Observations on and typification of *Navicula difficilis* Grunow and its transfer to the genus *Humidophila* R.L.Lowe & al. (*Diadesmidaceae, Bacillariophyta*)

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A large number of diatom taxa described by Albert Grunow (1826–1914) were published in Van Heurck's Atlas and Synopsis Diatomées de Belgique (Van Heurck 1880-1885), usually only by one or a few line drawings and a figure caption (Figs 1, 2). These drawings were cut-outs from Grunow's personal collection currently kept at the herbarium of the Naturhistorisches Museum (W) in Vienna, Austria (Figs 1, 3). Each drawing in the Grunow collection is accompanied by handwritten notes, mostly added by Grunow himself, indicating which sample was used for the drawing (Figs 1, 3). Although the original drawings that were assembled as plates for Van Heurck's Atlas (1880–1885) are currently conserved in the Van Heurck collection (Meise Botanic Garden, Belgium; BR), the remainder of the cut-outs (Fig. 3) and additional drawings at W are very valuable for those seeking to typify Grunow's many names. They contain the necessary information (e.g., Grunow sample number, occasionally locality, collector, and collection number) to retrieve the type material that is also kept in the Grunow collection at W. More information on the sample can be found in Grunow's accession books (also at W), which catalogue his entire sample collection (Fig. 4, entry for Grunow sample 1854). It is worth noting that Grunow's sample numbers are not necessarily collection numbers, as he received samples from numerous collaborators with their own numbering systems but represent an organisational system for his samples. Together, Van Heurck's Atlas and Grunow's drawings, cut-outs, accession books, and samples, facilitate the analysis of species described by him, though this requires a considerable time commitment, effort in the laboratory-and some good fortune-to succeed.

Navicula difficilis Grunow is illustrated only by two small drawings (Fig. 1) in Van Heurck (1880, pl. XII [12]: figs 16, 17). The figure caption (Fig. 2) did not mention any additional morphological details other than that fig. 16 was taken from a dry preparation ("préparé à sec") and fig. 17 from a balsam preparation ("préparé au baume"). Despite the lack of a description, the name is valid from 1880 by the provision of analytical figures (see ICN Art. 38.7 "For the purpose of Art. 38.5, prior to 1 January 1908, an illustration with analysis (see Art. 38.9 and 38.10) is acceptable in place of a written description or diagnosis."; Turland & al. 2018). Plate XII of the Atlas includes several Navicula taxa that were first transferred to Anomoeoneis Pfitzer (e.g., in Cleve 1895) and later to Brachysira Kützing (Round & Mann 1981, Hartley & al. 1986, Lange-Bertalot & Moser 1994). As Grunow's drawings of N. difficilis showed some similarity with these Anomoeoneis and Brachysira taxa, it was generally accepted that N. difficilis should also be transferred to Anomoeoneis. For example, Hustedt (1930: 265) suggested that N. difficilis showed some similarity to Anomoeoneis zellensis var. linearis Hustedt ["Vielleicht gehört hierher auch Navicula difficilis Grunow (V.Heurck, Syn. Taf. 12, Figs 16, 17)"]. Hustedt (1959: 753, no fig.) later recombined the species as Anomoeoneis zellensis f. difficilis (Grunow) Hustedt, recognizing however that the taxon is quite distinct and cannot be confused with any other ["Sonst wie die Art. Nav. difficilis Grun. kann auf keine andere Art bezogen werden."] Hamilton (in Hamilton & al. 1992: 29, table 4) finally transferred the taxon to Brachysira, probably based on Hustedt's conclusions, as B. zellensis f. difficilis (Hustedt) P.B.Hamilton.

During a revision of European *Brachysira* taxa, the morphology of the type material for *Navicula zellensis* Grunow (Grunow 1860: 521, pl. III [3], fig. 34 a-d) and *N. difficilis* were reinvestigated. In addition, the file of the original Grunow drawings for *N. difficilis* in the Grunow collection indicated that only Grunow sample 1854 was used as basis for these drawings (Figs 1, 2). Although there are two microscope slides in **W** made from sample 1854, the unmounted material for this sample is no longer present in **W** but was discovered in a small set of samples in the Van Heurck collection (**BR**). The entry in Grunow's accession books unfortunately did not yield more information other than that the sample was taken from *Calothrix* and the liverwort *Dumortiera hirsuta* (Swartz) Nees (Fig. 4), the latter is commonly found in Central America (Costa Rica, Panama; Forrest & al. 2011).

Here we present detailed observations on *N. difficilis* from a slide prepared from Grunow sample 1854, kept in **BR**, using light and scanning electron microscopy (SEM). Sample 1854 is also **designated as lectotype** for this taxon in accordance with ICN Art. 9.3 (Turland & al. 2018).

The morphology of these specimens also indicates that the species clearly does not belong to the genus *Brachysira*, but should be transferred to the genus *Humidophila* R.L.Lowe & al.

It should be noted that *Navicula difficilis* Pantocsek (Pantocsek 1892: 66, pl. 41, fig. 560), a Tertiary fossil diatom, is a later homonym and is thus illegitimate. The name was, however, replaced by Cleve (1895) as *Navicula hennedyi* var. *difficilis* Cleve.

