

## Micro-XRF Mapping Elucidates the Taphonomy of Two Early Cretaceous Paravian Fossils from Western Liaoning, China

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**ABSTRACT:** Paraves is the taxonomic group that includes Dromaeosauridae, Troodontidae and Avialae, and thus records the origin of birds, the evolution of pennaceous feathers, and their exaptation for flight. Non-destructive micro-X-ray fluorescence (micro-XRF) imaging technology was utilized to analyze two paravian fossil specimens, one referable to the Microraptorinae (Dromaeosauridae) and the other to the Yanornithidae (Avialae). Both fossils are from the Lower Cretaceous Aptian Jiufotang Formation, a Lagerstätte that records the youngest stage of the famous Jehol Biota in western Liaoning, China. The analyses show that the bones preserve high amounts of Calcium (Ca), Phosphorus (P), and Sulfur (S), with significant amounts of heavy elements such as Strontium (Sr) and Yttrium (Y) which may be related to biological apatite. The preserved feathers show high amounts of Cuprum (Cu), Nickel (Ni) and Titanium (Ti). The claw sheaths exhibit high levels of P and Ca, which suggests they were preserved through phosphatization. Notably, large amounts of Ferrum (Fe) occur at the intraskeletal joints in both specimens and in the trunk of the microraptorine. Further analyses indicate that the enrichment of Fe may be related to the presence of pyrite. The distribution of Fe indicates precipitation of this element was a postburial taphonomic process. This micro-XRF data reveals the distribution of elements in different tissue types in these two paravian fossils and provides information for reconstructing the taphonomic processes responsible for exceptional preservation in paravian fossils in the Jiufotang Formation.

## INTRODUCTION

The Jehol Biota has produced numerous well-preserved feathered dinosaur fossils including birds, many which preserve soft tissues.<sup>1-6</sup>

These fossil materials have made important contributions to our understanding of avialan origins, dinosaurian flight, and the early evolution of feathers.<sup>7-13</sup> The majority of feathered dinosaurs in the Jehol Biota belong to the group of Paraves, which includes Neornithes (crown avialans), the most diverse clade of living

tetrapods. Paraves consists of the Dromaeosauridae, Troodontidae and Avialae (the clade that includes fossil and living birds).<sup>14,15</sup> Most studies on fossil representatives of these groups from the Jehol deposits have focused on the superficial morphology of preserved skeletons and feathers, and their possible functions at the morphological level.<sup>16-21</sup> However, with the use of new imaging and analytical methods such as X-ray spectroscopy, X-ray fluorescence (XRF) analyses, secondary-ion mass spectroscopy, and Raman spectroscopy, interdisciplinary studies utilizing chemistry to investigate taphonomy are becoming increasingly common.<sup>22-34</sup> XRF is a method that uses X-rays to excite elements in a sample, providing compositional data that can be qualitatively and quantitatively analyzed to better understand taphonomic pathways in fossils. XRF has been applied to a wide range of fossil groups including vertebrates, invertebrates, and plants. XRF studies on paravians include *Jianianhualong tengi*, *Archaeopteryx lithographica*, *Confuciusornis sanctus*, *colliiformes* bird and others.<sup>30-41</sup> This method not only reveals elemental distributions within vertebrate fossils but also improves the understanding of taphonomic pathways responsible for the preservation of soft tissues, in order to better understand the evolutionary and taphonomic process.<sup>32,34</sup> Compared to Synchrotron-XRF, which is limited by sample size (large samples cannot be analyzed by most set ups) and not portable, micro-X-ray fluorescence analysis (micro-XRF) scanning has the benefit of being a non-destructive method that allows large-size (decimeter-scale) samples to be analyzed under air or vacuum conditions.<sup>32,34</sup> Recent application of this technology to the study of extinct paravians have provided insights into the fossilization process of bones, feathers, and other soft tissues.<sup>23,26-34</sup>

Soft tissues such as visceral organs and integument (*e.g.*, feathers and scales, *etc.*) preserved in exceptional specimens of feathered non-avian dinosaurs, fossil birds, and primitive mammals have provided irreplaceable information for understanding the biological and ecological evolution of these animals.<sup>23,42-47</sup> Taphonomic studies link the preservation of these soft tissues to the deposition of finely layered shale or mudstone interbedded with ash layers resulting from volcanic eruptions.<sup>48-50</sup>

However, comparative analysis of different types of fossils, and the different types of fossilized tissues, from the Jehol Lagerstätten are lacking. Collecting complete chemical data through XRF maps can provide new data concerning biology and taphonomy critical for better understanding the taphonomic processes at work in the Jehol Biota.

