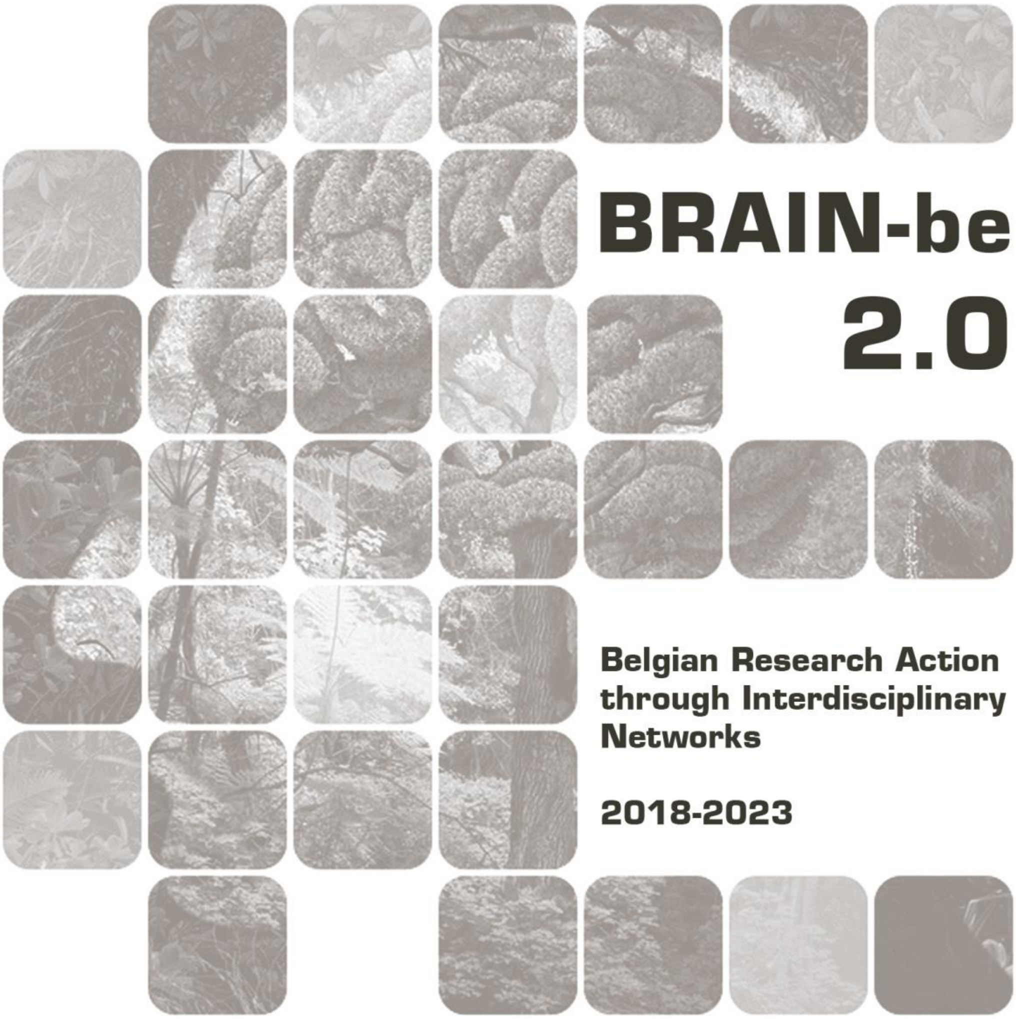

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Belgian Research Action
through Interdisciplinary
Networks

2018-2023

VERTIGO

Deciphering early stages of VERTebrate evolution: insights from long Ignored Belgian Devonian fossil Organisms.

Sébastien Olive (Royal Belgian Institute of Natural Sciences) – Aude Cincotta (Royal Belgian Institute of Natural Sciences) – Pierre Gueriau (Institut photonique d'analyse non-destructive européen des matériaux anciens & University of Lausanne) – Bernard Mottequin (Royal Belgian Institute of Natural Sciences)

Pillar 1: Challenges and knowledge of the living and non-living world

NETWORK PROJECT

VERTIGO
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Contract - B2/202/P1/VERTIGO

FINAL REPORT

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ABSTRACT

This project aimed at characterising fossils, including soft-bodied organisms, encountered in the Lower Devonian siliciclastic succession of the southernmost part of Belgium, in the Neufchâteau Synclinorium. The Warmifontaine locality recently yielded hundreds of enigmatic organisms. The latter were collected in slates of Pragian age and include cephalochordates, various groups of cephalopods, arthropods, echinoderms, 'worms' and many unidentified specimens. Thanks to state-of-the-art imaging and spectroscopy techniques, we identified and studied several of them. A new methodology has been established using fossil elemental composition as a complement to anatomy to help identifying specimens otherwise difficult to classify. Regarding the taphonomy of the Warmifontaine locality, the data show that the fossils are preserved as pyritized and carbonaceous compression fossils, decaying organic matter having participated to the trace elemental composition of the pyrite crystals. Preservation of the Warmifontaine fossils therefore closely resembles that of the coeval Hunsrück Slate fossils, Germany.

The Belgian locality of Warmifontaine constitutes a new Lower Devonian *Konservat-Lagerstätte*, with the preservation of both bio- and non-biomineralized organisms. Future identification of problematic taxa should shed more light on the biodiversity of that new fossil locality.

1. INTRODUCTION

After years hidden in historical collections of the Royal Belgian Institute of Natural Sciences (RBINS) and of the Centre Grégoire Fournier (Maredsous Abbaye), fossils from the Lower Devonian of Belgium were rediscovered by one of us (B.M.) in 2020. Those fossils were collected during the end of the ninetieth and beginning of twentieth centuries, from slate mines (named *ardoisières* in French) in the vicinity of Neufchâteau, in the southern part of Belgium. The old Belgian collections include corals, bivalve molluscs, trilobite arthropods, orthocone cephalopods, putative vertebrates (euphaneropids?) and enigmatic specimens reminiscent of Cambrian early chordates. One of these localities is called Warmifontaine and displays sedimentary facies very similar to those of the well-known Hunsrück Slate localities in Germany. The fossil record of early chordates and early vertebrates is extremely scarce as these organisms mostly consist of decay-prone soft parts (integument, organs, muscles) that are usually degraded and lost prior to the onset of fossilisation processes (Sansom et al., 2010). To date, no early chordates have been found outside Canada (Burgess), China (Chengjiang), and USA (Marjum; Lerosey-Aubril & Ortega-Hernández, 2024) in rocks younger than ca. 500 Myr (Cambrian). The occurrence of chordates in Lower Devonian deposits of Belgium would therefore greatly extend their stratigraphic range and could provide pivotal new insights into our understanding of chordate evolution.

2. STATE-OF-THE-ART AND OBJECTIVES

State-of-the-art

The fossil record offers unique and crucial insights into many important episodes in the evolution of life on Earth. The early steps of vertebrate evolution represent key episodes in that wider evolution of life. The onset of vertebrate evolution started in the early Cambrian (ca. 520 Myr), with the first occurrences of elongated and laterally flattened soft-bodied organisms known as early chordates. They lacked a backbone, paired fins or jaws but possessed a notochord (the precursor of the spinal column) (Gee, 1996; Holland & Chen, 2001; Donoghue & Purnell, 2005; Conway-Morris & Caron, 2012; Mallatt & Holland, 2013). After that, major anatomical changes occurred in early vertebrates with the acquisition of a skull, jaws, paired fins and, in some taxa, adaptations to terrestrial life (e.g., paired limbs, amniotic egg) (Janvier, 1996; Clack, 2012).

Early chordate and early vertebrate fossils provide critical information on the origin of vertebrates and on the evolution of their particular body plan. Fossils also bring information about when and in which order diagnostic characters of modern clades were acquired. Fossil taxa show improbable characters or set of characters that challenge decisions on polarity and homology based on extant taxa alone. This had in some cases led to radical reinterpretations of the evolutionary relationships of living groups.

Unfortunately, the fossil record of part of the early vertebrates (i.e., euphaneropids, in the scope of this project) and early chordates is extremely scarce as these organisms mostly consist of decay-prone soft parts (e.g., integument and muscles) that are usually degraded and lost prior to fossilisation (Sansom et al., 2010). Even in localities where they are considered ‘exceptionally preserved’, early vertebrate fossils are not anatomically complete, or their preservation is never pristine. They were subjected to complex decay and fossilization processes that make interpretation of their anatomy extremely challenging (e.g., Briggs, 2003; Donoghue & Purnell, 2005, 2009; Sansom et al., 2010, 2013a). The identification of fossilized organisms relies on how well they are preserved and whether diagnostic anatomical details are retained. Phylogenetically important characters are often those that do not resist decay and, by extent, fossilization (Sansom et al., 2010). Those characters are indeed composed of labile compounds (soft tissues) that degrade quickly under normal environmental conditions. It is only in very particular – exceptional – conditions that soft tissues are preserved through time. Sansom et al. (2010) showed with experimental decay that loss of chordate diagnostic characters is non-random: the most phylogenetically informative characters are lost first during decay whereas plesiomorphic characters are more resistant to decay. Phylogenetically important characters appear to be absent in Warmifontaine fossils when observed with the naked eye. VERTIGO project will attempt to provide more information on the anatomy of those enigmatic fossils using a range of analytical techniques.

