake Krossinsee and Lake Geneva samples (Fig. 4).

Of the 38 measured valves from Lake Geneva 23 valves had a width of 3.6–4.2  $\mu\text{m}$  (Fig. 4), corresponding to *N. cryptotenelloides*. Interestingly, all 23 valves had a striae density of 18.5–21.0 in 10  $\mu\text{m}$  (Fig. 4), which consistently exceeds 16–18 striae in 10  $\mu\text{m}$ , the given range for *N. cryptotenelloides* (LANGE-BERTALOT 2001, HOFMANN et al. 2011). One of the 38 Lake Geneva valves had a width of 5.5  $\mu\text{m}$  and 17 striae in 10  $\mu\text{m}$ . 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*. The remaining 14 valves (36.8%) of Lake Geneva had a width between 4.2–5.0  $\mu\text{m}$  (Fig. 4), contrasting the details given in LANGE-BERTALOT (1993, 2001), i.e. the width were in the range between *N. cryptotenella* and *N. cryptotenelloides*. These 14 valves had a striae density between 17.3 and 21.0 in 10  $\mu\text{m}$  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 labelling these valves as cf.



**Fig. 4.** Striae density and valve width from valves of the *Navicula cryptotenella* and *N. cryptotenelloides*-complex from 38 valves from Lake Geneva, Switzerland (black diamonds), and 38 valves from Lake Krossinsee (grey circles). Vertical dashed lines denote the valve width of *N. cryptotenelloides* (3.7–4.2  $\mu\text{m}$ ) and *N. cryptotenella* (5.0–7.0  $\mu\text{m}$ ), horizontal dashed line denotes the differentiating striae density of 16 striae in 10  $\mu\text{m}$  according to LANGE-BERTALOT (2011).

Of the 38 measured valves from Lake Krossinsee two valves had a width of ~3.7 and 4.0  $\mu\text{m}$  and a striae density of ~18 and 20 in 10  $\mu\text{m}$ , respectively (Fig. 4). Correspondingly, these valves were probably *N. cryptotenelloides*. 13 valves had a width > 5.0  $\mu\text{m}$  (Fig. 4), matching *N. cryptotenella*. Of these 13 valves 10 valves had ~14–16 striae in 10  $\mu\text{m}$  (Fig. 4) and are thus probably *N. cryptotenella*. Three of these 13 valves had ~17–18 striae in 10  $\mu\text{m}$  (Fig. 4) and are therefore only ambiguously *N. cryptotenella*. The remaining 23 valves (60.5%) from Lake Krossinsee had a width between 4.2–4.9  $\mu\text{m}$  (Fig. 4), 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  $\mu\text{m}$  (Fig. 4). Thus, it remains unclear if the valves belong to *N. cryptotenella* or *N. cryptotenelloides* and should also best be labelled as cf.

Despite these distinct deviations from the description of *N. cryptotenella* and *N. cryptotenelloides* of most valves from Lake Krossinsee and Lake Geneva (Fig. 4), only four of the 37 participants and one auditor labelled any valves with cf for Lake Krossinsee and only four other participants and the same auditor used cf. for these valves from Lake Geneva.

### ***Navicula reichardtiana* and *N. caterva* in Lake Geneva**

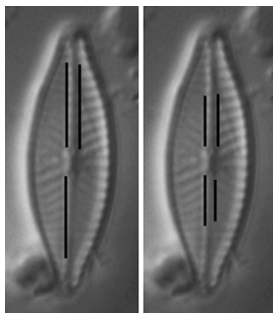
*Navicula reichardtiana* was detected by 25 of the 37 participants and two auditors in the Lake Geneva sample. One additional participant and the third auditor identified *N. cf. reichardtiana*. *N. caterva* was detected by five participants and no auditor. The sum of all *N. reichardtiana* and *N. caterva* from all participants and auditors was below 2.5%. Thus, not listing these taxa does not necessarily suggest an identification mistake or problem.