Humidophila difficilis (Grunow) Van de Vijver, Ector & T.M.Schuster comb. nov. (Figs 5–19)

- Basionym: Navicula difficilis Grunow (in Van Heurck), Synopsis des Diatomées de Belgique, Atlas, pl. XII [12]: figs 16, 17, 1880; non Navicula difficilis Pantocsek 1892, Appendix, pl. 41: fig. 560, nom. illeg., subsequently replaced by Navicula perennis Pantocsek 1905, Appendix, p. 82, pl. 41, fig. 560.
- Lectotype designated here: slide BR-4633, prepared from Grunow sample 1854, original dried material present in the Van Heurck collection (**BR**).
- Isolectotypes: slide W0127019 and slide W0127020, both from Grunow sample 1854 (W).
- Description: Frustules rectangular in girdle view (Figs 5, 17). Girdle composed of several broad, open copulae bearing one row of transapically elongated narrow, slit-like areolae (Fig. 17). Mantle rather large with broad abvalvar hyaline edge. Advalvar edge at the valve face/mantle junction covered by dense irregular siliceous pattern bordered by a series of small, rounded ridges. Valves linear to slightly linear-lanceolate with parallel to weakly convex margins and non-protracted, broadly rounded apices (Figs 6–16). Valve dimensions (n=10): length 19–24 μm, width 3.5–4.5 μm. Axial area narrow, linear. Central area small, rounded. Raphe straight, filiform with straight, inconspicuous central raphe endings. Terminal raphe fissures absent (Fig. 18). Internally, terminal raphe endings internally terminating on a weakly raised central nodule. Valve surface structure obscured by an irregular network of ridges forming a reticulate pattern covering the striae (Fig. 18). Striae parallel, narrow, composed on one, transapically elongated areola, ca. 45 in 10 μm, externally hardly visible due to the siliceous network. Internally, areolae covered by porous hymenes (not shown). Mantle areolae separated from valve face areolae by short ridges (Fig. 19, arrows).

The morphology of the specimens in the Grunow sample confirms that the species should be transferred to the genus *Humidophila* in accordance with the definition of the genus in Lowe & al. (2014: 352). The only feature that is not included in the original description is the presence of the reticulate pattern of the valve surface. Werum & Lange-Bertalot (2004) illustrated a similar taxon, originally described by Krasske (1932: 114, figs 3, 18) as *Navicula irata* Krasske, which was first

transferred to the genus *Diadesmis* as *D. irata* (Krasske) Gerd Moser, Lange-Bertalot & Metzeltin (1998: 146) and later to the genus *Humidophila* as *H. irata* (Krasske) R.L.Lowe & al. (2014: 358). The latter presents a similar irregular siliceous pattern on its valve face but has a shorter raphe and a less complex reticulate pattern compared to *H. difficilis*. *Humidophila difficilis* clearly does not belong to the genus *Brachysira* as all *Brachysira* species possess striae composed of several areolae, in contrast to *H. difficilis* with only a single, large, slit-like areola per stria.

Neither the entry in Grunow's accession book, nor the slide labels reveal where the sample was collected. However, the associated diatom flora (Fig. 4) indicates an origin from Central America. As stated above, the liverwort species from where the sample was taken is usually found in Costa Rica and Panama, although its range includes Europe and Asia, where it is said to be less frequent (Forrest & al. 2011). One of the more frequent species in sample 1854, is *Humidophila arcuatoides* (Lange-Bertalot) R.L.Lowe & al., a species originally described from Costa Rica (Werum & Lange-Bertalot 2004), although initially identified as *H. arcuata* (Heiden) R.L.Lowe & al. by Rumrich & al. (2000). Therefore, we speculate that Grunow sample 1854 was collected in Central America, possibly Costa Rica.

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Figs 1–19. *Humidophila difficilis* (Grunow) Van de Vijver, Ector & T.M.Schuster, *comb. nov.* **Fig. 1.** Original drawings of *Navicula difficilis* Grunow published in Van Heurck (1880: pl. XII: figs 16, 17). **Fig. 2.** Original caption in Van Heurck (1880) for pl. XII: figs 16, 17. **Fig. 3.** Original cut-out remainder of figure 17 from Van Heurck (1880: pl. XII) showing sample number 1854 and indicating '*Dumortiera*'. **Fig. 4.** Entry in Grunow's accession book for sample 1854. **Figs 5–16.** Cell diminution series cycle of *H. difficilis* (Grunow) Van de Vijver, Ector & T.M.Schuster *comb. nov.* showing the lectotype material (Grunow sample 1854). Fig. 5 shows a frustule in girdle view. **Fig. 17.** SEM external view of an entire frustule showing the open girdle bands and the typical ornamentation of the valve mantle. **Fig. 18.** SEM external view of an entire valve showing the raphe and the striae obscured by the typical reticulate pattern. **Fig. 19.** SEM internal view of an entire valve with the central nodule, the slit-like, transapically elongated areolae and the low ridges separating mantle areolae from the valve face areolae (arrows). Scale bars = 10 μm.