

# PHOSPHATIC MICROFOSSILS FROM YUKON, CANADA PROVIDE NEW INSIGHTS INTO THE EVOLUTION OF EARLY ANIMALS AND THEIR ENVIRONMENT

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## Abstract

The history of life on Earth is preserved in the rock record. As we search for evidence of life on other planets, it is likely to be preserved in the rocks. As such, it is critical that we understand how to accurately interpret the rock record. Here we describe fossils from Yukon, Canada that represent the earliest evolution of animals 540 mya. The goals of this project are to investigate potential drivers for this sudden explosion of diversity including ecological and environmental changes. To do this, we characterize the fossils from two rock units that encompass this increase in diversity and use stable oxygen isotopes as a proxy for paleotemperature. The increase in diversity of fossil organisms is closely tied to the appearance of reef forming metazoans, suggesting that reefs provided new habitat space for organisms to diversify. Oxygen isotope data have not been obtained yet but will provide new insight into temperature during this significant period in animal evolution. Additionally, this data will contribute to our knowledge of the formation of phosphatic crusts and their potential for recording paleotemperature. Beyond Earth, phosphatic crusts have been identified on Mars and may provide clues to the history of the red planet.

## Introduction

Earth is currently the only planet known to host life. Despite this apparent rarity, the conditions to support life have existed on Earth for at least 4 billion years of its 4.6 billion years of existence

(Schopf 2006). The fossil record provides the history and evolution of life on our planet. It is likely that any evidence we find of life on another planet will be fossil evidence (McMahon et al. 2018). As such, it is important for us to develop our understanding of geological record so that we can properly interpret the record of life and the environment on Earth, and also apply what we know to the search for life on other worlds.

Much of the history of life on Earth is occupied by single celled organisms (Knoll and Nowak 2017). Complex multicellular life, and animals in particular, only arose approximately 540 million years ago (Zhuravlev and Wood 2018). The question remains why did it take so long for complex life to evolve? Multiple drivers for this evolutionary event, termed the “Cambrian Explosion” have been suggested including biological mechanisms such as the development of HOX genes and Gene Regulatory Networks (Peterson, McPeck, and Evans 2005; Zhu et al. 2017), ecological processes such as predation and the evolution of biomineralization (Vermeij 1989; Erwin and Tweedt 2012; Kouchinsky et al. 2012), and environmental factors such as temperature changes, nutrient availability, and dissolved oxygen levels (Sperling et al. 2015; Zhang and Cui 2016; Wood, Ivantsov, and Zhuravlev 2017; Dahl et al. 2019).

Temperature is one of the mechanisms that is not well constrained for this time period (Song et al. 2019; Scotese et al. 2021). We know that seawater

temperature is a significant control on the distribution of modern marine animals, so it could have impact on the distribution and evolution of early animals (Stuart-Smith, Edgar, and Bates 2017; Oppenheim et al. 2019). Some current estimates for Cambrian seawater temperatures push 50°C, which would prove deadly to many modern sea creatures (Vinagre et al. 2019). A better understanding of temperatures during this time is critical to our understand of early animal evolution.

Here we will use stable oxygen isotopes as a proxy for paleotemperature.

#### Geological Setting

The rocks used in this study come from the Wernecke Mountains in Yukon, Canada. The stratigraphy here preserves a record of the breakup of the supercontinent Rodinia and the formation of a passive continental margin on the paleogeographically northern coast of Laurentia. This succession, known as the Windemere



Figure 1: Map of Yukon, Canada showing location of field site

Supergroup preserves rocks from the Tonian period (~1000 Ma) to the Cambrian period (~540 Ma) (Moynihan et al. 2019). Our field sites are located approximately 185 km northeast of Mayo on geologic features known as the Ogilvie Arch and Misty Creek Embayment (Scott et al. 2022). The Ogilvie Arch was a topographic high during the Cambrian that resulted in shallow water deposits. Here we collected material from the Ingta Formation. The Misty Creek Embayment was a topographic low that resulted in deeper water deposits in a relatively nearshore environment. Here we collected material from the Sekwi Formation.