Although initial stages of vertebrate evolution are occasionally clarified by discoveries of new key-taxa, mainly from China and North America, there are still major gaps in our understanding of the processes and timing of character evolution. It is therefore essential to find new fossil material in other stratigraphic units. Until then, palaeontologists need to focus on the quite limited material available for study. To overcome the issues coming from the poor fossil record of early chordates and early vertebrates, the VERTIGO project proposes to focus on the study of new findings of putative early chordates, early vertebrates (euphaneropids) and unidentified, enigmatic, taxa from the Lower Devonian of Belgium.

Early chordates

Given the poor fossil record of chordates, the affinities of some specimens including *Metaspriggina*, *Pikaia*, *Cathaymyrus* and yunnanozoans from the well-known Cambrian fossil localities of Chengjiang in China (Xian-Guang et al., 2017) and Burgess in Canada (http://www.burgess-shale.rom.on.ca; Royal Ontario Museum, 2011), remain highly debated (e.g. Holland & Chen, 2001; Conway-Morris, 2008; Shu et al., 1996; Conway-Morris & Caron, 2012; Mallatt & Holland, 2013).

A broad range of phylogenetic affinities was proposed for each possible chordate taxon, with little consensus being reached (Sansom et al., 2010; Fig. 1). One of the best-known examples remains the assumed chordate *Pikaia gracilens* from the middle Cambrian Burgess Shale (Canada). *Pikaia* has successively been interpreted as a polychaete, an early chordate, a cephalochordate, a potential myxinoid and even a specialised chordate (see Conway-Morris & Caron, 2012 for the historical aspect and Mallatt & Holland, 2013). The number of characters observed on the fossil specimens keep increasing and changing due to varied interpretations (Mallatt & Holland, 2013: table 1). A myomeral arrangement and the presence of a notochord, a dorsal nerve chord, a pharynx and an endostyle are characters that are all critical in the debate on chordate origin. Those diagnostic characters are still to be clearly identified in the fossil record. Preservation is often too poor to allow definite identification.

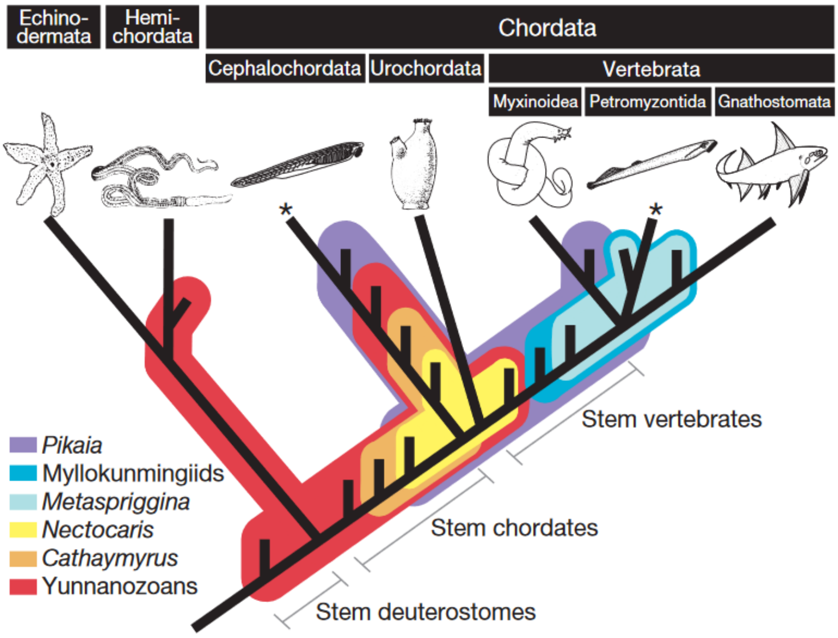

Figure 1. Deuterostome phylogeny with various affinities for several early chordates. From Sansom et al. (2010).

Euphaneropids

Euphaneropids (‘naked anaspids’) are early jawless vertebrates (Fig. 2) thought to be anaspids (basal-most stem gnathostomes; e.g., Blom, 2012) with a reduced or lost dermal skeleton (the scales and superficial head bones) (Stensiö, 1939). Because euphaneropids are generally preserved as imprints and give little anatomical information, interpretation of morphological characters remains challenging. Some adult specimens of the best-known ‘naked-anaspid’, *Euphanerops longaevus*, from the Late Devonian Miguasha *Lagerstätte* in Canada, preserve internal calcified structures thought to be mineralised cartilage (Janvier & Arsenault, 2007). Euphaneropid is the first vertebrate group that clearly showed (i) gill filaments enclosed by gill pouches (Janvier et al., 2006), (ii) paired anal fins (Sansom et al., 2013b) and pelvic fins (Chevrinais et al., 2018), and (iii) an intromittent organ (Chevrinais et al., 2018). Despite their scarcity in the fossil record and the usual poor preservation of their fossils, the study of this group of jawless vertebrates is crucial for our understanding of early vertebrate evolution.

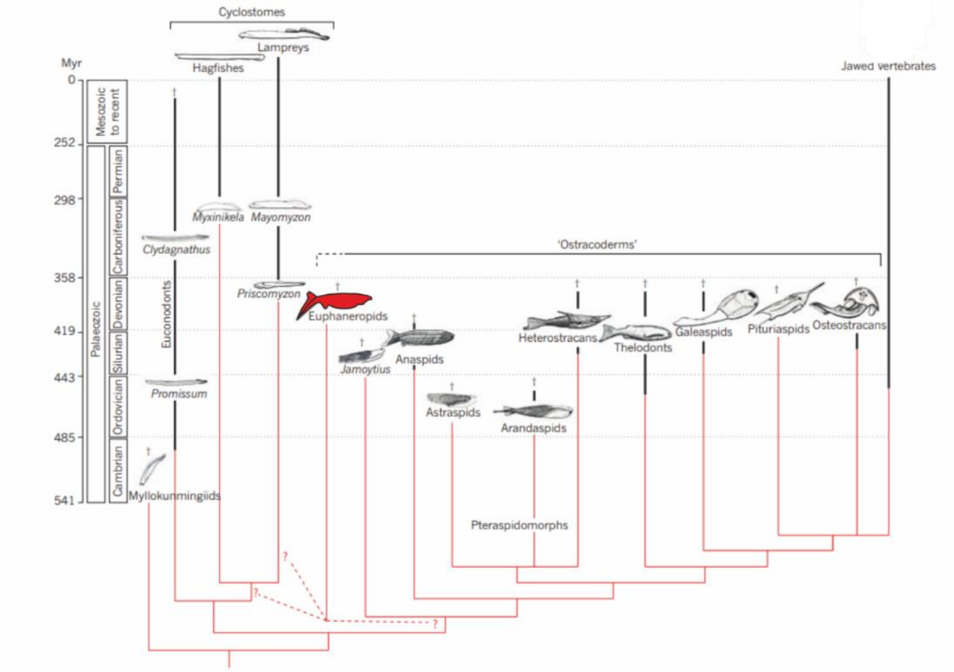

Figure 2. Interrelationships between major Palaeozoic jawless vertebrate groups and their extant relatives. Euphaneropids in red. Modified from Janvier (2015).

Objectives of the VERTIGO project

Enigmatic fossils from the Lower Devonian of southern Belgium were recently rediscovered in old collections of the Centre Grégoire Fournier of the Maredsous Abbey (Fig. 3A). A major step in the VERTIGO project will be to reassess the specimens previously labelled as cephalochordates. If the assessment is correct, this would significantly extend the stratigraphic range of those animals in the fossil record and would provide crucial new insight into our understanding of chordate evolution. Recently also, specimens of putative euphaneropids were found in the Lower Devonian collections of the RBINS. They resemble *Euphanerops* in that they also display elongate structures that cover gill pouches (Fig. 3B). Given the importance of euphaneropids and the recent advances in our understanding of vertebrate evolution, each discovery should be considered with a lot of attention. That is what we aim for in our project.

Figure 3. Examples of Early Devonian fossils recently rediscovered in Belgian collections. A. A putative early chordate collected during the 20th century at Warmifontaine, coll. number Maredsous II-228. B. A fossil from La-Roche-en-Ardenne resembling part of an euphaneropid (early vertebrate), coll. number IRSNB-Vert-09383-00001.

Fossils collected at the Lower Devonian locality of Warmifontaine are enigmatic because their preservation seems rather poor. Some specimens present similar, curved, shapes but their identification remains problematic. We do not know whether those specimens consist of well-preserved soft-parts or poorly preserved hard parts (or a mixture of both conditions). It is therefore critical to recover as many anatomical details as possible from those fossils for systematic, phylogenetic and evolutionary purposes. This project aims at bridging a gap in the understanding of early chordate evolution and, more generally, animal biodiversity during the Early Devonian.

