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**Neutron-based computed microtomography: *Pliobates cataloniae* and
Barberapithecus huerzeleri as a test-case study**

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27 KEYWORDS

28 X-rays, neutron radiation, neutron imaging, fossil catarrhines

29

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37

38 **Abstract**

39 **Objectives:** High-resolution imaging of fossils with X-ray computed microtomography
40 (μ CT) has become a very powerful tool in paleontological research. However, fossilized
41 bone, embedding matrix, and dental tissues do not always provide a distinct structural
42 signal with X-rays. Here we report on neutron radiation as an alternative to standard X-
43 rays for the μ CT of ‘problematic’ fossils.

44 **Materials and Methods:** We compare neutron with X-ray μ CT scans of fossils from two
45 Miocene catarrhines from the Vallès-Penedès Basin: the cranium (IPS58443.1, holotype)
46 of the putative stem hominoid *Pliobates cataloniae*, to discriminate between bone and
47 matrix; and two lower molars (IPS1724n,o, holotype) of *Barberapithecus huerzeleri*, to
48 discriminate among dental tissues.

49 **Results:** X-ray μ CT scans of these specimens fail to retrieve any contrast between
50 matrix/bone and enamel/dentine, whereas neutron μ CT scans deliver high-contrast
51 images, enabling a proper evaluation of the specimens’ internal anatomy.

52 **Discussion:** Low bone/matrix intensity difference with X-ray μ CT scans in IPS58443.1 is
53 due to the extreme similarity in chemical composition between the matrix and the fossilized
54 tissues, and the presence of high-density elements. In IPS1724, it is attributable to the
55 convergence of enamel and dentine compositions during fossilization. On the contrary,
56 neutron radiation returns very different contrasts for different isotopes of the same element
57 and easily penetrates most metals. Neutron-based μ CT scans therefore enable a correct
58 definition of the bone/sediment and enamel/dentine interfaces, and hence a better
59 segmentation of the images stack. We conclude that neutron radiation represents a
60 successful alternative for high-resolution μ CT of small-sized fossils that are problematic
61 with standard X-rays.

62

63 1 | INTRODUCTION

64

65 The use of computed microtomography (μ CT) in paleontological research has dramatically
66 increased during the last decade, in parallel to concomitant enhancements in image
67 detectors and computing power. The success of computed tomography in paleontology is
68 due to the fact that it enables the non-destructive study of internal anatomy, the virtual
69 extraction of sediment-embedded fossils, the virtual reconstruction of damaged specimens
70 (including the mirroring of antimeres), and even the retrodeformation of plastically
71 deformed fossils (Macchiarelli et al., 2004; Olejniczak & Grine, 2006; Olejniczak,
72 Tafforeau, Temming, Smith, & Hublin, 2007; Abel, Rettondini Laurini, & Richter, 2012;
73 Benazzi, Kullmer, Schulz, Gruppioni, & Weber, 2013; Faulwetter, Vasileiadou, Kouratoras,
74 Dailianis, & Arvanitidis, 2013; Macchiarelli, Bayle, Bondioli, Mazurier, & Zanolli, 2013;
75 Benazzi, Gruppioni, Strait, & Hublin, 2014; Cunningham, Rahman, Lautenschlager,
76 Rayfield, & Donoghue, 2014; Lautenschlager, 2016). Modern X-ray μ CT (especially based
77 on synchrotron radiation) can reach a very high spatial resolution (up to less than 1 μ m;

78 Gren et al., 2016), and the size of the specimens that can be scanned with a single
79 acquisition has recently increased considerably (currently in the order of some decimeters;
80 e.g., Tuniz et al., 2013).