As two auditors and most participants unambiguously identified *N. reichardtiana* and no auditor and only five participants identified *N. caterva*, we could assume that the latter are misidentifications. However, with the data at hand a misidentification cannot be verified. More taxonomic examinations would be necessary. The workshop that was conducted subsequent to the intercalibration exercise identified complicated taxonomic problems instead of simple misidentification leading to some participants determining *N. caterva* instead of *N. reichardtiana* and to two participants identifying both taxa in the same sample. Similarly, one auditor only identified *N. cf. reichardtiana* from this complex. In the following we will demonstrate these problems exemplarily by describing the identification approaches of two participants (A and B) when identifying *N. reichardtiana* and *N. caterva* (Fig. 5).

Both participants measured the striae density along the margin of the axial area, beginning at the end of the central area. Participant A counted the striae along a 5  $\mu\text{m}$  scale, while participant B used a shorter scale (Fig. 5). Both extrapolated to striae density in 10  $\mu\text{m}$  (Tab. 1). These different interpretations of striae counting methods and consequently varying measuring methods only led to small differences in the extrapolated number of striae in 10  $\mu\text{m}$  (16.2 and 15.1, respectively; Tab. 1), but have a great impact on the identification process (see below).

#### Interpretation and species identification by participant A:

Participant A argues that the width of the valve in the given example (Fig. 5) fits the range of *N. caterva* (4.2–5.5  $\mu\text{m}$  according to LANGE-BERTALOT 2001) and is too small for *N. reichardtiana* (5–6  $\mu\text{m}$  according to LANGE-BERTALOT 2001). The striae density (16.17 in 10  $\mu\text{m}$ ) only slightly exceeds the range for *N. reichardtiana* (14–16 in 10  $\mu\text{m}$  according to LANGE-BERTALOT 2001) and fits the range of *N. caterva* ((16)18–21 in 10  $\mu\text{m}$  according to



**Fig. 5.** Example of different striae-counting approaches (vertical lines) from participant A (left) and B (right) on a valve from the *Navicula reichardtiana* and *N. caterva*-group from sample Lake Geneva. Scale bar (horizontal line) = 5  $\mu\text{m}$ .

**Tab. 1.** Results of measurements of participant A and B of the valve shown in Fig. 5 of the *Navicula reichardtiana* and *N. caterva*-group from sample Lake Geneva. Participant A measured directly in  $\mu\text{m}$  while participant B measured in pixels and then converted into  $\mu\text{m}$ . In this given example 0.0549 pixels convert to 1  $\mu\text{m}$ .

Participant	Length ( $\mu\text{m}$ )	Width ( $\mu\text{m}$ )	Striae/ $\mu\text{m}$ (pixel)				Striae/ 10 $\mu\text{m}$
			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

LANGE-BERTALOT 2001). Participant A denotes the central area as small, which characterises both species (LANGE-BERTALOT 2001). The striae orientation changes only once abruptly (bottom right in Fig. 5) and three times gradually, which participant A thinks fits better to *N. caterva* than to *N. reichardtiana* (see discussion, i.e. remarks about 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. 5 as *N. caterva*.

Interpretation and species identification by participant B:

Participant B argues that the width of the valve in the given example (Fig. 5) 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  $\mu\text{m}$ ) 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. 5), which distinctly characterises *N. reichardtiana* according to participant B. In summary, participant B argues that the valve in Fig. 5 more resembles *N. reichardtiana* than *N. caterva*. However, as the width is slightly outside of the range of *N. reichardtiana* and the striae orientation changes only gradually on three sides, participant B identifies the valve in Fig. 5 as *N. cf. reichardtiana*.

Both participants considered very carefully all traits when identifying the valve in Fig. 5. Still, they arrived at different names for the very same valve. Based on the different approaches of the two participants it is not surprising that they also named many other valves differently from each other. For example, when identifying 15 different valves from the *N. reichardtiana* and *N. caterva*-complex, participants A and B agreed only in five cases and differed in the naming of the remaining ten valves.

