REFLECTED LIGHT MICROSCOPY AS AN EFFICIENT AND COST-EFFECTIVE METHOD FOR THE DETECTION OF CALCAREOUS MICRO-FOSSILS, AN EXAMPLE FROM THE MUCH WENLOCK LIMESTONE FORMATION, SHROPSHIRE, UK.

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Ovummuridae are calcareous egg-shaped microfossils of unknown biological affinity. There are limited observations in the literature due to their occurrence only within exceptionally preserved carbonate rocks. The first observation and subsequent erection of the family was via scanning electron microscope imagery; more recently standard light microscopy has been used successfully to observe these microfossils. In this study 124 polished and etched thin sections from various lithofacies from the Much Wenlock Limestone Formation were studied via light microscopy to investigate a population of Ovummuridae. During data collection, it was noted that many specimens were extremely difficult, if not impossible to detect via transmitted light microscopy. However, when observing polished and etched thin sections via reflected light microscopy specimens were conspicuous. This paper introduces reflected light microscopy as a cheap, time efficient method for the observation of Ovummuridae, and explores the wider uses of the technique where other calcareous walled microfossils may have the potential for detection.

Key Words: Ovummuridae, Wenlock Edge, Wenlock, Reflected Light Microscopy

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INTRODUCTION

The study of microfossils is extremely significant and useful in biostrati-graphy, geological correlation and palaeoenvironmental reconstruction, all of which are utilised within the petroleum, mining, environmental and engineering industries, as well as academia and are just some of the examples of their use (Tietjen, 1982; Haq, 1978; Olsson *et al.*, 1980 and Dorning, 1986). Observations of microfossils with the naked eye is virtually impossible as they are too small to detect and often require the magnification and resolution of a microscope. Microfossils are abundant within the rock record and are generally readily observable under a petrological microscope (preservation, diagenesis and neomorphism willing), however not all microfossils are easily detected and are limited to higher magnifi-cations through the use of a scanning electron microscope (SEM) or by certain preparations such as staining. The use of

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microscopy as a method for observing both siliceous and calcareous microfossils is a common practice within research, industry and academia.

Ovummuridae are calcareous, egg-shaped microfossils comprising two equal sized internal chambers separated by a septum like structure (see figure 1 for anatomical features) and are of an unknown biological affinity belonging to the family Ovummuridae MUNNECKE, SERVAIS and VACHARD 2000 (Minoura and Chitoku, 1979; Munnecke *et al.*, 2000). They have limited reportings within the literature due to their occurrence only within Konservat-Lagerstätten or exceptionally preserved limestones which have not undergone diagenetic alteration, neomorphism or dolomitisation (Munnecke *et al.*, 2000). The relatively recent erection of the family may also play a role in the limited number of observations to date. The size and morphology of these microfossils can vary but they are approximately 100 µm in diameter (Minoura and Chitoku, 1979) and have a known stratigraphical distribution from the Llandovery (mid-Silurian) to Guadalupian (Upper Permian; Munnecke *et al.*, 2001).

The discovery of Ovummuridae was first made under a SEM by Minoura and Chitoku (1979) within the Upper Pennsylvanian and Lower Permian limestones of Eastern Kansas, USA (see table 1). Later, Munnecke *et al.* (2000 and 2001) also observed Ovummuridae via SEM whilst investigating Silurian carbonates of Gotland, these observations resulted in the systematic establishment of the family Ovummuridae. It was not until 2006 when MacNeil and Jones reported that they had observed specimens of Ovummuridae using a petrological microscope within etched and slightly polished thin sections. These specimens came from the late Devonian carbonate ramp of the Upper Escarpment and Alexandra Formation (MacNeil and Jones, 2006). MacNeil and Jones (2006) also suggested that thin sections stained with Alizarin Red-S were more useful for the detection of ovummurid walls.

The Homerian-aged Much Wenlock Limestone Formation (MWL Fm.) is world famous and well-known for its exceptionally preserved fossil biota (Ray and Thomas, 2007). Indeed, it forms the type locality for the Wenlock Series

