Can Development of a Morphological Identification Key Simplify Determination of Early Paleogene Dinoflagellate cysts? Application to the Eocene of the Aquitaine Basin, Southwestern France, with Xper3
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Kan utveckling av en morfologisk identifieringsnyckel förenkla genusbestämning av cystor av dinoflagellater från tidig Paleogen? Tillämpning på Aquitainebassängen från Eocen, sydvästra Frankrike, med Xper3

Veronica Carlsson
This work was carried out in collaboration with Total in Pau, France.
Abstract

Can Development of a Morphological Identification Key Simplify Determination of Early Paleogene Dinoflagellate cysts? Application to the Eocene of the Aquitaine Basin, Southwestern France, with Xper³

Veronica Carlsson

Knowing the taxonomy of unicellular dinoflagellate cysts is important in reconstructing paleoenvironments, paleoclimatology and paleobathymetry. Different dinoflagellates tend to prefer different depths, salinity levels and climate. Species of dinocysts only occur during a certain interval in the geological record and are therefore an important tool for dating sediments. Biostratigraphic research uses age determination of sediments depending on first and last occurrences of different species, or co-occurrences of several species. However, it can be rather time consuming and difficult to identify dinoflagellate cysts for people that are not experts in this field. In an attempt to resolve this, a database was created for 145 genera of dinoflagellate cysts existing during Palaeocene and Eocene in the online accessible program Xper³. Morphological criteria or “descriptors” were added along with describing “states”. The number of morphological descriptors chosen were 13, with 3 to 15 states for each descriptor.

Each genus was described in a matrix and was given one or more unique states for each descriptor, which were defined from the literature by original morphological descriptions of dinocyst genera and photographs of holotypes. A morphological identification key was automatically created in Xper³ from this database, which enabled identification of genera by choosing unique states that were visible for the dinocysts being analysed.

A test using photographs with different levels of preservation of dinoflagellate cysts of Eocene age of Aquitaine basin was undertaken in order to assess the reliability of the morphological identification key. The aim was also to see which morphological criteria were more common and how many descriptors and states were needed to reach an end-result of 5 remaining genera, including the correct genus.

Errors that were caused in the identification key were immediately reviewed and re-tested. In 38 of 43 tries, a maximum of five genera were remaining, with the correct genus included. This confirmed that the identification key worked relatively well.

Another test demonstrated how the identification key worked for identifying dinocysts with an optical microscope in unknown samples, which are photographed and published in the present report, along with data showing how many descriptors and states were used, remaining genera and which states were chosen for each genus.

In general, only 1-8 descriptors were necessary and 5 the most common number used. The morphological character (state) “type of ornamentation” was the most widely used followed by “distribution of ornamentation”, “shape of the cyst” and “size of the ornamentation”. However, the archeopyle (excystment opening), was not always visible in the dinocysts, but is still considered a key morphological descriptor.

Keywords: dinoflagellate cysts, Xper³, morphology, Early Paleogene, Aquitaine basin, genus identification

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Populärvetenskaplig sammanfattning

Kan utveckling av en morfologisk identifieringsnyckel förenkla genusbestämning av cystor av dinoflagellater från tidig Paleogen? Tillämpning på Aquitainebassängen från Eocen, sydvästra Frankrike, med Xper³

Veronica Carlsson

Dinoflagellater är en typ av mikroskopiska plankton som kan bilda så kallade cystor eller vilosporer för att skydda sig vid kritiska miljöförändringar. Dessa cystor kan ligga vilandes i sediment under en längre tid. Det är också dessa cystor som bevaras som fossil. Eftersom det finns en sådan hög mångfald av dinoflagellatcystor i fossila sediment, liksom att de mikroskopiska, existerar över stora delar av jorden samt att de har en snabb evolution, så används de ofta till att datera sediment inom biostratigrafi.

För att kunna datera sediment med hjälp av dinoflagellatcystor, krävs det att man kan identifiera de olika arterna och veta i vilken tidsärder de existerade. Att klassificera dinoflagellatcystor kan därför vara mycket svårt och ta en hel del tid om man inte är någon expert. I denna studie har därför en databas och identifieringsnyckel skapats i ett onlinebaserat dataprogram kallat Xper³. Xper³ är primärt riktad till att hjälpa personer med grundläggande kunskaper i att beskriva utseendemässiga karaktärer hos olika typer av mikrofossil, inklusive dinoflagellatcystor.

I denna identifieringsnyckel använde jag mig av 145 olika dinoflagellatsläkten vilka alla existerade någon gång under tidig Paleogen, dvs hela Paleocen och/eller Eocen (66–34 miljoner år sedan). Dessutom användes 13 olika morfologiska beskrivningar ”descriptors” med 3–15 olika svar ”states” per beskrivning.

Målet är att få en identifieringsnyckel som unikt kan identifiera olika typer av dinoflagellatcystor på genusnivå, med ett förslag på maximalt fem återstående släkten (genera). Studien undersökde även vilka morfologiska beskrivningar som är mest användbara och hur många olika typer av beskrivningar som behövs för att analysera dinoflagellatcystor.

För att testa om identifieringsnyckeln fungerade, användes olika typer av kända och okända dinoflagellatcystor i ett så kallat ”Test 1: Kända dinoflagellatcystor” och ”Test 2: Okända dinoflagellatcystor.”

I det första testet, ”Test 1”, grupperades kända dinoflagellatcystor i olika typer av bevarande tillstånd så som ”välbevarade”, ”tveksamt bevarande” och ”väljukt dåligt bevarade” dinoflagellatcystor. Detta test förde statistik på hur många släkten av förslag som kom upp i slutändan och vilka morfologiska beskrivningar som användes mest och hur många olika beskrivningar som behövdes.

Det andra testet, ”Test 2”, undersökte hur denna identifieringsnyckel fungerade att analysera dinoflagellatcystor från okända prover under mikroskop. Fotograferade dinoflagellatcystor presenterades i denna rapport tillsammans med detaljer på hur de hade identifierats.

I de flesta fallen, kunde identifieringsnyckeln ge ett förslag på maximalt fem återstående släkten av dinoflagellatcystor, där det korrekta släktet var ett utav förslagen, vilket tyder på att identifieringsnyckeln fungerar. Den mest använda morfologiska beskrivningen var ”typ av ornament”, och antal morfologiska beskrivningar som behövdes var mellan ett till åtta, där fem var det antal beskrivningar som mest användes i att identifiera dinoflagellatcystor i identifieringsnyckeln.

Nyckelord: dinoflagellatcystor, Xper³, morfologi, Tidig Paleogen, Aquitainebassängen, genusidentifiering

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1. Introduction

The biostratigraphic study of microfossils is an important tool in oil exploration. It provides information on the age of the sediments drilled by exploration wells and on their paleoenvironment of deposition. In the biostratigraphic team of Total Exploration in Pau, France, three groups of microfossils are used: Foraminifera, nannofossils and palynomorphs. Palynomorphs are organic microfossils, and include spores, pollen and dinoflagellate cysts.

Another application of the palynomorphs is to provide information on thermal maturity of sediments. “Thermal maturity is the extent of heat-driven reactions that alter the composition of organic matter” (Peters et al., 2012) and the colour of the fossils can predict whether oil or gas can form (Singh, 2008).

Dinoflagellates, which are the subject of this project, are photosynthetic or, sometimes, heterotrophic microorganisms. They are mostly organic-walled, and their organic membrane is preserved by anoxic conditions.

There are many diverse morphological characteristics among dinoflagellate cysts. Fast evolving species, together with their relatively small size and high abundance in different strata enable dinocysts to be of great use in biostratigraphy (Evitt, 1985). The biostratigraphic use of dinocysts in petroleum exploration was first tested in the second half of the 20th Century (Stover & Williams, 1982). Jurassic and Cretaceous dinoflagellate cysts were calibrated with ammonite stratigraphy (Woollam & Riding, 1983; Riding & Thomas, 1992; Monteil, 1992), while Cretaceous and Cenozoic cysts used Northern Hemisphere planktonic foraminifera and calcareous nannofossil biozonations (Stover & Williams, 1982).

Dinoflagellate cysts are not only useful in biostratigraphy but also for paleoecology (Stover & Williams, 1982 & Stover et al., 1996). Identifying dinocysts taxa is the key for dating sediments but is time-consuming and requires a strong morphological expertise.

The development of a simple morphological description tool would therefore be very helpful for scientists or people who are not experts in dinocyst taxonomy but have the necessary skills to describe morphological characters in other unicellular microorganisms.

In the present project, the Xper³ software was used to create a morphological identification key. The Xper³ software was developed at Pierre and Marie Curie University in Paris, France in 2017 (Pinel et al., 2017). Xper³ is an online taxonomic descriptor software, which enables the user to search for taxa based on morphological characters. It was developed for describing taxa with a multi access key, meaning that one can enter all the criteria that are visible, without the difficulties that arise from an unanswerable question in a single access key (Pinel et al., 2017). The multi access key is preferable for use in instances of poor dinocyst preservation. People working with dinoflagellate cysts can then search for morphological characters of unspecified samples in the Xper³ software and eliminate...
dinocysts that lack characters of choice and come up with possible suggestions of taxa in order to save time for geochronological correlations, paleogeographical and paleoenvironmental studies. A morphological identification key has earlier been applied and tested in Xper³ on Cretaceous dinoflagellate cysts with a successful outcome by another master student for Total, Ivan Rodriguez-Barreiro (Rodriguez-Barreiro, 2018). This study is going to be about dinoflagellate cysts from the Early Paleogene, which was a time of very high dinocyst diversity. Although, the highest dinoflagellate cyst diversity occurred during the Cretaceous (Sluijs et al., 2005).

