

*Diploneis lecohuiana* sp. n., *D. fereparma* sp. n., and  
*D. parma* Cleve: Rare diatoms (Bacillariophyta) in  
Central European freshwater

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Appeared in  
Nova Hedwigia, Beiheft 146 (2017), pp. 32-42  
(Festschrift issue for René Le Coahu)

## Abstract

Numerous freshwater *Diploneis* species have been misidentified in the past, thus masking the high species-richness of populations by inadequately few species names. A striking example is *Diploneis parma* Cleve. *D. parma* sensu stricto is in fact a rare species known from Finland and Japan. It has recently also been encountered in a pond in a Central European region of medium altitude mountains of volcanic origin. Presumably equally rare are the similar taxa *D. Lecohuiana* and *D. fereparma*, both originating from oligotrophic Alpine lakes. They are proposed here as species new to science.

## Keywords

Freshwater, *Diploneis*, species-diversity, rarity, frequency, abundance, Europe, Alps.

## Introduction

Until recently it had been part of the common conception of the genus *Diploneis* that it exhibits its highest diversity mainly in marine habitats with only few species living in freshwater. This conception may have to be revised as recent studies begin to reveal; see e.g. Lange-Bertalot & Fuhrmann (2017, in preparation), Jovanovska et al. (2015) concerning the ancient lakes Ohrid in Macedonia and Hövsgöl in Mongolia, Kulikovskiy et al. (2015) concerning Lake Baikal. To put it briefly, the main impediment towards doing justice to the diversity of the genus *Diploneis* in freshwater habitats seems to have been its comparative rarity in a conjunction of two senses: most of its taxa occur neither frequently (i.e. not encountered in many places) nor abundantly (i.e. not encountered in large numbers). In this paper we present three taxa that are rare in this sense.

By way of a short historical survey, let us begin by mentioning that Hustedt (1930) distinguished no more than ten taxa for Central Europe. The last comprehensive treatise on *Diploneis* has been Hustedt (1937), including fifteen taxa which inhabit freshwater ecosystems of North and Central Europe. His conceptions of freshwater species have been perpetuated since then, i.e. over a period of eighty years without thorough actualisation. Cleve-Euler (1953) added new taxa from Scandinavia yet mainly extending extant species. Patrick & Reimer (1966) report fourteen taxa for North America, including many taxa from brackish habitats. More recently Germain (1981) counts eight taxa in South-West France. Krammer & Lange-Bertalot (1986) list seventeen freshwater and seven brackish water taxa for almost the entire Europe. In the Red List of endangered species in Germany and neighbouring countries from 1996 (Lange-Bertalot 1996) this number had not increased. In their recent atlas of diatoms, covering numerous large and small watercourses in the rather large monitoring area of the Rhône-Alpes region in France, Bey & Ector (2013) end up with only five established plus two non-identified species of *Diploneis*. Their studies pertain not only to the Rhône catchment area but also cover part of the Loire river area with its tributaries from the Eastern Central Massif mountains. Even though the geological and ecological variability of the investigated sites is thus very high, their survey results in a surprisingly low number of *Diploneis* taxa. This is so despite their taking into consideration new taxonomical results concerning the genus that have become available only after the appearance of the comprehensive "Süßwasser-Flora" (Krammer & Lange-Bertalot 1986). Since then new freshwater taxa of *Diploneis* had not only be

described from South America, Asia, and the paleotropical Pacific island of New Caledonia, but likewise from Europe, such as *Diploneis ovalis* ssp. *arctica* Lange-Bertalot in Lange-Bertalot & Genkal 1999, *D. krammeri* Lange-Bertalot & Reichardt 2000, *D. fontium* Reichardt & Lange-Bertalot 2004, *D. fontanella* Lange-Bertalot in Werum & Lange-Bertalot 2004, and *D. separanda* Lange-Bertalot in Werum & Lange-Bertalot 2004.