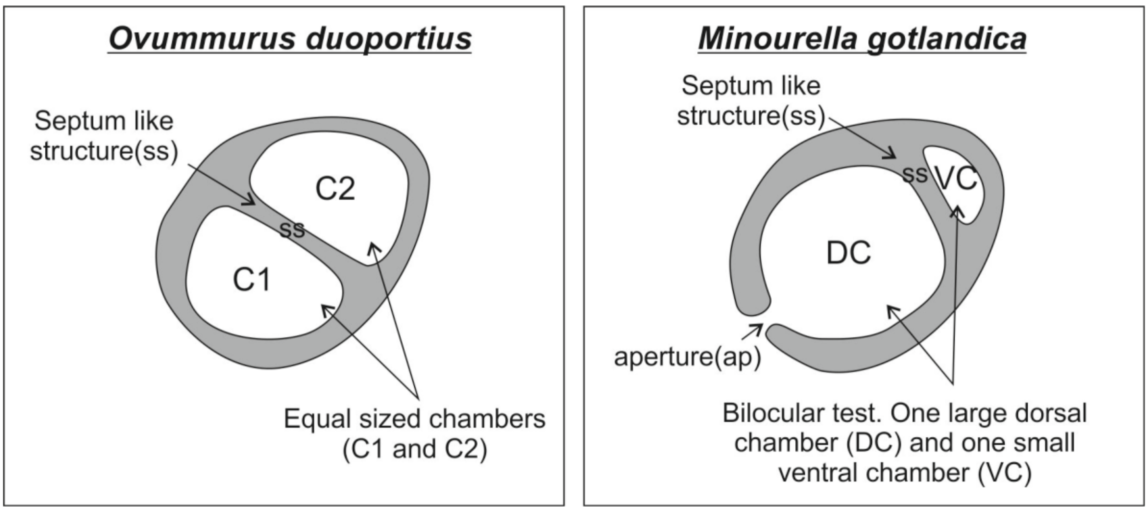


Figure 1: *2D cross-sectional sketch of Ovummuridae (Ovummurus duoportius and Minourella gotlandica) highlighting their characteristic anatomical features and what they may look like under both SEM and light microscopy.*

Table 1.0: A summarised history of observations of Ovummuridae and their methods of discovery.

Discovered By	Method	Location and Stratigraphy	Reportings
Minoura and Chitoku (1979)	SEM of etched and polished thin sections	Upper Pennsylvanian and Lower Permian Limestones from Eastern Kansas, USA	The first discovery and reports of Ovummuridae. Established the genus: Ovummurus Minoura and Chitoku and the type species *Ovummurus duoportius*.
Munnecke *et al.* (2000)	SEM (Cambridge Stereoscan and CamScan scanning electron microscope) of etched and polished thin sections	The limestone-marl alternations from the Silurian of Gotland	Established the family: Ovummuridae Munnecke, Servais and Vachard, 2000 and erected three new genera: Minourella, Arouxina and Samtlebenella and their type species: *Minourella gotlandica*, *Arouxina pluricellata* and *Samtlebenella circumcamerata*.
Munnecke *et al.* (2001)	SEM (Cambridge Stereoscan and CamScan scanning electron microscope) of etched and polished thin sections.	Investigated various locations of differing stratigraphical ages including: Pozary Formation from the Silurian–Devonian boundary, Czech Republic; Upper Carboniferous of the Donetz Basin in Ukraine; Lower Permian strata in the Ural Mountains, Russia and Upper Permian strata from Hindu Kush in Afghanistan.	Determined the current known stratigraphic extent of various genera and species of ovummurids found within various global locations. No observations of ovummurids within Mesozoic deposits.
MacNeil and Jones (2006)	Use of transmitted light microscopy (Leica DMLP microscope) with no thin section modification other than the use of Alizarin Red-S to stain thin sections that make Ovummuridae more identifiable and etching for SEM (JEOL 6301 field emission scanning electron microscope.)	The late Devonian aged carbonate ramp of the Upper Escarpment and Alexandra Formation from southern Northwest territories, Canada.	The first reports of Devonian aged ovummurids. Discovered that the magnification of SEM is not required to observe ovummurids and can be seen with a petrological microscope. Also, established the species: *Minourella cameroni*.

(Bassett, 1989 & Woodcock, 2000). The formation was deposited on the Midland Platform as a result of shallow subtropical seas that allowed a network of patch reefs to thrive within the photic zone and below the storm weather wave base (Ratcliffe and Thomas, 1999). The formation is well exposed around Wenlock Edge, Shropshire, UK, (see figure 2). An SEM investigation of the MWL Fm. off-reef tract limestones and marls by Rogers et al., (2017) highlighted that a substantial population of previously unobserved ovummurids were present. Due to very little diagenetic alteration within the MWL Fm., the sediments are exceptionally preserved allowing the observation of both macro and micro fossil biota.

Problematic calcareous microfossils such as Ovummuridae are difficult to detect using standard microscopic methods (due to their size). This paper introduces a new method allowing ease of identification of certain calcareous microfossils, focusing on the Ovummuridae in particular. Munnecke and Servais (1996) developed a method to study micritic limestones and marls under SEM using polished and slightly etched thin sections that were cut perpendicular to the bedding. However, SEM can be expensive, with limited accessibility and time consuming in cases where there is a vast data set. SEM analysis of thin sections is also better suited for 'precision analysis' (i.e. the analysis of specific features known to be present from reconnaissance work on a petrographic microscope). The use of SEM to analyse multiple thin sections for individual specimens/features is not efficient especially if the microfossil in question may not even be present.