In this study, two paravian fossils from the Lower Cretaceous Jiufotang Formation in western Liaoning were scanned using non-destructive micro-X-ray fluorescence (micro-XRF) imaging in order to explore the elemental characteristics of different structures in the same fossil individual and between different taxonomic groups (*i.e.*, dromaeosaurids and avialans) with the same structure (*i.e.*, feathers), and to investigate the taphonomy history of these specimens through the recovered distribution of related elements.<sup>26,32-33</sup> This work provides information for reconstructing the taphonomic processes responsible for exceptional preservation in paravian fossils in the Jiufotang Formation.

## MATERIALS AND METHODS

**Paravian fossils.** The studied specimens (130108-MHGU-F4281 and 130108-MHGU-F4282) are housed at the Museum of Hebei GEO University. Both specimens were discovered from deposits of the Lower Cretaceous Jiufotang Formation at Lamadong Town, Jianchang County, western Liaoning, China.

130108-MHGU-F4281 (hereinafter referred to as F4281) can be assigned to the Microraptorinae (Dromaeosauridae) based on the presence of the following characteristics: unserrated premaxillary teeth; elongated chevrons and prezygapophyses of the caudal vertebrae; supracoracoid fenestra of the coracoid; semilunate carpals preserved in both wrists; severely constricted proximal end of metatarsal III diaphysis; large and recurved raptorial ungual of pedal digit II; sum of the lengths of metacarpal I and digit I phalanx-1 less than metacarpal II.<sup>51,52</sup>

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**Fig. 1** Optical images of 130108-MHGU-F4281 (a) and 130108-MHGU-F4282 (b). The scale bar is 5 cm.

**Fig. 2** Overall elemental distribution of microraptorine 130108-MHGU-F4281 revealed via micro-XRF imaging. ((a) Light photo of 130108-MHGU-F4281 (black box shows the actual scanning area of the specimen); (b)–(l) micro-XRF false color elemental distribution maps. Each map corresponds to the following elements: (b) Ca; (c) P; (d) Sr; (e) S; (f) Fe; (g) K; (h) Ti; (i) Cu; (j) Ni; (k) Mn and (l) Si. The scale bar is 10 cm.)

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130108-MHGU-F4282 (hereinafter referred to as F4282) is referable to the Yanornithidae (Ornithuromorpha) by possessing the following characteristics: toothed upper and lower jaws with each dentary containing approximately 20 teeth; subparallel dorsal and ventral margins of the dentary; rostrum 55–60% of total skull length; elongated cervical vertebrae; completely fused synsacrum consisting of nine sacrals; completely fused pygostyle less than one-third of the length of the tarsometatarsus; proximal pedal phalanges longer than the distal ones.<sup>53,54</sup>

Microraptorine F4281 is preserved in a tuffaceous shale slab, with the bones not entirely flattened. Yanornithid F4282 is preserved in a tuff slab, with the sternum showing 3D preservation. These slabs were broken into several pieces while being collected and were glued together when the fossils were prepared. F4281 and F4282 both preserve the nearly complete and articulated skeleton, including feathers (Fig. 1). The skeleton of F4281 is light brown. Two layers of the matrix can be observed on the slab of F4281: an overlying white layer (WL) and an underlying black layer (BL) possibly rich in organic matter that preserves the fossil. The areas surrounding the skeleton, possibly including soft tissue remains (SR), form a hollow of more brightly colored matrix

around the fossil due to preparation (Fig. 1a). The skeleton and feathers of F4282 are black and preserved in dark-gray tuff. During the fossil preparation, some of the feathers on the body trunk were removed in order to expose the bones (Fig. 1b).

