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Using diatom analysis in paleoecology: A case study from the *du Loup Bourrou* spring at the Bibracte archaeological site in France

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BOTANICKÝ ÚSTAV AV ČR v.v.i.

OTEVŘENÁ VĚDA
AKADEMIE VĚD ČR

Abstract

This work is one part of a multidisciplinary study led by a team of Czech archaeologists. Its goal is to reconstruct the aquatic biotope of the *du Loup Bourrou* spring at the Bibracte site on Mont Beuvray hill in Burgundy, France. The author took part in this multidisciplinary study by conducting a diatom analysis, which involved identifying the species of diatoms present in samples and using them as bioindicators to obtain data about substrate characteristics and environmental quality conditions at Mont Beuvray in the past.

This work presents the results of an analysis of a soil profile taken at the site of a spring. This sample reached a depth of 29 cm and was dated to a period stretching from 1747 to 1999. The outcomes of the analysis were the creation of a palaeoecological diatom diagram and six photographic plates presenting chosen identified species.

In analyzing the samples, the author identified 66 species from 28 genera. Substrate characteristics have not changed significantly over the course of time. The site is characterized by oligotrophic to dystrophic conditions. The water was circumneutral to slightly acidic. Electrolyte levels were always low, but the water was highly oxygenated. The identified species of diatoms indicate siliceous soil, which is consistent with the silica-rich granite bedrock. The number of species in the samples declined from 1747 to 1999, from an initial 36 species to 23, a 38% decrease. This fact demonstrates that a clear change in environmental conditions occurred at the spring in this period.

In recent years, interest in the reconstruction of ecosystems and aquatic habitats has grown, and this study contributes to their understanding. Further research on the *du Loup Bourrou* spring should focus on determining the causes of the observed decline in diatom abundance. Gaining an understanding of how water quality and the distribution of water bodies evolved in past centuries is critical for developing effective and environmentally friendly water management methods in the present.

Keywords

Diatom analysis, diatoms, paleoecology, *du Loup Bourrou* spring, Bibracte, substrate characteristics

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1. Introduction

Studying water, water quality, and in particular the source of water pollution is fundamental for guiding today's society toward more environmentally friendly and less risky water management practices. The increased attention being paid to water explains the great number of methods that have been developed to study it. It is, however, impossible to determine the degree of anthropogenic impact on the environment, or more specifically on water, if we do not know about the environment's past, when humans had only minimal or even no impact on the environment. Therefore, assessing historical water quality is essential if we wish to better evaluate data obtained today. Diatomology is one of few disciplines that provides a way for gaining an overview of how water bodies developed over time and about past environmental conditions.

In the Czech Republic, diatoms have been used as bioindicators since only the late 20th century. Many studies examining diatoms focus on studying geological eras and the transitions between them and above all concentrate on analyzing aquatic biotopes. Rarely are diatoms used to study more recent historical periods and human settlements.

However, in the present study, diatoms are used to determine the environmental changes that have occurred since the mid-18th century at the *du Loup Bourrou* spring, which is located at the site of the former Bibracte oppidum in Burgundy, France. The spring area was altered, most likely paved over, and studying older sediments from the time of the Celtic settlement of the site would be possible only after removing the layers of stone. The aim of the larger multidisciplinary palaeoecological study of this site, of which the present diatom analysis is a part, is to determine past changes in environmental conditions there since the alteration in its structure.

2. Theoretical introduction

2.1. Diatomology

Diatoms belong to the SAR supergroup, the Stramenopila (syn. Heterokonta) infrakingdom, and the Bacillariophyta (syn. Diatomea) superclass (Adl et al., 2012). Diatoms are the most widespread group of algae and one of the most abundant groups of living organisms on Earth. According to a 1996 estimate (Mann & Droop), the total number of diatom species is 200,000. A more recent study, however, indicates that the real number is probably somewhere around 100,000 (Mann & Vanormelingen, 2013). Thus far, only a mere 12,000 species have been described (Guiry, 2012).

Diatoms primarily live in aquatic habitats. They are a fundamental component of phytoplankton (communities of cyanobacteria and algae living freely in water) and of phytobenthos (communities of cyanobacteria and algae attached to bottom surfaces in standing and running water). Diatoms can be found in fresh, salt, and brackish water (Kalina & Váňa, 2005). They also live in terrestrial ecosystems and are capable of surviving aerophytically as components of aeroplankton (Tormo, Recio, Silva & Muñoz, 2001).

Diatom size ranges from 4.2 to 653 µm (Snoeijs et al., 2002). One of their defining features is their silicone shells, known as frustules. This inorganic part of the organism is made up of the slightly larger epitheca and the smaller hypotheca, which fit inside of each other like a box and lid and thus form a solid cover. In most substrates, these shells are preserved for many centuries and can therefore be used as bioindicators when studying past conditions at a site.

Diatoms were first used in environmental archaeology in the 1940s (Miller & Florin, 1989), and the incorporation of diatomology into archaeological studies was primarily initiated by Scandinavian research teams (Battarbee, 1988). Diatoms have been used more extensively for several decades now. However, other methods than diatom analysis are often applied to them (Cameron, 2013).

2.2. Bibracte

Bibracte, an oppidum and the capital of the Aedui, a Celtic tribe, was located on Mont Beuvray in what is today Burgundy, France. Its origins date to the second century BCE, and it played a critical role in the society of that time (Romero, 2006).

Before 150 BCE, the Aedui had signed a treaty of alliance with Rome, probably for economic reasons. The treaty was all the more significant because if Rome maintained relations with other Celtic tribes, they were unfriendly relations. Moreover, two of the most important figures in the Gallic Wars visited Bibracte: Julius Caesar and Vercingetorix. The fact that the Aedui maintained good relations with Rome, however, does not mean that they had completely broken away from the Celts. When the first clashes between Caesar and the Celtic tribes occurred, it was the Aedui who forced the Roman army to withdraw (Romero, 2006).