Over many decades the European watercourses have suffered from strong eutrophication and organic pollution. Since *Diploneis* is rather intolerant to such conditions the genus has become increasingly infrequent in the huge rivers. Indeed, these rivers are nowadays widely devoid of *Diploneis* species. Where the ecology is conducive to *Diploneis* taxa, most taxa of the genus occur inabundantly in a given locality and are thus easily overlooked. Where specimens are found they appear to be too few to merit closer examination and often find themselves listed under the catch-all names known from the literature.

*Diploneis parma* Cleve (from Lake Lojo in Finland) is an historical example of underrating the rarity of *Diploneis* species. In Hustedt (1937, fig. 1066) it is represented by two line drawings, one of them (the one on the right) in fact depicting a different taxon, viz. *D. hinziae* Lange-Bertalot & Fuhrmann 2016. Hustedt's flora may have been the basis for many subsequent further misidentifications. For all we know, *D. parma* is a much rarer taxon than the literature suggests; see our discussion below.

Two further rare species of *Diploneis* are here proposed as new to science. Both may serve as examples of how a closer examination of catch-all *Diploneis* taxa like *D. parma* (*sensu* Hustedt) coerce a differentiation. The first, *D. fereparma*, is -- as the name is meant to suggest -- indeed quite similar to *D. parma* in the strict sense, i.e. that of Cleve, and may have been identified as such in the past. The second, *D. lecohuiana*, is similar to the first (and also to *D. elliptica*). In both cases we point out below characteristics, clearly visible in LM view, that separate the taxa discussed.

The three species described and discussed in this paper originate from stagnant oligo- to slightly eutrophic waters. The two new species are so far only known from the type locality and occur only in few specimens within that locality. For all we know, they are, in this sense, very rare.

## Materials and Methods

Our observations on taxonomically critical *Diploneis* populations have been carried out during fifteen years since 1999. Samples have been taken sporadically over a longer period from 1982 up to 2014. Colleagues, friends and former students have collected from mainly stagnant freshwater habitats in various regions of the Holarctic. We have also taken fresh looks at old type material used by P.T. Cleve, Hustedt, Petersen and others. In the course of time, data of questionable *Diploneis* specimens in slides were recorded or marked with coordinates. Gradually we have thus accumulated a basis for rewarding comparative taxonomical study which allows, in particular, informed estimates of the relative rarity of taxa.

The following light microscopes were used: Leitz Dialux, Leitz Diaplan, Zeiss Standard Universal, and Nikon Eclipse E600. All micrographs were taken with apochromatic lenses of aperture no less than 1.3. Brightfield illumination and digital photographic equipment (ImagingSource) was used throughout. The material prepared by us (*D. parma* from Westerwald and *D. fereparma*) was cleaned by standard methods (see e.g. Hustedt 1937) and mounted in Naphrax with a refractive index of ca. 1.7. The slide from the Krasske collection (*D. Lecohuiana*) is a Styrax mount; no further information as to preparation is available.

We regret that there are no studies of the fine structure of the two new taxa discussed here -- as frequently is the case with rare diatoms. For *D. Lecohuiana* we have so far only a historical slide from the collection of Georg Krasske; similarly, for our study of *D. fereparma* only LM slides were available. However, differentiating features appear so clearly in LM view that identifications can be made with confidence. For a comparison with the findings in Idei & Kobayasi (1988) SEM studies of specimens of *D. parma* from the Westerwald region in Germany would be very desirable. However, the taxon occurs so rarely in our samples that all attempts at finding specimens for SEM observation have so far proved futile.

## Results

*Diploneis fereparma* sp. nov.

Figures 10--15

Valves broad-elliptical with bluntly cuneated ends, roughly resembling in shape *D. parma* in the strict sense. Length 33--41  $\mu\text{m}$ , breadth 22--25  $\mu\text{m}$ ; ratio length-to-breadth 1.65--1.75. Raphe filiform with indistinct distal ends and distinctly expanded proximal ends lying in a narrow axial area of 1.5--2  $\mu\text{m}$  width. Central area is 4--5  $\mu\text{m}$  wide, rhombical, forming short appendices (horns, visible by appropriately focusing). The lanceolate canal system is 6--9  $\mu\text{m}$  broad around its central inflation. The entire axial system is slightly to moderately arcuate from pole to pole. Flanking apical depressions appear with appropriate focusing on the internal valve side. Striae radiate throughout but more strongly so closer to the valve ends, 12--13 in 10  $\mu\text{m}$ . Areolae of the longitudinal canal doubled around central nodules, then single towards the valve ends. Other areolae of the valve face and mantle uniseriate and separated by very narrow virgae.