**Micro-XRF mapping.** The specimens were analyzed using the Bruker M6 Jetstream mobile X-ray fluorescence (XRF) scanner (Bruker Nano GmbH, Berlin, Germany) at the Institute of Earth Sciences, China University of Geosciences (Beijing, China). The Bruker M6 Jetstream consists of a mobile measuring head used a Rhodium (Rh) target X-ray source (50 kV, 0.6 mA) and polycapillary optics, which could generate a spot size smaller than 50  $\mu\text{m}$  for Mo-K $\alpha$ . The X-ray beam was perpendicular to the sample surface. The silicon drift detector (SDD) had an active area of 50 mm<sup>2</sup> with an energy spectrum resolution of 145 eV for Mn-K $\alpha$  at 275 kcps. The scanner is capable of scanning the surface of a sample that is mounted on a motorized XY stage. The specimens were positioned horizontally on a table and aligned accurately before the scanner proceeded to scan the entire specimen.

Full-area XRF elemental maps were acquired for F4281 (Fig. 2). The acquisition conditions were 2190  $\times$  1140 pixel for a total of

**Fig. 3** Overall elemental distribution of yanornithid 130108-MHGU-F4282 revealed via micro-XRF imaging. ((a) Light photo of 130108-MHGU-F4282 (white box shows the actual scanning area of the specimen); (b)–(l) micro-XRF false color elemental distribution maps. Each map corresponds to the following elements: (b) Ca; (c) P; (d) Sr; (e) S; (f) Fe; (g) K; (h) Ti; (i) Cu; (j) Ni; (k) Mn and (l) Si. The scale bar is 6 cm.)

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2.49 million pixel/map, and the acquisition time per pixel was 15 ms with a pixel size of 300  $\mu\text{m}$ . The entire F4281 scan took 12 hours 57 min. Full-area XRF elemental maps were also acquired for F4282 (Fig. 3). The acquisition conditions were  $1421 \times 901$  pixel for a total of 1.28 million pixel/map, and the acquisition time per pixel was 10 ms with a pixel size of 200  $\mu\text{m}$ . The F4282 scan took a total of 5 h 40 min. After data collection, chemical elements were identified based on the peaks in the XRF spectra corresponding to the characteristic X-ray emission lines of each element. Elements were identified using the peak values through the spectrogram to eliminate interfering elements. Using the characterization software provided by Bruker Micro Analytics to analyze elemental data from the specimens and generate distribution (Figs. 2 and 3) and heat maps (Figs. S1 and S2). The color of the elemental distribution map has no practical significance and is only used to display the presence of elements. Conversely, the elemental heat map uses a series of colors to display the enrichment and distribution of individual elements more clearly.

## RESULTS

**Overall elemental distribution revealed by XRF imaging.** After measuring the peak values in the spectrogram, interference elements such as Vanadium (V), Cobalt (Co), Argon (Ar) and Rh were excluded. The XRF measurements provide an overall distribution of at least 16 recognizable elements in both F4281 (Fig. 4a) and F4282 (Fig. 4b).

Sorting of the elements by their main phase show that the light rock where the fossil is embedded is rich in Silicon (Si), Kalium (K), Titanium (Ti), Aluminium (Al), and Ferrum (Fe) (Figs. 2 and 3; Figs. S1-S3). A few small regions are enriched in Manganese (Mn). This is consistent with the previous interpretations of the lithology. The elemental distribution and heat maps of the two specimens reveal the presence of greater amounts of Strontium (Sr), Calcium (Ca), Phosphorus (P), and Sulfur (S) in the skeleton relative to the matrix. Based on elemental distribution maps and

F4281, reflecting the poor preservation of feathers in this specimen. Ti is mainly distributed close to the body in the feathers on the trunk and the tail (Fig. 3; Figs. S2 and S4).