The method used here utilises the practise of reflected light microscopy as opposed to transmitted light microscopy (Williams *et al.*, 1982; Winchell, 1957). The use of reflected light has proven significant for the observations of Ovummuridae within the 124 thin sections from the MWL Fm. This study highlights the proven difficulties of observing Ovummuridae in transmitted light as opposed to the use of SEM and therefore, has opted to adopt reflected light as a quicker, more efficient and cheaper method for the detection of Ovummuridae.

There has been no reported method of using reflected light as a practice to identify calcareous microfossils from thin section, the closest method is to observe loose fossil specimens under a binocular microscope, for example foraminifera (Heinz *et al.*, 2005). Results highlight an abundance of Ovummuridae in the studied samples however, these are mostly impossible to see when thin sections are observed under transmitted light whereas under reflected light they are conspicuous. The use of SEM for such a quantity of thin sections for the observation of such microfossils would be economically and temporally inefficient.

MATERIALS AND METHODS

124 thin sections obtained from various localities (see appendix for locations and grid reference) around the off-reef tract of the MWL Fm. exposed around Wenlock Edge were analysed to detect the presence of Ovummuridae.

The following samples used for this study were previously collected and investigated by Blackburn (2016), who established the microfacies, lithofacies and environments of deposition across the off-reef tract of the MWL Fm. The samples were collected from various lithofacies (the crinoidal grainstone, nodular limestone and tabular mudstone and limestone) from the MWL Fm. found in six localities around Wenlock Edge. The majority of the thin sections were sampled from 5 localities around the off-reef tract and an additional three samples were obtained from Lea Quarry within the reef tract (Rogers *et al.* (2017). The thin sections were systematically sampled from the following locations as