## Discussion

The taxonomic problems that occurred in the intercalibration exercise for benthic diatoms and that we discuss in the following sections based on a few examples represent only a fraction of the problems that occur during routine diatom-counts in practise. Interviews and discussions during the workshop of the intercalibration exercise identified several reasons for deviating counting results. Sometimes participants differed in the interpretation of the species specifications in the identification literature or their results differed due to insufficient use of taxonomic specifications. In other cases the results differed due to partly ambiguous species descriptions and to misleading recommendations about which traits to use for species identification or differentiation in the identification literature and also due to currently insufficient taxonomic and ecological knowledge of some diatom taxa.

### 1. Using different identification literature

The problem of using different identification literature was especially apparent when identifying the small *Amphora*-species and the varieties of *Cocconeis placentula*. For example, *Amphora pediculus* and *A. indistincta* occurred roughly with similar abundances in Lake Krossinsee. Still, *A. indistincta* was detected only by five of the 37 participants and by two auditors. One important reason for this discrepancy is the fact that some participants did not use the current and mandatory identification literature (e.g. LEVKOV 2009, HOFMANN et al. 2011). Some participants only used KRAMMER and LANGE-BERTALOT (1986–2004), which do not incorporate *A. indistincta*. Thus, these participants did not find any *A. indistincta*. Instead *A. indistincta* valves from Lake Krossinsee were counted as *A. pediculus* or *A. inariensis*. This problem can be resolved fairly easily by using the new identification literature (e.g. LEVKOV 2009, HOFMANN et al. 2011 or 2013).

Similarly, participants used various identification literatures when identifying the varieties of *Cocconeis placentula*. This is especially problematic, because several morphological concepts exist for distinguishing the varieties of *C. placentula* (see e.g. HUSTEDT 1930, GEITLER 1982, KRAMMER and LANGE-BERTALOT 2004, JAHN et al. 2009, ROMERO and JAHN 2013) that are concurrently in practical use. Thus, in this case counting results can only be comparable, if the same literature is used or if the used literature is detailed and the concept is documented with pictures and a comprehensive and detailed accompanying text.

### 2. Morphological variability

Another problem apparent from the intercalibration exercise is the relatively frequent presence of valves from certain diatoms that are outside the given morphometric ranges of the species in one or more traits. Thus, identification without ambiguity is not always possible. This phenomenon occurred commonly in all four taxonomic groups presented here

from the Lake Krossinsee and Lake Geneva samples. For example, the valves labelled *A. cf. indistincta* (Pl. 1: 5–7) and *A. cf. pediculus* (Pl. 1: 13–15) were distinctly too small and had striae that were too dense compared to their description in LEVKOV (2009). Most rapheless valves from the *Cocconeis placentula*-aggregate from Lake Krossinsee had a striae density of ~24–26 in 10 µm (Pl. 2: 32–45), corresponding to *C. placentula* var. *placentula* according to KRAMMER and LANGE-BERTALOT (2004). However, the arrangement and the shape of the areolae rather correspond to *C. placentula* var. *euglypta* or *C. placentula* var. *lineata* according to KRAMMER and LANGE-BERTALOT (2004). Also, about 37% of the valves of the *Navicula cryptotenella* and *N. cryptotenelloides*-complex from Lake Geneva had a width between 4.2–5.0 µm and ~ 61% of the Lake Krossinsee valves from this complex (Pl. 3: 62–68, Fig. 4). This width is exactly between the width of *N. cryptotenella* and *N. cryptotenelloides* and should not occur according to LANGE-BERTALOT (1993, 2001). On the contrary, the width is supposed to be a very good criterion to discriminate the two species (LANGE-BERTALOT 1993, 2001). Additionally, most valves of the *N. cryptotenella* and *N. cryptotenelloides*-complex had a higher striae density than specified in LANGE-BERTALOT (2001) (see Fig. 4).

At least two reasons may be responsible for the frequent occurrence of diatom-valves that are outside the given morphological description in some traits but that otherwise fit well to the species specifications: (1) Some described species have a greater morphological range than in the given species specifications (see e.g. WOLF et al. 2002, DRESSLER and HÜBENER 2006). (2) Some species specifications describe species-groups instead of species, i.e. they include several similar species that have not been examined sufficiently neither taxonomically nor ecologically so far (see e.g. EVANS et al. 2008, JÜTTNER et al. 2013). Both reasons indicate some challenges for taxonomists that will improve diatom identification in practise.

