Investigation of carbon and nitrogen stable isotopes in a vertebrate coprolite assemblage from the Late Triassic of continental equatorial Pangaea

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Abstract

Coprolites, fossilized faeces, provide evidence of ecological interactions in the fossil record and provide a rare window into the biology of extinct taxa (e.g.[1]). Ecological interactions, such as trophic level and carbon source, can be gleaned from carbon and nitrogen stable isotope compositions of excrement from extant and recent vertebrates[2,3]. However, the utility of stable isotopes for reconstructing ancient ecological dynamics from fossil vertebrate coprolites is little-known. Here we investigated the carbon and nitrogen isotope compositions of an assemblage of coprolites collected from a single horizon (**PFV 456 - representing a marginal lacustrine paleoenvironment in the humid equatorial paleotropics of continental central Pangaea**) in the **Upper Triassic (~ 220 Ma) Chinle Formation of Arizona**. The exact taxonomic affinity of these coprolites to their makers were unknown due to their association with disarticulated skeletal elements from at least 53 vertebrate taxa. We organized the sample set of 52 coprolites into 13 discrete morphotypes based on size, shape, color, inclusions, and internal and external structures, and analyzed their organic carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope compositions. **$\delta^{13}C_{org}$ of the coprolites ranged from –29.9‰ to –23.6‰**. This range is consistent with both the range of $\delta^{13}C$ from modern C3 plants and other coprolites, and living tetrapod excrement, which suggests the $\delta^{13}C_{org}$ in these coprolites represent the original isotopic compositions of the excrement from the source animals. Low nitrogen contents limited the number of nitrogen isotope compositions we were able to collect. However, **two coprolites yielded $\delta^{15}N$ from 2.2‰ to 3.5‰**. Reconstruction of trophic interactions based on these results is limited by the paucity of nitrogen values; however, analysis of relationships between the isotopic results and characteristics of the coprolite morphotypes such as diameter, color, and presence of inclusions allows for some insights to be drawn. For example, that fish were common to the diets of the makers of several coprolite morphotypes, and the distribution of $\delta^{13}C_{org}$ values suggests the presence of a range of coprolite-producer diets with the exclusion of terrestrial apex predators given the relative lack of trophic isotope enrichment.