81 Depending on the taphonomic processes occurring during fossilization, not all
82 vertebrate fossil remains are amenable to internal anatomy analysis based on standard X-
83 ray μ CT. The latter may fail to retrieve an adequate contrast between the fossil bone and
84 the matrix or between different dental tissues because of two main problems: (1) the
85 embedding matrix and the fossilized tissues have a very similar composition, due to
86 element exchange resulting from permineralization (Zanolli, Grine, Kullmer, Schrenk, &
87 Macchiarelli, 2015; Beaudet et al., 2016); (2) a considerable amount of high-density
88 elements is present in the embedding sediment or in the fossil itself (Spoor, Zonneveld &
89 Macho, 1993; Abel et al., 2012). Either of these problems hinders and might even entirely
90 preclude the segmentation of the fossil, thus preventing the extraction of essential
91 paleobiological evidence (e.g., Schwarz, Vontobel, Lehmann, Meyer, & Bongartz, 2005;
92 Smith et al., 2009; Zanolli et al., 2017a). For these reasons, an alternative to X-ray μ CT is
93 needed. Neutron radiography and tomography, respectively developed in the 1950s and
94 1970s (Kardjilov et al., 2003; Schwarz et al., 2005; Winkler, 2006), constitute a potential
95 alternative. However, so far neutron-based μ CT (n- μ CT) has only sporadically been used
96 in paleontological and paleoanthropological research (Schwarz et al., 2005; Sutton, 2008;
97 Zanolli et al., 2013, 2017a; Beaudet et al., 2016; Laaß & Kaestner, 2017; Schillinger,
98 2017)., Although the reliability of n- μ CT for investigating the internal anatomy of fossils has
99 previously been demonstrated by previous researchers (Schwarz et al., 2005; Beaudet et
100 al., 2016; Zanolli et al., 2017a), here we test further the applicability of this method to
101 specimens that cannot be properly analyzed by means of X-ray μ CT. In particular, by
102 focusing on the fossil remains of two European Miocene catarrhines, we address two
103 common problems in CT-based paleoprimatological research: difficulties in discriminating

104 fossilized cranial bone from the surrounding or embedding matrix (as exemplified by the
105 putative stem hominoid *Pliobates*); and the inability to discriminate well between enamel
106 and dentine in fossil teeth (exemplified by the pliopithecoid *Barberapithecus*).

107

108 **2 | MATERIALS AND METHODS**

109

110 **2.1 | Studied sample**

111 The remains of two Miocene catarrhines from the Vallès-Penedès Basin (NE Iberian
112 Peninsula), housed at the Institut Català de Paleontologia Miquel Crusafont (Sabadell,
113 Spain; ICP), were investigated: (1) the partial cranium of *Pliobates cataloniae*
114 (IPS58443.1, holotype), a putative stem hominoid from the stratigraphic series of
115 Abocador de Can Mata (ACM) locality ACM/C8-A4 (Alba et al., 2015: Figs. 1, 4), with an
116 estimated age of 11.6 Ma (middle to late Miocene boundary; Alba, Casanovas-Vilar,
117 Garcés, & Robles, 2017); and (2) two lower molars (right M₂ and left M₃) of a single
118 individual of *Barberapithecus huerzeleri* (respectively IPS1724n,o, holotype), a
119 pliopithecoid from Castell de Barberà (Alba & Moyà-Solà, 2012: Figs. 4F–G, 5D, 10D–I,
120 12F, 13C), with an estimated age of 11.2–10.3 Ma (late Miocene; Casanovas-Vilar et al.,
121 2016). These specimens are part of the holotypes of their respective species, that of
122 *Pliobates* consisting of a partial skeleton, and that of *Barberapithecus* consisting of
123 associated upper and lower teeth of a single individual. The former is thus far the only
124 known individual of this species, which represents the only small-bodied ape currently
125 known from the Miocene of Europe (Alba et al., 2015), whereas the hypodigm of
126 *Barberapithecus* is restricted to two additional isolated teeth and a fragment of radius from
127 its type locality (Alba & Moyà-Solà, 2012; Moyà-Solà, Alba, & Almécija, 2013). The
128 cranium of *Pliobates* consists of two main parts that are very crushed but not plastically
129 deformed, enabling the virtual reconstruction of its external appearance based on X-ray

130 μ CT scanning. However, the poor discrimination between cranial bone and the embedding
131 matrix precludes a clear ascertainment of the morphology of inner cranial structures such
132 as the carotid canal, which in *Pliobates* apparently displays an orientation uniquely shared
133 with extant hylobatids (Alba et al., 2015). In turn, the teeth of *Barberapithecus* are well
134 preserved; however, unlike for other Vallès-Penedès pliopithecoids (Zanoli et al., 2017b),
135 standard X-ray μ Ct do not enable to adequately discriminate between enamel and dentine.
136 This precludes ascertaining the endostructural dental morphology—in particular, the
137 enamel-dentin junction (EDJ) morphology—or to compute 3D relative enamel thickness in
138 this taxon, with potential implications for further clarifying its taxonomic/phylogenetic
139 affinities as well as its paleodietary adaptations, respectively.