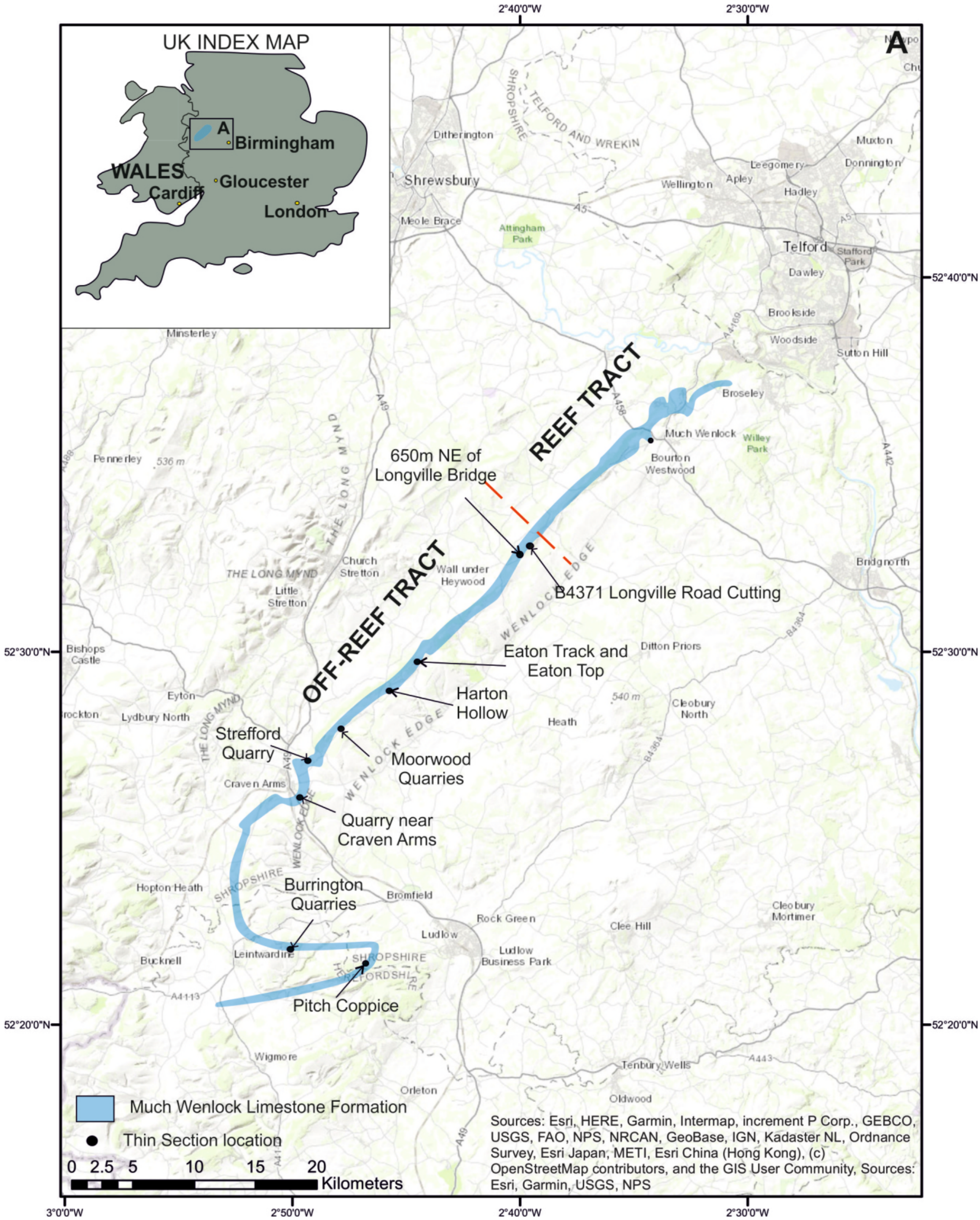


***Figure 2:** A location map highlighting the exposure of the Much Wenlock Limestone Formation in and around Wenlock Edge, Shropshire, UK and the sample locations of the thin sections observed (see appendix for thin section grid references) (Modified from Blackburn, 2016). Arrows indicate sample locations.*

shown by figure 2 from NE-SW (see appendix for thin section details): B4371 road cutting near Longville, above Eaton Track, Harton Hollow, Lower Moorwood Quarry, Moorwood Quarry, Strefford Quarry, Craven Arms, Pitch Coppice, Ludlow Anticline, Burrington, Lower Dinchop-Stefford Lane and Upper Burrington Quarry.

The thin sections were made into 4.75cm x 2.75cm polished thin sections by Blackburn (2016) and were later etched during this study. The thin sections, in a similar manner to Munnecke and Servais (1996), were prepared and submerged into a solution made up of diluted 0.1M hydrochloric acid of a 1:10 ratio of distilled water for approximately 7 seconds. The thin sections were mounted at a thickness of 60µm (double the thickness of 'normal' thin sections; Blackburn, 2016 & Rogers *et al.*, 2017).

Following etching, the thin sections were observed under a light/polarising petrographic microscope (model: Nikon Eclipse LV100N POL with digital sight DS-Fi-2 camera) using both transmitted and reflected light mode to produce several photomicrographs of every specimen of Ovummuridae observed. The photomicrographs generated from the petrological microscope using reflected light allows for the recording of several measurements in various loci and determination of the taxonomical position based on the morphotypes of each specimen using the same method as Munnecke *et al.* (2000). SEM was later used to observe the details of the ultra-structure of the wall.

RESULTS

The results highlight the benefit of using reflected light allow for easy identification of Ovummuridae in comparison to the transmitted light and SEM (see figure 3) and highlights the distinct shape of Ovummuridae without difficulty (figures 4 and 5). The distinct egg shape is compartmentalised into two chambers that can be symmetrical or asymmetrical in morphology (Minoura and Chitoku, 1979 & Munnecke *et al.*, 2000). The chambers are separated by a septa and often these features are impossible to identify in non-reflected light as seen in figures 4 and 5 (Minoura and Chitoku, 1979 & Munnecke *et al.*, 2000). The two chambers are anatomically referred to as the dorsal (larger) and ventral (smaller) chambers based on cross-sectional cuts if the chambers are asymmetrical as shown previously by figure 1.0 (Minoura and Chitoku, 1979 & Munnecke *et al.*, 2000).

The method has allowed for the detection of over ~6000 specimens of ovummurids within the MWL Fm. in particular, morphotypes of *M.gotlandica,* visible in a short time (approximately 6 weeks) with limited accessibility to the microscope and SEM (see figures 4C and 5A). A single specimen of Ovummuridae was found within 3 hours using a SEM, whereas, within the same time frame approximately 300 ovummurids were observed using reflected light.

Observations in reflected light show the detailed anatomical features of Ovummuridae such as the characteristic two chambers, septum like structure (see figure 3 and 4 for more details) and in certain cases, the aperture is perfectly visible (see figure 4D). The characteristic calcareous brick like wall is observable and the method does not detract from the detail of the tablet or slab like arrangement of the calcite crystals (see figure 4B and 5A), indeed due to the 3D nature of the polished and etched sections they are more prominent than in observations using transmitted light (see figures 4C and 5B) where the specimens are completely obscured.