Geologic and Paleobiological Context

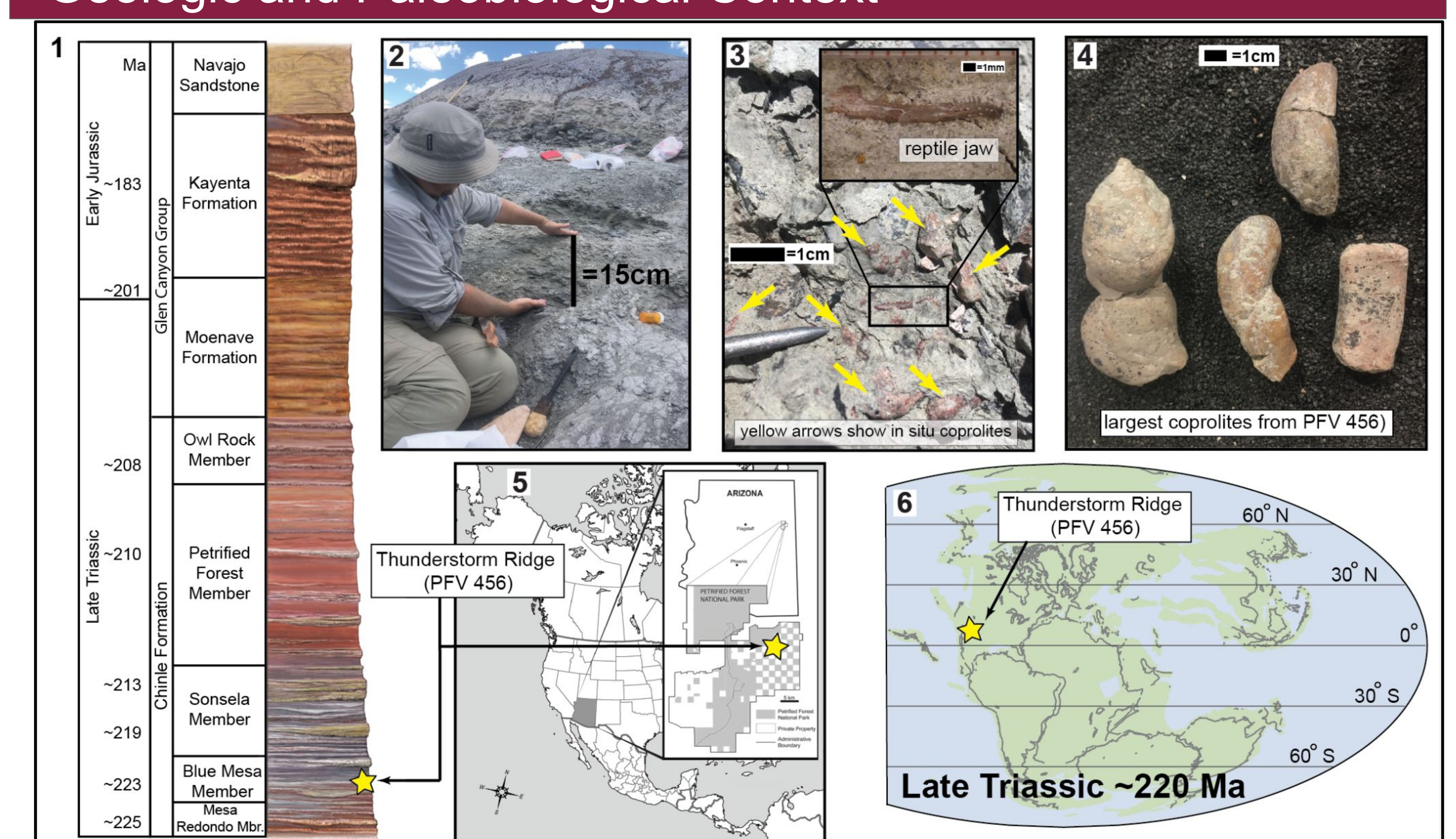

Figure 1. Stratigraphic context of PFV 456, with approximate radioisotopic ages[4] (1.1); photograph of fossiliferous horizon in outcrop (1.2); photograph of in situ coprolites and bones (1.3); photograph of largest coprolites from PFV 456 (1.4); map showing location of PFV 456 in Petrified Forest National Park (1.5); paleogeographic map showing approximate Late Triassic location of PFV 456 (1.6).

- Depositional regime of Upper Blue Mesa Member: northwest-flowing fluviolacustrine system on the western margin of equatorial Pangaea at a paleolatitude of ~6° N in a humid monsoonal climate[5] (Fig. 1.6)
- Thunderstorm Ridge locality (PFV 456), 15-cm-thick poorly sorted siltstone horizon, bearing a dense concentration of carbonate nodules, angular intraformational clasts, and micro- and macro-vertebrate bones, and coprolites (Fig.