1.1. Hypothesis

Can development of a morphological identification key simplify determinations of Early Paleogene dinoflagellate cysts? Our hypothesis was that the Xper³ software could be a useful tool for palynologists.

Scientific questions to further answer throughout the report:

- Can the chosen descriptors and states (characters) inserted in Xper³ determine the correct taxa of dinoflagellate cysts? How many suggestions of genera come up? What is the optimal number of descriptors and which ones best describe the genus in question?
- How accurately can the selected descriptors and states in Xper³ decide the taxon, especially for badly preserved dinoflagellate cysts? If the degree of accuracy is not satisfactory, how can we improve it?
- Will it work to identify dinocysts genera from unknown samples?

1.2. Aims

This project will focus on building up a database and a morphological identification key to simplifying determination of 145 Early Paleogene (Palaeocene to Eocene) dinoflagellate cysts genera, and a small number of acritarchs. The idea for this project is to have a working identification key and determine which morphological descriptors, inserted into the Xper³ program, are more relevant and, how many descriptors are necessary to use in order to get a maximum of five likely remaining genera as a result after submitting visible criteria in the dinocyst identification key.

The multi access key-based identification in Xper³ is structured to identify dinocysts by choosing morphological characters for any visible criteria of the analysed dinocyst without inserting a hierarchy, which could help identifying even the worst preserved dinocysts.

1.3. Implementation

Morphological states will be chosen for each dinocyst mainly based from original descriptions of holotypes. Visible characters in the dinocyst being analysed can then be selected in order to find the
correct dinocyst genus. The identification key will be created and tested in the Xper$^3$ software. The Xper$^3$ software is adapted to help determine the identity of damaged/folded/obscured dinocysts, up to at least genus level. In theory, for dinocysts, the genus name (let alone a species name) cannot be determined if one cannot clearly see the opening. With Xper$^3$, secondary descriptors such as shape, presence or absence of ornamentation, type and distribution of the ornamentation when present etc. can be used instead. These can then be combined and Xper$^3$ will process the data entered and suggest a manageable number of possible solutions.

1.3.1. Test

In order to evaluate the efficiency of the identification key, it must be tested with analysing dinoflagellate cysts from both known and unknown samples from the Eocene of the Aquitaine basin of France. Known dinoflagellate cysts will be tested and divided into groups of different levels of preservation and defined criteria. Statistical analyses will be done based on the first test of already known dinocysts and the dinocysts from the unknown samples will be photographed and published in the report, to see how this application works in reality. Data on each photographed dinocyst will show how many descriptors and states were used and how many genera were remaining, along with the correct genus name as determined by Total’s dinocyst specialist. The main goal is to end up with maximum of about five possible genera, of which the correct genus should be one solution.
2. Background

2.1. Dinoflagellates in general

Dinoflagellates are microscopic unicellular eukaryotic microorganisms, living in marine and freshwater environments. Their general size is about 15-100 µm in diameter and they have been abundant since the Upper Triassic, about 200 million years ago (Ma), recorded in the fossil record. The Greek word *dinos* means whirling and *flagellate*, whip or scourge. Their general morphology is described of having an anterior epitheca, central cingulum, sulcus, flagellum, posterior hypotheca and plates (Fig. 1). The most important criteria for an organic microfossil to be called a dinoflagellate cyst is the presence of a cingulum and archeopyle. Many acritarchs, organic organisms with unknown affinities, do look very similar to some dinoflagellate cysts but lack these most important characteristics for being considered a dinoflagellate cyst.

![Figure 1. Sketch of a motile dinoflagellate (Evitt, 1985).](image)

2.2. Life cycles

The most common life cycle (Fig. 2) in dinoflagellates are haplontic, meaning that their reproduction is asexual by mitosis (Fensome et al., 1993). Some even have sexual reproduction (Pfiester & Anderson, 1987) when a zygote is formed from fusion. Mobile zygotes are called planozygotes, which might form a resting cell, a hypnozygote. Dinoflagellates produce cysts in resting stages to protect themselves from different environmental changes like increased salinity levels or temperature changes (Anderson & Wall, 1978; Dale, 1983). These dinoflagellate cysts or dinocysts are preserved in sediments and studied for biostratigraphic and ecological purposes. Most fossils from the Mesozoic (252-66 Ma) – Cenozoic (66-0 Ma) are organic-walled and hypnozygote cysts (Stover et al., 1996).
2.3. Morphology and classification

There are different ways to classify dinoflagellates. In palaeontology, dinoflagellate cysts are classified based only on morphology, whereas classification of living dinoflagellates is based on morphology and molecular studies. Merging of these different classifications could cause problems since the same dinoflagellate could have different names.

Living dinoflagellates (Fig. 3a) possess plates and have two flagella which they use to move around in the water. Fossil dinoflagellate cysts (Fig. 3c) reflect the position of tabulation by crests, processes or other types of ornamentation (Fig. 3b). They also have an opening, called an archeopyle. Both living forms and cysts can have horns. However, the horns of the cyst are often shorter and more rounded than those of the motile stage. The most important morphological descriptor for dinocyst classification are the shape of the cyst, type of ornamentation, including the wall structure, paratabulation (even though it is difficult to detect it in many types of dinocysts) and the location of the archeopyle. The archeopyle is an excystment opening from where germinating cells leave the cyst. The type of archeopyle is one of the most important criteria for identifying and classifying dinocysts (Evitt, 1985 & Boltovskoy, 1973). It is most often located on the anterior-dorsal side (with one possible exception for the genus *Caligodinium*, where the position of the archeopyle, apical or antapical, is still debated (Stover and Evitt, 1978)).

The archeopyle helps orienting the cysts as well as classifying a dinoflagellate cyst at family and genus level (Matsouka & Fukuyo, 2000). Other ways of finding the position of the dinocysts when the archeopyle is difficult to see, is for example that the dinocysts might have three horns. Then, it is very likely that the two horns next to each other are on the posterior position. Finding the horizontal cingulum can determine the posterior or anterior position either to be up or down, since the cingulum is encircling the entire dorso-ventral part of the dinoflagellate cyst. The presence of distinct polar
processes in genera such as *Achilleodinium*, *Diphyes*, *Hystrichokolpoma* and *Oligokolpoma* will also be very useful to determine the posterior and anterior position of the dinoflagellate cyst.

Criteria such as shape, ornamentation, type of archeopyle can be determined without knowing the orientation of the dinoflagellate cysts. Dinocysts with for instance parasutural crests, penitabular ornamentation or intratabular processes often indicate the paratabulation. It is then possible to count the number of plates and even further identify the specific dinoflagellate cyst. The two main groups of Early Paleogene dinoflagellate cysts, Peridinioids and Gonyaulacoids, can be separated by their different types of archeopyles, paratabulation and most often the shape of the cysts.

### 2.3.1. Paratabulation

In the early 20th Century, an American biologist, Charles Kofoid (Kofoid, 1907 & 1909), devised a numerical nomenclatural scheme to describe the series of plates forming the outer wall of dinoflagellates, taking as morphological reference points the apex and the cingulum.

- The apical series, so called because they contact the apex of the dinoflagellates, are labelled 1', 2', 3', 4'.

- The plates directly above the cingulum are called precingular, numbered 1'', 2'' … up to 6'' or 7'' depending on the dinoflagellate group.

- After the discovery of an additional series of plates between the apical and the precingular plates, Kofoid introduced the term “anterior intercalary”. These plates are numbered 1a, 2a, and 3a.

- The nomenclature for plates making the cingulum uses an alphanumerical scheme based on the letter “c” for cingulum, preceded by a number (1c, 2c, … up to 6c).

- The plates below the cingulum are called postcingular, and numbered 1''', 2''', 3''', up to 6'''.

- The plates at the antapex are called antapical. This series often have a lower number of plates, one or two, and are numbered 1'', 2''.

- Finally, a plate called posterior intercalary, called 1p, is often present between the postcingular and antapical plate.

![Figure 3](image1.png)

**Figure 3.** Sketch over a living *Gonyaulax* and comparison with a chorate gonyaulacoid cyst. a) A subspherical and sharp edged shaped *Gonyaulax* with plates, cingulum and sulcus. b) Gonyaulax merged with a gonyaulacoid cyst. Each process corresponds to one paraplate, whereas the processes corresponding to the cingulum paraplates are smaller and more aligned. c) Spherical shaped gonyaulacoid cyst (Evitt, 1985).
2.3.2. Ornamentation
Description of ornamentation includes the presence of horns, processes, trabeculae or crests and, also the wall structure. Dinocysts with horns (normally between 1-5 horns) are usually described as having a polyhedral, ceratioid, fusiform or drop shape. Dinoflagellate cysts lacking horns most often have a spherical, subspherical, ovoid or ellipsoidal shape. Since the presence or absence of different number of horns are already describing the shape of the cyst, it is then not necessary adding horns as a type of ornamentation in Xper\(^1\). Crests are, as already mentioned, most often located at the sutures of the paraplates. Several other descriptors can be used to describe dinocysts with processes, including process number, interior structure, shape, and connection of tips and shafts. If processes are hollow, then they could either be closed or open to the vesicle. Hollow processes are also often described as tubular, if they are not thick, in which case they would be described as having box-like processes.

2.3.3. Size and distribution of ornamentation
The size of the ornamentation is extremely variable among dinocysts. It varies from short granules to very long processes. The ratio process length: diameter of the cyst is used to make three broad categories. This is measured compared to the smallest diameter of the cysts. If the ratio is less than 0.1 of the smallest size of the diameter of the cyst, then the cyst is called proximate. If the ratio is between 0.1 and 0.3, the cyst is called proximochorate. If the ratio is 0.5 or more, the cyst is called chorate (Fig. 4).

![Figure 4](image)

**Figure 4.** Size of the ornamentation in relation to the shortest diameter of the cyst. a) Proximate, b) proximochorate and c) chorate (Evitt, 1985).