**Differential diagnosis:** *Diploneis parma* in the strict sense of P.T. Cleve (i.e. excluding all misidentifications from Central Europe) possesses the same valve outlines. It is mainly distinguished by a conspicuously higher density of striae and areolae, 15--17 and 18--19 in 10  $\mu\text{m}$  respectively (vs. 12--13 in *D. parma* for both parameters). As a second distinguishing characteristic *D. parma* has a differently shaped central area of lower width; it lacks the arcuate inflation of the central part of the canals (compare Figs 1--9 to 10--15).

Also resembling species is *Diploneis lecohuiana* sp. n. with a somewhat similar valve shape but distinguished consistently by its rhombic-elliptical outline and less broadly cuneated ends. The central area of *D. lecohuiana* is wider in comparable cell cycle stages, and it is simply elliptical instead of rhombic.

The valves of *Diploneis elliptica* are simply elliptic, i.e. neither broad- nor rhombic-elliptic. They also differ from *D. fereparma* by a coarser pattern of striae and areolae, 10 or less in 10  $\mu\text{m}$  for both parameters (see three valves depicted from the type slide in Lange-Bertalot & Reichardt 2000, p. 668, figs 7: 1--3). The sternum, raphe and adjacent canals appear almost linear rather than arcuate.

**Holotype** (designated here): Slide Seen-205 in Coll. Lange-Bertalot, Naturmuseum Senckenberg, Frankfurt am Main (FR); represented by Figs 10--12.

**Type locality:** Lake Weitsee near Reit im Winkel, Upper Bavaria, Germany, 753 m a.s.l., eastern shore of the lake, epilithic; leg.

Dr. G. Hofmann, August 2000.

**Etymology:** Latin *fere* means almost, here indicating the resemblance to *D. parma*.

**Distribution and ecology:** As yet observed with few specimens exclusively from a single sample, epilithic in the type location, absent from other samples from this or other lakes in the region. Possibly misidentified as *D. elliptica* in former records from Bavarian lakes. Weitsee, close to the Austrian border, is a comparatively small and shallow lake, rich in calcium bi-carbonate, with drinking-water quality. It belongs to a group of Bavarian lakes that have recently been put under the control of state authorities monitoring water quality. There may thus be an increased chance to find more *D. fereparma* specimens in the future.

***Diploneis Lecohuiana* sp. nov.**

Figs 16--26

Valves rhombic-elliptical, with largest stages of the cell-cycle having a tendency towards a broadly elliptical-lanceolate outline. Broadly cuneated and finally obtusely rounded ends. Length 34--56  $\mu\text{m}$ , breadth 20--26  $\mu\text{m}$ . Ratio length-to-breadth 1.3--2.2. Raphe filiform with slightly expanded proximal and indistinct distal ends, lying in a 2--3  $\mu\text{m}$  wide axial area. Central area 4.5--6  $\mu\text{m}$  wide with elliptic shape in the apical axis, forming short appendices (horns). Longitudinal canal narrowly lanceolate but with its central part arcuate around a central nodule of 7--10  $\mu\text{m}$  diameter. The entire axial area is straight or at most slightly arcuate from pole to pole. Flanking apical depressions parallel to the raphe are visible as faint lines by focusing correctly (Figs 17, 19). Striae radiate throughout, 11--13 in 10  $\mu\text{m}$ . Areolae of the canal system doubled in the arcuate part around the central nodule, gradually merging to single towards the ends. Other areolae of the valve face and mantle uniseriate and crossed by undulate apical lines, 10--12 in 10  $\mu\text{m}$ .