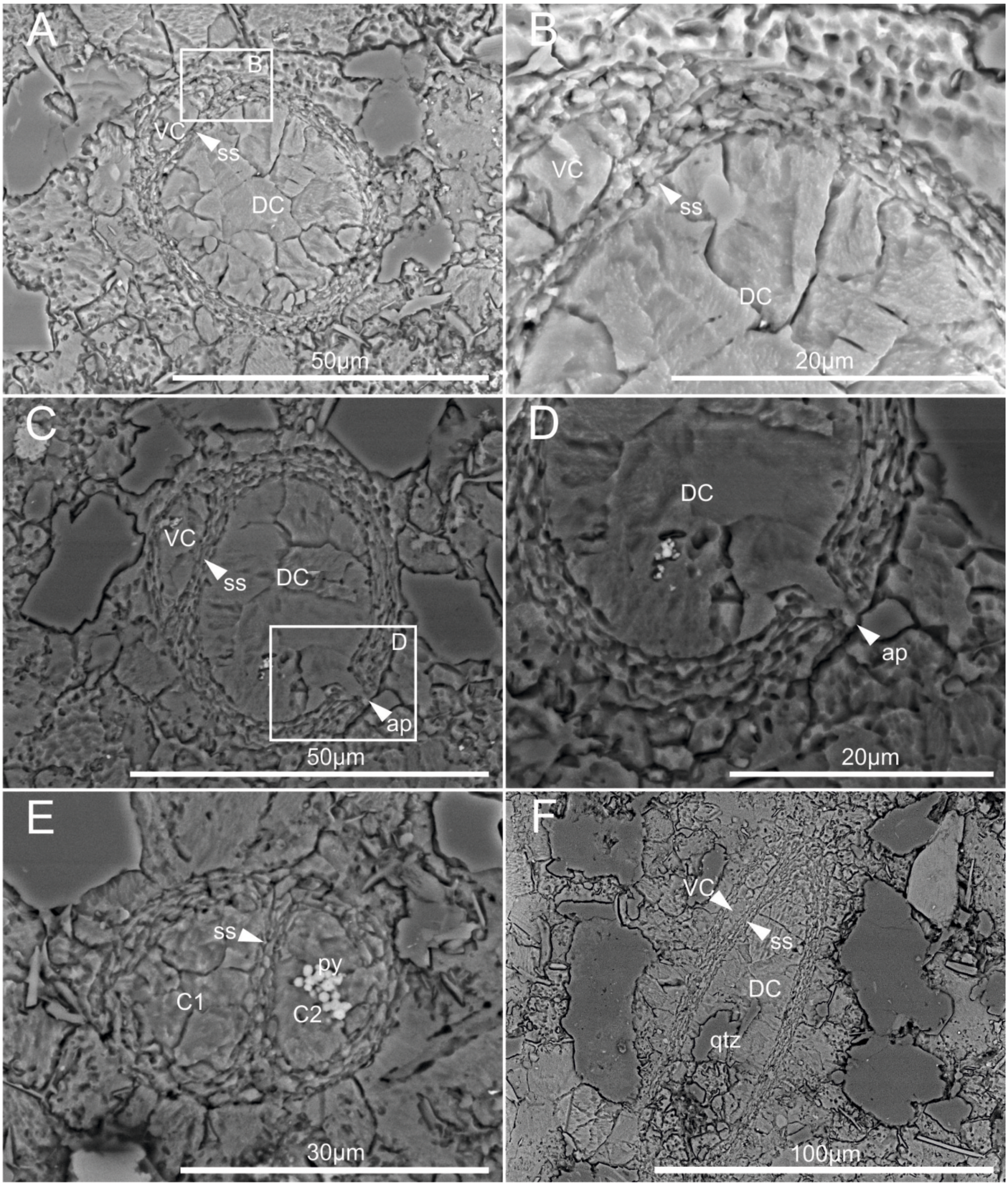
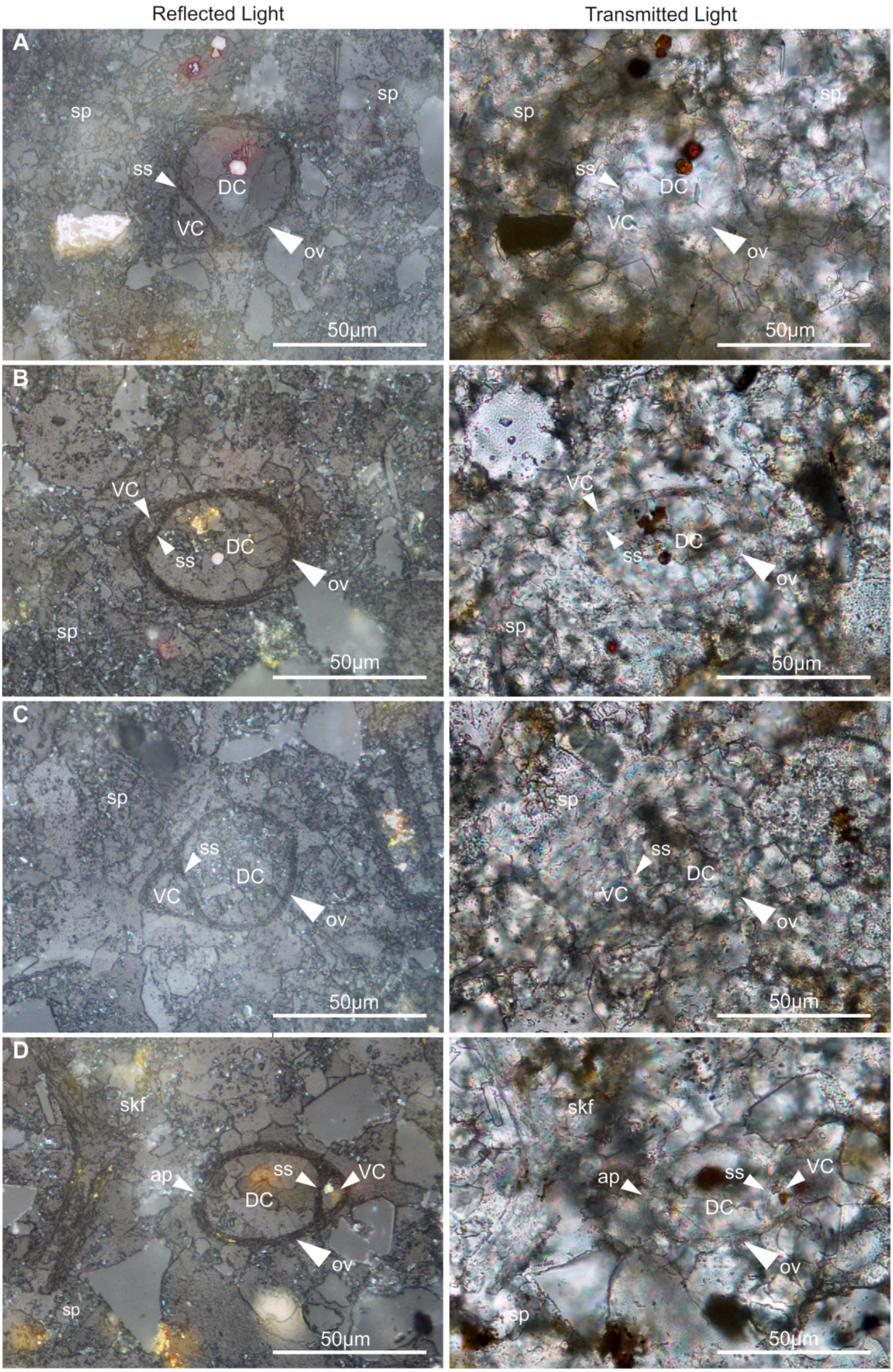