The distribution of ornamentation (Fig. 5) depends on where on the paraplates these ornamentations are located. The distribution of the ornamentation is called parasutural if the ornamentation is located at the sutures of the paraplates, which often is the case for crests.

Other types of distributions are penitabular, meaning that the ornamentation is located inside the paraplate but very near the sutures. Intratabular distribution means that the ornamentation is evenly distributed on the paraplates. The term gonatal is used for ornamentation (often processes) located at the junction of sutures. Intergonal ornamentation is located between two such junctions. In an intergonal pattern, processes are located on the sutures separating two plates. Nontabular means that the distribution is random or simply covers the whole cyst.
2.3.4. Paracingulum expression

The paracingulum is the expression of the cingulum of the living dinoflagellate on the dinocyst. It can be expressed in many different ways. Tabulation in the cingulum of living dinoflagellates is often expressed by centrally aligned elongated plates. The paracingulum can be expressed by processes. Processes within the cingular plates can often be different in shape or smaller in size than the rest of the cyst. They can be perfectly aligned, and their number of processes on cingular plates can differ from the rest of cyst. Sometimes, there is a complete lack of cingular processes. Other ways to recognize the cingulum could be lateral horns, horizontal folding, thinner elongated paraplates which are materialised by parasutural crests, and concavity or convexity of the cyst outline.

2.3.5. Wall layers and cavations

Dinoflagellate cysts can have different types and numbers of wall layers (Fig. 6). If the wall layer is made of a single layer, it is called an autophragm. When the cyst has two layers, the inner layer is called the endophragm, the outer layer the periphragm. Sometimes these layers are in very close contact and can be difficult to distinguish.

An extra outer layer, the ectophragm, can also be present above the autophragm or periphragm. The ectophragm can appear as a membrane or trabeculae distally connecting the process tips. The ectophragm is most often supported by some types of processes or projections.

A mesophragm is an extra thin wall layer that can be recognized in some Peridinioid cyst as a layer between endophragm and periphragm (Evitt, 1985).

The term cavation is used when a dinocyst shows visible separation of the wall layers (Fig. 6). Dinocysts can be distinguished by their types of cavation. This feature has taxonomic significance at genus level and is particularly useful for identifying Peridinioids. A cavity separating two wall layers is called a pericoel. Shape and distribution of the pericoel define the type of cavation. The main terms are defined as follows:

- Epicavate: the wall layers are separated over the epicyst
- Hypocavate: the wall layers are separated over the hypocyst
- Bicavate: the wall layers are separated over both the epicyst and the hypocyst
- Holocavate: the wall layers are separated over the entire cyst, there is no contact in any direction.
- Circumcavate. In this configuration, some separation of the wall layers is visible, depending on the orientation. If the dinocyst is centred in a dorso-ventral view, it looks similar to holocavation. But in fact, is connected to the outer layers middorsally and midventrally, which can be seen in a side view and in a posterior and anterior view.
- Camocavate corresponds to a cavation on one side of the cyst.
- Cornucavate: cornucavate cysts have cavation restricted to the horns or other types of protrusions.
- Suturocavate: corresponds to cavation at the base of the crests or ridges
- Apiculocavate: this is a term used for dinocysts where cavation is restricted to the processes (Evitt, 1985).

![Figure 6](image)

Figure 6. Cavities and their respective wall layer. a) Acavate dinocyst with one wall layer, autophragm, b) holocavate dinocyst with two wall layers, autophragm and ectophragm, c-g) two wall-layered dinocysts with inner endophragm and outer periphragm. c) circumvcavation, d) bicavation, e) cornucavation, f) epicavation and g) camocavation (Evitt, 1985).

2.4. The main groups of dinoflagellate cysts

There are two main groups of Early Paleogene (66-34 Ma) dinoflagellate cysts, the Gonyaulacoids and the Peridinioids. The names refer to their similarity with the living genera *Gonyaulax* and *Peridinium*. These two groups are separated by their plate arrangement, or tabulation. The Gonyaulacoids (4',6",6c, 6", 1p and 1") have four apical (') plates, six precingular (') plates, six cingular (c) plates, six postcingular ("') plates, one posterior intercalary (p) plate on the ventral surface and one antapical ("') plate, which sometimes can be easy recognized by a single enlarged or outstanding process. The tabulation for Peridinioids (4',3a, 7",4c, 5" and 2") differs in having three anterior intercalary (a) plates on the dorsal side, seven precingular (') plates, four cingular (c) plates, five postcingular ("') plates and two antapical ("') plates, but still have the same amount of apical plates as the Gonyaulacoids.
2.4.1. Gonyaulacoids
Within the Gonyaulacoids, the most common genera are separated by having proximate (short) ridges/crests, for example like the genera Impagidinium or Leptodinium, or having chorate (long) processes like Hystrichosphaeridium. Further distinguishing between Impagidinium and Leptodinium is done by identifying the shape and contact of specific plates: the first and fourth apical plates 1’ and 4’, and the sixth precingular 6”. For Impagidinium, the 6” is triangular and does not have any connections to 1’. The 1’ and 4’ are often fused together and the cingulum is offset. However, in Leptodinium, the 6” is pentagonal in shape and have a connection to 1’. 1’ and 4’ are very distinctive and the cingulum is straight (Stover & Evitt, 1978).

These are very fine morphological differences that are only applied to separate these two genera and should therefore be used as secondary criteria and so are not necessary in the morphological identification key. The main archeopyle types existing within Gonyaulacoids cysts are the apical type, an opening involving all four apical plates, and the precingular type, involving one or more plates from the precingular series. The operculum in an apical configuration can remain attached, which is not the case for precingular archeopyle Combination archeopyles are made of plates from different series, apical and precingular.

2.4.2. Peridinioids
The archeopyles of the Peridinioids are different from Gonyaulacoids, since the opening always involves the intercalary paraplates, either alone or with a combination of intercalary, precingular and apical.

The simplest type of archeopyle formation corresponds to the removal of the second intercalary plate (type I/2a). A type 2I, where two intercalaries are removed, is very rare. The 3I/3a type, where all three intercalary plates are lost, is common.

Combination archeopyles in Peridinioids involve plates from different series, in varying numbers: One intercalary plate together with a precingular plate, three intercalary together with three precingular plates or one apical with three intercalary and three precingular plates as one opening. To simplify this, the states used are just intercalary or combination archeopyle.

Two of the most important Peridinioids groups in the Cenozoic are the Wetzeliella and Deflandrea complexes. Genera in the Wetzeliella complex are: Dracodinium, Gochtodinium, Kisselevia, Rhombodinium, Wetzeliella and Wilsonidium and genera of the Deflandrea complex within the Cenozoic are: Alterbidinium, Deflandrea, Isabelidinium, Lejeunecysta, Palaeocystodinium, Senegalinium and Svalbardella. This group typically have a hexa-type (six-sided) 2a intercalary plate or archeopyle. Whereas the Wetzeliella complex have quadra-type (four-sided) shape in the 2a plate. Less common is it to have a combination of one intercalary and one precingular opening (Evitt, 1985).
3. Material & Methods

3.1. Sample preparation

Samples were already prepared but a brief summary of how palynological preparations are done at Total is as follow: The sample is “deoiled”, meaning removal of hydrocarbon products present in the drilling mud by solvents. This is specific to the oil industry. Carbonates are removed by using hydrochloric acid (HCl). A similar procedure is used to remove silicates, with hydrofluoric acid (HF). The action of HF creates unwanted fluoro-silicates, which are removed by heating the residue in HCl. Nitric acid (HNO3) is then used at low concentration to remove some of the organic matter and make palynomorphs lighter in colour.

Heavy inorganic remains still present undergo heavy mineral separation with a bromoform solution. The final residue is sieved with 10 µm sieves and mounted in Canada Balsam between a glass slide and a coverslip. Glycerine jelly is sometimes used at this stage in other laboratories, but this product deteriorates with time, and Canada Balsam is the preferred mounting medium in Total, because of its durability.

3.2. Literature

In order to give unique states for each genus descriptor, literature had to be studied of original descriptions and photographs of holotypes that were compiled into a WORD document by Dr Daniel Michoux, senior palynologist with Total. Photographs of all the holotypes as well as pictures of morphological characters such as ornamentation and archeopyle were also added for each genus in Xper3. These pictures are also useful to identify and recognize certain morphological criteria in different genera. The morphological descriptions of each genus were mainly taken from the book “Analyses of Pre-Pleistocene Organic-walled genera” written by Stover and Evitt 1978, from which detailed descriptions of each genus are available. Other genera descriptions were from Bujak et al., 1980; Heilmann-Clausen & Van Smaeys, 2005; Fensome et al., 2007; Fensome et al., 2009; Slimani, 1994; Sluijs et al., 2009; Marshall & Patridge, 1988; Stover & Williams, 1987; Stover & Williams, 1995; Wrenn & Hart, 1988; Head & Wrenn, 1992 and Wrenn, 1988.

3.3. Data input into Xper3

The first step in creating a morphological identification key is to add and log in to an account in Xper3 (http://www.xper3.com). A box (Fig. A.1) will appear showing “My Knowledge Bases”, where already existing identification keys are visible that either the user has created or been invited to. There is also a tab called “Create Knowledge Base” which must be clicked on in order to create a base.
3.3.1. Genera

The genera used in the identification key for Xper³ were all present at some time in either the Palaeocene (66-56 Ma) and/or Eocene (56-34 Ma) (Tab. 1). Genera already present in the Cretaceous but having their last occurrence within the Palaeocene or Eocene were also included. The same goes for genera having their first occurrence within the Early Paleogene and persisting into the Oligocene. All the selected Early Paleogene genera that were described, were added as “Items” in Xper³ (Fig. A.2), from the first tab (of a total of five) in the main page of the software. Photographs (Fig. A.3) and description were also added for all genera, especially descriptions focusing in separating very similar genera, for example the genera Achilleodinium and Hystrichokolpoma, which look very similar apart from the archeopyle which is precingular in Achilleodinium and apical in Hystrichokolpoma.
Table 1. Genera of all dinoflagellate cysts described in Xper.