#### Ingta Formation

The Ingta Formation is a mixed clastic and carbonate unit approximately 250 m thick (Aitken 1989). The unit is mostly made up of green to brown siltstone. The uppermost part of the section contains peloidal and micritic beds and carbonate nodules. These carbonate features are topped by a phosphatic crust, which also contains phosphatic small shelly fossils (Pyle et al. 2006). These fossils place the Ingta formation as earliest Cambrian in age (Conway Morris and Fritz 1980; Nowlan, Narbonne, and Fritz 1985). The Ingta Formation also contains many trace fossils that corroborate this age (MacNoughton and narbonne 1999). The Ingta formation unconformably overlies the dolomitic Risky Formation and is overlain by the quartzitic Backbone Ranges Formation (Aitken 1989).

#### Sekwi Formation

The Sekwi Formation is a predominantly carbonate unit that ranges from 500 m to over 1000 m thick (Handfield 1968). The unit consists of gray bedded

limestone, orange weathering dolostone, and gray silty limestone (Fritz 1976). The rocks were deposited in a deep-water carbonate ramp environment (Dilliard et al. 2010). The Sekwi formation contains abundant fossil material including trilobites, archaeocyaths, brachiopods, chancelloriids, and abundant small shelly fossils first described here (Fritz 1972; Stelck and Hedinger 1975; Randell et al. 2005). Trilobite biostratigraphy places the Sekwi Formation in the *Bonnia-Olenellus* zone, which correlates to Cambrian Stage 4 (514-509 Ma) (Fritz 1972). The Sekwi formation conformably overlies the shale of the Vampire Formation and is unconformably overlain by the shales of the Hess River Formation (Fritz et al. 1991).

#### Methods

##### Sample Collection

Samples were collected from phosphatic layers of the Ingta Formation during the 2022 field season. Phosphatic material from the Sekwi Formation was collected during the 2023 field season.

Microprobe polished thin sections were made for material containing small shelly fossils and phosphatic crusts.

##### Fossil Extraction

Material containing small shelly fossils was dissolved in buffered 10% acetic acid following modified methods of Jeppsson, Anehus, and Fredholm (1999). The rocks were placed in 2 gallon or 5 gallon buckets and submerged in 10% acetic acid buffered by spent acetic acid – leftover from previous macerations. The time to dissolve the rocks varied by the lithology and the size of the sample from 2 to 6 months. The material remaining after the dissolution was sorted using sieve size fractions of >710  $\mu\text{m}$ , 710-355  $\mu\text{m}$ , 355-

210  $\mu\text{m}$ , 210-149  $\mu\text{m}$ , and <149  $\mu\text{m}$ . The sieved material was viewed under a light microscope, and fossil material was picked out and sorted by morphology. The fossils were mounted on stubs with carbon tape and imaged using a Hitachi TM3000 tabletop Scanning Electron Microscope (SEM).

##### Oxygen Isotope Analysis

In order to measure the oxygen isotopes of the phosphatic materials, the phosphate must be isolated from the other materials in the sample. To do this, I used a method modified from O'Neil et al. (1994) where the phosphate is chemically isolated as silver phosphate. The samples are crushed into a powder and dissolved using nitric acid. Then potassium fluoride is added to the solution to pull out calcium and other cations. Silver amine is added to the remaining solution to precipitate silver phosphate. The crystals can then be rinsed and removed for isotope analysis on the isotope ratio mass spectrometer. Samples were sent to the Center for Stable Isotopes at the University of New Mexico for analysis.

#### Results

##### Petrographic Analysis

The rocks that contain the phosphatic microfossils and crusts are calcitic grainstones (figure 2). The dominant grains are rounded quartz grains, carbonate peloids, calcitic fossils, and phosphatic fossils and grains. Most grains are less than 100  $\mu\text{m}$  in diameter. Fossil grains can be over 1 mm in length. Phosphatized fossils are often internal molds. In some cases the calcitic shell is still visible around the mold in thin section. Phosphatic molds may contain quartz grains. Phosphatic crusts appear laminated and contain abundant quartz grains. Fossil shells are visible in the phosphatic crust in low