Nonetheless, 15 BCE marked the beginning of the end of this important town, as the aristocracy gradually moved to the more modern newly established Roman town of Augustodunum, today's Autun (Romero, 2006). Bibracte thus ceased to exist and disappeared from historical sources. Until the end of the 19th century it was even believed that the oppidum that Julius Caesar mentions in his writings was located on the site of today's Autun and that Bibracte and Augustodunum were two names for the same settlement (Lemarchand, 2017).

Written sources from 1220, however, mention the "du Beuvray" annual market, which was held every year on the first Wednesday of May on the site of the former La Chaume oppidum. The du Beuvray market was held until the early 20th century (Romero, 2006). Around 1400, a Franciscan monastery was established on Mont Beuvray (Bibracte.fr), perhaps on the site of an older Benedictine estate. The monastery was inhabited by a small community of Franciscans, who probably established gardens and fields, changing the way land was managed on this hill. The altering of the springs around the monastery, including the *du Loup Bourrou* spring, was connected to these activities.

3. Methods

3.1. Field methods

Soil core sampling was conducted at the *du Loup Bourrou* spring site on 17 August 2020 by Petra Goláňová's research team. Two profiles were taken. Profile P1a reached a depth of 36 cm. Profile P2a, the subject of this study, reached a depth of 29 cm.

Soil profile P2a was then divided into 12 samples based on the following depths: 0–3; 3–5; 5–7; 7–9; 9–11; 11–13; 13–15; 15–17; 17–19; 19–21; 21–23; 23–29. At the upper and lower boundaries, organic materials were sampled for radiocarbon dating. The samples were labeled and then sent to the Institute of Botany of the Czech Academy of Science in Brno, where they were further processed.

3.2. Preparing permanent slides

Permanent slides were prepared in June 2021. Organic compounds were removed from the samples following van der Werff's (1995) method using a 30% solution of hydrogen peroxide. To mount the samples on permanent slides for diatom analysis a synthetic resin called Pleurax was used. This brown viscous material that results from the reactions between phenol and sulfur in the presence of sodium carbonate anhydrous (Vojíř, 2017) is used because it has a high refractive index, which is necessary for ensuring ample contrast between diatom shells under a microscope (Bešta, 2007).

3.3. Identifying diatoms based on shells

The mounted diatom slides were observed using an Olympus BX53 optical microscope with an immersion lens at a magnification of 1000x. To accurately measure the length and width of the diatoms and to photograph them, a digital camera was attached to the microscope.

To identify the species, nine identification reference books were used: Krammer & Lange-Bertalot (1986, 1988, 1991a, 1991b), Krammer (2000, 2002, 2003), Lange-Bertalot (2001), and Lange-Bertalot et al. (2017). To identify species, the shape of the diatoms, the direction and length of their striae, the presence of raphes, the length and width of each organism, specific formations on the thecae, and so forth had to be carefully examined. A semi-quantitative scale was used to determine the abundance of each species in the samples:

Label	Taxon abundance in the sample	Relative abundance of the taxon (%)
1	Sporadically occurring species	to 0.1 %
2	Very rare species	0.1–1 %
3	Rare species	1–5 %
4	Rather abundant species	5–20 %
5	Abundant species	20–50 %
6	Very abundant species	50–90 %
7	Massively abundant species	90–100 %

Table 1: Semi-quantitative scale

Every species mounted on a slide was given a number based on its estimated abundance in the sample. The data were entered into a spreadsheet, which was used to create a diatom diagram indicating the abundance of each species and how abundances changed (Figs. 7 and 8).

3.4. Processing the results

Graphs depicting the results were created using Excel. The diatom diagram was created for the Institute of Botany in the program PolPal.

It was not possible to apply commonly used equations to calculate trophic indices, electrolyte levels, and pH because the exact number of shells for each species was not determined in each sample. Therefore, these equations were modified so they would be applicable.

The calculations were made by dividing the sum of assigned numbers from the semi-quantitative scale of all indicators of a certain feature (e.g., oligotrophy) by the sum of the numbers from the semi-quantitative scale of all species indicating the characteristics of a certain level of biological productivity (i.e., oligotrophy, dystrophy, eutrophy, etc.). I created graphs using the obtained values.

The graph depicting the number of pollution-sensitive species (Figure 5) displays such species as a percentage of the total number of diatoms in the sample.

3.5. Taking and processing photographs

To create the plates depicting selected identifying species (Appendix 1), an Olympus BX53 microscope and QUICK PHOTO MICRO 2.3 software were used.

4. Results

4.1. Dating

The lower boundary of the profile was dated to 1747, and the upper boundary to 1999. Intermediary samples were dated using an age-depth model and marked in the diatom diagram.

4.2. Species and genus composition

Sixty-six species of diatoms from 28 genera were identified in the 12 analyzed samples contained in the profile taken from *du Loup Bourrou* spring. The shells were relatively well preserved, and their condition did not prevent from identifying what species they belonged to.

The taxonomic classification of 14 diatoms were limited to the genus level. There were three organisms from the genus *Pinnularia*, two from the genus *Fragilaria*, and one each from the genera *Achnantes*, *Achnantidium*, *Caloneis*, *Diploneis*, *Encyonema*, *Eunotia*, *Hantzschia*, *Humidophila*, and *Neidium*. The fact that these species were only roughly identified had no impacts on the results. These are diatoms that either occurred only in the single digits or are from genera in which ecology does not differ significantly from species to species (e.g., species in the genus *Pinnularia*).