One problem is that some type material for species descriptions contains only a few valves and that species descriptions are often only based on one or few sites or species populations. Thus, some species descriptions include just a small part of the true morphological variability and ecological range of a species. Consequently, it would be desirable if the species descriptions and details in the identification literature included measures that enable the applied limnologist and other diatomists to better assess the morphological and ecological variability of a taxon. For example, it would be desirable to know on how many valves from how many populations, samples and inland waters a species description is based on.

### 3. Ambiguous taxa descriptions and documentations in the identification literature

Besides the morphological variability of taxa described above, diatom-identification is further complicated by ambiguous or relatively short taxa-descriptions in some cases. For example, in the German Phylib-method for assessing water quality (SCHAUMBURG et al. 2006, 2011) HOFMANN et al. (2011) is listed as the mandatory and main identification literature. They (HOFMANN et al. 2011) refer to KRAMMER and LANGE-BERTALOT (2004) for the differentiation of the *Cocconeis placentula* varieties. Additionally, they recommend not differentiating the *C. placentula* varieties for now, when assessing the water quality with the German Phylib-method (except *Cocconeis pseudolineata* (Geitler) Lange-Bertalot). Thus, this latter comment could be responsible for just listing *C. placentula* in the intercalibration

exercise in Lake Krossinsee by 14 laboratories, next to the morphological problems. However, when using the German Phylib-method (SCHAUMBURG et al. 2006, 2011) a separation of the varieties is essential, as the indicator values of the *C. placentula*-varieties differ from one another (German water quality assessment software Phylib, version 4.1; October 2012). Thus, using the mandatory German water quality assessment software, the lumping or splitting of *C. placentula* (and varieties) will affect the results of the ecological water quality assessment.

According to KRAMMER and LANGE-BERTALOT (2004) the differentiation of the varieties should be based on the fine structures of the rapheless valves that are visible in the light microscope. Accordingly, the varieties *placentula* and *tenuistriata* can be easily distinguished based on the striae density (KRAMMER and LANGE-BERTALOT 2004). Problems occur with the most common varieties, i.e. *lineata* and *euglypta*, as the striae density and number of areolae per stria of *euglypta* (19–22 striae in 10 µm and 3–5 areolae per stria) are entirely within the range of *lineata* (16–23 striae in 10 µm and 3–10 areolae per stria) (KRAMMER and LANGE-BERTALOT 2004). Additionally, 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 and LANGE-BERTALOT 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) (KRAMMER and LANGE-BERTALOT 2004). Overall, it remains difficult to clearly distinguish the varieties *euglypta* and *lineata* based on these rather vague traits. Thus, these vague descriptions probably explain some of the inconsistent results of the intercalibration exercise with respect to the *euglypta* and *lineata* varieties.

Another problem is the mismatch between pictures and species description in the identification literature for the varieties *euglypta* and *lineata* (e.g. KRAMMER and LANGE-BERTALOT 2004). For example, the valves on Plate 53, Figures 17–18 (page 354) depict *C. placentula* var. *euglypta* in KRAMMER and LANGE-BERTALOT (2004), but show distinctly more than five areolae per stria and a pattern of the longitudinal lines of areolae that can be called irregular or an arrangement in a zigzag pattern (which should both be typical for *lineata* not *euglypta* according to KRAMMER and LANGE-BERTALOT 2004).

Based on the currently inadequate state of information about the taxonomy and ecology of the varieties of *C. placentula*, it is difficult to make recommendations for the practical use (except: document your choices with pictures and text). Further taxonomic and ecological work is necessary for a distinct differentiation of the *C. placentula* varieties.