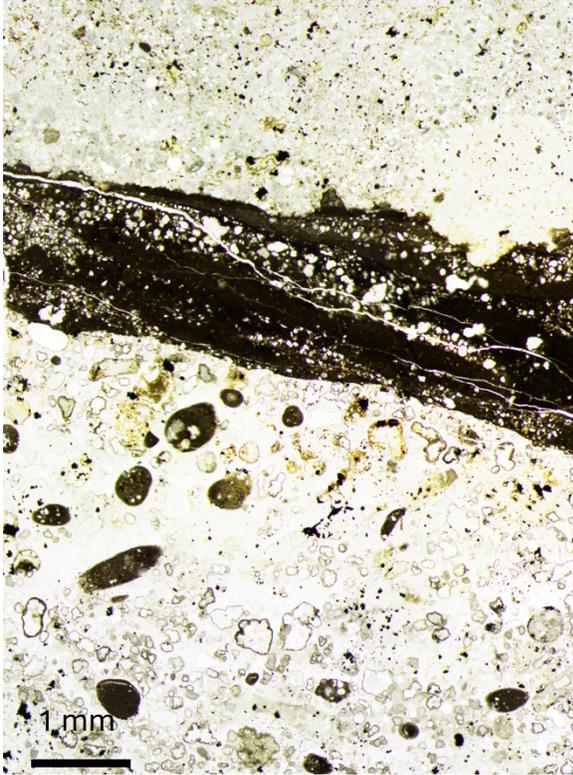


Figure 2: Petrographic micrograph of sample from the Ingta Formation showing calcite matrix (white) with abundant quartz sand grains (also white) and phosphatic crust (black). Note the lack of fossil material above the phosphatic crust and the abundant fossils, often phosphatized, below the crust

abundance. Layers without fossil material consist of mostly quartz grains, with few carbonate grains.

#### Extracted Fossils

Ingta Formation The most common fossil from this unit is the conical form *Anabarites trisulcatus* (figure 3). These fossils are preserved as both internal molds and phosphatized shells. The molds are typically black in color. Shells can range in color from black to blue to white. The external texture of the shell is often smooth or with very fine annulations. Other fossils found in this unit include annulated tubular forms (e.g. *Hyalithellus* sp.), protoconodonts, embryos, and the unique form *Zhenjiangorhabdion* sp.

While the protoconodonts are inferred to be at least partially original phosphatic material (Szaniawski 1982), many of these fossils are likely secondarily phosphatized.

Sekwi Formation The fossils from the Sekwi formation are much more diverse and contain many forms attributable to recognizable crown groups (figure 4). These fossils include reef building organisms such as archaeocyathids and chanceloriids. Other fossils include hyoliths, trilobites, brachiopods, and monoplacophorans, as well as other plates belonging to unknown metazoans. Brachiopods are known to produce phosphatic shells, but all other material is likely secondarily phosphatized.

#### Oxygen Isotopes

Samples were sent for analysis, but I have not yet received the results. I sent preliminary samples including the phosphatic crust, an embryo, an internal mold of *Anabarites*, and an original shell of *Anabarites* from the Ingta Formation, and a brachiopod from the Sekwi Formation. I will update this report when I have those results.

#### Discussion

##### Phosphatic Preservation

Phosphorus is the limiting macronutrient for life on geologic timescales. As such, the preservation of fossil material in phosphate, which requires abundant phosphorus, is a rare taphonomic process (Schiffbauer et al. 2014). During the Cambrian, for reasons that are still not fully understood, phosphatization was much more common (Porter 2004). Preservation by phosphate is known to be able to preserve subcellular detail