***Figure 3**: SEM photoplates highlighting the resolution and details of the wall structure that is characteristic of the family. (A) Minourella gotlandica with two asymmetrical chambers. The larger dorsal chamber (DC) is separated from the smaller ventral chamber (VC) by a septum like structure (ss), (B) detailed septum like structure from specimen observed in (A), (C) Minourella gotlandica with an aperture (ap) present within its DC. (D) Detailed view of the aperture within the DC (E) Ovummurus duoportius with equal sized chambers (C1 and C2) that are separated by ss, (F) Ovummuridae specimen still difficult to determine in SEM, especially as their walls blend into the matrix and fabric of the sections.*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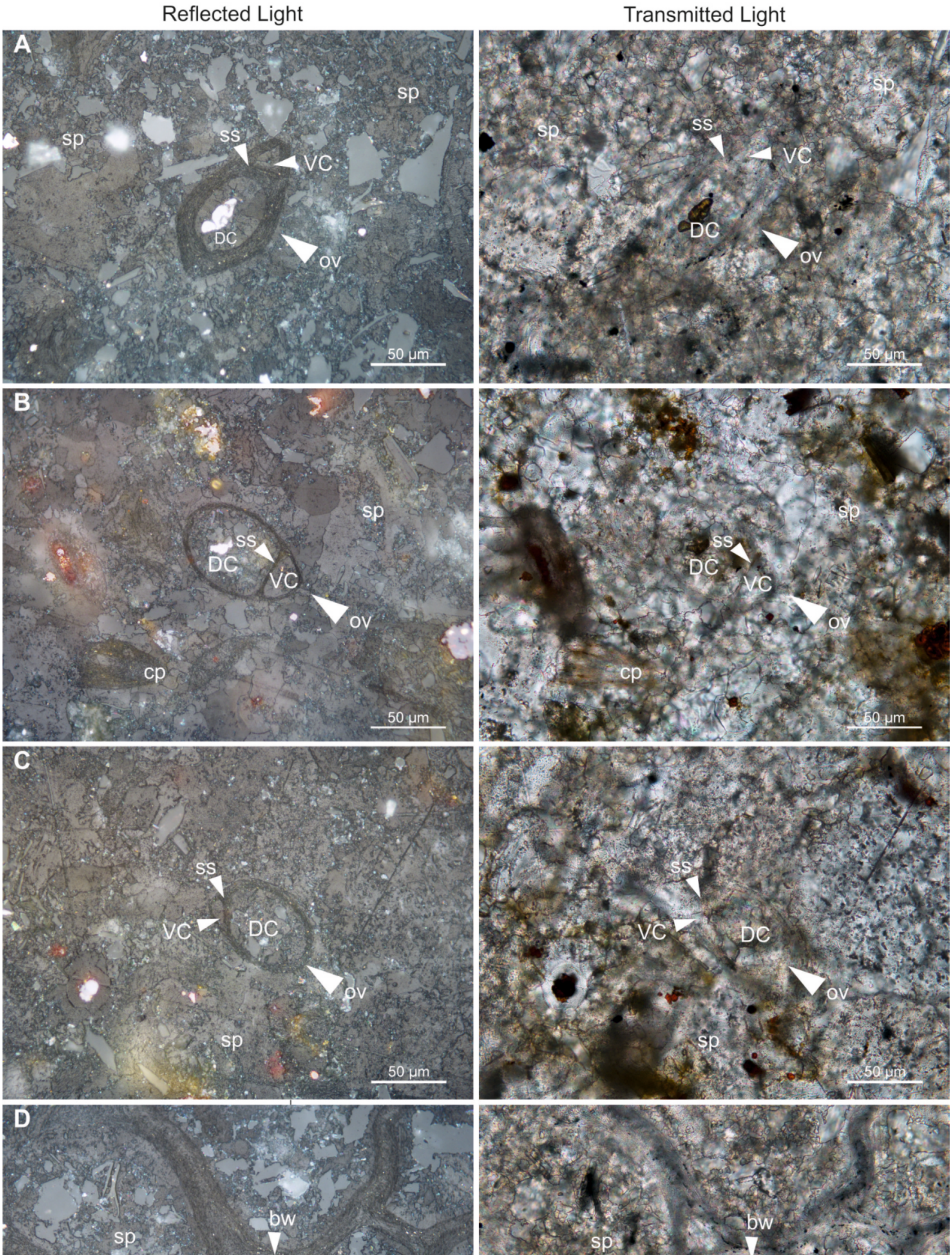
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***Figure 4**: Photomicrographs highlighting the difficulty of identifying Minourella gotlandica (OV) in reflected light (left) in contrast to transmitted light (right) through a polarising light microscope. A, B and C = Minourella gotlandica within a sparitic (sp) matrix with a distinct larger dorsal chamber (DC) and a smaller ventral chamber (VC) separated by a septum like structure (ss) and D = A Minourella gotlandica specimen with a visible aperture (ap) within its DC, Sp matrix with occasional skeletal fragments (skf) present.*