The fewest species were identified in the uppermost 0–3 sample (23 species) and the most in samples 23–29 and 19–21 (37 species). On average, each sample contained 32 species. Figure 1 depicts the number of taxa present in each sample. The trend line clearly demonstrates how the number of species declined from the greatest to the shallowest depth.

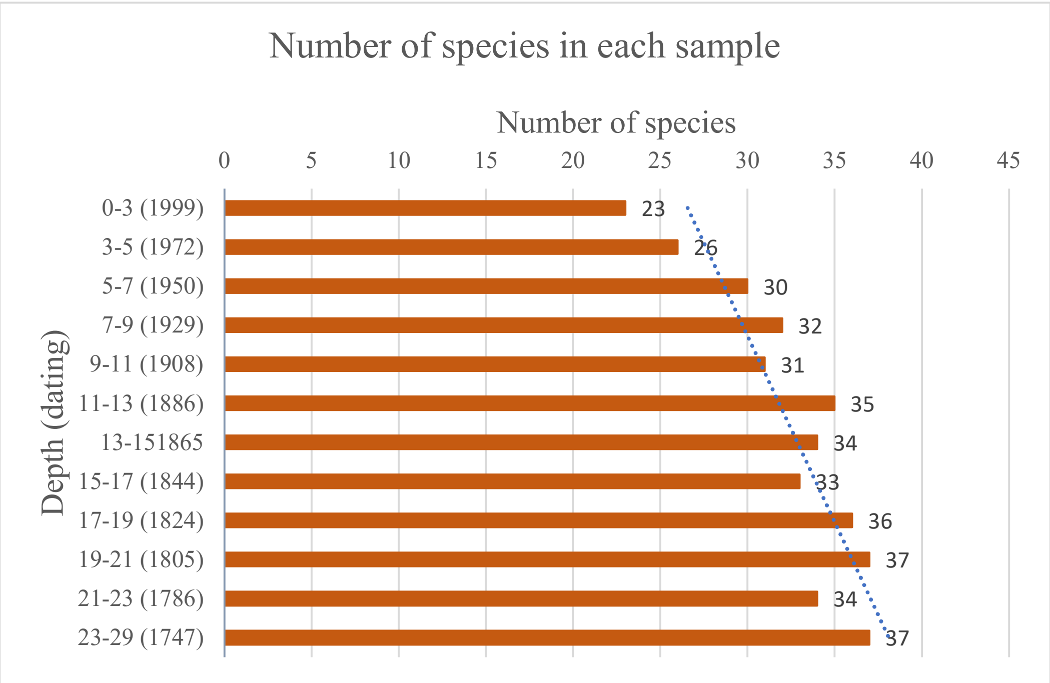


Figure 1: Number of species in each sample

The ten most abundant species in the entire profile are *Fragilariforma virescens*, *Eunotia minor*, *Pinnularia sinistra*, *Planothidium lancoleatum*, *Gomphonema productum*, *Neidium bisulcatum* var. *bisulcatum*, *Fragilaria gracilis*, *Sellaphora pseudopopula*, *Psammothidium* cf. *Daonense*, and *Cymbopleura naviculiformis*.

The most abundant genera were *Pinnularia*, *Eunotia*, *Gomphonema*, and *Neidium*. The relative abundance of each genus in percentages is noted in Table 2.

Pinnularia	20,6 %
Eunotia	8,7 %
Gomphonema	7,4 %
Neidium	7,1 %
Fragilaria	4,7 %
Planothidium	4,5 %
Fragilariforma	4,2 %
Surirella	4,1 %
Sellaphora	3,6 %
Stauroneis	3,6 %

Encyonema	3,5 %
Cymbopleura	3,3 %
Caloneis	3,2 %
Psammothidium	2,3 %
Achnantidium	2,1 %
Meridion	2,1 %
Navicula	2,1 %
Achnantes	1,6 %
Nitzschia	1,5 %
Odontidium	1,2 %

Cavinula	1,2%
Placoneis	1,2%
Adlafia	1,1%
Diploneis	1,1%
Frustulia	1,1%
Hantzschia	1,1%
Humidophila	1,1%
Pseudostaurosira	1,1%

Tables 2, 3 and 4: Relative abundances of each observed genus

4.3. Trophic state

Figure 3 depicts the relative abundance of species indicating certain trophic states. The samples predominantly contained indicators of oligotrophic to dystrophic water bodies. Only three species were not indicators of oligotrophic conditions. The species *Surirella angusta* and *Pinnularia grunowii* are typical of eutrophic habitats. *S. angusta*, however, was recorded only once and only in the form of a broken shell, which could have been brought to the site. The species *Eunotia minor*, which is abundant in the profile, is an indicator of dystrophic waters. Its presence thus confirms that this is an oligotrophic to dystrophic spring. Trophic conditions did not change significantly over time. The greater relative abundance of eutrophic diatoms in layers 9–11 to 15–11 is due to the increased presence of just one species, *Pinnularia grunowii*.