## COMPARISONS AND DISCUSSION

### Information revealed by micro-XRF chemical imaging.

Applying large-area micro-XRF on the two paravian fossils characterizes the surface elemental distributions of the fossils and their surrounding sedimentary matrices. Ca and Sr are significantly enriched in the bones, producing clear images of the skeleton that can assist in observing morphology. Although the observed features are not significantly different from those under natural light, these elemental maps assist in observing details such as bone sutures and outlines. Among them, Ca is not only present in the skeleton but also in the soft tissue remains. In contrast, Sr seems heavily and uniformly present in the skeleton, and only occurs at low levels in other regions (Figs. 2 and 3; Figs. S1 and S2). This may be related to significant Sr participation in bioapatite mineralization throughout the life of an animal.<sup>55</sup> Comparisons between the distribution of Ca and Sr may also provide insight into the taphonomy and fossilization process.<sup>32</sup> Several other heavy elements such as Sr, Y, and Zr also appear to be enriched in the fossil over the sediment. This enrichment may be related to the presence of original bioapatite (the composition of bones and teeth, mainly composed of Ca and P), where elements such as Sr and Y are strongly enriched in apatite minerals, and these elemental distributions are consistent with the skeletal morphology. The distribution of Sr and Y in F4282 is consistent with the skeleton and consistent with the tendency for these elements to replace Ca in calcium phosphates like bioapatite.<sup>37, 56, 57</sup>

Cu, Ti, and Ni are associated with preserved feathers of F4281 and F4282. The distribution of Cu and Ni reveals the orientation of the body feathers and secondaries. Ti displays the orientation of the tail feathers, but it is not found in the secondaries. Ca and P are also distributed in feathers of F4282, but in lower concentrations compared to the bone. The content of these two elements varies in different feather regions, both showing a stronger signal in the remiges. The enrichment of Ca and Cu in feathers is associated with melanin pigmentation.<sup>22, 26, 30, 58</sup>

**Comparisons with chemistry of other fossil materials.** Previous studies have concluded that the elemental signature of vertebrate fossils was tissue-specific and controlled by many biological and environmental factors, meaning that the similarities and differences in the elemental distribution in the feathers and bones can be compared with findings for other vertebrate fossils.<sup>27, 32, 34</sup> Due to S, P, and Ca being common elements in living organisms, they are also the most commonly examined elements in chemical studies based on fossil materials.<sup>32, 59</sup> Consistently, the micro-XRF

**Fig. 4** Spectrogram of the micro-XRF scanning area of 130108-MHGU-F4281 (a) and 130108-MHGU-F4282 (b). (The actual scanning area of these specimens is shown in the boxes of Fig. 2a and Fig. 3a; the main scale of the y-axis increases exponentially at a rate of 10.)

semi-quantitative heat maps, it is found that the enrichment of Ca and Sr are more obvious than other elements (Figs. 2 and 3; Figs. S1 and S2). The enrichment levels between Sr, Ca, and S in F4281 are similar, with P having a slightly lower enrichment than Ca, but still concentrated in the skeleton. The enrichment level of P in F4282 is similar to that of Ca, and the distribution of these two elements is slightly lower in the feathers than in the skeleton (Fig. 3; Fig. S2). The distribution of S in the two specimens is different: S is only distributed in the skeleton of F4281 (Fig. 2; Fig. S1), but S is more obvious in the feathers than in the skeleton of F4282 (Fig. 3; Fig. S2). Elemental heat maps of F4282 show that Yttrium (Y) and Sr are similarly present in the skeleton, and the distribution of Zirconium (Zr) in bones is similar to that of P in bones (Fig. S2). In contrast, concentrations of Y and Zr in the skeleton of F4281 are much lower (Fig. S1). Both specimens reveal significant enrichment of Nickel (Ni) in the bones relative to the matrix. (Fig. S1 and S2).

**Elements associated with the soft tissue and feathers.** Fe is mainly distributed in the body trunk in F4281 and at various intraskeletal joints in F4281 and F4282 (Figs. 2 and 3; Figs. S1, S2, and S6). The claw sheaths of the manual and pedal distal phalanges preserved in F4281 have elemental configurations similar to those of the bones and have higher levels of Ca, P, Sr, Y, and S compared to the matrix (Fig. 5; Fig. S5). The feathers of F4282 are well-preserved, and show clear signals for S, Cuprum (Cu), Ni, and Ti (Fig. 3; Fig. S4). Ca and P are also weakly distributed in the feathers. These signals are much weaker in

**Fig. 5** Light photo and micro-XRF detail elemental maps of the claw sheaths in microraptorine 130108-MHGU-F4281. ((a) Light photo of the F4281 manual claw sheath and its element distribution maps (b)-(h) and its elemental heat maps (i)-(l). (b) Ca; (c) Sr; (d) S; (e) P; (f) Y; (g) Th; (h) Fe, and heat maps: (i) Ca; (j) Sr; (k) S; (l) P. The scale bar is 2 cm.)