***Figure 5**: Photomicrographs highlighting the difficulty of identifying Ovummuridae (OV) in transmitted light (right) compared to reflected light (left) through a polarising light microscope. A = Minourella gotlandica comprising of a larger dorsal chamber (DC) and a smaller ventral chamber (VC) separated by a septum like structure (ss). The overall specimen has thickened walls within a sparitic matrix (sp), B = Minourella gotlandica found within a sp matrix. Note the presence of a concentric peloid (cp), C = Minourella gotlandica with an unusually smaller VC than the DC, D = Bryozoa (Bry) are observable both under reflected and transmitted light. The distinctive wall (bw) is prominent in both and can easily identify to partly sparitic and miciritc (mi) infill, however more resolution is observed in reflected light.*

The use of both reflected and transmitted light also highlights the mineralogy of the internal clasts that are often incorporated within the chambers and walls. These can be further investigated using SEM. It should be noted that the thicker the walls of the Ovummuridae specimens and their subsequent overgrowths, the easier their detection under transmitted light (although this is still not obvious as highlighted by figures 4A/C and 5A) and in SEM (figures 3F).

DISCUSSION

Although it is not a common or known practice within research to actively search for, and analyse, calcareous microfossils under reflected light, thin sections that have undergone polishing and slight etching allows for the detection of microfossils as long as they are distinguishable from the matrix material, possibly due to the nature of their prominent wall structure. The polishing and etching of thin sections can cause chemical erosion of the micro-topography of the thin sections which results in flattened crystals allowing for the visible detection of the ovummurid walls. Alternatively, observations can be due to the nature of their walls which have a brick-like wall appearance similar to those of a bryozoa (Minoura and Chitoku, 1979). Observations of bryozoans under reflected light show the same detail of resolution as seen with Ovummuridae (See figure 5D). We suggest that the similarities in wall structures could be the reason for this.

The benefits of using such a method not only allows ease of detection of specific microfossils but does not require high-tech specialist equipment; a petrological microscope that can emit reflected light is much cheaper and easier to maintain than an SEM. It is a cheap and easily accessible method that requires no preparation other than the polishing and etching of thin sections. It also does not require the use of staining. SEM can provide high resolution images that show immaculate details of the ovummurid walls (figure 3A/B), however, finding them initially is problematic especially when their walls blend in to the matrix and fabric of the thin sections (see figure 5F). With reflected light there is no initial problem of finding a specimen and for users who lack experience on a SEM, can easily miss these microfossils. It is also noted, where several ovummurids were observed in a single thin section under reflected light, they were not always detectable in SEM.