During the identification of valves from the *N. reichardtiana* and *N. caterva*-complex two participants (A and B) carefully considered the whole combination of characters (see Tab. 1) to identify the valve in Fig. 5. However, they differ in their identification of the valve in Fig. 5 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 about which measuring approach is more appropriate. Ultimately, striae density should be measured 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 (Pl. 31, Fig. 38, striae top left and bottom left). The same valve is also depicted in LANGE-BERTALOT (2001) (Fig. 7, Pl. 33) and also named *N. caterva*. Similarly, valves of *N. reichardtiana* are depicted with gradually changing striae orientation towards the poles in HOFMANN et al. (2011) (Pl. 31, Figure 29 and 30, for both: top striae) and in LANGE-BERTALOT (2001) (Pl. 13 Fig. 26) (gradual 3-times except bottom right). Thus, for identifying *N. reichardtiana* and *N. caterva* the question arises what to do with the mismatch between describing text and 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 partly explains the different approaches of participants A and B (see Tab. 1). Again, it would be key to know how the describing authors interpreted this trait.

There are more mismatches of pictures and species descriptions similar to the *C. placentula*-varieties and to *N. reichardtiana* / *caterva* in the identification literature. For example, the areolae on the dorsal striae of *A. pediculus* should typically be visible in a light microscope according to LEVKOV (2009) and HOFMANN et al. (2011), but are not discernable on most valves labelled *A. pediculus* in HOFMANN et al. (2011; page 785).

Overall, we recommend always using all three: the pictures, the measurable dimensions (e.g. width, length, striae density) and also the verbal species descriptions when identifying diatoms. In case of mismatching pictures and descriptions the applied limnologist should document with text and pictures the approach and reasoning taken to identify the diatom. The taxonomist may correct the current mismatches and avoid them in future by choosing the pictures more carefully or by providing appropriate explanations.

#### 4. Marking identification uncertainties

Another substantial problem became apparent in the groups of *Navicula cryptotenella* / *N. cryptotenelloides*, *N. reichardtiana* / *N. caterva* and the small *Amphora*-species. For example, 37% (Lake Geneva) and 61% (Lake Krossinsee) of the measured 38 valves had a width between the width of *N. cryptotenella* and *N. cryptotenelloides*. Still, only eight of the 37 participants and one auditor labelled the *Navicula cryptotenella* / *N. cryptotenelloides* results with uncertainties by using cf. in either lake. Similarly, part of the *Amphora*-valves in the Lake Krossinsee sample deviated distinctly in their morphology from the descriptions in HOFMANN et al. (2011) and LEVKOV (2009) (see Pl. 1: 5–7, 13–15, 16–17). However, only four laboratories and two auditors indicated any uncertainty about the identified small *Amphora*-valves by using »spec.« and »cf.«.

Overall, it seems very likely that *A. indistincta* and *A. pediculus* have wider morphological ranges than given in the identification literature (LEVKOV 2009, HOFMANN et al. 2011). Thus, for example the valves 5–7 (Pl. 1) (*A. cf. indistincta*) and 13–15 (Pl. 1) (*A. cf. pediculus*) may actually belong to their respective species. However, as long as further taxonomic and morphological examinations do not clarify the dimensional ranges, these and other valves that are outside their species specifications, should be counted with a »cf.« and be documented with a picture.

One reason for submitting results with more certainty than actually present was identified during the workshop of the intercalibration exercise. Several participants reported that they noted deviations of some valves from the descriptions without labelling them with a cf. They reasoned that the German method for implementing the Water Framework Directive (SCHAUMBURG et al. 2006, 2011) only allows a maximum of 5.0% diatom objects that could not be determined (sp., spp.) and/or could not unambiguously be determined (cf.). Otherwise the diatom-indices are assumed to be unreliable and the sample cannot contribute to the water quality assessment. Thus, the participants wanted to avoid the exclusion by indicating ambiguity (using cf.) as little as possible.