data for F4281 and F4282 show that S, P and Ca are associated with the skeleton and soft tissues, while the Ca and S levels are slightly higher in the skeleton, which is slightly different from the previous studies where only Ca or Ca and P content is higher than other elements.<sup>22,32,33,58</sup> Relatively, the S content also differs between F4281 and F4282. Based on the elemental heat maps, it is found that S in F4281 is more enriched than in F4282, but the contrast of the element distribution maps of both is poor. Sr is strongly present in the bones preserved in many fossil bone materials, which is related to its involvement in the formation of bone bioapatite.<sup>32,55,60,61</sup> F4282 and *Jianianhualong* show a similar and consistent distribution of Sr and Y; together with the signal for Ca and P, this indicates preservation of the skeleton's original calcium phosphate ( $\text{CaPO}_4$ ), with the additional presence of Sr ( $\text{SrPO}_4$ ) and Y phosphates ( $\text{YPO}_4$ ).<sup>32,59,61</sup> This suggests that during permineralization, calcium ions in bioapatite were replaced by Sr and Y ions dissolved in groundwater.<sup>62</sup> However, further research is needed to determine the specific reasons for this phenomenon.

Ca, Cu, and Ni are associated with melanin pigmentation in modern and fossil feathers.<sup>22,29,30,39,58</sup> As previously documented in other paravian fossils such as *Jianianhualong*, *Anchiornis*, *Archaeopteryx*, and *Gansus*, as well as other extinct and living birds, Cu levels usually vary between different feather samples within an individual and are generally higher in the feathers than in bone.<sup>26,28,32,39,58</sup> Cu is distributed fairly evenly throughout the preserved feathers of F4282 and is relatively enriched in the tail feathers, but does not show any significant between feather tracts. In contrast, the preserved feathers of both specimens show high Ti levels, but this element is only enriched in the body feathers and noticeably absent in the remiges and rectrices. Ti has also been documented in the fossil feathers preserved in specimens of *Gansus* and *Jianianhualong*, but has not been found in the feathers of living birds.<sup>32,58</sup> This suggests the presence of Ti in fossil feathers is due to fossilization processes after death. In addition, unlike *Archaeopteryx*, *Confuciusornis*, and fossil coliiiformes, but

similar to *Jianianhualong*, the skeletons and feathers of F4281 and F4282 do not show enrichment of Zinc (Zn) (Fig. S3).<sup>26,32,33,39</sup> Unlike other documented in paravian fossils, there is no relative enrichment of Mn in the skeletons and feathers of F4281 and F4282.<sup>26,32,33,39,58</sup>

**Structure and chemistry of the claw sheath.** The claw sheaths of the manual and pedal ungual phalanges are preserved in F4281 (Fig. 5; Fig. S5). Micro-XRF reveals that the claw sheaths have a similar elemental composition to the bones, and have higher levels of Ca, P, Sr, Y, and S compared to the matrix. However, the level of these elements in the claw sheaths is slightly lower than that in the skeleton with the exception of Ca. High levels of P and Ca in claw sheaths were also found in chemical analyses of *Archaeopteryx*, *Shuvuuia*, *Citipati*, *Confuciusornis*, and *Jianianhualong* indicating that the claw sheaths of F4281, like these fossils, was preserved in the form of calcium phosphate.<sup>32,33,63-65</sup> The claw sheath could be preserved through keratin calcification. Although claw sheaths and bones are composed of different tissues in vivo, they have similar elemental compositions in fossils, indicating that they may have been preserved through similar fossilization processes. This speculation is consistent with previous research.<sup>65</sup> In contrast to *Jianianhualong*, there is no enrichment of Thorium (Th) in the claw sheath of F4281.<sup>32</sup>

The claw sheath of F4281 is preserved through phosphatization, similar to interpretations based on analyses of specimens of *Jianianhualong* and *Confuciusornis*.<sup>32,65</sup> This previous study found that Ca and P in the claw sheaths of living birds were relatively low, being formed primarily from alpha- and betakeratins.<sup>65</sup> This suggests that the presence of Ca and P in the fossils may be due to post-mortem fossilization processes, and that these structures were preserved through phosphatization processes; it is also possible that the degradation and loss of organic matter may lead to a relative increase in the concentration of these elements, which requires further research to verify these



hypotheses in the future.