To achieve this, we propose a methodology that focuses on investigating the structure and chemical composition of the fossils. The latter is useful to detect biological structures that are hidden to the naked eye and/or under visible light. Besides classical optical microscopy and camera lucida drawings, we used state-of-the-art imaging and spectroscopy techniques to obtain better morphological contrasts and to be able to identify some fossils. We studied the (geo)chemistry of the fossils using band-pass emission macroscopy (BEM) and Synchrotron-based micro-X-ray fluorescence, major-to-trace elemental mapping (Synchrotron-based µXRF). In addition to those two techniques, we also used Energy-dispersive X-ray spectroscopy (EDS) and Raman spectroscopy to study the elemental and mineralogical composition of the fossils.

Other important objectives of the VERTIGO project were to (i) prospect public and private Devonian collections from Belgium (both vertebrate and invertebrate collections, as early vertebrates have often been misidentified in the past) in search for additional similar soft-bodied chordate specimens and identify other enigmatic fossils from Warmifontaine, (ii) organise new field work in Belgian historical localities and prospecting for new coeval localities in order to collect more fossil material, (iii) identify the rest of the non-bio and biomineralized fossil fauna from the historical collections and new collected material, and (iv) highlight the historical and geological importance of slate mining in Belgium.

3. METHODOLOGY

The material discovered so far in the old collections of the Centre Grégoire Fournier (Maredsous Abbey) and the RBINS (Brussels) comes from Pragian (Lower Devonian) slate deposits located in two distinct areas of southern Belgium. The first locality, which yielded putative early chordates, is situated in the Neufchâteau Synclinorium and corresponds to an old, underground roof slate quarry located in the village of Warmifontaine (Remacle, 2007). A late Pragian age (ca. 410 Myr) was assigned for this lithostratigraphic unit (Stainier in Godefroid et al., 1994; Bultynck & Dejonghe, 2002; Denayer & Mottequin, 2024). The slate horizons were extracted there from La Roche Formation from ca. 1865 to 2006. After the end of all extraction activities in the slate mine, slate waste deposited outside the mine for decades was progressively removed from the location to be processed and transformed into gravel. It is in those huge piles of slate waste that we looked for new fossil specimens during field work, the original underground quarry being flooded. The second fossil locality (La Roche-en-Ardenne) is situated ca. 50 km from Warmifontaine and lies in the Ardenne Anticlinorium. It displays laterally equivalent deposits to those at Warmifontaine, although those from La-Roche-en-Ardenne experienced less metamorphism.

One of the objectives of the project was to investigate the locality, i.e. Warmifontaine, that yielded most of the fossils (Fig. 4) found in the historical collection of the Centre Grégoire Fournier in Maredsous, including the putative early chordates. The success of the research project relied partially on the hope of new fossil finds, which would help for the identification of putative early chordates. Two **field campaigns** of two and five days, respectively, were organised in 2021 and 2022. A team of 5–10 palaeontologists from the Royal Belgian Institute of Natural Sciences (RBINS), Institut photonique d’analyse non-destructive europééens des matériaux anciens (IPANEMA, Paris-Saclay), University of Lausanne (UNIL), Vrije Universiteit Brussel (VUB, Brussels) and National Museum of Natural History (MNHN, Paris) participated in those two fieldworks, which resulted in the collection of more than 250 new fossils. A selection of those specimens was chosen for analyses. All the fossils collected during fieldwork are now curated in the palaeontological collections of the RBINS.

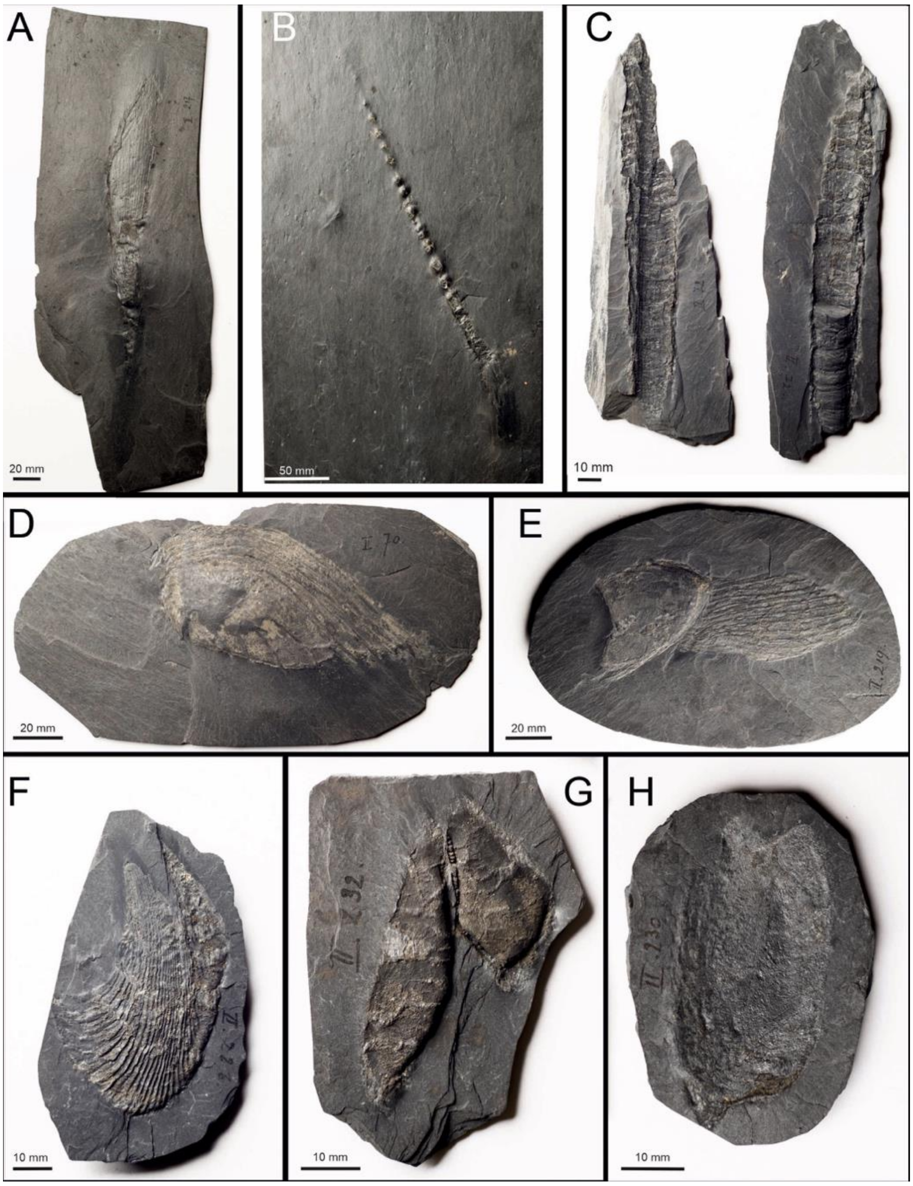

Figure 4. Fossils from Warmifontaine rediscovered in the historical collection of the Centre Grégoire Fournier at Maredsous. The collection includes corals (A and F, A=spe II-217, F=spe II-226), cephalopods (orthocones) (B-C, B=spe II-222, C=spe II-72), arthropods (trilobites) (D–E, D=spe II-70, E=spe II-219), bivalve molluscs (G, G=spe II-232) and unidentified specimens (H, H=spe II-230).

Given the rather poor preservation of the fossils collected at Warmifontaine, it was decided to **prospect for fossils of similar age in museum collections,** in order to have comparison material. The fossil collections from two major institutions in Germany – Natural History Museum of Mainz (NHMM) and the University of Bonn (UniBonn) – were visited to obtain data about the fossil fauna found in the Lower Devonian strata of the Rhenohercynian Zone. The Hunsrück and Mayen sediments were deposited in the same sedimentary basin as Warmifontaine – to the South of the Old Red Sandstone continent – and they are characterized by similar depositional environments. Although Warmifontaine slates are slightly older (late Pragian) than those of Hunsrück (early Emsian), their fossil content is comparable. The observation of fossils with distinct levels of preservation from Hunsrück and other related German localities will help identifying the less well-preserved fossils from Warmifontaine and

La-Roche-en-Ardenne. Three fossils from NHMM (Curator: Manuela Aiglstorfer) resembling fossils from Warmifontaine were borrowed by the RBINS to be studied and might bring new important information about the faunal diversity in the Rhenohercynian Zone during the Early Devonian.

Besides the field prospections and the visit of comparison collections, it was also decided to **screen palaeontological collections of Belgian institutions**, i.e RBINS, CGF Maredsous, Ghent and Liège universities, in search for early vertebrate/chordate fossils from Warmifontaine and La Roche-en-Ardenne, but also contemporaneous localities. Both vertebrate and invertebrate collections were investigated, as early vertebrates have often been misidentified in the past. Moreover, the collections of the Musée d'histoire naturelle de Lille (musée Gosselet, France) (Goemaere et al., 2020), and the Musée national d'Histoire naturelle du Luxembourg, that include fossils from the Neufchâteau area, were also screened.