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3.3.2. Morphological descriptors

From the tab “Descriptive model” (Fig. A.4), different morphological descriptions were added as well as alternative options which were added in the third column, “States”, inside the second “Descriptive model” tab (Fig. A.5). Three dimensional (3D) digital images were added into Xper, as well as a composition of photos of very distinctive characters seen in the holotypes or other dinoflagellate cysts.
and copies of schematic sketches from the literature (Evitt, 1985). All of the inserted data (genera, descriptors and states) where combined into a matrix, were a selection of unique states were provided for each genus descriptors (Fig. A.6-A.8). A flow chart (Fig. 7) visually presents data input of genera, descriptors and states into Xper®. This was used to create a morphological identification key inside the matrix.

**3.3.2.1. Shape**

Concerning overall shape, the descriptor “shape of the cyst” was added; with states such as spherical, subspherical, ellipsoidal, lenticular, drop-shaped, fusiform, ceratioid, rhomboidal, pentagonal, hourglass shaped, trilobate, quadrilobate, square/rectangular and triangular (Fig. 8). The “Number of horns or protrusions” is already mostly described based on the shape, for example a pentagonal shaped dinocyst normally has five horns, whereas rhomboidal shapes have four horns, or possibly one very reduced antapical horn. Other selections of horns are: zero, one, two, three, four and five.

**Figure 7.** Flow chart of genera, descriptors and states inserted into Xper®. Followed by giving unique states to each genus descriptor, based on original descriptions and holotype studies, which was modified in the matrix.

**Figure 8.** Digital images created with 3D paint of different shape of the cyst that is inserted for the different states into Xper®. From left to right: Spherical, subspherical, ellipsoidal, ovoid, lenticular, drop-shaped, fusiform, ceratioid, rhomboidal, pentagonal, hourglass shaped, trilobate, quadrilobate, square/rectangular, triangular. The green figures also show examples of number of horns. The first green figure from the left as “one horn” or tear drop-shaped, followed by “two horns” or fusiform, “three horns” or ceratioid, “four horns” or rhomboidal and “5 horns” and pentagonal.
3.3.2.2. Archeopyle

The “Archeopyle” descriptor, corresponds to the opening of the cyst which is described depending on which row of plates it belongs to. It could be either apical, precingular, intercalary, combination or possibly antapical (Fig. 9).

![Figure 9](image)

**Figure 9.** Archeopyles or openings. a) Apical, b-c) intercalary d) precingular, e-f) combination archeopyles, e) epicystal, g) antapical and h) location and name of the different plate rows (Evitt, 1985).

3.3.2.3. Ornamentation

“Ornamentation” addresses whether the dinocysts have processes and/or crests, along with their other wall structures. To be specific, the cyst could be described as having processes, spines or crests, which are long wall-like networks often following the parasutures of the reflecting tabulation or having penitabular distribution. Rugulate and reticulate ornamentations are similar to crests but are shorter and appear more irregular or nontabular over the entire cyst. Fibrous and spongy wall features are also combined with the reticulate and rugulate wall features, since it can be more difficult to see what type of ornamentation a cyst has if it does not have crests or processes. Granular ornamentation describes small grains with a positive relief, whereas perforated ornamentation is similar but has instead a negative relief (Fig. 10). Depression describes other types of negative relief which could be mistaken as crests, since they can be located around at the sutures and the paracingulum. It is also possible to use combination options for the ornamentation, for example “Processes and crests” etc… “Surface of crests, processes or trabeculae” is another description that should be applied if it is distinctive. It could be described as: fibrous, smooth, perforated, striate, (spiny), granular, reticulate/rugulate etc…

![Figure 10](image)

**Figure 10.** Different types of ornamentation. a) Processes, b) crests, c) trabeculae/membrane, d) depressions, e) granules, f) perforations, g) smooth/low relief and h) fibrous/reticulate/regulate/spongy (Evitt, 1985; Manum & Williams, 1995; Drugg, 1970).
The “Paracingulum expression” descriptor depends mostly on the cyst ornamentation. Several options can be used, for example the processes can describe the paracingulum in many different ways. It could either be an alignment of processes, absence of processes, different types of processes, or different number of processes compared to the rest of the plates. Other ways of describing “Paracingulum expression” could be: none, poorly, parasutural or penitabular crests or features or lack of it (including paraplate shapes), folds, concavity or convexity as well as the location of lateral horns (Fig. 11).

**Figure 11.** Digital images created with 3D paint of different types of paracingulum expression. From left top to right bottom: None, poorly, crests, folds, lateral horns, concavity/convexity, alignment of processes, absence of processes, different types of processes, same amount of processes as the rest of the plates, different numbers of cingular plates than the rest of the cyst.

“Size of ornamentation to the shortest diameter of the cyst”, can be chorate (high relief), proximal (low relief) or intermediate as proximochorate. “Distribution of ornamentation”, describes where ornamentation is distributed in relation to the paraplates. It could either be nontabular, intratabular, parasutural, penitabular, gonal or intergonal (Fig. 12).

**Figure 12.** Digital images created with 3D paint of distribution of ornamentation in relation to the paraplates. From left to right: Nontabular, intratabular, parasutural, penitabular, gonal and intergonal distribution of ornamentation.

### 3.3.2.3.1. Processes

Processes are a very important part of the ornamentation that many dinocysts possess. They are also very variable. It is therefore essential to add further descriptors describing their appearance. “Aspects of shaft of the processes”, describes the shape of processes and could be anything from slender, thick, flat, irregular, tubular, conical, cylindrical, round, branching, box-like or square, vase-shaped/funnel, hollow, solid, and if they are proximally connected (Fig. 13).

**Figure 13.** Shaft or shape of the processes. a) Solid, b) hollow, c) flat, d) thick, e) tubular or cylindrical, f) conical, g) vase-shaped, h) round, i) box-like/square, j) irregular, k) branching and l) proximally connected (Fauconnier & Masure, 2004; Evitt, 1985, Damassa, 1979, Stover, 1975, and Eaton 1976).
“Aspects of the tips of the processes”, describes whether the process tips are open or closed, along with their shape which can be rounded, sharp, bifurcate, trifurcate, multifurcate or paintbrush-like or distally connected (Fig. 14).

**Figure 14.** Tips of the processes indicated by the red box. a) Open, b) closed, c) sharp, d) rounded, e) expanded, f) bifurcate, g) trifurcate, h) multifurcate/paintbrush-like and i) distally connected (Evitt, 1985; Fauconnier & Masure, 2004).

### 3.3.2.4. Wall layers and cavation

Cysts can have several separate wall layers and the space between layers is called a pericoel and is the same as a cavation. Cavation is expressed by the descriptor “Number of layers”. The number of layers inserted into the identification key in Xper® is: one; two; three; and four or more. In favourable cases, when the separation of the wall layers is clearly visible, and when it is possible to link it with a particular position on the cyst, the descriptor “Type of cavation” can be used. The types of cavation options possible are: Acavate (no cavation), circumcavate, camocavate, cornucavate, bicavate, epicavate, suturocavate, holocavate or vesicular autophragm (Fig. 15).

**Figure 15.** Digital images created with 3D paint of different types of cavation. From left to right: Acavate, circumcavate, camocavate, cornucavate, bicavate, epicavate, holocavate, suturocavated and vesicular autophragm (Williams et al., 2004).

### 3.3.2.5. Other characteristics

The “Other characteristics” brings up characters that are present but did not fit any of the descriptors already created. This is useful if there are only one or a few processes per plate (intratabular processes), any missing rows of processes (most often in paracingulum but other cysts can have missing rows of processes or crests in the pre- or postcingular, apical or antapical plates), more than one type of processes (not only for paracingulum, it is very common that the polar processes are dissimilar). In some cysts, only a few processes could be connected while the others are commonly isolated. The shape of intercalary archeopyles, as described above, can be either quadra- (four-sided) or hexa-types (six-sided), and this is included in the “Other characteristics” descriptor. This is also the case for combination archeopyle of the epicystal type. The last state corresponds to unusual shapes that are difficult to describe with the “Shape of the cyst” descriptor.
3.4. Aquitaine basin
Dinocysts that were going to be tested based on morphological criteria in the Xper³ identification key were from samples coming from the Eocene of the Aquitaine basin. The Aquitaine basin (Fig. 16a) was a site of sediment deposition caused by the Iberian microcontinent and European plate collision which also gave rise to the Pyrenees mountain belt (Angrand et al., 2005) from the Campanian (83-72 Ma) to middle Miocene (34-23 Ma) (Puigdefàbregas & Souquet, 1986). The southern Aquitaine basin consist of marine facies reaching from deep ocean to shelf environments (Sztrákós et al., 1998).

Sediments in the area of Gan, south of Pau (Fig. 16b) are between 60 – 50.7 million years (Myr) old and consist of sand and gray marls which are rich in microfossils, mostly calcareous nannofossils and foraminifera, including larger foraminifera transported by turbiditic currents. Several faults are present in the area and studies of sequence stratigraphy reveal sea level changes which have been affected by Pyrenean tectonics, especially in the Gan region (Steurbaut & Sztrákós, 2002).