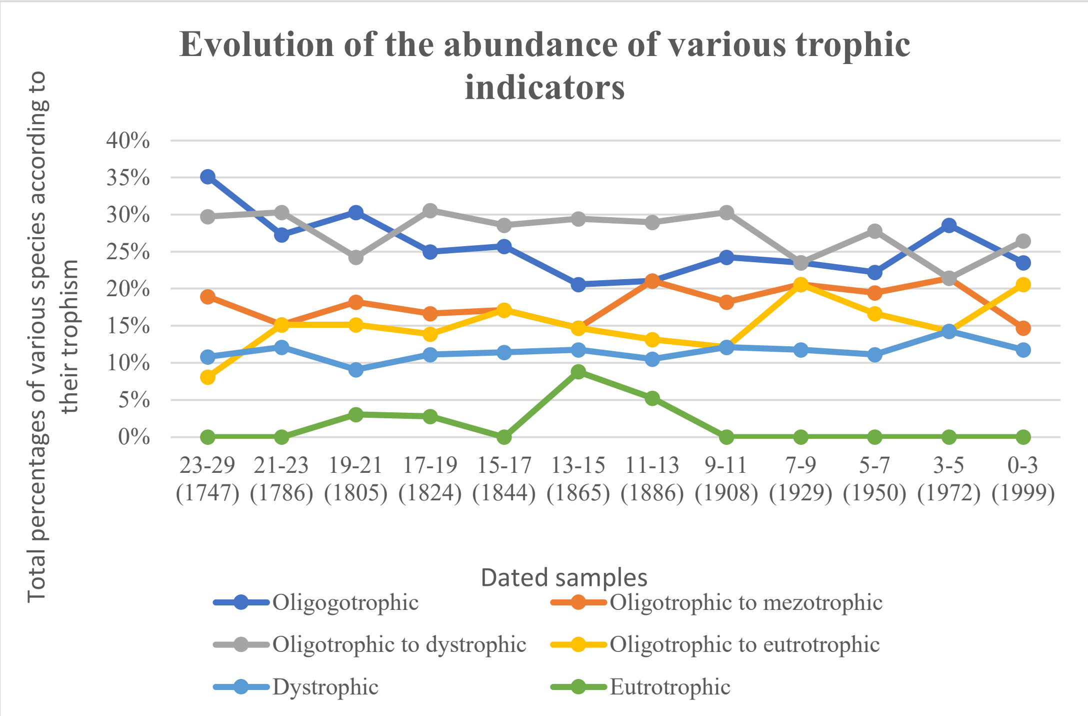


Figure 2: Evolution of the abundance of various trophic indicators

4.4. Electrolyte levels

Of all the diatoms identified in the samples, 36 species enabled me to determine the electrolyte levels in the spring environment (Fig. 4). The greatest percentage of diatoms indicate low electrolyte levels across all layers of the profile. The most abundant species indicating low electrolyte levels include *Pinnularia sinistra*, *Eunotia minor*, *Fragilaria gracilis*, *Fragilariforma virescens*, *Neidium bisulcatum* var. *Bisulcatum*, and *Gomphonema productum*.

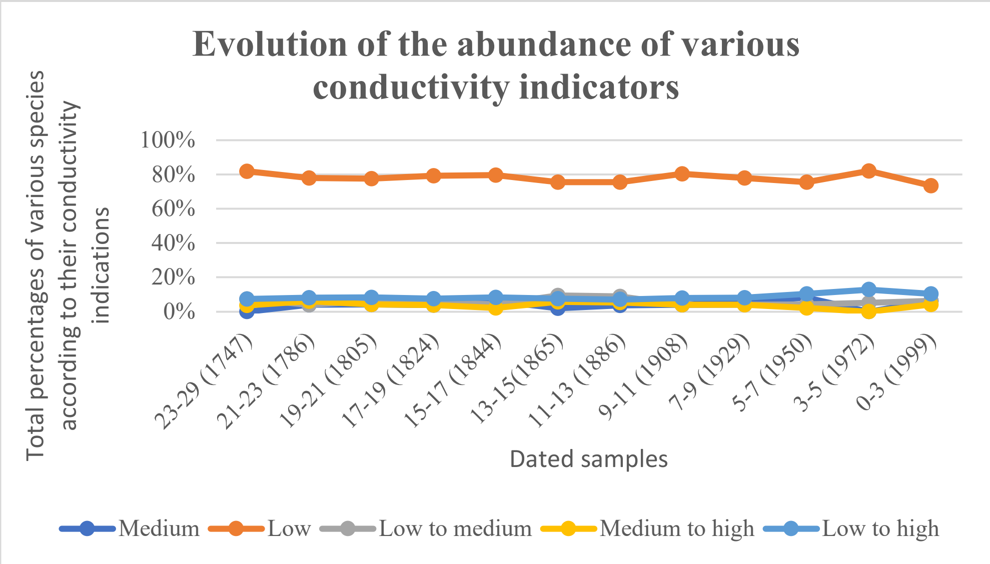


Figure 3: Evolution of the abundance of various conductivity indicators

4.5. Substrate characteristics

For 15 of the diatoms present, the ecology of the substrate in which they usually occur is known, and all point to a substrate of a siliceous nature. The species *Pinnularia gibba* is typically found in habitats very poor in calcium bicarbonate; its presence, however, is very low, and therefore, it cannot influence the results too much.

4.6. Pollution

Figure 5 depicts the percentage of pollution-sensitive species out of the entire diatom sample. The most pollution-sensitive species are found in sample 11–13 dated to 1886.

The species *Pinnularia sinistra* and *Pinnularia perriorata* tolerate anthropogenic pollution. In contrast, the species *Encyonema minutum*, *Eunotia nymanniana*, *Pinnularia schoenfelderi*, *Pinnularia stomatophora*, and *Psammothidium daonense* are sensitive to human impact and only occur where there is minimal anthropogenic pollution. Moreover, *Adlafia suchlandtii* is sensitive to organic pollution. However, it does not occur in great enough abundance to make any conclusions about its presence.