The following analyses were performed on several fossils of the VERTIGO project: (1) Synchrotron-based micro-X-ray fluorescence (**µXRF**) elemental mapping coupled to X-ray absorption spectroscopy (XAS) elemental speciation at Synchrotron SOLEIL (Gif-sur-Yvette, France), (2) Band-pass emission macroscopy (**BEM**) at IPANEMA (Gif-sur-Yvette, France), (3) Energy-dispersive X-ray spectroscopy (**EDS**) and (4) **Raman spectroscopy**. Images of the fossils were also acquired using **photography**, scanning electron microscopy (**SEM**), **surface scanning**, X-ray **radiography** and computed-tomography (**CT**) scanning.

Analyses

(1) **Synchrotron-based µXRF** elemental mapping is a technique permitting to highlight anatomical features based on their chemical signature. X-rays are used as the excitation source that will interact with material (here the fossils) and cause electronic transitions. Each element is characterized by the electronic structure of its atoms. This method is also used to improve contrast between the fossil and the surrounding sedimentary matrix, as they usually have a different elemental composition. Our results will provide significant information on the chemistry of the fossils, which will help for characterizing anatomical features otherwise not observable. The µXRF methodology is similar to that of EDS, which was used successfully on the early chordate *Pikaia* (Conway-Morris & Caron, 2012). The main advantages of synchrotron-based µXRF compared to EDS are that (i) the synchrotron setup can accommodate much larger fossils (up to 100×60 cm^2, Bergmann et al., 2010, 2012; Wogelius et al., 2011; Edwards et al., 2018), with a spatial resolution in the micrometre range, and (ii) due to the high brightness of the synchrotron radiation, it is sensitive to trace elements including trace metals and rare earth elements (REEs; yttrium and lanthanides) up to a few part per million (Bergmann et al., 2010, 2012; Wogelius et al., 2011; Gueriau et al., 2014, 2015, 2016, 2018). Comparatively, the detection limits of EDS are typically 0.1–5.0 wt%. Another great advantage of Synchrotron-based µXRF is that it is usually non-destructive.

We obtained, through a competitive call for proposals, 15 shifts (5 days) of beamtime to conduct such experiments at the PUMA beamline of the SOLEIL Synchrotron (Gif-sur-Yvette, France). This allowed us to map 10 specimens of purported early chordates and vertebrates from the Lower Devonian of

Belgium. We mapped them at 60-150 µm lateral resolution, and collected 3 detailed, higher resolution maps (10–20 µm lateral resolution) of regions of interests for two of them (heads and notochord of an early chordate – part and counterpart). We also mapped another 12 specimens of invertebrates with 50–200 µm resolution depending on their size. The elemental distributions were reconstructed through a full spectral decomposition using the PyMCA data-analysis software (Solé et al., 2007).

(2) **Energy-dispersive X-ray spectroscopy** (EDS) and XRF are based on the same principle, but the excitation source differs. In EDS, electrons are the source particles that cause electronic transitions in atoms. EDS gives information about the elemental composition of material, that is which elements are present. Based on the interpretation of EDS spectra and SEM images it is possible to identify (although in an indirect way) minerals present in fossil material. EDS is widely used to identify elements in fossils from *Konservat-Lagerstätten* and identify their mode(s) of preservation (Anderson et al., 2021; Bezerra et al., 2020; Saleh et al., 2020; Whitaker et al., 2022).

EDS analyses were performed using an environmental FEI Quanta 200 scanning electron microscope at the RBINS.

(3) **Raman microspectroscopy** is a non-destructive spectroscopy technique – i.e., using the properties of light and the results of its interaction with matter – that gives information about the chemical composition of the analysed material. Raman uses a laser as light source (from UV to visible and the near-infrared range) that, when interacting with matter, generate molecular vibrations in the material that are characteristic of certain functional groups and therefore of the composition of that material. The results of Raman measurements are displayed through Raman spectra, representing the intensity of scattered light for each energy of light (measured in wavenumbers). Spectra feature spectral bands that give information about the structure of compounds in the material (e.g. crystallinity, amount of material). The technique has been widely used in palaeobiology to understand the taphonomy of ancient organisms in *Konservat-Lagerstätten* (Butterfield et al., 2007; Marshall et al., 2012; Olcott Marshall & Marshall, 2015; Bezerra et al., 2020; McCoy et al., 2020). Raman microspectroscopy is complementary to EDS and usually allows to confirm the presence of minerals in the fossil and sedimentary matrix.

Raman analyses for the VERTIGO project were performed using a Senterra Raman micro spectrometer (at RBINS) using 785 nm laser radiation for excitation. The fossils were placed on a microscope stage (Olympus BX51). Raman spectra were acquired at atmospheric pressure using a 10 mW laser power and a 50x long distance objective (~2µm laser spot size, ~5mW remaining laser power at the sample, resulting in a ~16mW/cm² irradiance). Three spectra were accumulated in the 155–3800 cm^{-1} region for 10 s exposure time each. All spectra were analysed in SpectraGryph 1.2 spectroscopy software (Menges, 2017). No preparation of the fossils was necessary prior to perform the analyses.

(4) **Optical photography**. Classic optical photographies of the fossil specimens from Warmifontaine were taken with a Panasonic Lumix GX80 digital camera combined with a 14–45 mm Panasonic lens and a 50 mm PENTAX lens. Image settings were adjusted using Adobe Lightroom. All

the specimens from the historical collection and 57 specimens collected in 2021 and 2022 were photographed.

(5) **Scanning electron microscopy** (SEM) was performed on fossil specimens and on thin/polished sections with an environmental FEI Quanta 200 SEM equipped with a backscattered electron (BSE) detector, using a working distance of 10–26 mm, accelerating voltage of 15–30 kV and probe current of x–x. The specimens were not coated. SEM analyses were performed under both secondary electron (SE) and BSE modes.

(6) **Band-pass emission macroscopy** (BEM) is a new imaging technique based on UV photography and ‘multispectral imaging’ – i.e., the collection of multiple images in different excitation and emission wavelengths with the use of different excitation sources and band-pass filters. Those two techniques are combined to generate a BEM image, that is the most contrasted image of an object. BEM has easily and effectively been applied in palaeontology by exposing fossils to UV, white light, and narrowed visible wavelengths such as the green light of laser (546 nm; Haug et al., 2009), or blue light by filtering the light source with the cyan filter of 3D red/cyan stereo glasses (Haug et al., 2011). The latter authors demonstrated that applying different excitation wavelengths to the same specimen can produce very different images and enhance distinct features (Haug et al., 2009, 2011). Laser-stimulated fluorescence (Kaye et al., 2015) is another emerging technique using powerful lasers to image palaeontological material that remains dark under UV light or other wavelengths. Only a few excitation/emission combinations are possible with laser-stimulated fluorescence, which results in low spectral resolution (because the emitted light is collected in large regions of the visible spectrum).

The BEM technique overcomes most of those limitations; it is based upon the collection of reflectance or luminescence images within small regions of the visible and near-infrared spectrum (defined by narrow band-pass filter) using different excitation wavelengths from the UV-A (365–400 nm) to the near-IR (~800 nm). Fifteen to 20 combinations are easily available, depending on the camera sensitivity to the different wavelengths. False-colour RGB images can then be produced to enhance morphological contrasts. This novel approach shows great promise for improving and revealing invisible anatomical details in a wide range of fossils (Brayard et al., 2019; Klug et al., 2019).

Reflection and luminescence images in specific band-pass spectral ranges were collected using an innovative imaging setup under development at IPANEMA (a detailed description of the system is currently in preparation, including applications to a wide range of fossil specimens). The setup consists of a low-noise 1-megapixel Si EMCCD camera (Qimaging Rolera EMC2) with a sensitivity ranging from 200 to 1100 nm, fitted with a UV-VIS-IR 60 mm 1:4 Apo Macro lens (CoastalOptics) in front of which is positioned a filter wheel holding 8 Interference band-pass filters (Semrock) to collect images in specific spectral ranges. Illumination is provided by 16 LED lights ranging from 365 up to 700 nm (CoolLED pE-4000), coupled to a liquid light-guide fibre fitted with a fibre-optic ring light-guide. This setup provides a homogeneous illumination of the region of interest. High resolution imaging of the entire fossils is produced by stitching a dozen images with a smaller but high-resolution field of view.

Twenty specimens of putative early chordates and invertebrates from the Lower Devonian of Belgium (Warmifontaine and La Roche-en Ardenne) were analysed thanks to band-pass emission macroscopy.

(7) **Surface scanning**. Surface scanning was realised to obtain high-resolution 2D images of the surface. The 2D images were taken using an optical profilometer (Keyence VR-5000 series). That imaging technique is well suited for flat material or material with very low relief, like the Warmifontaine fossils. We used magnification of 12–120x.