However, the limitations of reflected light technique results in average quality photomicrographs, this can be easily resolved using image editing (Adobe photoshop and CorelDraw) to reduce brightness and glare from any reflecting minerals present by the addition of filters that reduce the contrast. In contrast, SEM produces high quality images due to the higher magnification and resolution of detail, in which images produced by reflected light cannot compare to. As a result, the detailed study of the ultra-structure of the walls is better suited for SEM rather than reflected light. Another limitation of reflected light is that it cannot determine the intra-wall mineralogy whereas SEM can with Energy Dispersive Spectroscopy (EDS) analysis. For the purpose of detection and observation of calcareous, determining the mineralogy of certain structures is not first-priority and if required, SEM can be used conjunctively for the determination of the intra-wall mineralogy. Reflected light is a technique purely suited for the detection of microfossils whereas SEM is a suite technique for detailed studies.

Previous reportings of Ovummuridae have all been observed using SEM and transmitted light (see table 1.0) (Minoura and Chitoku, 1979; Munnecke *et al.*, 2000; 2001; MacNeil and Jones, 2006 & Rogers *et al.*, 2017). However, this study clearly highlights that detection under transmitted light does not allow for the resolution required to identify or observe Ovummuridae specimens, neither does SEM in which the walls of Ovummuridae are often concealed by the matrix. We suggest this could be a reason for the lack of observations or reportings of ovummurids within the literature as they go undetected and could appear far more common within the geological record than we previously know.

The applicability of the technique to detect other microfossils is still under investigation. Evidence from the MWL Fm. not only show the detection of Ovummuridae under reflected light but the detection of other microfossils such as: bryozoans (see figure 5D), corals, algae, calcispheres and foraminfera. The detection of other microfossils, especially those that have a different wall structures and chamber arrangement to that of Ovummuridae highlight the vast potential this technique has within the field of micropalaeontology. However, this study has not considered the applicability of the technique to siliceous microfossils such as Radiolaria, purely due to the calcareous nature of the samples. Whether, siliceous microfossils are detected under reflected light in the same manner as calcareous microfossils is indefinite, as the composition of their skeletons is different to those of calcareous microfossils. The extent to which reflected light microscopy is suitable for the detection of certain microfossils will require further investigation.

The wider application of this technique is extensive, as it will allow for the comparison of the data from the MWL Fm. to that of the Silurian of Gotland whose strata are of the same age and similar sedimentology but of a different palaeogeographical setting. This may add further data towards the affinity of Ovummuridae and provide further details into the geological and diagenetic history of the MWL Fm. This new method may allow the detection of ovummurids that have previously gone undetected and could increase their stratigraphic distribution and their palaeogeographical significance. It also presents the opportunity of investigating if ovummurids are present regionally across the West Midlands where the MWL Fm. is exposed and its possible correlation to the Wenlock Edge area based on the occurrence of Ovummuridae.

CONCLUSIONS

This study has highlighted the difficulties of observing Ovummuridae specimens within polished and etched thin sections using transmitted light and a SEM when compared to reflected light through a petrological microscope aswell as highlighting some of the restrictions of using an SEM. The use of reflected light has allowed the detection and observation of several ovummurids within the MWL Fm., in particular noting the distinct characteristic wall structure and its two chambered anatomy in a short amount of time. The limited reportings of Ovummuridae within the literature is possibly due to the previous methodical approach of finding these problematic calcareous microfossils, to which reflected light resolves. Reflected light is an alternate method that is a quick and cost-effective and easily accessible as opposed to SEM for the detection of difficult to observe calcareous microfossils, in this case Ovummuridae. This new technique is potentially also applicable to the detection of other microfossil groups.

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Appendix:

Location	Thin Sections	Grid Reference
B4371 road cutting near Longville	8 samples (LS0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4)	SO 540575 66639
top of the edge on the B4371 near Longville	7 samples (LST1-7)	SO 545569 66173
Eaton Track	5 samples (ET1-5)	SO 506343 72980
Eaton Top	4 samples (EG 1-4)	SO 506343 72980
Harton Hollow	23 samples (1-23HH)	SO 48043 87628
Lower Moorwood Quarry	11 samples (1-11LMQ)	SO 46563 80622
Moorwood Quarry	15 samples (1-15MQ)	SO 46563 80622
Stefford Quarry, Lower Dinchop-Stefford Lane	6 samples (SQ1-5)	SO 454775 81349
Craven Arms	14 samples (CA 1-14)	SO 43606 84729
Pitch Coppice	1 sample (PC)	SO 342427 77593
Ludlow Anticline, Burrington, Herefordshire	17 samples (BMQ 1-17)	SO 44259 72549
Upper Burrington Quarry	5 samples (BUQ A-E)	SO 44143 72672