**Differential diagnosis:** *Diploneis elliptica* differs from *D. Lecohuiana* by the former's simple elliptic valve outline without any tendency towards a broadly rhombic-elliptic shape. Margins taper gradually from the middle to obtusely rounded ends. In specimens of similar size the distal parts of *D. Lecohuiana* are more cuneately shaped than in *D. elliptica*. The density of striae and areolae is significantly higher (above 10 in 10  $\mu\text{m}$ ) in *D. elliptica*.

*Diploneis fereparma* sp. nov. and *Diploneis parma* differ from *D.*

*Lecohuiana* by more compact outlines and by a higher density of striae of areolae.

*Diploneis ostracodarum* (Pantocsek) Jovanovska et al. 2013 possesses a similar valve outline but a considerably coarser system of striae and areolae, 7--8 and 6--10 (instead of 11--13 and 10--12) in 10 µm respectively.

**Holotype** (designated here): Slide B IV 58 (1959) in Coll. Georg Krasske, Museum für Naturkunde im Ottoneum, Kassel, Germany; represented by Fig. 17.

**Type locality:** Schwarzsee / Lai Nair (''black lake''), near the village Tarasp, Engadin, Kanton Graubünden, Switzerland, 1546 m a.s.l.; leg. Fr. Meister, July 1919.

**Etymology:** Dedicated to Prof. René Le Cohu on the occasion of his 80th birthday.

**Distribution and ecology:** The diatom assemblage from Lai Nair consists of both calciphilous and acidophilous taxa. This may be due to the contact of the lake to neighbouring peat bogs. From the latter group *Eunotia arcus* (in the strict sense) is the most abundant example. Disregarding its lower striae- and areolae density, the new taxon has probably been mistaken for *D. elliptica* in the past. Thus, e.g., Krammer in Krammer & Lange-Bertalot (1986) identified figs 108: 1--2 both as *D. elliptica* from Central Europe while the latter is likely to represent *D. lecohuiana*. On closer examination, and from the perspective of present day taxonomy, three of the five resembling specimens depicted by Krammer in figs 108: 1--4 do not represent *D. elliptica*.

***Diploneis parma*** P.T. Cleve 1891

Figs 1--9

Valves broadly elliptical, sometimes weakly rhombic-elliptical. Ends obtusely, more or less cuneately rounded. Length 26--46 µm, breadth 17--27 µm; length-to-breadth ratio ca. 1.3--1.7, i.e. rather low and with a low variance in comparison to other taxa of the genus. Raphe filiform, curved and together with the internal flanking depressions gradually expanding to the central ends. Distal ends appear indistinct in LM. Central area ca. 3--4.5 µm wide, variable in shape from narrowly elliptic to moderately broad-elliptic, sometimes slightly constricted dependent on focus. Appendices (horns) are present as proximal raphe ends protude into the central area. Canal

system lanceolate, ca. 6--8  $\mu\text{m}$  broad proximally, marked by two, rarely three canal areolae set off from the striae areolae. Striae radiate throughout, 15--16 in 10  $\mu\text{m}$  in specimens from Finland and Germany while 12-14 in 10  $\mu\text{m}$  in specimens from Lake Ladoga (Russia) and from Japan. Areolae may appear at first uniseriate in LM but can be resolved as oppositely biseriata with appropriate focusing, 18--20 in 10  $\mu\text{m}$ . This can be confirmed by SEM and TEM observations (see Idei & Kobayasi 1988, pl. 1--3). The areolae become alternating biseriata in the marginal parts.

**Distribution and ecology:** *Diploneis parma* is a very rare species among freshwater *Diploneis*. Many supposed records have turned out to be misidentifications. In ascertained habitats its abundance is very low, including in syntype locations and in the lectotypified population from Lake Lojo, Finland. No record from Central Europa could be confirmed prior to this study. It was thus surprising when two specimens of the true *D. parma* were identified in samples from the Westerwald region, medium altitude volcanic mountains in Germany. They conform to the Finnish rather than the Japanese and Russian specimens. The habitat is a moderately eutrophic, oligosaprobic artificial lake hosting an uncommon diatom assemblage with rare species. A shallow small eutrophic lake in Finland has also been mentioned by Idei & Kobayasi (1988) as one of the locations where *D. parma* has been collected in recent time.