(8) **X-ray radiographies**. Radiographies of six specimens were taken using the RBINS micro-CT RX EasyTom 150.

(9) **CT scanning**. Slate slabs including fossil specimens were scanned using the RBINS micro-CT RX EasyTom 150. Computed tomography scans were collected using a voxel size of 0.048–0.085 mm. After scanning, extraction into 16-bit TIFFs was performed with X-Act software. 3D-rendering and segmentation into polygon meshes was done with Dragonfly ORS software or Mimics software. The raw data of these specimens are/will be stored on Belspo’s LTP platform after completion of the project and publication of the data. Consultation of the scans and radiographies can be made on request (to the RBINS paleontology collection manager). 3D-meshes of segmented regions of interest can be consulted via the RBINS Virtual Collections Platform (http://virtualcollections.naturalsciences.be/).

The fossils collected in the field have been studied by the scientists hired for the project and some particularly interesting specimens have been studied by external collaborators. Experts in diverse palaeontology fields were contacted to help identifying problematic taxa. Two experts in arthropods and echinoderms, respectively, visited the Warmifontaine collections at the RBINS. Those visits allowed the identification of fossil groups that were unknown from the locality. The goal of the project is also to look at the taxonomic diversity at Warmifontaine. Besides early chordates, there are many interesting fossil specimens that will bring important information on the faunal diversity in the marine realm, along the southern border of the Old Red Sandstone continent during the Early Devonian.

4. SCIENTIFIC RESULTS AND RECOMMENDATIONS

4.1. Field campaigns

Research and prospection were conducted to look for the original location of the fossils that were rediscovered in the collections of the Centre Grégoire Fournier of the Maredsous Abbey. It appeared that the fossils came from an underground slate mine in the vicinity of Neufchâteau, in the Luxembourg Province.

We organised two field campaigns, in the summer of 2021 and 2022, on the location of the old slate mine (Fig. 5). We collected slates from large slate heaps still present outside the mine and broke big slate pieces along schistosity planes using chisels. Our team of palaeontologists collected more than 200 fossil specimens over a week of work in total. The first year, the accent was put on finding additional early chordate specimens, which resulted in the collection of dozens of new specimens. The second year, it was decided to collect all fossil material that could bring information about the palaeoecology of the area. The team collected the second year more than a hundred specimens. All the fossils collected in 2021 and 2022 are now part of the palaeontological collections of the RBINS. Prospection for fossils in other localities (Herbeumont and Bertrix) was not successful.

Figure 5. Field campaign of 2022 in Warmifontaine Slate Mine. A-B. Opening slates for finding fossils. C. Several fossils on a slab.

4.2. The Warmifontaine Slate Mine

Extraction of roof slate at Warmifontaine started in 1865 following geological prospection in the area from 1845 (Asselberghs, 1924). The first maps of the mine show a quite limited number of extraction chambers. By 1901, ca. 60 chambers had been quarried. Following an incident in 1912 that resulted in the collapse of five houses in the village, the slate mine closed. A few years later, another company bought the mine and the extraction work resumed in 1923. The Warmifontaine slate mine was once producing 12 million roof slates per year, making it the biggest roof slate manufacturer in Belgium (Asselberghs, 1924). The competition with the Italian, Spanish and then Chinese slates during the second half of the twentieth century combined to high production costs progressively put an end to the activity in Belgium. Warmifontaine slate mine permanently closed in 2002. From 2010 until recently, the remaining slate waste at the surface of the site were moved away and reused to produce gravel. That activity ended about a year ago and the future of the site is still unknown.

There is already mention of fossils from the Warmifontaine slate mine in a report of 1891 from the *Annales de la Société géologique de Belgique* (Dewalque, 1891). G. Dewalque (1857–1891) – geologist and professor at the University of Liège – presented at a meeting of the association some fossils, including bivalves, orthoconic cephalopods, rugose corals and trilobites, and fossils he interpreted as being segments of trilobite thoraces. In 1912, Etienne Asselberghs (1889–1959) – professor of geology at the Catholic University of Leuven – published a paper dedicated to the discovery of several fossils by Mr Duvigneaud. The fossils are described in that report as “highly compressed imprints” and. Asselberghs (1912) identified one of them as a rhynchonellide brachiopod. Fossils are quite rare in the deposits extracted from the mine.

The slates extracted from the mine and that yielded fossils come from a specific lithostratigraphic unit called the Martelange Member located at the base of a broader unit, the La Roche Formation (LAR). The LAR Formation consists of dark grey slates with minor silty sandstones (Dejonghe, 2013). Millimetre-sized euhedral pyrite is common in the slates from the Martelange Membre. The deposits from LAR Formation are late Pragian in age (ca. 408 Myr) (Dejonghe, 2013; Dejonghe et al., 2017).

Boreholes were made at several locations in the vicinity of the mine and the cores were described by the Geological Survey of Belgium. They consist of very fine slate with good cleavage, without any sedimentary structures or macrofossils. Pyrite is usually dispersed in the rock and quartz veins are present. Some thin sandy layers are present in some places. The location of those boreholes is not known.

4.3. A shark in the Late Famennian of Belgium

During the visit of the Devonian palaeontological collections of the RBINS, several fossils of interest were found. Among them the lower jaw of a shark that has been published in 2021 in *Journal of Vertebrate Paleontology* (Fig. 6, Wilk et al., 2021). Here is the abstract:

Herein, we describe the first occurrence – other than teeth and scales – of a possible ctenacanthid shark (right Meckel’s cartilage) from the Upper Devonian (Famennian) of the Liège Province, Belgium.

It is the first fossilized element of the internal skeleton found in the Upper Devonian of continental Europe. Despite the lack of characteristic features which could allow an accurate specific assignment, it is likely that the examined specimen belongs to one of the species that have already been described from Belgium.

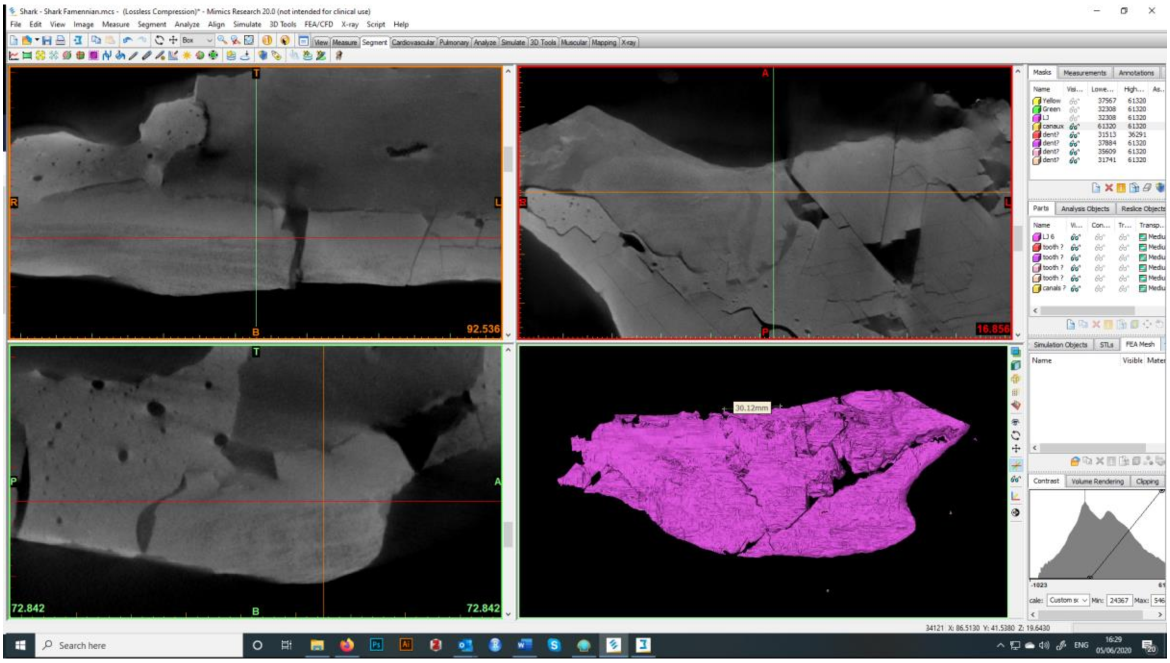

Figure 6. Screenshot of the Mimics software displaying the reconstruction in 3D of the lower jaw of a Famennian shark (specimen IRSNB P 9956).