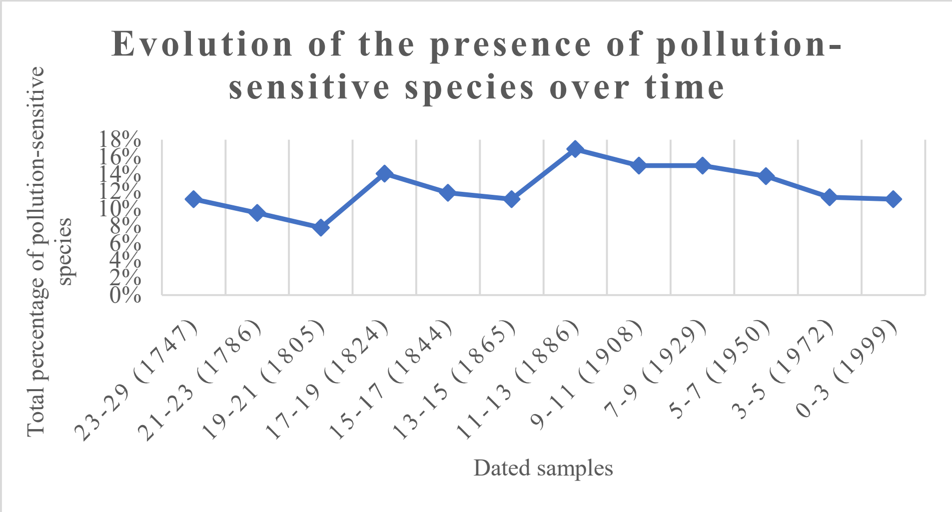


Figure 4: Evolution of the presence of pollution-sensitive species over time

4.7. pH

The substrate is circumneutral (pH approx. 7) to slightly acidic (Fig. 6). The most abundant species indicating a circumneutral environment are *Eunotia minor*, *Psammothidium daonense*, and *Planothidium lancoleatum*. The species *Sellaphora pseudopopula* and *Gomphonema acidoclinatum* indicate a slightly acidic environment. *Fragilariforma virescens*, *Fragilaria gracilis*, and *Neidium bisulcatum* var. *bisulcatum* occur at acidic or circumneutral sites. The species *Stauroneis acidoclinata* and *Stauroneis silvahassiaca* occur together with acidophilic diatoms.

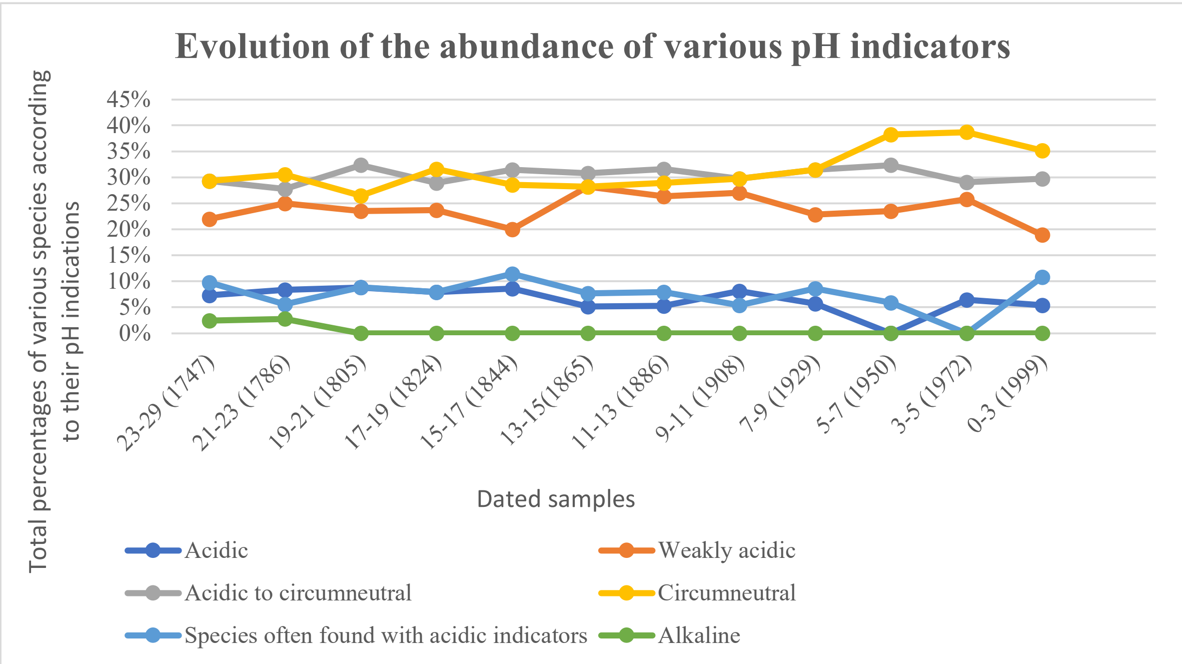


Figure 5: Evolution of the abundance of various pH indicators

4.8. Photographs

Photographs were taken in every step of analyzing the samples from the entire profile. In total, 43 of the 66 identified species were photographed and classified by their taxonomic order. Appendix 1 includes a plate with the first four species of these microphotographs.

4.9. Diatom diagram

The data obtained from diatom analyses is usually used to create a palaeoecological diagram that depicts the abundance of different species over time. A diagram created using the program PolPal is displayed on the following pages (pp. 13, 14). Along the x-axis are the species names, and the y-axis is dedicated to a timeline derived from radiocarbon dating and the age-depth model.