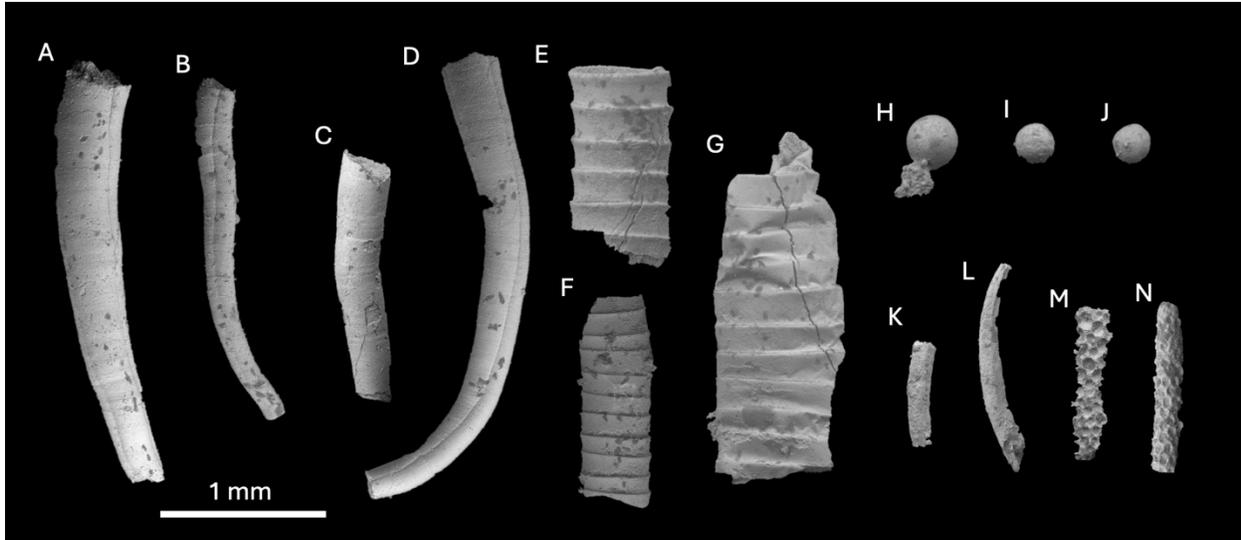


Figure 3: Small shelly fossils from the Ingta Formation. A-D *Anabarites trisulcatus*, E-G *Hyolithellus* sp. note the wrinkled texture indicating a soft tube that was secondarily mineralized, H-J embryos, *K* *Carinachites* sp., *L* *Protohertzina* sp., M-N *Zhenjiangorhabdion* sp.

(Xiao, Zhang, and Knoll 1998). Because of this exceptional preservation, the “phosphatization window” during the Cambrian allows for the detailed study of the first major radiation of animal life. The phosphatized fossils from northwest Canada include shells inferred to be originally phosphatic, phosphatized internal molds, and phosphatic casts of organic or biomineralized structures. Soft tissue fossils that are preserved, must be phosphatized before they succumb to decay, whereas phosphatic internal molds could potentially form at any point after burial (Briggs 2003). While these fossils are all preserved in phosphate, the processes that led to that preservation may vary.

#### Petrographic Analysis

Thin section analysis reveals that some fossils are preserved as internal molds while others are preserved as casts of the original shell. Further, shells may be only partially replaced or partially infilled with phosphate. This preservation can result in what appear as fragmented fossils when extracted through acid macerations. Often these broken bits

are taken to represent material that has been transported before burial, or reworked (Creveling et al. 2014). Care must be taken when assessing the taphonomic history of these fossils. The phosphatized fossils are directly associated with, and sometimes fully contained within, the phosphatic crust. The timing of phosphate formation is critical to interpreting the geochemical data. The direct association might suggest that phosphatic molds found in the phosphatic crust formed at the same time that the crust was deposited. It is difficult to assign taxonomic identification to many of the fossils in thin section because there is not enough detail in a single cross section. Therefore, inferences about taphonomic history can only be applied to different preservational modes and not to distinct taxa. While most taxa can be assumed to be preserved through only one taphonomic mode (i.e. original phosphatic shell, cast, or mold) there are some taxa that exhibit multiple taphonomic modes (e.g. *Anabarites* is preserved as both cast and mold).