A lot of newly described species of dinoflagellate cysts were described from another locality, Monfort-en-Chalosse in Landes, France, located northwest from Pau and Gan. The species described were *Hystrichokolpoma globula*, *Hystrichokolpoma? incerta*, *Impagidinium brevisulcatum*, *Impagidinium gibrensis*, *Membranophoridium bilobatum*, *Phthanoperidinium chalossensis* and *Phthanoperidinium delicatum* (Michoux, 1985). At another location north of Biarritz and southeast of Monfort-en-Chalosse, species belonging to the *Wetzeliella* complex were first described within marls and calcareous marls. Those were *Kisselovia aculeata*, *K. columna*, *Rhombodinium translucidum*, *R. rugosum*, *Wetzeliella unicenata* and *Wilsonidium compactum* (Michoux, 1988).

![Map of the Aquitaine basin in relation to the Pyrenees mountain belt by Sinclair et al., 2005. Map of Gan, south of Pau in Southwestern France (Steurbaut & Sztrákós, 2002).](image-url)

**Figure 16.** a) Map of the Aquitaine basin in relation to the Pyrenees mountain belt by Sinclair et al., 2005. b) Map of Gan, south of Pau in Southwestern France (Steurbaut & Sztrákós, 2002).
3.5. Microscopy and Photography

A Leica DMRB microscope was used, coupled with a digital camera and image capture software (Axone), designed by the Newtec Company, and based in Toulouse, France. This software uses a “stacking” module to combine different fields of view into a single photograph.

Slides containing unpublished dinoflagellate cysts from Early Ypresian to Lutetian samples from the Southwestern Aquitaine basin were provided by a Total dinocyst specialist and used to practice dinocyst recognition under the microscope as well as selecting criteria for testing Xper³. The dinocysts were photographed and published in this thesis. These pictures were inserted in the result along with tables of identification of number of descriptors and states used and the genera left remaining as well as the correct genus name.

3.6. Interactive key in Xper³ and identification

Here are details and instructions about the identification process inside the interactive key created in Xper³. The first step of identifying dinocysts with this tool was to enter recognizable criteria into the Xper³ software in the “Interactive key identification” tab (Fig. A.9). All morphological “descriptors” are displayed (Fig. A.10) on the screen and it is up to the user to choose which descriptors will be used, depending on the morphological criteria visible in the dinocysts. In each of the “states”, a number is visible in parentheses (Fig. A.11), which is the number of genera that will be remaining after pressing the “Submit” button (Fig. A.12-A.13). The ”History” tab enables one to go back and see which morphological descriptors have been selected and possibly change certain states in one descriptor or to remove an entire descriptor (Fig. A.14). It is possible to continue typing more morphological descriptors (Fig. A.15) after submitting. In that case Xper³ uses equations that will automatically remove descriptors that are not necessary in order to find the targeted genus. It is also possible to choose several states in one descriptor. However, two states in the same descriptor cannot be combined. The result will be that all dinocysts genera that have either one of the mentioned states in the same descriptor will appear in the result as remaining genera and not only the ones with the combination of these states. Combining more than one state into one descriptor is preferable when there is some doubt about the state or characters observable. For example, if it is difficult to decide whether one dinocyst is spherical or subspherical. Choosing both as possible choices will mean that the final selection will combine all genera having a spherical or subspherical shape.

When a small number of states selected with a high degree of confidence have been entered, they were submitted to see how many genera were left as possible identifications. The remaining genera could thereafter be compared with each other’s from photographs and descriptions that was added inside the Xper³ (Fig. A.16), or the user could look up further literature.

Some descriptors are more general than others. Shape is a good example. Many genera have in common a subspherical shape and an apical archeopyle/opening. It is then necessary to add more descriptors. Conversely, some characters are found in very few genera. This is the case for camocavate
dinocysts, where cavation is only present on one side of the cyst. In that case, selecting “camocavation” is enough to bring up the proper genus.

Adjustments in descriptors or states for Xper\(^3\) were made from the first test of known samples to work better for the unknown samples in the last test (Fig. 17). States that exist in some genera but are not visible or described in the holotypes were also taken into account as possible options since it is important to have open minds and also think about how other would analyse it.

**Figure 17.** Flow chart showing the way dinocysts gets analysed into the interactive identification key in Xper\(^3\) with different outcomes and solutions.

## 3. Results

### 4.1. Testing the module

To see how well the morphological identification key works, tests must be performed that will analyse a number of dinocysts in the Xper\(^3\) identification key. The idea is to use dinocysts that are already known and analyse them in order to figure out if the identification key will come up with the targeted genera in question, and how many and which types of descriptors were more used. Unidentified dinocysts are thereafter also useful to test.

There were two different kinds of testing. One was on known dinoflagellate cysts from well documented samples, “Test 1”. This can be considered as a control testing to see how well and accurate the identification key in Xper\(^3\) worked. Photos were provided by Daniel Michoux. Those pictures were divided into three different sets depending on preservation and how well certain criteria were visible.
The first part consisted of 17 morphologically different and well-defined dinoflagellate cysts. Most of them had visible archeopyles and their shape and ornamentation were clear. The other part of the test consisted of 21 more challenging specimens, which could be the result of blurry photos, fragmentation, or a bit more compression or deformation. The last part consisted of 5 very poorly preserved specimens or just very small fragment pieces like a simple process for example.

The other test identified dinoflagellate cysts from unknown samples which were studied under a microscope and photographed. Photos that were saved were analysed based on their morphological characters with help of the identification key in Xper³.

The samples from “Test 2” were from Ypresian (56-47.8 Ma)-Lutetian (47.8-41.2 Ma) age strata (labelled 1, 43 and 61) from localities in the southwestern Aquitaine basin and were analysed with the morphological identification key in Xper³ that was created for this project and reviewed in the first test. All this was performed to see how well Xper³ works in reality when studying samples directly under the microscope. No statistical analyses were made on this part of the test. The focus was to suggest a genus name for the dinocysts that were found. The number of “descriptors” and “states” that were used was recorded, as well as how many genera were left remaining under or next to each photograph. Additionally, the states that were used in order to find the correct genus name were noted. The objective here was to find the correct genus in the remaining genera from the interactive key identifications.

The goal with all analyses was to stop when five or less genera were left if possible. The reason for having a maximum of five remaining genera was decided in communication with Daniel Michoux, to easier find the selected genus among the remaining ones. If that was not likely, then the analysis went as far as it could, leaving more than five genera. All data were recorded on how many descriptors and states were used, which ones were saved and later used for building up diagrams for the “Test 1”.

4.2. Test 1: Known dinocysts
The first test was divided into three sub-parts depending on the preservation and how well-defined certain characters of dinoflagellate cysts were. Those were “Well-defined dinoflagellate cysts”, with a total of 17 tests on dinocysts, “Not so well-defined dinoflagellate cysts”, with 21 tests and “Poorly defined dinoflagellate cysts”, which only consisted of 5 tests. Calculations were done on each test to see which morphological descriptors were more used, which one were more important, how many descriptors and states were used and how many genera normally comes up in the end after submission. These were presented in four different diagrams and “Test 1” part one, part two, and part three where done individually and then combined.

4.2.1. Statistical analyses
The first diagram focuses on which morphological descriptors were more commonly used to identify dinocysts in the identification key (Fig. 18). Ornamentation is clearly the most used one for all
different categories, followed by distribution of ornamentation, shape of the cyst and size of ornamentation.

The second diagram (Fig. 19) shows the number of needed descriptors to arrive at a maximum of five remaining genera. Between one and eight descriptors was used in total, and five, three and four descriptors were most commonly used to identify dinocysts in the identification key. The case where only one descriptor was needed, was for a dinocyst with a unique state of the descriptor “type of cavation” that only five genera possess, and it is “camocavation”.

Figure 20 shows similar results, the only difference is that the number of necessary states (answers to the different descriptors) selected was displayed. Four, three, six and eight states were the most chosen number of states used in Xper3.

The last diagram (Fig. 21) demonstrates how many genera were left after inserting all possible criteria or until a maximum of five genera were remaining. Results show that the majority of the tests ended up with between 1-5 genera, only a few with between 6-10 remaining genera and nothing over 10 remaining genera.

![Figure 18](image_url) **Figure 18.** Diagram showing the most common descriptors used for identifying dinocysts sorted in “Well defined dinoflagellate cysts” from 17 tests, “Not so well-defined dinoflagellate cysts” from 21 tests, “Poorly defined dinoflagellate cysts” from 5 tests and the total of the 43 tests.
Figure 19. Diagram showing how many descriptors were necessary to use in order to get a maximum of five remaining genera if possible.

Figure 20. Diagram showing how many states were used in order to get a maximum of five remaining genera if possible.

Figure 21. Diagram showing how many genera remained after applying the tests.
4.3. Test 2: Unknown dinoflagellate cysts

Here photographs of different dinoflagellate cysts from unknown samples from the Ypresian–Lutetian age of the Aquitaine basin will be presented, along with tables of how many descriptors and states were used to find the genus name. In addition to this, which states were used and how many genera remaining were recorded.

4.3.1. Peridinioids

The dinocyst of the genus Wetzeliella (Fig. 22a-b), is described with states such as five horns, processes as ornamentation, which have a nontabular distribution, and closed processes. This was enough to get the genus of interest. It was not necessary to use the other characters present, such as pentagonal shape, lateral horns as paracingulum expression, two layers, cornu- and circumcavation. Wetzeliella species also have cylindrical and solid processes (Fig. 22b). Charlesdowniea (Fig. 22c-d) is very similar to Wetzeliella, and the descriptors and states used were the same. The only significant difference is that the intercalary archeopyle is visible in the pictures of Charlesdowniea, not on the pictures of Wetzeliella. From the pictures, both genera can be considered as having a nontabular tabulation. Charlesdowniea actually have intratabular or penitabular distribution of processes, but this is not strongly indicated here. Therefore, the option of having nontabular distribution is accepted for Charlesdowniea in the identification key. The processes in Charlesdowniea are also furcate in the ends and distally connected (Fig. 22d).