Figure 6: Palaeoecological diagram of diatom analysis (part 1)

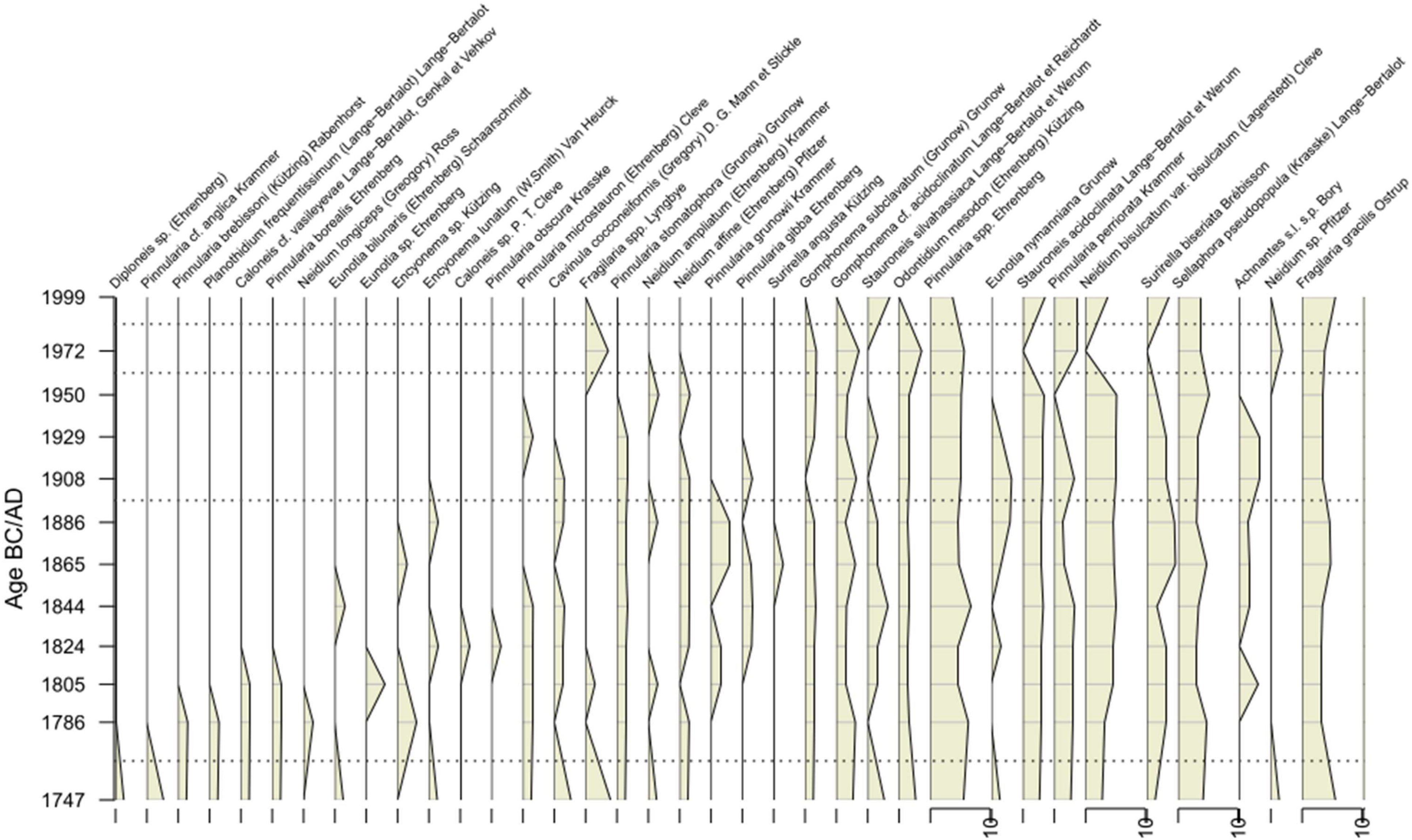
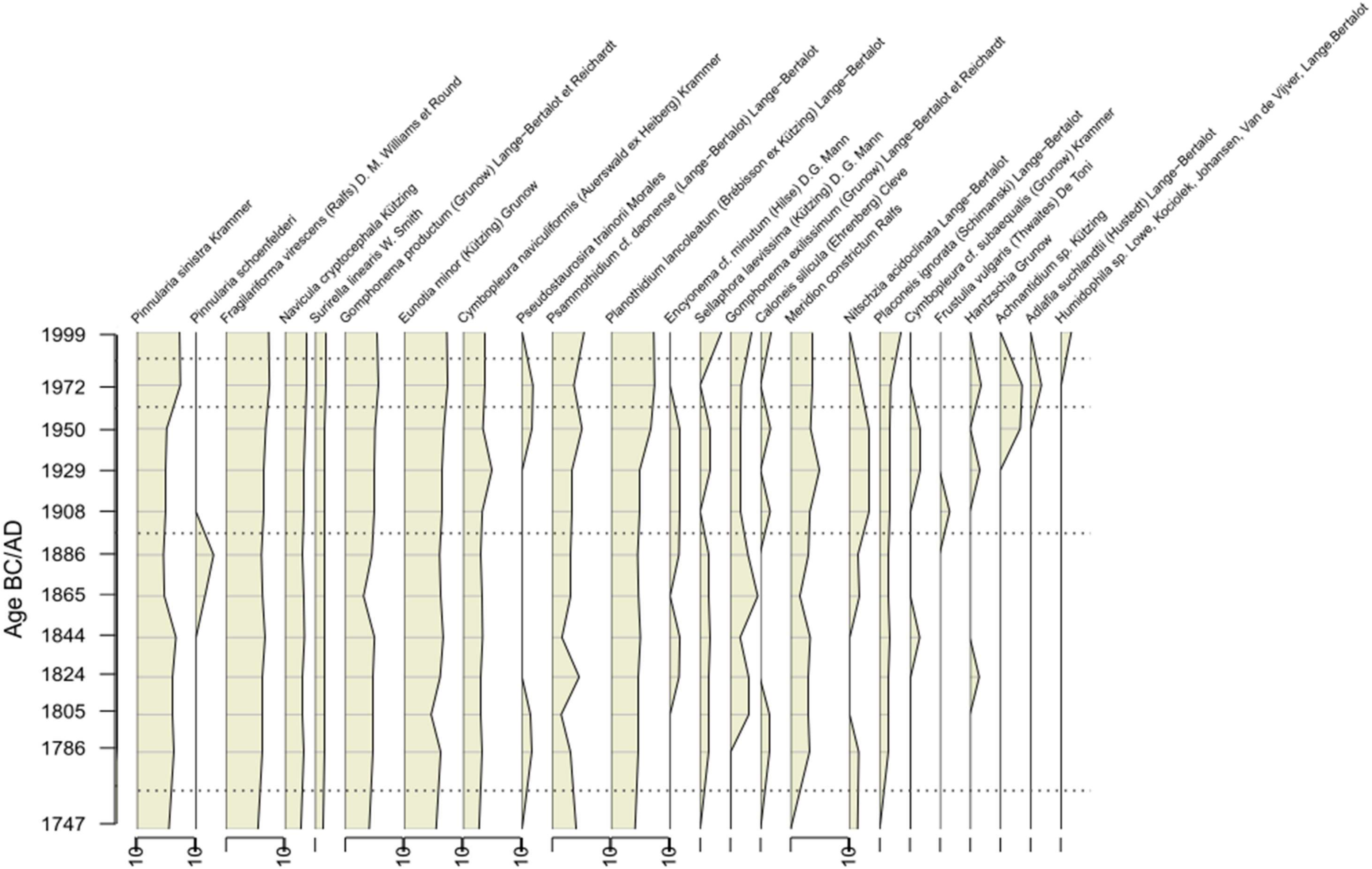