**Taxonomical comments:** Specimens Russia (Lake Ladoga) and from Japan, as shown in Idei & Kobayasi (1988), are distinguished by a lower striae density (see above). It can thus not be excluded that they represent an infraspecific separate taxon. It should also be mentioned that other authors have counted striae near the valve margin as opposed to the more usual counting along the median axis. After the recognition of *D. hinziae* Lange-Bertalot & Fuhrmann 2016 and further resembling taxa to be published soon we expect that *D. parma* will no longer give rise to misidentifications.

## Discussion

On close examination *D. parma* turns out to be a very rare diatom and apparently restricted to the northern (boreal) zones of Eurasia. Even in these regions it occurs infrequently and in very low abundance which accounts often for misidentifications. (Thus, for example, on finding only a single specimen of what we now would clearly identify as *D. parma* in a lake of low alkalinity/conductivity in northern Finland, Lange-Bertalot &

Metzeltin (1996, fig. 43: 6) conjectured *D. elliptica*.) The discovery of specimens of the true *D. parma* in Central Europe 125 years after its first description is thus surprising and remarkable from the biogeographic point of view.

*Diponeis fereparma* resembles the true *D. parma* Cleve more than what Hustedt (1937, reprinted 1959, fig. 1066) identified as *D. parma* from the Davos Alps in Switzerland. The latter is now an independent taxon, *D. hinziae* Lange-Bertalot & Fuhrmann 2016. *D. hinziae* is in fact easy to distinguish by its different valve outlines in the cell division cycle and its considerably lower striae and areolae density. But while *D. hinziae* occurs frequently and abundantly in Europe, *D. fereparma* has so far been found only in single location.

*Diploneis lecohuiana* likewise appears to be a very infrequent taxon. It is unlikely to be confused with the aforementioned taxa, though it may have been misidentified in the past as *D. elliptica* (Kützing) Cleve. Specimens are moderately abundant in a sample taken by F. Meister in the late summer of 1919 in a lake near Tarasp, a village in the Engadin Valley, Swiss Alps. The lake is probably in contact with neighbouring bogs and fens, since it hosts diatom species tolerating both moderately high and low pH values and water conductivity. Although *D. lecohuiana* may occur even now, a century later, in fair abundance in appropriate habitats, we were unable to encounter it in two decades of research focused on freshwater *Diploneis*. Krammer in Krammer & Lange-Bertalot (1986, fig. 108:2) illustrates a single specimen from southern Germany under the collective name of *Diploneis elliptica*.

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**Captions for four tables**

**Figs 1--9.** *Diploneis parma* from three habitats. 1--3. Same valve at different focus, only specimen found in a voluminous sample from dystrophic Julma Ölkky ("Devil's Lake"), Finland, near the Polar Circle. 4--6. Two specimens from Ala Kuurtonen, another slightly dystrophic lake in Finland. 4--5. Same valve at different focus. 7--9. Two specimens from a slightly eutrophic pond in the volcanic mountains Westerwald, Germany, with close-up of marginal part in 8 at doubled magnification. Scale bar 10  $\mu\text{m}$  (except for close-up of 8.)

**Figs 10--15.** *Diploneis fereparma*. 10--12. Both valves of the same frustule at different focus. 13. A larger cell-cycle stage. 14--15. Both valves of a smaller stage, all from the type locality, Weitsee, Bavarian Alps, limestone. Scale bar 10  $\mu\text{m}$ .

**Figs 16--20.** *Diploneis lecohuiana*, select specimens from type locality near Tarasp, Swiss Alps. Close-up (doubled magnification) of marginal part in 16, displaying uniseriate mantle areolae. Scale bars 10  $\mu\text{m}$ .

**Figs 21--26.** *Diploneis lecohuiana*, more specimens from type locality. Scale bar 10  $\mu\text{m}$ .