4.4. Reproductive strategies within chondrichthyans

Also, during the visit of the Devonian palaeontological collections of the RBINS, the Liège University and the Centre Grégoire Fournier, fossils assigned to the oldest known chondrichthyan egg case have been re-discovered and subjected to micro-CT-imaging. This study, published in 2021 in *The Science of Nature* (Mottequin et al., 2021), revealed the erroneous assignment of these fossils and explored the implications for our knowledge on the evolution of the reproductive strategy within chondrichthyans (Fig. 7). Here is the abstract:

Spiraxis interstrialis*, and its junior synonym* Fayolia mourloni*, an uppermost Famennian (Upper Devonian) fossil first described as algae and subsequently interpreted as the oldest known chondrichthyan egg case, is reinvestigated based on the discovery of several additional specimens in Belgian collections. New data, in particular from micro-CT imaging, allow to refute S. interstrialis, and by extension also Spiraxis major (the type species of* Spiraxis *Newberry, non Adams) and* Spiraxis randalli *from the Famennian of New York and Pennsylvania, as chondrichthyan egg cases. Alternative interpretations of these enigmatic helicoidal fossils are discussed. The first occurrence of oviparity in the fossil record of chondrichthyans is thus not as old as previously thought and is close to the first occurrence of viviparity in this group, both being recognised now in the Mississippian. The question of which of both conditions is plesiomorphic within chondrichthyans, and more widely within vertebrates, is discussed. Also, the presence of the genus* Spiraxis *in both the USA (east coast) and Belgium* *reinforces the strong faunal resemblance already observed in both palaeogeographical areas. It suggests important faunal exchanges between these regions of the Euramerica landmass during the Famennian.*

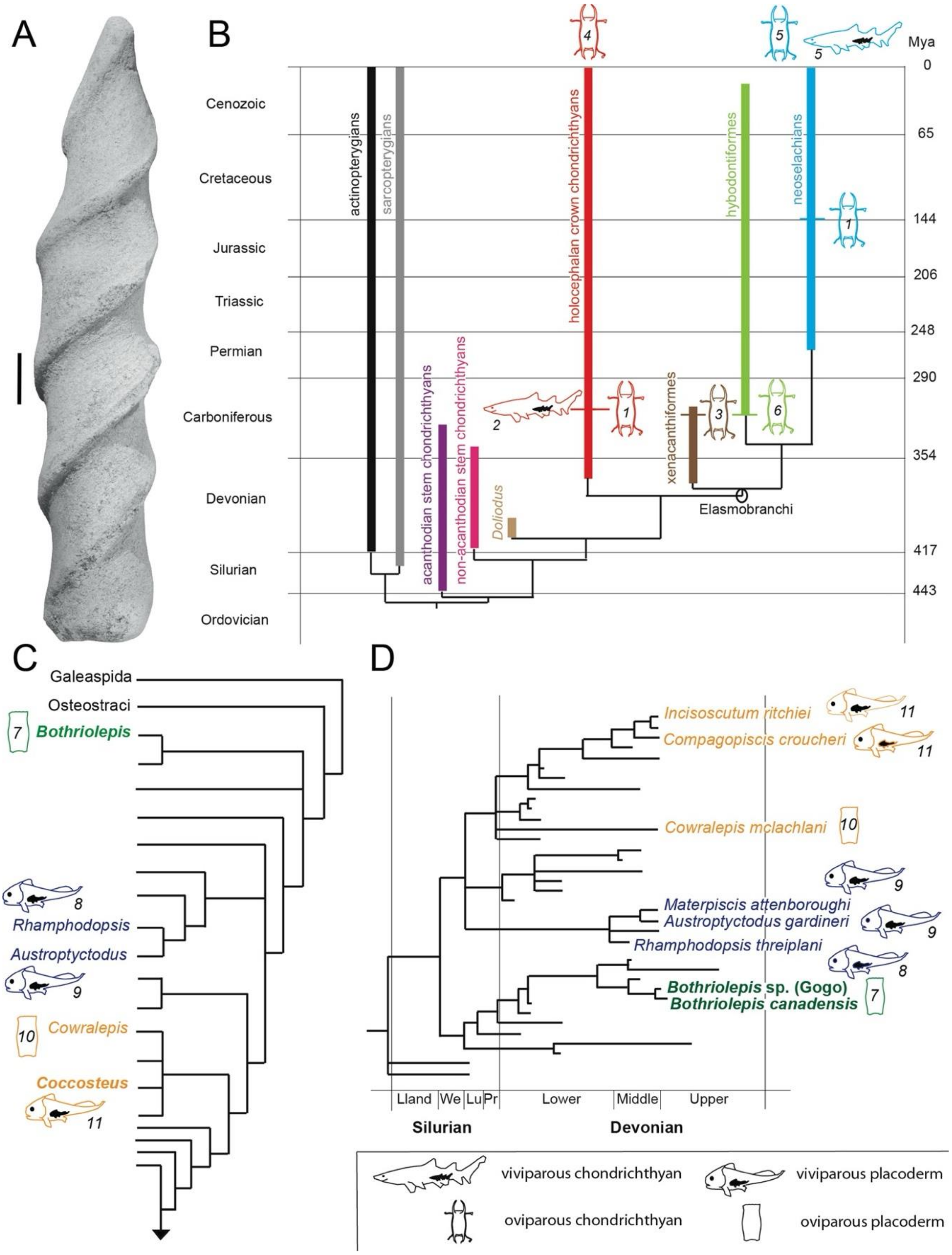

Figure 7. Distribution of oviparous and viviparous modes of reproduction in chondrichthyans and placoderms based on the reappraisal of the Belgian "egg case". A. *Spiraxis interstrialis*, from the Bois de la Rocq Formation (latest Famennian), specimen PA.ULg.2020.11.19/1, B. chondrichthyan phylogeny, C. placoderm phylogeny (monophyletic), D. placoderm phylogeny (paraphyletic). From Mottequin et al., 2021.

4.5. Chondrichthyan egg capsules inventory from the Carboniferous of Belgium

The palaeontological collections of the RBINS delivered an important number of chondrichthyan egg capsules. All the Carboniferous occurrences from Belgium have been listed, figured and described in a paper published in 2022 in *The Science of Nature* (Mottequin et al., 2022). Here is the abstract:

Records of chondrichthyan egg capsule morphotypes from the paralic deposits of the Belgian Coal Measures Group (Pennsylvanian; Bashkirian–Moscovian; Namurian B–Westphalian B according to the traditional subdivision) are presented and discussed. These include several species of the hybodontiform type Palaeoxyris *as well as the putative holocephalian types* Vetacapsula *and* Crookallia*. Furthermore, the type specimens of* Scapellites cottoni *and* S. minor*, two additional putative and enigmatic egg capsules from the same lithostratigraphic unit, are figured and discussed. Altogether, a highly diverse egg capsule assemblage documented from the Belgian deposits implies the presence of at least eleven different Carboniferous chondrichthyan species using the ancient aquatic environments for spawning and as nurseries. The absence of the xenacanthiform morphotype* Fayolia*, known from surrounding coeval Coal Measures areas of northern France, the Netherlands, and Germany, is conspicuous. This lack may be a result of collecting bias and does not reflect a real pattern.*

4.6. "Euphaneropids"

Recently, specimens of putative euphaneropids were found in the Lower Devonian collections of the RBINS. They resemble *Euphanerops* in that they also display elongate structures that cover gill pouches (Fig. 3B). Several imagery techniques and chemical analyses (SEM, BEM, X-ray radiographies, µXRF, EDS and Raman) were applied on this material to confirm or infirm the nature of the material.

Unfortunately, several analyses (i.e. BEM, X-ray radiographies and µXRF) were not efficient on this material and did not allow a better identification of the specimens. On the contrary, SEM imaging of the fossil specimens studied herein allows the identification of ornamental features. The highlighted chevron-like ornamentation would better correspond to the ornamentation of a possible arthropod abdominal segment (i.e., tergite) and are reminiscent of some phyllocarid crustaceans (Vannier et al., 1997: fig. 10B; Fig. 8). Thus, in the absence of more convincing euphaneropid features and because of the arthropod-like ornamentation, we decided to focus our investigations on other specimens. Nevertheless, the recognition of this specimen as a phyllocarid crustaceans is definitely not without value, as it adds to recent discoveries of several other phyllocarid remains in several Devonian and Carboniferous localities of Belgium, including Warmifontaine, which all together will be described in a scientific publication.