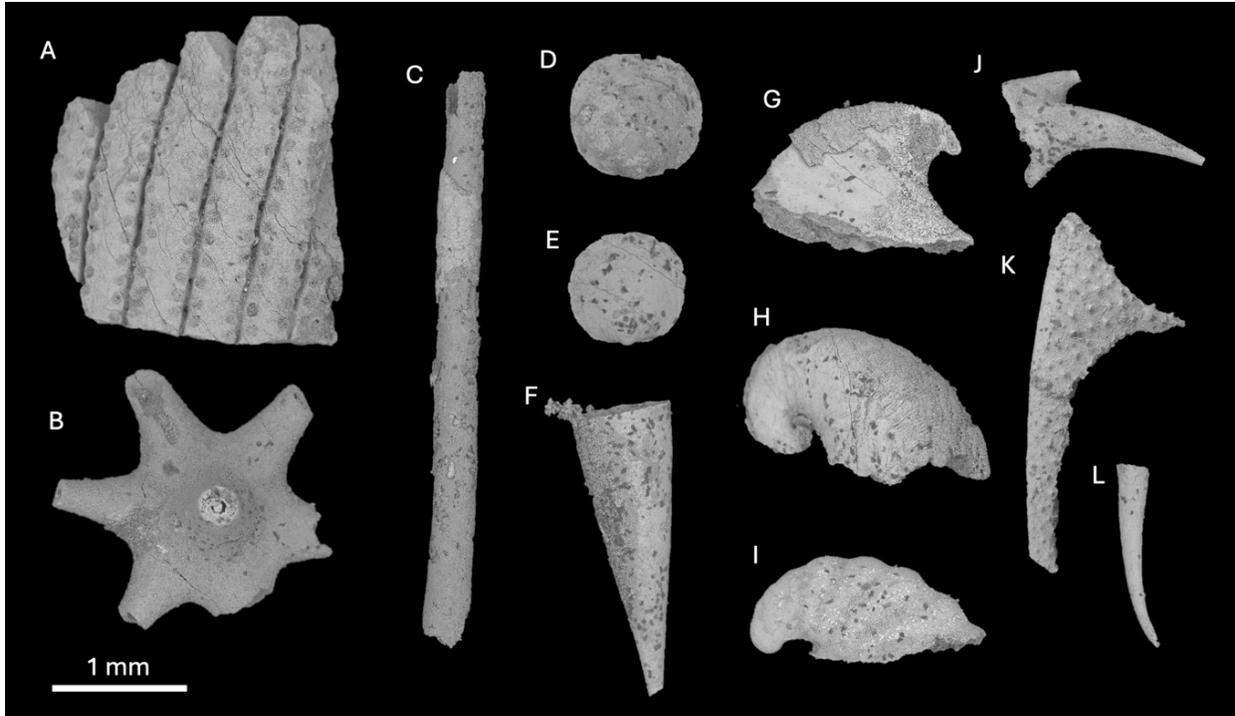


Figure 4: Small shelly fossils from the Sekwi Formation. A archaeocyathid, B chancelloriid, C unidentified tubular fossil, D-E brachiopods, F hyolith, G-I mollusks, J-K trilobites, L Protohertzina

### Oxygen Isotopes

The stable isotopes of oxygen ( $^{16}\text{O}$ ,  $^{17}\text{O}$  and  $^{18}\text{O}$ ) will fractionate between seawater and oxygen-bearing mineral phases based on temperature (Sharp 2017). Commonly, the ratio of the two most abundant isotopes –  $^{16}\text{O}$  and  $^{18}\text{O}$  – are used. The ratio of the two isotopes is defined using delta notation which is generated from the following formula:

$$\delta^{18}\text{O} = \left( \frac{\frac{^{18}\text{O}}{^{16}\text{O}}_{\text{sample}}}{\frac{^{18}\text{O}}{^{16}\text{O}}_{\text{standard}}} - 1 \right) \times 1000 \quad (1)$$

At colder temperatures, the heavier isotope is favored in the mineral matrix resulting in a higher  $\delta^{18}\text{O}$  ratio. At warmer temperatures, more of the lighter isotope is incorporated into the mineral phase, resulting in a lower  $\delta^{18}\text{O}$  ratio. Both carbonate (containing  $\text{CO}_3^{2-}$ ) and phosphate (containing  $\text{PO}_4^{3-}$ ) bearing minerals are used as a paleotemperature proxy with oxygen isotopes. Carbonate minerals (e.g.

calcite) are some of the most common minerals precipitating in the ocean and used by animals to make their shells. This makes them incredibly useful as a recorder of paleotemperature throughout earth history. Unfortunately carbonate minerals are quite susceptible to dissolution and diagenetic alteration meaning that older carbonate rocks may not be a reliable recorder of paleotemperature. Phosphatic minerals (e.g. apatite) can also record paleotemperature and are much more resilient to chemical alteration (Yang et al. 2021). Much of our record of temperature through the Paleozoic is based on phosphatic fossils, while more recent temperature estimates are obtained through a combination of phosphatic and calcitic fossils (Song et al. 2019). An equation has been empirically determined from data from modern phosphatic organisms including brachiopods and sharks to calculate

seawater temperature from oxygen isotope values (Lécuyer et al. 2013):  
$$T(^{\circ}C) = 117.4(\pm 9.5) - 4.50(\pm 0.43) \times (\delta^{18}O_{phosphate} - \delta^{18}O_{seawater}) \quad (2)$$

In the early Cambrian, when animals were first starting to diversify, there were not as many organism producing shells as in the modern oceans. As such, this makes it challenging to utilize Cambrian biominerals as paleothermometers. The limited temperature reconstruction we have for the Cambrian is based on phosphatic brachiopod shells (Grossman 2012). Only one previous study has investigated the isotopic composition of Cambrian small shelly fossils (Chen et al. 2007). Their findings support moderate temperatures for the early Cambrian. Importantly the fossils they examined are interpreted to be secondarily phosphatized, so further investigation is necessary to verify these results (Chen et al. 2016). My work will reveal whether there are differences in the record of originally phosphatic shells and those that are secondarily replaced. Additionally, it will demonstrate the effectiveness of abiogenic phosphatic crusts as a paleotemperature proxy. Previously, Archean (~3500-3200 Mya) phosphatic crusts have been shown to record temperatures of 26°C to 35°C for the ocean at the time, however more work is needed to demonstrate the reliability of abiogenic phosphate (Blake, Chang, and lepland 2010).