**Fe variation in different body structures.** Interestingly, enrichment of Fe is found in F4281 and F4282. Fe is mainly concentrated in the body trunk of F4281 and at various intraskeletal joints in F4281 (Fig. 2; Fig. S6) and F4282 (Fig. 3; Fig. S6). This phenomenon has not been found in previously studied paravian fossils analyzed through micro-XRF.<sup>32</sup> The matrix in F4281 also contains relatively abundant Fe, which is distributed in a spotted, aggregation pattern throughout the slab (Fig. 2). High Fe content distribution is also observed in broken fish bones preserved on the slab (Fig. S6). Fe is evenly distributed in the matrix of F4282.

Observation under the microscope reveals that the Fe enriched regions in the body trunk of F4281 are associated with yellow-brown precipitates (Fig. S6); these yellow-brown precipitates are also observed in the matrix, and accumulated around the skeleton. Fe is not present in the skeleton, but rather accumulated outside and around it. We hypothesize this precipitate formed during the fossilization process. Although the association of Fe with the skeleton superficially suggests it could at least partially represent permineralized soft tissues, the overall distribution of Fe throughout the slab does not support this interpretation. The intraskeletal joints in F4281 with enriched Fe appear the same reddish-brown color as the broken fish bones, which is darker than the bone color and suggests it could represent a hematite mineral (Fig. S6). Fe enrichment in F4282 is far less widespread and limited to intraskeletal joints. In ordinary optical photographs, these regions appear the same yellow-brown color as observed in the trunk of F4281, but different from the reddish-brown color at the intraskeletal joints of F4281 (Fig. S6). In addition, Fe in the matrix of F4282 is also distributed in a sheet-like but uneven, patchy pattern. The results indicate that the Fe enrichment at intraskeletal joints in F4282 is located outside the skeleton, possibly due to mineral precipitation during fossilization. In addition, the distribution of Fe could be related to the growth and weathering of pyrite around the skeleton of F4281. This was a symptom of secondary pyrite mineralization in the bones after diagenesis and/or collection. The distributions of S and Fe are not consistent, which may be due to the subsequent transformation of pyrite into iron oxide during fossil formation.<sup>66,67</sup> The enrichment of Fe in two specimens could also be due to the reducing environment during the decomposition of organic matter, leading to the presence of pyrite, with the pyrite decomposing into hematite during diagenesis and weathering. The distribution of Fe in the two specimens indicates that Fe is not closely related to the skeletons themselves, and the enrichment of Fe may be related to pyrite formation. Further research is needed in conjunction with new mineralogy, taphonomy and sedimentology data to determine whether Fe-containing minerals are pyrite and their specific formation process.

## CONCLUSION

Micro-XRF obtained elemental distribution and heat maps from a microraptorine and a yanornithid from the Jehol Biota in western Liaoning, reveal the relative distribution of elements in different regions of the slab, and provide insights into the taphonomic history of these two specimens. Analyses of these elemental distribution patterns in the bones, feathers, and claw sheaths, as well as the surrounding matrix, reveal that the bones primarily consist of Ca, P, S, and Sr, with additional bone related elements such as Y. The enrichment of Ca, P, Sr, and Y may be related to biological apatite. The feather related elements are Cu, Ni, and Ti. The claw sheaths exhibit high levels of P and Ca, indicating preservation through phosphatization. In addition, a large amount of Fe is found in the two specimens, and the comparisons reveal that Fe is not related to the skeleton, and the enrichment of Fe could be related to pyrite. This is the first time elemental distribution patterns have been compared between a non-avian dinosaur and sympatric bird, in order to explore the distribution of elements in different tissues of the same individual and in the same tissue between different individuals and taxa. This greatly increases our knowledge concerning the chemical characteristics of paravian fossils and provides new information for reconstructing the taphonomic processes of paravian fossils in the Jiufotang Formation.

## ASSOCIATED CONTENT

Supporting information (Figs. S1-S6) is available at [www.at-spectrosc.com/as/home](http://www.at-spectrosc.com/as/home).

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## Notes

The authors declare no competing financial interest.

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