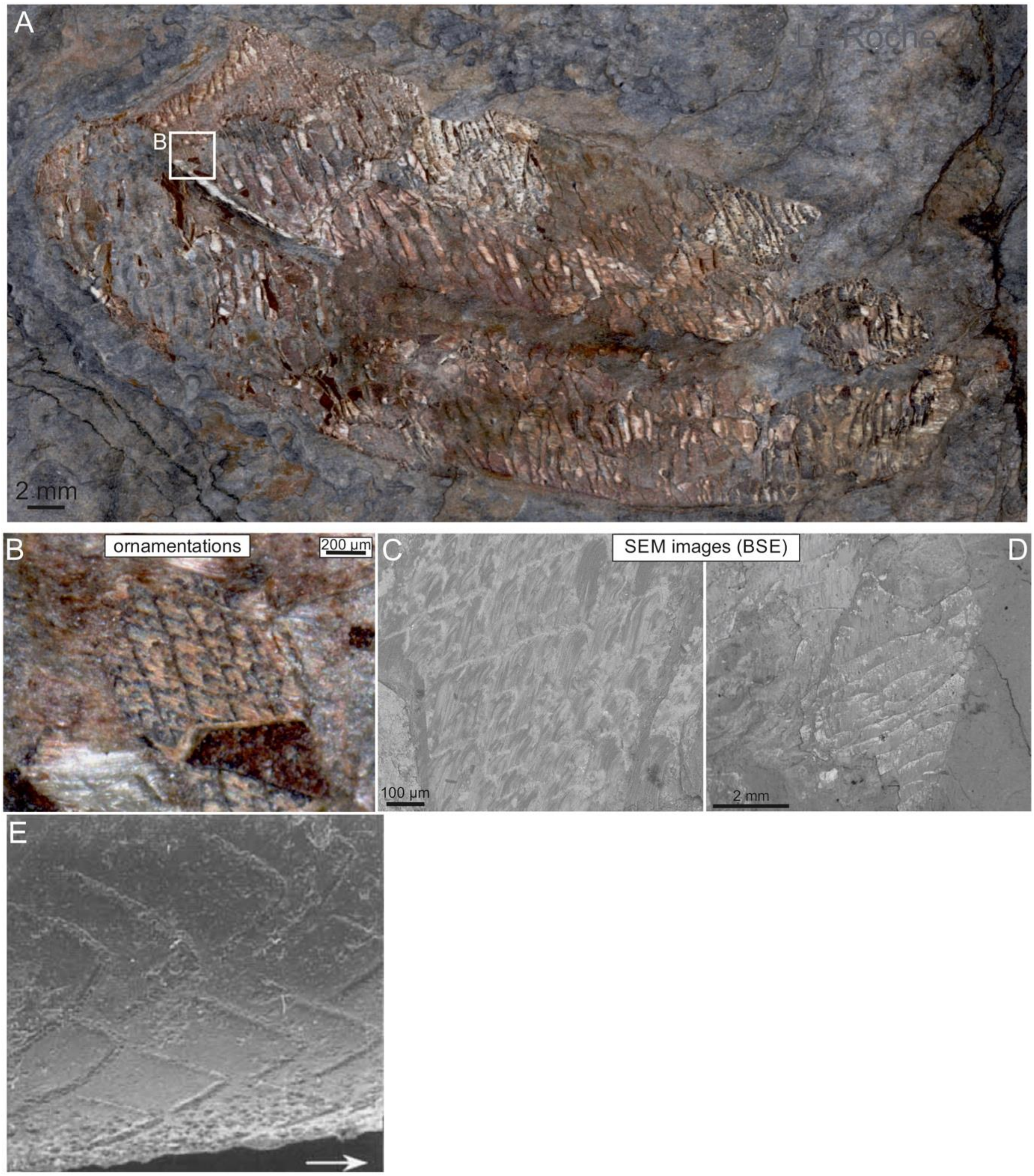

Figure 8. Reappraisal of the ‘euphaneropid’ material from the Lower Devonian of Belgium. A. Fossil from La-Roche-en-Ardenne. B. Close-up on the ornamentation. C. SEM images. E. Similar ornamentation on a phyllocarid from La Chevrolière, France (Vannier et al., 1997).

4.7. Early chordates from the Lower Devonian of Belgium

Several techniques have been applied to the material of putative early chordates (historical and recently collected material), i.e. UV, SEM, tomography, raman, MEB, but the most efficient ones have been µXRF and BEM to highlight morphological features. During our 15 shifts of beamtime at the PUMA beamline of the SOLEIL Synchrotron, we mapped the 10 specimens of purported early chordates and vertebrates from the Lower Devonian of Belgium we intended to analyse at 60–150 µm lateral resolution, and collected 3 detailed, higher resolution maps (10–20 µm lateral resolution) of regions of interests for two of them (heads and notochord of an early chordate – part and counterpart).

µXRF permitted to gain a better understanding of the anatomy of the chordate fossils, and to exclude several specimens, previously identified as such (e.g. IRSNB 34369-2, Fig. 9). Indeed, the general shape and overall features of several specimens let us think mistakenly they could belong to this group, but µXRF highlighted the absence of chordate diagnostic features on those specimens.

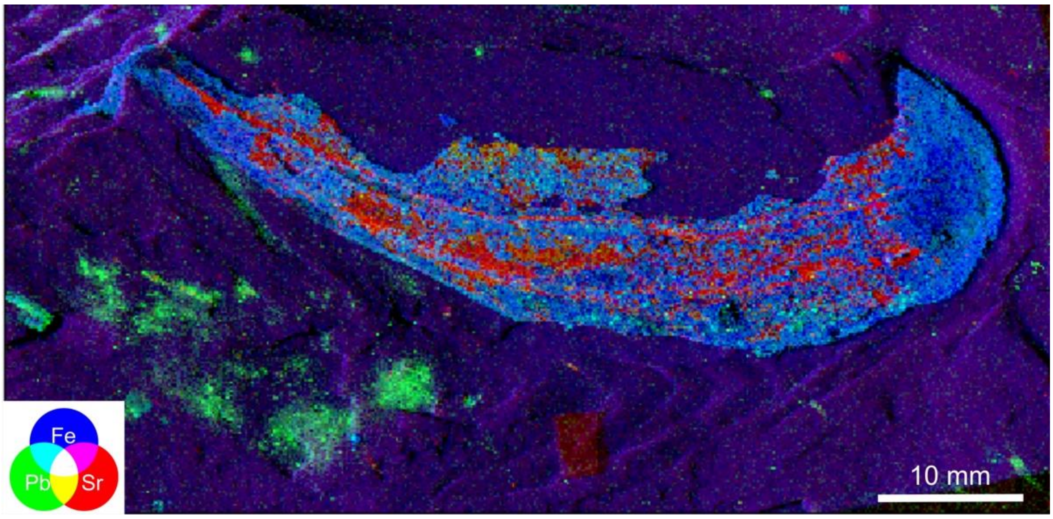

Figure 9. Reappraisal of one of the 'early chordate' specimens from the Lower Devonian of Belgium. IRSNB 34369-2 being likely part of an arthropod animal. False colour overlay of strontium (red), iron (blue) and lead (green) distribution using µXRF data.

Moreover, we established a chemical characterisation of each specimen that could help to gather (and exclude) hardly identifiable fossils together with identified fossils with a known chemical identity (Fig. 10). In other words, we use the fossil elemental composition as a complement to anatomy to help us identify specimens otherwise difficult to classify.

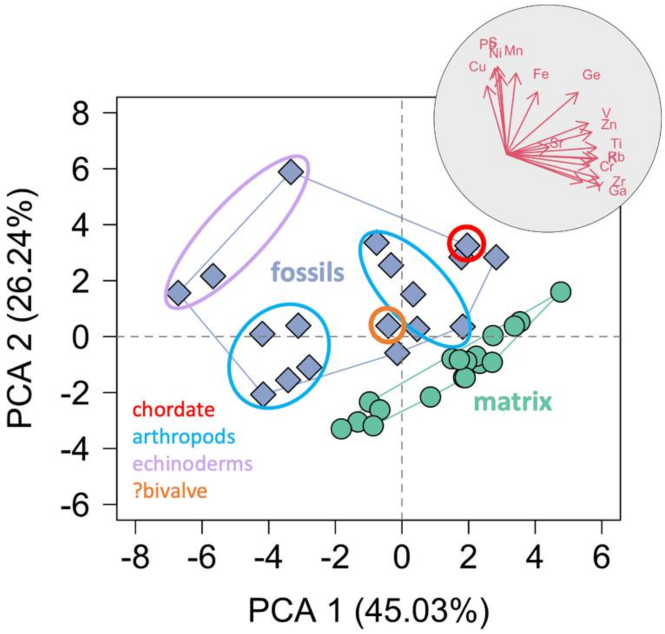

Figure 10. Preliminary multivariate exploration of the µXRF data using principal component analysis. The elemental composition of the fossils is clearly distinguished from that of the sedimentary matrix, and fossils belonging to the same taxonomic group appear to cluster to at least some extent.

Using µXAS, we further investigated the speciation of elements exhibiting specific distributions in the specimens that likely are early chordates. We targeted particularly areas of the notochord and the head, which are the most diagnostic. We collected XAS at the Cu and Ni K-edges but based on the obtained µXRF we also collected data at the Fe and Sr K-edges. The obtained spectra have not yet been fully processed and interpreted but they clearly reveal variable speciation between different anatomical features of the fossils and the surrounding sedimentary matrix. It is too soon for us to tell, but this data will provide either interesting complement to anatomy for the precise identification of some features or at least inform us on the fossilization processes at play at the site.