#### Applications to Extraterrestrial Systems

There are three fundamental questions that underly all NASA Astrobiology research: 1. How did life begin and evolve on Earth? 2. Does life exist elsewhere in the universe? and 3. How do we search for life elsewhere in the universe? This research addresses both the first and the third question most directly.

The fossils from the Ingta Formation represent some of the earliest recognizable animals. They show that the tubular form was a very common early solution for metazoan life, which has been demonstrated in Ediacaran forms as well (Surprenant and Droser 2024). Additionally, most of these organisms were likely sedentary living on the seafloor. The fossils of the Sekwi Formation record and explosion of diversity and the expansion of recognizable crown groups. This increase in diversity is associated with the development of the first metazoan reefs built by archaeocyathids. This relationship could represent the expansion of ecospace provided by reefs. Reefs are known as hotspots of biodiversity in modern times (Reaka-Kudla 1997). These fossils suggest reefs have provided expanded habitat for animals for over 500 million years. The study of phosphorite deposits on Earth also has implications for the search for life on other planets. Phosphorus is a key nutrient for all life on Earth, so it has been suggested that finding phosphorus on other planets is the first step to identifying extraterrestrial life (Weckworth and Shidlowski 1995; Mojzsis and Arrhenius 1998). Thanks to multiple rover missions, we know that phosphatic minerals are present on Mars (Maciá, Hernández, and Oró 1997). Through weathering processes, these minerals can dissolve, producing aqueous phosphate (Adcock and Hausrath 2015). This dissolved phosphate would be available for organisms to use and could result in the precipitation of phosphatic crusts. The Curiosity rover has identified phosphorus on Mars (Maurice et al. 2016) and has detected phosphorus rich nodules which may be relics of a

previously phosphatic phase (Lanza et al., 2021). These materials would be excellent candidates for oxygen isotopic analysis to reconstruct past climates on Mars. Additionally, the Perseverance rover has increased elemental analytical abilities with the SuperCam and PIXL tools (Anderson et al., 2022). SuperCam allows for the remote analysis of mineral samples by firing a laser to vaporize the sample and measuring the spectra emitted. PIXL uses x-ray fluorescence to identify the chemical composition of materials and is also capable of taking high resolution microscopic images. Together these tools could allow for the identification and targeting of phosphatic samples. Currently the limitations of the rovers sent to Mars do not allow for equipping a mass spectrometer that could measure the stable isotopes of samples. The Perseverance rover is caching samples to be sent back to Earth during a future mission. Until that time, we must learn as much as we can about similar samples on Earth so that we can accurately interpret the history of Mars and identify any signs of ancient life.

### Conclusions

The fossil record of the early Cambrian preserves the evolution and radiation of complex multicellular life. The drivers for this sudden appearance of nearly all animal phyla are not well understood. Here we investigate the phosphatic small shelly fossil record of Yukon, Canada to provide insight into suggested mechanisms. The fossil record suggests that the arrival of reef forming archaeocyathids played a key role in providing new habitat, facilitating the diversification of many animal lineages. Phosphatic fossils also provide a potential record of paleotemperature through stable oxygen isotopes.

Associated phosphatic crusts may also provide a record of temperature conditions at the time of deposition. Phosphorus is a critical nutrient for all life on Earth, and phosphatic deposits on other planets are an important target in the search for life on other planets. The lessons we learn from studying the fossil record on Earth will help us in our search for extraterrestrial life.

### Acknowledgments

All fossil material comes from land of the First Nation of Na-cho Nyäk Dun and has been collected with their permission. I thank my advisor, Shuhai Xiao, for guidance at every step of this project. I thank Justin Strauss, Scott Evans, and Reina Harding for assistance with collecting fossil material in the field. This research is funded by grants from the National Science Foundation, the Paleontological Society, Virginia Tech Department of Geosciences, and the Virginia Space Grant Consortium.

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