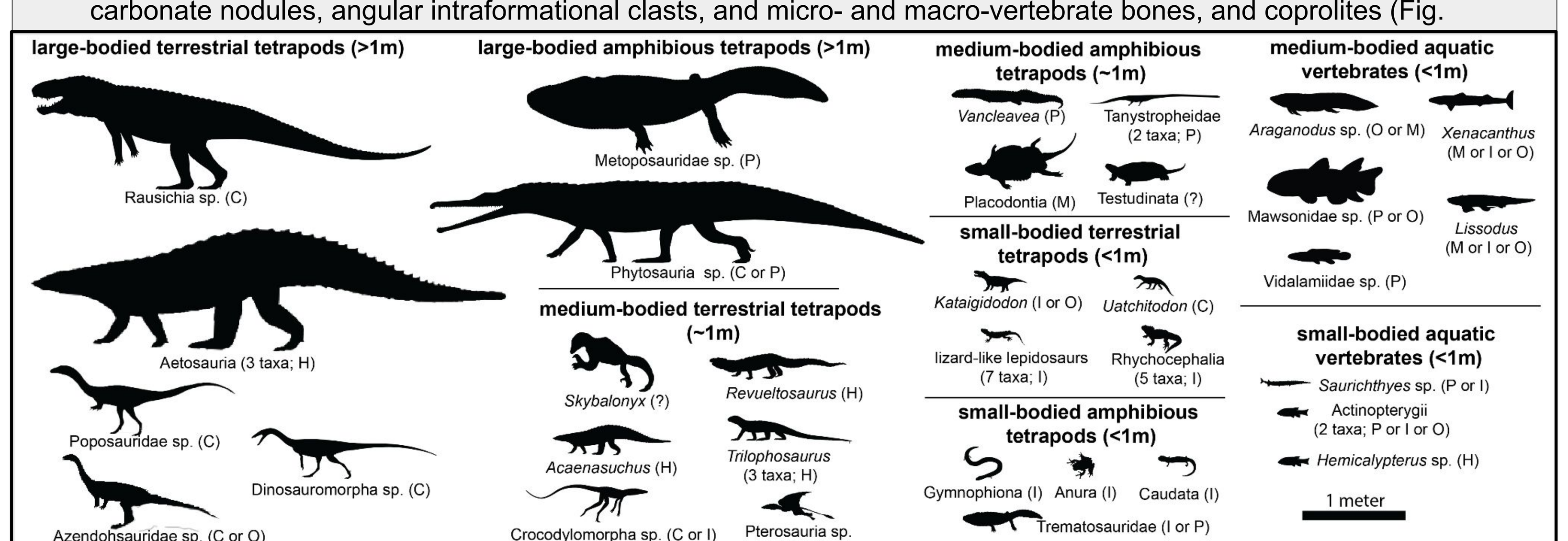

Figure 2. PFV 456 vertebrate assemblage; taxa grouped by body size (>1m, ~1m, <1m) and life habit (terrestrial, amphibious, aquatic); diets listed in parentheses after taxon name (C, carnivore; H, herbivore; O, omnivore; P, piscivore; I, insectivore; M, molluscivore; ?, unknown), diets inferred from tooth/jaw morphology, body size, and other paleobiological indicators.

- 52 vertebrate taxa present, including a wide range of body sizes, life habits, and inferred diets
- Direct links between coprolite morphotypes and their respective coprolite producing taxa are unknown

Methods

Sample Collection and Morphotype Grouping

- Specimens were collected in 2018-2019 and 2021 at PEFO as bulk fossiliferous sediment from PFV 456
- 54 Coprolites were separated following screenwashing into 3 morphotypes **(Figure 3)**

Sample Preparation for Isotope Analysis

- **Cleaning samples**
- **Powdering and decarbonation:**

Samples > 2g	Powdered with a ball mill following pre-contamination	Treated with 2 M HCl overnight	Neutralized and dried over 3-4 days in an oven
Samples < 2g		Treated with 2 M HCl overnight	Neutralized and dried over 3-4 days in an oven

Isotope Analysis

- **Data measured:** $\delta^{13}C$ and $\delta^{15}N$
- **Equipment:** Isoprime 100 isotope ratio mass spectrometer coupled to an Elementar vario ISOTOPE cube elemental analyzer at the Virginia Tech Stable Isotope Geochemistry Lab
- **Standards used:** CH6, CH7, USGS25 and USGS26
- **Error calculation:** +/- 0.521 ‰ for $\delta^{13}C$ and +/- 0.463 ‰ for $\delta^{15}N$