4.8. A selkirkid worm from the Warmifontaine locality

Until the recent description of a species from the Early Ordovician Fezouata Biota (Nanglu & Ortega-Hernández, 2014) selkirkiids were exclusively known from the early and mid Cambrian and are classically regarded as typical examples of Cambrian 'Burgess Shale-type' organisms. During the recent excavations made in Warmifontaine, two selkirkid specimens have been revealed and described thanks to µXRF and EDS imaging. These new finds add to the extremely scarce post-Cambrian scalidophoran fossil record and extend the range of Selkirkiidae by 70 Myr, giving the group a longevity of at least 110 Myr, a remarkable testament to their persistent ecological and evolutionary success well beyond the Cambrian.

The results of this study will be very soon submitted under the lead of Peter van Roy, a worldwide recognized specialist in the field.

4.9. First report of two classes of echinoderm in the Lower Devonian of southern Belgium

Two specimens from the Warmifontaine Biota are described and assigned to two distinct classes of echinoderms. The first one corresponds to a dendrocystitid solutan, which is tentatively identified as

Dehmicystis? sp., based on similarities with *D. globulus* from the Hunsrück Slate (Emsian) of Germany. This comparison has been made possible thanks to the visit of paleontological German collections (Mainz in September 2022 and Bonn in November of the same year) by Aude Cincotta. The second echinoderm specimen is a well-preserved, flattened, fully articulated and almost complete kirkocystid mitrate, morphologically close to peltocystidans from the Lower and Middle Devonian of Germany.

The study of the echinoderm specimens from Warmifontaine is led by Bertrand Lefèbvre from the University Claude Bernard, Lyon 1, France and will be very soon submitted to *Geologica Belgica*.

4.10. Physico-chemical characterization of the fossils and sedimentary matrix - taphonomical implications

The X-ray diffraction characterization of four slate (sedimentary matrix) samples from Warmifontaine indicates that three of them are composed of muscovite, quartz and chlorite. The fourth sample shows a slightly different composition, with quartz, chlorite, illite and anorthite. The sedimentary matrix around the specimen from La Roche is mainly composed of muscovite, chlorite and quartz, with less plagioclase. Those mineralogical compositions are in line with previous analyses of rocks from the LAR Formation (Berger et al., 1994; Duchesne et al., 2018).

The main elements found to be present in our specimens using EDS and µXRF are C, S, Ca, Fe and Ni, while Mg, Mn, Cu, As, Sr and Pb are present at lower levels. We clearly observe a co-occurrence relationship between S and Fe, which is indicative of pyrite (FeS_2), and between C, Ca and Sr that is indicative of calcite ($CaCO_3$) in which Sr substituted for Ca during diagenesis.

Raman microspectroscopy performed on one specimen of putative early chordate (Maredsous II-228, Fig. 3A), two isolated parts of arthropods, one echinoderm and two unidentified specimens also reveal the presence of disordered carbonaceous matter as another source for C in most of the fossils. The so-called D and G bands characteristic of disordered carbonaceous matter differ between specimens indicating the presence more or less “evolved” carbonaceous matter. Besides the bands characteristic of carbonaceous matter, most of the fossils exhibit the expected bands indicative of pyrite, but also iron oxides. Calcite and quartz bands were observed in three out of five specimens, including the putative early chordate shown in Fig. 3A. Chlorite is a common component of the analysed specimens, probably inherent to the sedimentary matrix, which is mainly composed of that clay mineral. Another interesting result of the Raman analyses is the possible presence of the phyllosilicate mineral palygorskite in the La-Roche-en-Ardenne specimen, now interpreted as a fragment of a phyllocarid crustacean (see above). This mineral was not detected in any of the Warmifontaine specimens analysed with Raman spectroscopy. Such difference in mineralogy might be related to local geological or taphonomical history differences (e.g. sediment-fluid interactions).

The distribution of Pb seen using µXRF follows the gross morphology of the soft parts in several fossils, and could represent a signature to identify soft tissues. Pb in pyrite occurs mainly in the form of inclusions of other Pb-bearing minerals such as galena. Preservation of organic matter as strongly suggested by Raman spectroscopy could hold clue to the presence and origin of Pb and Pb-bearing mineral inclusions in the fossils. Indeed, decaying organic matter tends to precipitate/chelate heavy metals such as Pb onto its surface through microbial action, which would have concentrated and made Pb available for the precipitation of Pb-bearing mineral inclusions.

On the morphological point of view, X-ray radiographies show that only a little part of the specimens is exposed to the surface when found on the field, with more material remaining embedded with the sedimentary matrix. Pyrite crystals are associated to all the studied fossils and provide good contrast for radiographies, leading to the identification of several specimens, e.g. trilobites and bivalved arthropods. Conversely, pyrite crystals can sometimes be large and obliterate anatomical features, making the identification of the fossils. Further characterization of the pyrite crystals using optical and scanning electron microscopy show that pyrite is ubiquitous in the Warmifontaine and La-Roche-en-Ardenne fossils and presents a range of morphologies and textures, including euhedral crystals.

Altogether our data show that the fossils are preserved as pyritized and carbonaceous compression fossils, decaying organic matter having participated to the trace elemental composition of the pyrite crystals. Preservation of the Warmifontaine fossils therefore closely resembles that of the coeval Hunsrück Slate fossils before manual preparation where X-ray radiographs reveal soft parts that have been pyritized more delicately through replacement or infilling by a groundmass of < 20 µm crystals with occasional larger crystals that grew at a later stage (Briggs et al., 1996).

5. DISSEMINATION AND VALORISATION

5.1. Dissemination to scientific community

Results of the VERTIGO project have been widely released to the scientific community through 8 international and national meetings (see the list below). Also, 3 papers were published (see paragraph 6) and others are in preparation or almost submitted (echinoderm, selkirkid, chordates and taphonomy of the Warmifontaine locality).

Cincotta, A., Lefebvre, B., **Gueriau, P.**, **Olive, S**. & **Mottequin, B**. 2023. A possible peltocystid mitrate (Stylophora) in a new Praguian (Early Devonian) Konservat-Lagerstätte from Belgium. 11th European Conference on Echinoderms. (poster)

Cincotta, A., Mottequin, B., Gueriau, P., & **Olive, S**. 2023. A new *Konservat-Lagestätte* with putative early chordate from the Lower Devonian of Belgium. 4th International Congress on Stratigraphy (Strati 2023). (poster)

Olive, S., **Cincotta, A., Gueriau, P.,** Lefebvre, B., Van Roy, P., & **Mottequin, B.** 2023. Un nouveau Konservat-Lagerstätte du Dévonien inférieur de Belgique. Congrès Annuel de l'Association Paléontologique Française. (talk)

Cincotta, A., Mottequin, B., Gueriau, P., & **Olive, S**. 2022. Taphonomy of a unique assemblage of putative early chordates from the Lower Devonian of Belgium. 66th Annual Meeting of the Palaeontological Association 2022. (poster)

Olive, S., **Gueriau, P., Janvier, P.,** & **Mottequin, B**. 2022. Enigmatic early chordates from the Lower Devonian of Belgium. 16th International Symposium on Early Vertebrates/Lower Vertebrates. (talk)

Olive, **S**. 2022. How soft-bodied chordates and early vertebrates from the Devonian of Belgium can help to decipher early stages of vertebrate evolution? Geosciences seminars of the Laboratoire de Géologie de Lyon – Terre, planètes, Environnement. March 7th.

Olive, S., **Gueriau, P., Janvier, P.,** & **Mottequin, B**. 2021. How soft-bodied fossils from the Lower Devonian of southern Belgium can help to decipher the early stages of vertebrate evolution. 65th Annual Meeting of the Palaeontological Association 2021. (talk)

Olive, S., **Gueriau, P., Janvier, P**., & **Mottequin, B**. 2021. Deciphering early stages of vertebrate evolution thanks to long ignored soft-bodied fossils from the Early Devonian of Belgium. 7th International Geologica Belgica Meeting. (talk)

5.2. Dissemination into popular science media

At the beginning of the project, a description of the VERTIGO project has been published on the website of the Royal Belgian Institute of Natural Sciences:
https://www.naturalsciences.be/en/science/research/ecosystems-over-time/projects/vertigo

The discovery of the shark mandible and the scientific paper about it caused a sensation in Belgian media (cf. press review attached to this report).

6. PUBLICATIONS

MOTTEQUIN, B., FISCHER, J., GOOLAERTS, S., & **OLIVE, S**. 2022. Revisiting the chondrichthyan egg capsules inventory from the Pennsylvanian (Carboniferous) of Belgium: new data and perspectives. *The Science of Nature* (peer review) 109: 39.

WILK, O., **OLIVE, S.**, PRADEL A., DEN BLAAUWEN, J., &. SZREK, P. 2021. The first lower jaw of a ctenacanthid shark from the Late Devonian (Famennian) of Belgium. *Journal of Vertebrate Paleontology* (peer review) 41(3): e1960537.

MOTTEQUIN, B., GOOLAERTS, S., HUNT, A. P., & **OLIVE, S**. 2021. The erroneous chondrichthyan egg case assignments from the Devonian: implications for the knowledge on the evolution of the reproductive strategy within chondrichthyans. *The Science of Nature* (peer review) 108: 36.

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