As a consequence of this German method it is common practise to pool certain ambiguous taxa with other taxa to avoid labelling objects with »spec.« or »cf.« for the ecological water quality assessment to allow for a seemingly reliable assessment according to some participants. Ultimately, this issue is also a psychological problem. A contractor may increase (or think to increase) the chances of future assignments without further quality control (such as participating in an intercalibration exercise), 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, there is the danger that a low number of ambiguous taxa becomes an alleged quality attribute, especially for the German method. Nonetheless, ambiguous taxa should be labelled with »cf.« or »spec.« also in practise (i.e. by the applied limnologist) and be documented with pictures to facilitate or allow a later comparison and verification of counting results. In principle, quality assessments of counting results are very useful. However, the < 5%-cf cut-off is problematic, as there are samples in practise with less than 5% ambiguous taxa that contain only very few indicative taxa (WERNER and DRESSLER 2007). Thus, they would still be considered reliable according to the German method (SCHAUMBURG et al. 2006, 2007). In this respect a new quality assessment was introduced for lakes in the German method, whereby samples with < 60% indicative taxa are deemed unreliable (SCHAUMBURG et al. 2011). However, it is problematic that they retained the < 5%-cf cut-off in this context, because samples are still deemed unreliable that may have a high abundance (>> 60%) of indicative taxa.

## 5. Ecological references and species identification

Another problem for the applied limnologist is the recommendation in the identification literature to use the ecological preferences of a taxon as an additional criterion for taxa identification for some taxa. For example, in contrast to *A. pediculus*, *Amphora indistincta* is supposed to live in nutrient-poor waters (HOFMANN et al. 2011). This may lead to the preclusion or disregard of certain taxa during the identification process. However, in some cases the ecological preferences are not verified sufficiently. For example, *A. indistincta* and *A. pediculus* lived concurrently in Lake Krossinsee, despite their different ecological amplitude described in HOFMANN et al. (2011).

Diatom assemblages are used to infer the ecological class status of a water body in the routine counts for the European Water Framework Directive. It would thus be a circular argument to use ecological preferences for identifying the diatom taxa, which in turn, are used to determine the water quality. Thus, taxa that are not supposed to occur in a certain water body or lake/river-type should not be disregarded during the identification process.

## Conclusions

The first German intercalibration exercise for benthic diatoms identified the following five major issues and thus generated the subsequent recommendations.

- 1) Use of different identification literature led to different species names of the same taxa.
- 2) Some diatom valves of the intercalibration exercise had morphological dimensions outside the given species ranges to a certain degree in one or more traits.
- 3) Participants and auditors also allocated different names to the same taxa due to ambiguous species descriptions and certain recommendation of traits that should be used to differentiate species in the mandatory identification literature. Also, the pictures do not always correspond to the species description in the literature, which led to further deviation among counting results.
- 4) Some 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 not labelled with a »cf.«.
- 5) Insufficient knowledge about the ecology of some species with misleading recommendations based on this ecology for some diatoms led to different naming.

Recommendation for diatom counts by applied limnologists:

During routine diatom counts in practise we recommend to carefully consider all traits defining a species and to use the mandatory identification literature. Also, for problematic taxa or ambiguous taxa the actually used traits for identification should be specified and the used identification books should be specified to the contracting authority, as it may make a difference if you, for example, only use HOFMANN et al. (2011) or if you can additionally use KRAMMER (1997a, b) when identifying *Encyonopsis* ssp. or *Encyonema* ssp.. Additionally, we recommend a photographic documentation of ambiguous taxa (spec., aff., cf.) for a better comparability of counting results among diatomists and for the possibility of a later adjustment of the results according to new research results. Ambiguous diatom valves should be labelled with »spec.«, »cf.« or »aff.« in the counting results. In the German method for water quality assessment using diatoms (SCHAUMBURG et al. 2011) the relative abundance of 5% ambiguous taxa (or more) should not make the results of a count unreliable. Instead, this measure should be replaced by other measures, such as the percentage of indicative taxa in a sample that contributed to the water quality assessment that should be above a certain threshold.

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, 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 the taxonomist needs to provide basic data, i.e. on how many valves are the given ranges in the identification literature 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). Also important is the method description that was used to generate the measured ranges, espe-

cially of striae density. Surely, these recommendations have often already been followed, but this intercalibration exercise demonstrates that there is room for improvement.

Overall, the recommendations for the applied limnologist may help to reduce the error when assessing the water quality with diatoms. An improved method that already works well will remain an important tool for water managers and may thus be used even more often. Also, the intercalibration exercise revealed the need for funding fundamental diatom research, as the taxonomist can help to further improve the use of diatoms as bioindicators by investigating species specifications.

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