Results - Gross Morphology

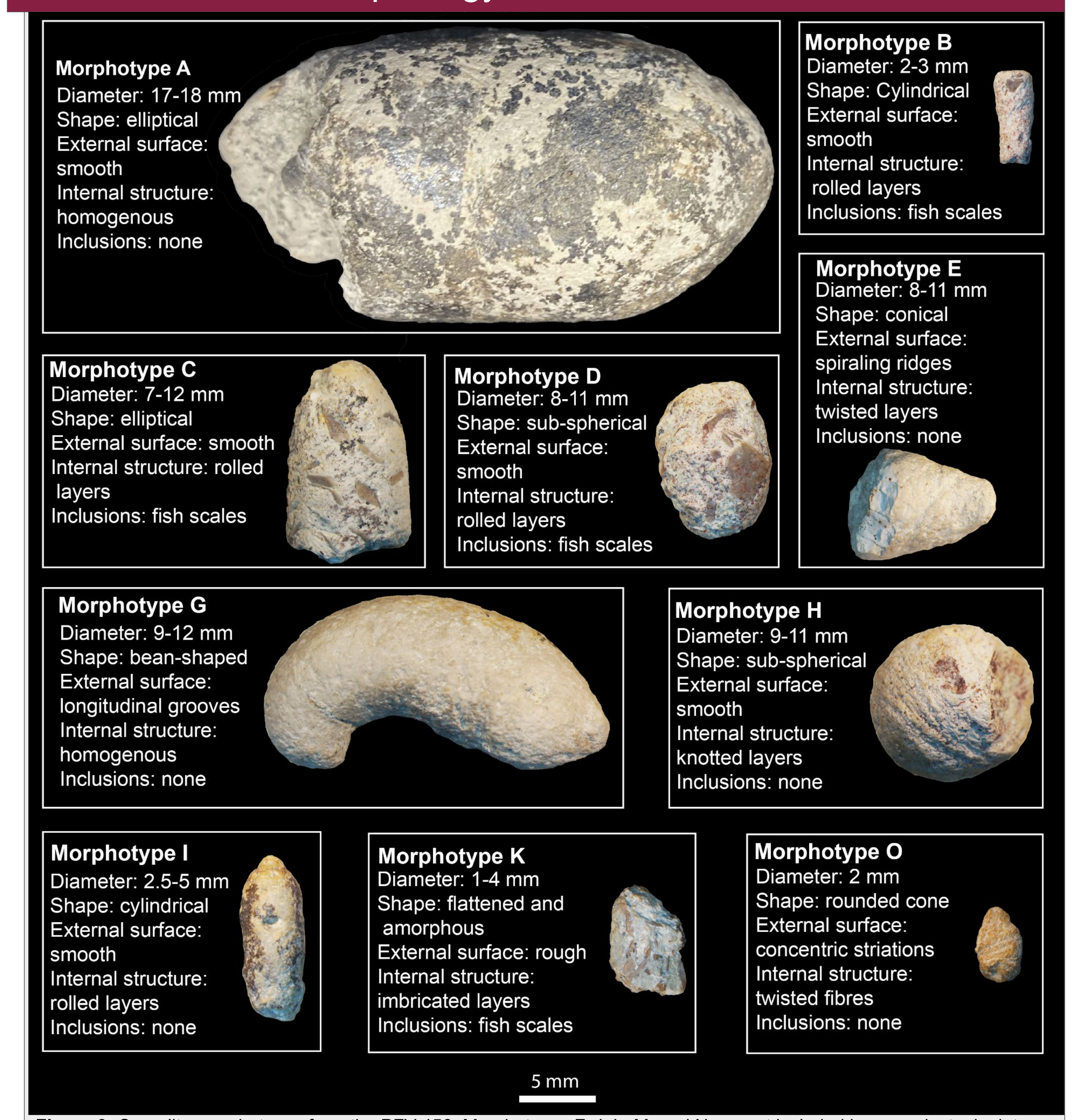

Figure 3. Coprolite morphotypes from the PFV 456. Morphotypes F, J, L, M, and N are not included because isotopic data were not recoverable from those specimens. The morphotypes are categorized by size, shape, layering, presence of inclusions, and surficial texture. These coprolites represent a large variety of producers (likely ranging in mass from .01 to 100 kg) and associated diets. The presence of ganoine scale inclusions from actinopterygian fish suggests these fish were a part of the diet of several of the coprolite producers. While not visible in these profiles,the internal colors of the coprolites ranges from beiges, to reds, to browns, to yellows.