Figure 7: Palaeoecological diagram of diatom analysis (part 2)



5. Discussion

5.1. Effectiveness of the selected methods

The method selected for preparing samples is considered to be the gentlest. However, it has been found to be inadequately effective when processing sediments containing a large amount of organic material (Bešta, 2007). Nonetheless, it was satisfactory for analyzing the samples from the *du Loup Bourrou* spring, and it can be used in the future for further diatom analysis at the Mont Beuvray site.

5.2. Interpreting the results of the reconstruction

As a result of the analysis, I identified 66 species of diatoms.

The number of species was stable in the 18th century, fluctuating around 36 species per sample. In the 19th century there was a decline in species, which was, however, not as significant as the decline that occurred in the 20th century. Over the course of two centuries, diatom-species diversity decreased from 37 species in 1747 to 23 species in 1999. The difference of 14 species means a 38% decrease in diatom diversity, which strongly suggests that in this period environmental conditions around the spring or in the spring itself changed.

Overall, substrate characteristics did not change significantly between 1747 and 1999. Trophic conditions ranged from oligotrophic to dystrophic. Dystrophic conditions indicate a high amount of humic acids, whereas oligotrophic conditions indicate low nutrient content, particularly nitrogen and phosphorus. Oligotrophic water bodies are species-diverse biotopes in which, however, individual taxa are not very abundant. Electrolyte levels were low in all samples, ranging from 50 to 100 μS/cm. The identified diatoms are also indictive of the siliceous geology underlying the spring, which is consistent with the geology of the entire Morvan region, which is made up of granite, of which quartz is an important component.

Based on an analysis of diatom indicators, I found that the substrate has a neutral to slightly acidic pH. In older layers the number of circumneutral and slightly acidic bioindicators is similar. From 1929 onward, however, I observed an increase in circumneutral diatoms, which outnumber acidophilic species.

5.3. Possible explanations for declining species diversity

If we compare my findings with the events and history of activities at Mont Beuvray between 1747 and 1999, several possible connections that we can hypothesize about emerge. Conditions and species diversity, however, may have changed at this site for completely different reasons. A multidisciplinary study that would compare the findings of my diatom analysis with data obtained through other methods could describe the reasons for these changes in greater detail.

Franciscan monks began inhabiting the hill in the 14th century, and their farming activities had a significant impact on the natural conditions at the site. The *du Loup Bourrou* spring was probably modified by the Franciscans. Their departure in the 18th century would explain the gradual increase in the abundance of diatoms sensitive to human activity. The decrease in diatom-species diversity could also be related to the departure of the Franciscans from Mont Beuvray. If the monks modified the spring, we can also assume that they took care of it. When they left, however, no one looked after the spring, and we can assume that vegetation began to take over the site. Denser vegetation led to greater shade, which would explain the lower diatom-species diversity, which is typical of areas receiving less light.

The reason for the fluctuating number of taxa and diatoms sensitive to anthropogenic impact, however, could be entirely different. The changes could be linked with the archaeological work led on the site at the turn of the 20^{th} century, when sediments began to indicate the greatest decline in species, which lasted until 1999. Deforestation in the wider Morvan region could have also had an impact on the area of Mont Beuvray, even though the analyzed site itself did not experience deforestation. Increasing temperatures since the early 20^{th} century (NASA), which have affected agriculture in southern France, including Burgundy (Le Roy, Daux & Luterbacher, 2006), could be another explanation for the observed changes.

5.4. The advantages and disadvantages of using diatom analysis in paleoecology

After completing the diatom analysis, I considered the advantages and disadvantages of this method.

Conducting such analyses is very time consuming. Identifying individual species can take a long time and requires up-to-date specialized literature covering taxonomy and other knowledge, which is constantly changing in this discipline. Another obstacle could be broken shells, which can, in the best-case scenario, be used to classify diatoms only at the genus level. Researchers starting out with conducting diatom analysis need advice from experts with greater taxonomic knowledge and experience.