140

141 **2.2 | Neutron-based computed microtomography**

142 Radiographic contrast is generated using the attenuation and scattering of a beam that
143 passes through an object. The main difference between X-ray and neutron-based
144 tomography lies on the particles that interact with matter (photons produced by the kinetic
145 variation of the electrons in the former, and neutrons in the latter). X-rays and synchrotron
146 radiation interact with the electron cloud that surrounds the atoms, and thus are more
147 scattered or attenuated by elements possessing a large number of electrons. Being
148 uncharged, neutrons only interact with the nuclei via very short-range forces (Schwarz et
149 al., 2005). The probability of absorption depends on the number of nucleons, thus showing
150 major differences between neighboring elements or even isotopes of the same element
151 (Schillinger, 2017). The attenuation coefficient of X-rays rises monotonously with the
152 number of protons of the elements, while neutrons show a decreasing trend (Schillinger,
153 2017). This allows high penetration power for heavy mineral elements, which are
154 commonly present in paleontological specimens (Beaudet et al., 2016). On the other hand,
155 neutrons are strongly scattered by hydrogen and some other light elements, such that

hydrogen-rich materials (i.e., organic materials, glues and resins) are easily detected (Schwarz et al., 2005; Winkler, 2006; Schillinger, 2017).

Most samples become activated if irradiated with neutrons. The standard decay time for the radioactivity ranges between some days and few weeks, after which the specimens can be released (Schillinger, 2017; Schulz et al., 2017). In addition, specific elements (such as europium and cobalt) may achieve hazardous levels of radioactivity if activated by the neutron beam (Sutton, 2008). However, this issue can be easily avoided by running preliminary tests of short-time irradiation and consecutive gamma scan (Schillinger, 2017).

Even if both X-ray and neutron-based radiography produce a shadow image of the sample, the beams used for the analysis differ considerably. X-ray tubes generate a cone beam that magnifies the projection of the sample, while a neutron beam is approximately parallel and does not magnify (Schillinger, 2017). The quality of the image thus depends on the collimation ratio and on the distance between the detector and the sample, while the limit for the resolution of a parallel beam is constrained by the detector's resolution. Using thinned detector screens (5–20 μm), a resolution of 10–20 μm is achieved (Schillinger, 2017; Schulz et al., 2017).

172

2.3 | Scanning settings

The dimensions and scanning parameters for the studied samples have been reported in Table 1. They were scanned with n- μCT at the imaging facility ANTARES, which is located at the cold neutron beam port of the reactor of the Forschungs-Neutronenquelle Heinz Maier-Leibnitz (FRM II; Garching bei München, Germany). It allows different detector positions and two different chambers, according to the requirements of the sample size, beam size, neutron flux and spatial resolution (for the specifics of the facility, see Schulz et al., 2017). Specimens were placed in chamber two, on a XY-Phi-table with an additional high precision 5-axes HUBER table. Measurements were carried out using the parallel

182 neutron beam originated from the cold source of the FRM II reactor with an energy range
183 of 3–25 meV and a collimation ratio of 500. Three different scans were obtained for
184 IPS58443.1: a general one (877 projections for 2,400 slices) with a final isotropic voxel
185 size of 19.90 μm ; and two close-ups, for the temporal (1,105 projections for 2,518 slices)
186 and maxillary (876 projections for 2,486 slices) areas, with a voxel size of 14.22 μm . In
187 turn, due to the reduced size of the specimens ($< 1\text{ cm}$), IPS1724n,o were scanned with a
188 single cumulative acquisition (2,221 slices) that yielded a final isotropic voxel size of 17.98
189 μm . The histograms of the images stacks were computed with Fiji (Schindelin et al., 2012).