Deflandrea (Fig. 22e) is described as having a ceratioid shape with proximate parasutural crests and paracingulum expression as well as parasutural crests as well. It has three distinctive horns, two layers and visible cornu- or circumcavaties. Lejeunecysta (Fig. 22e-f) has horns, which are not that well defined in this cyst and no cavation. Also, the parasutural crests are very clear in comparison to Deflandrea which looks more like it has a smooth or low relief ornamentation.
Figure 22. Peridinioid dinoflagellate cysts. a-b) *Wetzeliella*, from sample 61. b) In zoomed picture of the framed wall structures from figure a, showing cylindrical, solid processes. c-d) *Charlesdowniea*, from sample 1. d) In zoomed picture of the frames wall structures from figure c showing solid, furcate, distally connected processes. e) *Deflandrea*, from sample 1, f-g) *Lejeunecysta* from sample 43. f) Ventral view of the second *Lejeunecysta*. g) Dorsal view.

4.3.2. Gonyaulacoids without processes

The following figure 23, are three different dinocysts belonging to the genus *Impagidinium*, which have been described with slightly different descriptors. All of them have a subspherical shape with parasutural crests. In the first two cysts (Fig. 23a-d) a precingular archeopyle (opening) exists, located just in connection to the paracingulum. In the first cyst (Fig. 23a-b), seven descriptors and states were needed in order to arrive at six remaining genera, with *Impagidinium* being one of them. States such as subspherical, crests, parasutural distribution, precingular archeopyle, one layer, proximate and smooth crests were used.

In the second cyst (Fig. 23c-d), it was only necessary to use suturocavation and precingular archeopyle since it appears very clearly.
The last cyst (Fig. 23e) did not have a clearly visible archeopyle and therefore had that feature ignored. However, this type of *Impagidinium* was described as having proximochorate crests (higher relief in ornamentation than the others) and could only be described with six descriptors, ending up with eight remaining genera.

**Figure 23.** a-e) *Impagidinium* a-b) Proximate parasutural crests, from sample 61. a) Dorsal view. b) Ventral view. c-d) Suturocavated and proximate parasutural crests, from sample 61. c) Dorsal-lateral view. d) Ventral-lateral view. e) Proximochorate parasutural crests, from sample 1.
The dinocysts analysed in figure 24, possibly shows *Caligodinium* (Fig. 24a), which was one of the remaining genera from inserting states such as subspherical, smooth or low relief, apical, proximate and none or poorly expressed paracingulum. Other genera were excluded because of too many morphological dissimilarities, leaving this as an only option. It must be noted that *Caligodinium* might have an antapical archeopyle (whether it has an apical archeopyle or an antapical type is still debated). In that case, the image may be upside down.

The other dinocysts shown is a *Danea* (Fig. 24b-c) which is easily identified by its fusiform (lemon) shape and penitabular distribution of crests. However, crests were not needed in this identification key in order to get to this targeted genus.

![Figure 24. a) Caligodinium? From sample 1 b-c) Danea, from sample 61. b) Dorsal view. c) Ventral view.](image)

The dinocysts illustrated in figure 25 are a bit fragmented and have a low relief. It would be natural for an untrained eye to interpret figure 25a as a dinocyst with an apical opening. However, since the orientation is not clear and pieces of its outer wall layer are missing, this assumption should be ignored for now. What is observable is that the cyst is subspherical, has a smooth or low relief, two layers, is proximate and has no horns or protrusions. Within these remaining genera, *Thalassiphora* is one of them, and fits best into this photography (Fig. 25a).

The second dinocyst (Fig. 25b) used the states: subspherical, reticulate ornamentation, precingular archeopyle, proximate and nontabular distribution.
4.3.3. Gonyaulacoids with gonal and intergonal processes

The genera *Spiniferites* and *Spiniferella* are very similar (Fig. 26) and are described with the same criteria for this test, which were subspherical and ellipsoidal shape, crests, gonal and intergonal distribution of processes with trifurcate tips. They also both have a combination of crests and processes which can be described as proximal connections between the processes or just as gonal and intergonal distribution. Both of them also commonly have suturocavation, meaning they have hollow/empty crests. The processes are also branching. The main difference between *Spiniferites* (Fig. 26a-b) and *Spiniferella* (Fig. 26c-d) is that *Spiniferella* has a subquadrangular to pentagonal sixth precingular plate and an apical horn or protrusion while *Spiniferites* has a triangular shaped sixth precingular plate. The same differences in plate configuration exists between *Impagidinium* and *Leptodinium*, as discussed above in the “Early Paleogene dinoflagellate cysts” section.

Figure 25. a) *Thalassiphora*? from sample 63 b) *Xenicodinium*? from sample 63.
Figure 26. Gonyaulacoids. a-b) *Spiniferites*, a) from sample 1, b) from sample 43. c-d) *Spiniferella*, from sample 1. c) Dorsal view. d) Ventral view.

4.3.4. Gonyaulacoids with intratabular processes

All dinocyst from figure 27 are *Hystrichokolpoma* and are described in the same way, although there might be differences within them in a species level. The focus here is the identification on a genus level. All cysts have the states: subspherical, processes, intratabular, more than two types of processes which one type of them are box-like or square in shape.

Figure 27. a-d) *Hystrichokolpoma*, a-b) from sample 43, c-d) from sample 61.

Figure 28 shows cysts belonging to the genus *Homotryblium*. The descriptors used to interpret the three images are slightly different. In figure 28a, the combination archeopyle is visible and was further only described with processes and intratabular distribution. Whereas the other two cysts of *Homotryblium* were described with the states: subspherical, processes, intratabular, tubular and open processes and one process per plate.
**Homotryblium**

<table>
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</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>Remaining genera</td>
<td>4</td>
</tr>
<tr>
<td>Inserted states</td>
<td>Combination archeopyle and striated processes.</td>
</tr>
</tbody>
</table>

**Homotryblium**

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>States used</td>
<td>6</td>
</tr>
<tr>
<td>Remaining genera</td>
<td>6</td>
</tr>
<tr>
<td>Inserted states</td>
<td>Sub-tubular processes, tubular processes, processes, /plate</td>
</tr>
</tbody>
</table>

**Melitasphaeridium** (Fig. 29a-b) is described with the states: subspherical, processes, intratabular, tubular and closed processes and have distinctively more processes than *Cordosphaeridium* (Fig. 29c-d). *Cordosphaeridium* are described with having a subspherical shape, intratabular distribution of processes, which are fibrous and have expanded tips. It also has about one process per plate.

**Cordosphaeridium**

<table>
<thead>
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<th>Descriptors used</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>States used</td>
<td>7</td>
</tr>
<tr>
<td>Remaining genera</td>
<td>6</td>
</tr>
<tr>
<td>Inserted states</td>
<td>Sub-apertural processes, processes tips, processes, /plate</td>
</tr>
</tbody>
</table>

**Enneadocysta** (Fig. 30), which differ in length and thickness of the process tips. The genus *Enneadocysta* is unique in possessing this type of strongly recurved and spiny tips. However, short and thick processes (Fig. 30a) are not described in the literature and had to be

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**Figure 28.** a-c) *Homotryblium*. a) Visible combination archeopyle and striated processes. From sample 1. b-c) Tubular, hollow and open processes, from sample 43. b) Chorate processes. c) Proximate to proximochorate processes.

**Figure 29.** a-b) *Melitasphaeridium*, a) from sample 63, b) from sample 1. c-d) *Cordosphaeridium* c) from sample 61, d) from sample 1.
added afterwards in the identification key. This is likely to be a new undescribed species of *Enneadocysta*.

Both figure a and b where described in having a subspherical shape, intratabular processes, bifurcate tips, solid processes and about one process per plate. The only differences are that the apical opening is visible in the first cyst, and the second cyst has chorate as a chosen state.

![Figure 30. a-b) Enneadocysta from sample 1. a) Proximate to proximochorate thick processes and visible apical archeopyle. b) Chorate and slender processes.](image)

### 4.3.5. Gonyaulacoids with nontabular processes

The rest of the figures are Gonyaulacoids with interpreted several processes, often nontabular. In the following image (Fig. 31), *Adnatosphaeridium* (Fig. 31a), *Glaphyrocysta* (Fig. 31b) and *Chiropteridium* (Fig. 31c) are described.

*Adnatosphaeridium* is described with subspherical shape and trabeculae as ornamentation, which is also another name used for distally connected processes. The processes are also proximally connected.

Processes in *Glaphyrocysta* are not connected proximally, the terms used are subspherical shape and trabeculae as ornamentation, with an apical archeopyle and three wall layers. In addition, there is also no ornamentation in the midventral and dorsal area, just like *Chiropteridium*.

Processes in *Chiropteridium* are not connected either distally or proximally, but are described within Xper³ as subspherical, with processes, apical archeopyle. It is also described with more than two types of processes and furcate tips.
Operculodinium and possibly Impletosphaeridium are described in figure 32. All dinocysts have numerous nontabular processes, but with different shapes and sizes.

The first Operculodinium (Fig. 32a) is described as subspherical, processes as ornamentation, nontabular, proximochorate and precingular archeopyle.

The second Operculodinium (Fig. 32b) is described similarly, without the precingular archeopyle and with expanded tips instead, which is not a characteristic for the first species described of Operculodinium.

The last dinocyst could either be identified as Operculodinium or Impletosphaeridium here. Since the processes, solid and slender with bifurcate tips are typical of both genera, it could only be distinguished if the archeopyle was visible in the present case. If the archeopyle is precingular, then it would be an Operculodinium, and if it was apical, Impletosphaeridium.
Figure 32. a-b) *Operculodinium*. a) Visible precingular archeopyle, proximochorate and sharp/rounded tips, from sample 1. b) Thicker longer processes, little expanded tips, from sample 43. c) Could be either *Operculodinium* or *Ipletostosphaeridium*. Archeopyle is not visible, which is crucial in knowing which genus it belongs to, from sample 61.