Acknowledgements

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Results - Isotopic Values

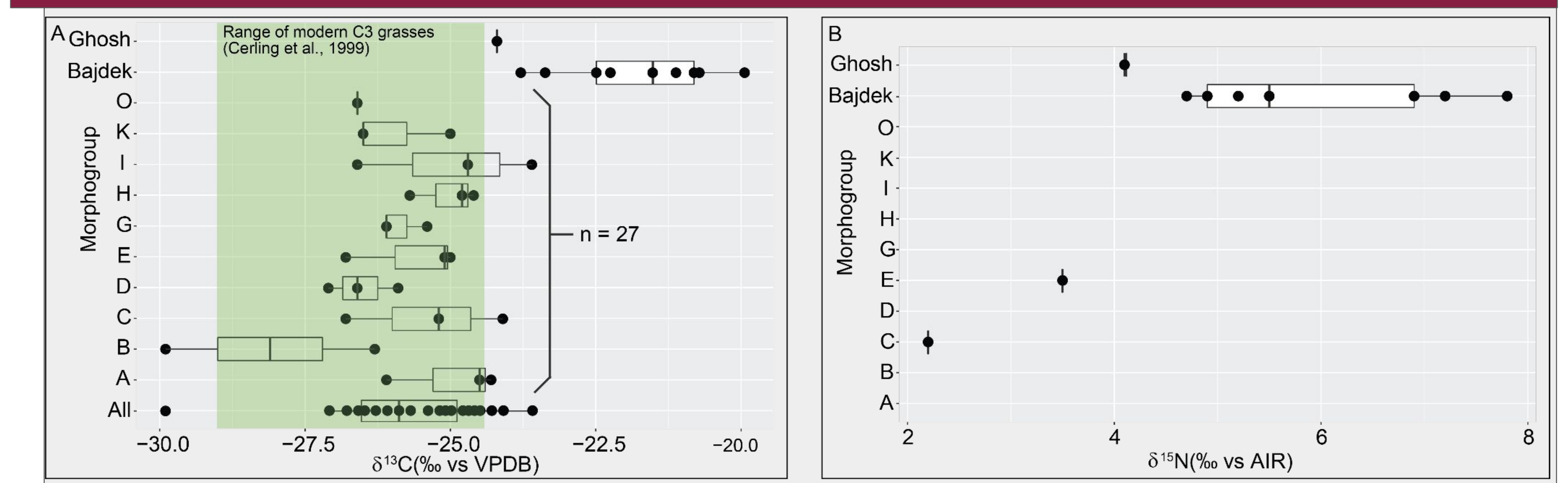

Figure 4. $\delta^{13}C$ and $\delta^{15}N$ of PFV 456 coprolites. A. $\delta^{13}C$ of the PFV 456 coprolites and from a Cretaceous dinosaur[6] and Triassic dicynodonts[7]. The PFV 456 coprolites are less enriched in ^{13}C than the other Triassic coprolites, but the observed values are consistent with diets based on C3 plants - the predominant terrestrial vegetation during the Triassic, before the proliferation of C4 plants. Different morphotypes do not form statistically distinct groups of isotope values (only D and H were significantly different from each other). B. $\delta^{15}N$ of the PFV 456 coprolites and from a Cretaceous dinosaur[6] and Triassic dicynodonts[7]. The two observed PFV 456 coprolite nitrogen values are less enriched in ^{15}N than the other reported coprolites. This may be due to the coprolite producers being trophically lower, and thus less enriched in heavier nitrogen than the other Triassic coprolites. Our $\delta^{13}C$ are outside of the range of enriched values in the excrement of extant terrestrial apex carnivores[7], suggesting that our dataset may not include the coprolites of terrestrial apex carnivores known to be present in the PFV 456 paleocommunity such as rausuchians, dinosauromorphs, and azendohsaurids. This absence may be a result of the fully terrestrial lifestyle of these taxa; their excrement may be less likely to be preserved in a lacustrine depositional environment.