However, diatom analysis is an effective method that can be applied at many types of sites because diatoms can be found everywhere in the world in practically all types of habitats, especially in aquatic ecosystems. Moreover, environmental changes and human impacts are directly reflected in diatom communities, and when diatomology is combined with other disciplines, it is possible to determine with much greater certainty what changes occurred. It is also a cost-effective method that does not require cutting-edge technology. An optical microscope is all that is needed. Due to the time and experience required, however, financial considerations must also be taken into account for covering the costs necessary for conducting this work. Once permanent slides have been created, they can be analyzed whenever needed. Permanent slides together with the photographs taken as part of the study can also be used as reference materials for identifying diatoms.

Diatom analysis is therefore an effective method, which becomes even more effective when combined with other disciplines that complement it.

6. Conclusion

In this work, I focused on assessing profile P2a from the *du Loup Bourrou* spring at the Bibracte site in France. Based on diatom analysis, I found that environmental conditions at the *du Loup Bourrou* spring did not change much between 1747 and 1999. There was, however, a significant decrease in species diversity, evidence of a change that must have occurred at this site, as well as a fluctuating number of species sensitive to human impact.

The next step will be to analyze profile P2a using a different method for recording the abundance of each taxonomic group—by counting the exact number of shells of each species in the samples. Another task will be to analyze the other profile, profile P1a, which was taken in the vicinity of profile P2a. It will then be possible to compare the newly calculated indices with the results presented here to determine if profile P1a confirms them.

It will also be interesting to compare the species found in both profiles and determine whether diatom communities so close to each other can differ significantly or, conversely, whether there is no large difference in species.

After both palaeoecological profiles have been assessed, it will be possible to publish the research findings in a multidisciplinary study led by archaeologist Petra Goláňová from the Department of Archaeology and Museology of Masaryk University as part of the project "Oppidum as an urban landscape: multidisciplinary approach to the study of space organization 'intra muros.'" In an interdisciplinary study, it will be possible to compare the findings of researchers in other fields with the results of my diatom analysis and perhaps determine what changes occurred at this site within the past two centuries.

When all the findings from the diverse disciplines included in this multi-proxy study will have been analyzed together, it will be possible for archeologists to remove the stone layer from the 14th century. This way, older sediments hidden under this layer will be uncovered, and it will therefore be possible to take a new profile dated to an older period. Conducting the same multidisciplinary research on this profile will enable us to reconstruct the evolution of environmental conditions at the site deeper into the past.

Bibracte and the entire Morvan region are part of a protected area, and the results presented here may in the future contribute to managing Mont Beuvray hill. They may serve as reference points that can be used to evaluate future changes in environmental conditions and pollution levels at the site.

7. Literature

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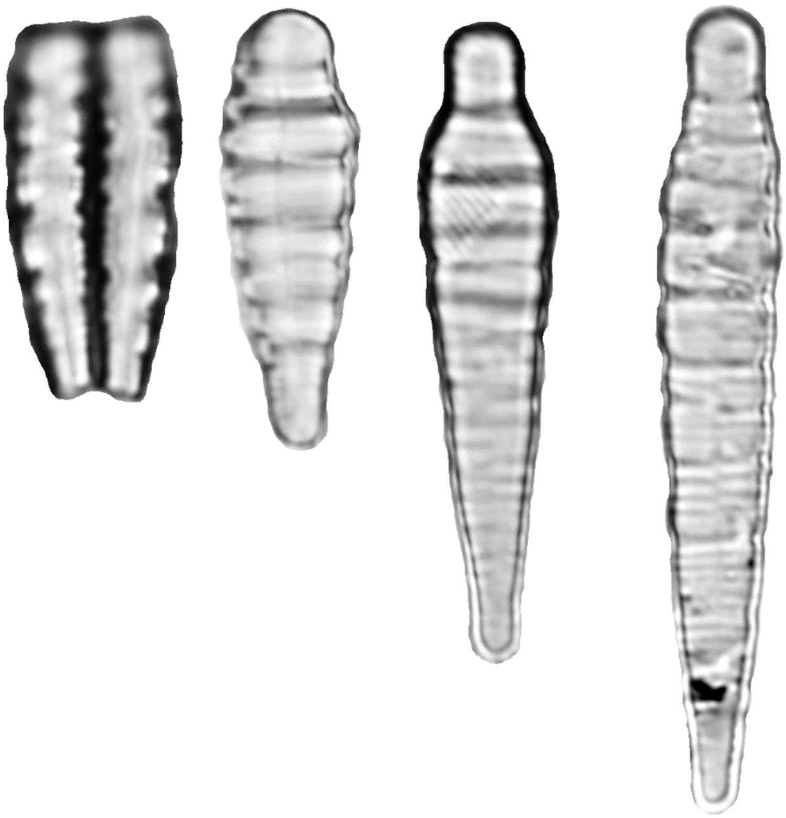
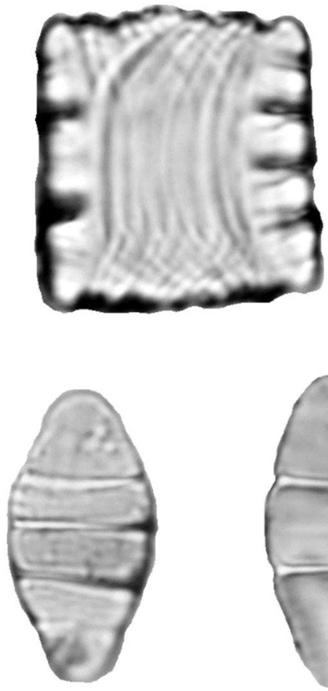
9. List of figures and tables

Appendix 1: Plate 1 (4 species)

Figures 1–4: ***Meridion constrictum*** Ralfs
Figures 5–7: ***Odontidium mesodon*** (Ehrenberg) Kützing





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