The last figure (Fig. 33) also shows different types of dinocysts with nontabular ornamentation. Figure 33a-b both have sharp tips, the difference being that *Lingulodinium* (Fig. 33a) are slender and have more numerous processes, but have the chosen states solid processes, whereas *Eocladopyxis* (Fig. 33b) has the chosen state: proximochorate and has also more thick and conical shaped processes.

*Polysphaeridium* (Fig. 33c) has more tubular and cylindrical shaped processes. It also has an epicystal (combination) archeopyle, which in this case it is hard to tell. Otherwise, the same descriptors are used for *Polysphaeridium* and for *Eocladopyxis* except for the tips which are described as expanded rather than sharp.
5. Discussion

5.1. Choices of morphological descriptors and states

The concept with the identification key inside the Xper3 is being able to choose from a multiple number of morphological descriptors to identify dinocysts without introducing a hierarchy. The aim of the work presented in this thesis was to apply this philosophy to Early Paleogene dinocysts at genus level, with the idea of using as few descriptors as possible, ideally no more than five. This implied finding the right balance, ideally, it would be easiest and less time consuming to arrive at a low number of genera with few descriptors. However, depending on the morphology of the dinocyst, its orientation, or state of preservation, it can be difficult to decide which morphological descriptors to use, and necessary to look for secondary, less obvious features.

Determining which morphological descriptors and states are best suited for creating an interactive morphological key for Early Paleogene dinoflagellate cysts can both be easy and challenging. In many cases several descriptors and states that were used meant practically the same thing and were unnecessary. For example, if there is a subspherical or spherical dinoflagellate cyst, then it is most likely that they do not have horns. Those with one horn (apical horn) are described as drop shape, two
horns as fusiform shape, three horns as ceratioid shape etc. There might also be several species within one genus that might have a different number of horns. There are also those Peridinioid dinoflagellate cysts that have a highly reduced antapical horn and can be described both as rhomboidal and pentagonal shapes.

The basic morphological descriptions and states work very well in general. There are however some morphological descriptors that can have several features. Most of the dinoflagellate cysts have one main type of ornamentation. There are however some cases with several types of ornamentations on the same cyst. For example, high parasutural crests and lower nontabular granules. These features cannot be combined uniquely in Xper3 and have to be entered as individual characters combining two states for example: “Processes and crests”, “Crests and granules”. However, it is preferable to also still use the single states as options if one of these characters cannot be identified. There are also other examples where there is no need to mention two types of ornamentation. This applies to “intergonal and gon al processes” and “proximally connected by parasutural crests”. If processes are described as gon al or intergonal, they are by definition connected proximally by parasutural crests. Horns were not selected as a state for the descriptor “ornamentation” in Xper3, considering that the concept of horns was already covered by the descriptors “shape of the cyst” and “number of horns”. In the same way, the morphological character apiculocavation, which corresponds the presence of a hollow space (pericoel) inside the process, is referred as hollow processes and is not included in the cavation descriptor.

The safest way to describe the shape of a rounded dinocyst would be to say that it is subspherical, even if strictly speaking it is ovoid, rounded, ellipsoidal or lenticular. The perception of the shape of an object can differ between biostratigraphers. It would then have been easier to put all of this into a category called “rounded” cysts, and to still keep the other states of shapes. The archeopyle is a reliable descriptor. However, it could be developed to become more specific, especially when working with a database including a larger number of dinocyst genera. Some obvious details about the archeopyle can be found in the “Other characteristic” descriptor.

A morphological feature which would greatly benefit from a more in-depth study is the processes. Improvements could be made in giving more details about process tips, with better illustrations and detailed descriptions, providing more options to choose from. Description of the structure of processes, open or closed, solid or hollow, would also add resolution. This additional work is worth undertaking, even if it means adding more descriptors.

5.2. Interactive identification key and Xper3

It is preferable to start first by selecting the clearest morphological descriptors, submit the results and see how many genera are remaining, and then decide if it is necessary to add more descriptors. Since the identification key is still developing, adding too many descriptors in the beginning is risky, since a single error might mean that it will not be possible to arrive at the correct genus. It is also possible for
the user to misinterpret a character in the identification process. While doing the test, adding processes as ornamentation seemed unnecessary since it is possible to directly enter the other aspects about the processes. The “ornamentation” field for processes was almost always filled.

After entering the first test, there were some small errors in some states of the descriptors as well as choosing the wrong parameters in the identification to find the correct dinocyst genus. However, that was easily adjusted and the accuracy of both the identification key and the art of identifying genera was improved over time.

Dinocysts that lack ornamentation were more difficult to describe since the other ornamentation descriptors could not be further filled.

If a single access key had been used instead of a multi access key, it would not have been possible to identify some dinocysts that are not so well defined and badly preserved, for example, if the shape must be described and there are only fragments of dinocysts available. Then the dinoflagellate cysts cannot be further identified (Fig. 34). The aim of this multi access key identification type is that secondary criteria should in many cases help in identification in order to better find the correct genus.

Figure 34. Single access key versus multi access key (Hagedorn et al., 2019).
5.3. Statistical analyses

The morphological interactive key works well in general since the morphological differences between each genus are distinctive. Many genera are distinguished from a similar genus by either the type of archeopyle, tips of the processes, distribution of ornamentation, shape of the cyst or number of horns, different kinds of processes, different set-up or type of processes as paracingulum expression etc.

To really understand what is meant by the different levels of “defined morphological criteria”, an example of photographs (Fig. 35) displays certain characteristic from each category. The “well-defined dinocyst” (Fig. 35a), exhibits clearly morphological descriptors such as shape, archeopyle, ornamentation, distribution of ornamentation and cingulum. A minimum of five descriptors can be used with certainty.

A fragmented dinocyst, however, belonging to “Not so well defined dinocysts” (Fig. 35b), only displays morphological descriptors such as ornamentation, aspects of processes shafts and tips. Only three descriptors can be used, perhaps four if distribution of ornamentation is decided to nontabular and intratabular.

The last dinoflagellate cysts, from “Poorly defined dinocysts” (Fig. 35c), can only be described by its ornamentation of processes and aspects of the shaft. The process tips are not well defined. Only two descriptors can be used for certain.

![Figure 35. Example of photographs of dinoflagellate cysts from “Test 1”. The different grades of morphological defined characters marked within the dinocysts. a) A “well-defined dinoflagellate cyst” b) A “not so well defined” fragment of a dinoflagellate cyst c) A “poorly defined dinoflagellate cyst” process.](image)

Ornamentation is by far the most used morphological descriptor in the identification key and is used 38 of 45 times, followed by types of distribution of ornamentation, used 27 of 43 times, and shape, 26 of 43 times. The shape is a relatively easy descriptor to describe. However, the reason why shape does not appear higher in the list of most used descriptors is that it is not possible to identify the entire cysts shape, based on fragments. This is visible from the diagram from figure 18 of “Poorly defined dinoflagellate cysts”, where there is a total absence of how many times the shape has been used as a descriptor.

Ornamentation is relatively easy to identify, it is most likely to be found in both whole cysts and broken pieces. The descriptors being most used from the Cretaceous dinocyst identification key made by Rodriguez-Barreiro, 2018, were “Relief”, which in this case means the same as ornamentation, then
“Size of the relief” and “Shape of the cyst”. Size of the ornamentation is the fourth most used descriptor for this interactive key, whereas number of horns and paracingulum expressions are used more widely than the distribution of ornamentation from the tests of the Cretaceous dinocysts (Rodriguez-Barreiro, 2018). In this case, it is not always necessary to use the number of horns when the shape of the cyst has already been described. The same applies to distribution of ornamentation and paracingulum expression. Very often, the paracingulum expression is similar to the distribution of the ornamentation, for example, if a dinocyst has parasutural crests, then the paracingulum will most likely be expressed be parasutural crests.

However, the most “important” descriptor that immediately removes a lot of genera cannot really be determined, since it really depends on what “states” are chosen. It is preferable to go for the most used descriptors in this case. An example of having a particular state in one descriptor, leading to having another particular state in another descriptor, making one of these descriptors unnecessary is for example if “Acavate” is being chosen as cavation (meaning that there is no cavation), then the cyst probably in most cases has one layer. Meaning that one could either chose the descriptor cavation and the state “acavation” or choosing the descriptor “number of layers” to the state “1”, leading to the same result.

What was notable for doing the test in the identification key, before reviewing user errors was that, very often, the archeopyle was misinterpreted by the user or just not used, which caused more than five genera to remain. When the archeopyle became correctly interpreted, it automatically removed a lot of unnecessary remaining genera in the end from earlier stages, with less descriptors being used than before. The literature also mentions that the archeopyle is considered to be most unique to the cysts (Evitt, 1985).

The number of descriptors used were between 1-8 and the most common number of descriptors being used was 5, closely followed by 3 and 4 out of a total of 13 descriptors. The majority of the “Not so well defined dinocysts” (21/43) and “Poorly defined dinocysts” (5/43), from “Test 1”, required the use of 5 descriptors. Only 4 descriptors of the “Well defined dinocysts” (17/43) were needed in most cases. The diagram showing how many states were more used were 4, closely followed by 3, 6 and 8 states. The reason why the number varies from the “number of descriptors used” is that more than one state can be selected for the same descriptor.