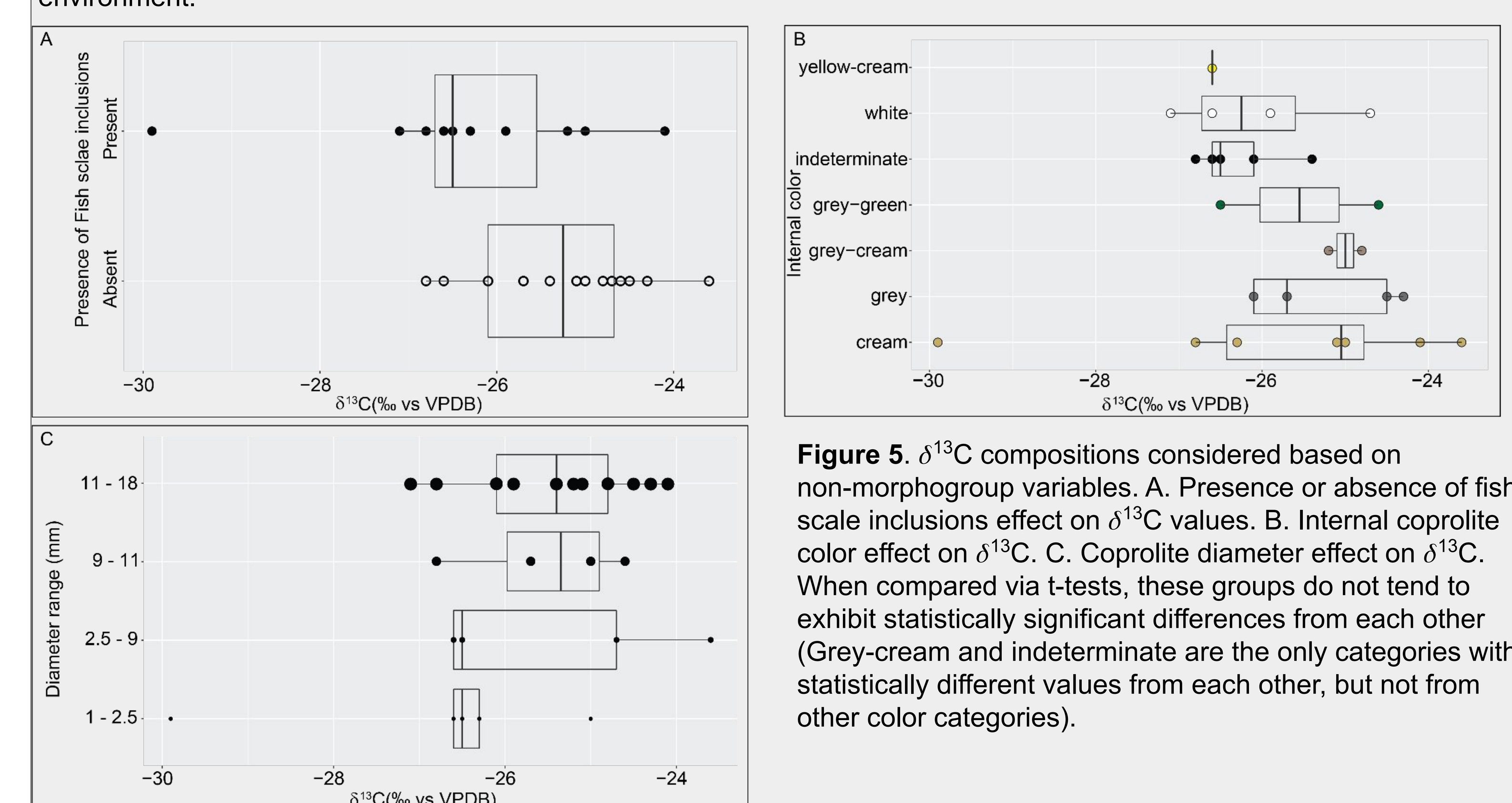

Figure 5. $\delta^{13}C$ compositions considered based on non-morphogroup variables. A. Presence or absence of fish scale inclusions effect on $\delta^{13}C$ values. B. Internal coprolite color effect on $\delta^{13}C$. C. Coprolite diameter effect on $\delta^{13}C$. When compared via t-tests, these groups do not tend to exhibit statistically significant differences from each other (Grey-cream and indeterminate are the only categories with statistically different values from each other, but not from other color categories).

Takeaway Points

For the limited sample size (52 coprolite samples, 13 morphotypes) we found that,

- Coprolite morphotypes do not correlate to specific $\delta^{13}C$ and $\delta^{15}N$ signatures.
- Larger coprolites do not have significantly different $\delta^{13}C$ values from smaller ones.
- Gross morphology and carbon and nitrogen isotope compositions alone may be insufficient to accurately reconstruct ecological dynamics from a coprolite assemblage.
- $\delta^{13}C$ values are consistent with a C3 plant base of the food chain, which was expected, though fish were clearly part of the diets represented by fish scale inclusions in the coprolites.
- The lack of isotopic differentiation among morphotypes suggests either the specific coprolite makers had dietary variability within species, or there is large convergence in coprolite morphology between coprolite producers of differing diets.

Future Work

- Refine techniques to measure the $\delta^{13}C$ and $\delta^{15}N$ signatures of small coprolite specimens that weigh less than 2 g.
- Examine the $\delta^{13}C$ and $\delta^{15}N$ signatures of the matrix encasing the coprolites.
- Collect and examine more coprolite specimens from PVF 456.
- Conduct additional analyses, including: 1) microscope and SEM observation of polished coprolite thin sections to search for microbial structures, micro-inclusions, and diagenetic overprinting, 2) microscope and SEM observation of acid-digested coprolite residues to search for plant cuticle fragments, palynomorphs, and insect exoskeleton fragments, and 3) EDS observation of polished coprolite thin sections to determine elemental composition.

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