Even if 13 different descriptors exist, no more than eight descriptors or states were necessary. Some descriptors were automatically removed after the first submission of only a few descriptors that Xper³, saw as unnecessary to get the correct genus in the end. The test made by the Cretaceous identification key, showed a range of 4-12 descriptors (compared to a range of 2-8 in the present work) where seven and eight were the most common number of descriptors used (Rodriguez-Barreiro, 2018). Compared to the Cretaceous key, the “Early Paleogene dinoflagellate cysts” identification key used less descriptors with more states, one reason might be that the Cretaceous key has more genera, 279, compared to the Early Paleogene identification key which only has 145 described genera.
The last diagram looks at the number of genera remaining. In most cases it is never reaching over 5 remaining genera and it is also never exceeding more than 10. Only 6 analysed dinocysts in the identification key ended up in having between 6-10 remaining genera, two from each group of “Well defined dinocysts”, “Not so well defined dinocysts” and “Poorly defined dinocysts”. If this was expressed in percentages, then the lowest percentages to get between 6-10 genera would be in “Not so well defined dinocysts” with about 9.5 % (21 divided by 2), followed by 11.8 % (17 divided by 2) from “Well defined dinocysts” and the highest percentage would correspond to “Poorly defined dinocysts” with about 40 % (5 divided by 2). No assumptions based on percentages should be made, especially not based on the low number of tests conducted for “Poorly defined dinocysts”.

5.4. Dinoflagellate cysts from the unknown samples

The dinoflagellate cysts that were photographed from the Ypresian-Lutetian samples were tested in how to apply the identification key from the reviewed first test period. Almost all dinocysts were successfully determined with their respective genus name. Small differences in similar dinocysts could be noticed in Charlesdowniea and Wetzeliella which were separated by the characteristic of their process tips. The tips in Charlesdowniea are bifurcate and almost, but not quite distally connected, while they are simple in Wetzeliella.

One detail, the archeopyle separating Operculodinium from Impletosphaeridium was missing in figure 32c. Therefore, the genus classification remains unclear. There were also some differences noticed within the same genus, concerning Impagidinium for example (Fig. 23). The second dinocyst of Impagidinium showed a clear suturocavation (hollow sutural crests), that could be mistaken for penitabular crests (see Fig. 24b-c for comparison with penitabular crests in Danea), and the third Impagidinium had a higher relief, or a more proximochorate crest size.

Hystrichokolpoma also display different process shapes, which is due to their great species variation. Dinocysts with poor or no ornamentation were and are more difficult to classify, like Caligodinium? (Fig. 24a), Thalassiphora? and Xenicodinium? (Fig. 25).

5.5. Other identification keys for dinoflagellate cysts

Another identification key from modern dinoflagellate cysts of Quaternary period are found in the webpage http://www.marum.de/dinocystkey.html (Zonneveld & Pospelova, 2015). That identification key is very dissimilar to the one created for this project in Xper. That identification key is very dissimilar to the one created for this project in Xper. It uses a single access key determination, where one progresses in a linear way through a series of morphological criteria in order to arrive at the correct taxa.

Since identification is made at a species level and not genus, the criteria are more specific and detailed. There is also the risk of not being able to distinguish important criteria due to preservation or non-adequate orientation of the dinocysts. This web site can be considered as an electronic catalogue rather than a true expert system. It was designed for the Quaternary where the number of genera is
limited and is not adapted to the biostratigraphic interpretation of older sediments containing much more genera.

5.6. Difficulties

It is difficult to describe “odd looking” dinocysts or fragments and to select consistent criteria for all the genera. When working with Xper3, visible criteria are inserted, but sometimes, choosing the wrong character without keeping other options open will cause the correct genus to be excluded from the results. However, being too open and having too many different suggestions, will result in a high number of genera being selected. One must find a balance between what information is available in the original descriptions and what the observer can see, and this must be taken into account in a test case.

The morphology found in the literature can be very detailed, but it is always more difficult to recognize criteria under the microscope. It is also possible that dinoflagellate cysts present in the samples for “Test 2” were not recognized and ignored, which could have caused bias in the report. It must be noted that this report was not aimed at biostratigraphic determinations and instead focus on creating and testing a morphological identification key. The fact that there is no English version of Xper3 added difficulties.

5.7. Further studies and improvements

Similar morphological identification keys could also be created and developed for Later Cenozoic and Jurassic dinoflagellate cysts.

Regarding processes, it is necessary to create better descriptions for their shape, as well as detailed nomenclature for the morphology of their tips, for improving identification in the morphological identification key.

The number of remaining genera was selected to be a maximum of five. The reason why five was kept as a good outcome of maximum remaining genera, was that in many cases, some dinocysts could not be described and had to be left with an “unknown” answer. Those dinocysts would therefore also be selected automatically when filling in any of the states in those particular descriptors where the answer is “unknown”. If there were more time, a better reviewed and precise identification key could have been created. Giving a result of a maximum number of one remaining genera, with the correct option. Then, more sub-descriptors of already existing descriptors would have to be created along with more detailed descriptors that wasn’t included in this project.

6. Conclusion

- Can the chosen descriptors and states inserted in Xper3 determine the correct taxa of dinoflagellate cysts? How many suggestions of genera come up? What is the optimal number of descriptors and which ones best describe the genus in question?
In most cases, the correct genus is within the remaining genera for the test. The first test basically focused on reviewing all errors of incomplete data or error made by the users themselves which caused the correct genus to not show up as one of the remaining genera.

All analyses from the second test gave the correct genus if the characteristics of the dinoflagellate cyst were interpreted correctly by the user. In most cases, in 37/43 times, a maximum of five genera are suggested by Xper³, which was the ultimate goal for this project. The remaining tests (6/43) ended up with between 6-10 remaining genera, which can be considered as an acceptable result. No tests reached over 10 remaining genera.

The most common number of descriptors that were being used was five, also closely followed by three and four and the descriptors that were more widely used were: type of ornamentation, distribution of ornamentation, shape of the cyst and size of the ornamentation.

The most important descriptor to use in order to uniquely describe genera is not easy to identify and probably depends on which unique “state” is being chosen. However, the archeopyle is described as one of the most unique descriptor for each dinocyst by authors, which also strengthens the assumption of whenever the archeopyle was picked as a descriptor, no matter the type of state, a lot of remaining genera were removed after submission in the interactive key.

- How accurately can the selected descriptors and states in Xper³ decide the taxon, especially for badly preserved dinoflagellate cysts? If the degree of accuracy is not satisfactory, how can we improve it?

By combining morphological descriptors, the interactive key in Xper³ selects a limited number of possible genera sharing the morphological states observed by the biostratigraphers, who can then assign the microfossil observed to the proper genus. The aim of the tests was not to arrive directly at correct genus, but to suggest a limited number of genera (ideally no more than five). The biostratigrapher can then go into the literature and make the correct choice.

The key works well for describing poorly preserved dinocysts or fragments, as it allows searches on all the visible morphological criteria. It could of course be improved. For example, the details of the processes can be reviewed and divided into more descriptors. It was not necessary in this project, which was aimed at determination at a genus level, but would be very useful when moving to determination at species level.

- Will it work to identify genera from unknown samples?

Finding dinocysts with the microscope and identifying the genera from the unknown sample worked relatively well. A total of 57 individual dinocysts were recognized and most of them analysed with Xper³ to identify the correct genus in the genus list selected by the morphological interactive key. This work was more successful than the ones from the first test, where selected photographs from Daniel Michoux were provided with different levels of preservation states. In the second test from the
unknown samples, the identification key had time to be reviewed and improved from the first test. Some errors caused by the user occurred but were fixed immediately after revision. However, there are still some dinocysts left with uncertain genus names, either because they lack ornamentation or because one specific feature that distinguishes a specific genus from another was not visible.

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Appendix: Workflow in Xper³

Following images or print screens, are describing the workflow from how to create a knowledge database of dinoflagellate cysts and how to use the identification key.

Figure A1. The first thing to do in Xper3 is to a “Create Knowledge Base”.

Figure A2. The first tab is called “Items” which are the genera. By filling in the “Add a new item”, a new genus can be entered to the data base. Where you can add descriptions and alternative names.
Figure A3. It is also possible to upload photographs of the dinocysts from the tabs “Choose from Dropbox” or “Add from Url”.

Figure A4. In the second main tab, “Descriptive model”, morphological descriptors can be added along with definitions and figures in a similar way as adding genera in the “Items” column.
Figure A5. It is possible to select states for each descriptor by having a descriptor chosen from the description list. In the third tab “States”, all states that could describe the descriptor in the dinocysts can be added.

Figure A6. In the fifth tab, “Tools” and “View Description Matrix”, all genera and the morphological criteria (states for each descriptors) can be combined in a matrix.
Figure 7. In the matrix, you are able to fill in unique states for each genera’s descriptor.

Figure A8. An example of how to fill in states for the descriptor "Shape of the cyst” for the genus *Achilleodinium.*
Figure A9. To test the identification key, the forth tab, “Identification” and the “Interactive identification” will open up the identification process.

Figure A10. Inside the identification key, the main eight descriptors are visible and can be selected with different states. In the right column are all the remaining genera.
Figure A11. By pressing on one of the descriptors, the states will appear. Next to each state is a number which is the number of genera that will come out after pressing the submit button.

Figure A12. Select for example “Apical” archeopyle, and all 55 dinocysts with that character (state) will appear after submission.
**Figure A13.** After the submission, 55 remaining genera are present and then it is possible to continue filling more morphological criteria (states for each descriptor).

**Figure A14.** The "History" tab, can show which descriptor that has been chosen. It is also possible to remove or change inside of the descriptor.
Figure A15. In here, there has been a selection of five descriptors that lead to having four remaining genera.

Figure A16. All remaining genera that have an “apical” archeopyle, “spherical” shape, “processes” as ornamentation with “expanded” process tips with “intratabular” distribution, can be easily be compared by the figures of the holotypes that is inserted into Xper® and read further descriptions on the genera to see what characters (states) are separating one genus from another.