190 X-ray μCT were performed for the same specimens. IPS58443.1 was scanned at the
191 American Museum of Natural History (New York, USA) using a Phoenix v|tome|x s180
192 system, using 160 kV voltage, 1.4 mA current, 0.2 mm Cu filter, and magnification of 2.10,
193 obtaining 1,600 slices (virtual cross-sectional images) of 0.2 mm in thickness and a pixel
194 size of 95.23 μm . In turn, IPS1724n,o were scanned in a single acquisition at the TomoLab
195 of the Multidisciplinary Laboratory of the International Centre for Theoretical Physics
196 (Trieste; ICTP), with 13 kV voltage, 72 μA current, and a 1 mm Al filter, obtaining 1800
197 slices and a voxel size of 7.56 μm .

198

199 **3 | RESULTS**

200 The resulting images of X-rays and neutron radiography are very different. Standard
201 radiography provides sharper air-specimen boundaries and uniform dark background,
202 whereas neutron radiography shows more diffuse background noise, especially for
203 IPS58443.1, probably due to the presence of the acrylic resin (Paraloid® B72, 5% diluted
204 in acetone) and nitrocellulosic glue (Imedio® Banda Azul) used in the preparation process.
205 However, the contrast provided by X-ray μCT for IPS58443.1 is insufficient to perform an
206 accurate analysis of the internal anatomy.

207 The partial cranium of *Pliobates* is filled with a mudstone matrix that is firmly stuck to the
208 fossilized tissues and was only partially removed during the preparation process. Electron-
209 dense elements fill some of the fractures along the specimen or are found dispersed in the
210 sediment, resulting in bright spots on the images stack that led to the underexposure of the
211 radiographies. The histograms calculated for the whole images stack show a low-shifted
212 distribution of the intensity curve with two extremely steep peaks (Fig. 2E). The lowest one
213 belongs to the air background, while the other includes both sediment and fossilized bone.
214 Due to the spot-like accumulation of the denser minerals, their peak is diluted within the
215 histogram and becomes visible only when cropping the image to the exact region of the
216 bright spots. Even if contrast is very low, segmentation between outer sediment and
217 fossilized tissues is possible (Alba et al., 2015). However, contrast is insufficient to
218 properly discern the inner matrix from internal bone boundaries of cranial cavities (Fig.
219 1E), especially close to the bright spots. In contrast, n- μ CT produced more balanced
220 images (Fig. 1F). This is clearly visible in the distribution of the intensities in the
221 histograms (Fig. 2F), where the second peak (corresponding to the embedding-
222 sediment/fossilized tissues compound) is lower and more distributed along the intensity
223 axis. The fossilized tissues appear in lighter gray, as the permineralization process is likely
224 not complete (or affects different areas of the fossil in a differential way) and their mineral
225 composition still differs from the embedding matrix, which appears darker and richer in
226 heavy elements (Fig. 1F). This enables the distinction and manual segmentation of the
227 borders of the inner cavities, such as the bony labyrinth, the inner ear nerves, or the
228 carotid canal, inter alia.

229 The two molars of *Barberapithecus* (IPS1724n,o) appear as a uniform gray-to-white
230 mass in the X-ray scans, such that there is no discernible EDJ (Fig. 1A,C). Probably this is
231 the result of deep mineralization of the dentine during the fossilization process, which
232 caused it to converge in chemical composition with the enamel. The histograms calculated

for each tooth, on the whole slices stack (Fig. 2A,C), show that there is a shift of the intensity curve towards higher intensity values for the standard X-ray μ CT images stacks. The right end of the curve shows a peak that corresponds to the brighter areas of the dentine, in which elements with a high attenuation coefficient have penetrated. The steep black background peak occupies the lowest intensity range. These two peaks flank a lower one (ranging from ca. 155 to 240 of the grayscale) that corresponds to the enamel-dentine compound, visible as an indistinct gray to white mass (Fig. 1A,C). In contrast, n- μ CT images stacks have more balanced histograms, in which the dental tissues (i.e., dentine and enamel) are better differentiated (Fig. 2B,D). Apart from broader lower peak, due to a lighter background, the curves displays a different intensity for the enamel (darker) and the dentine (lighter). Inside the pulp cavity, the presence of lighter and hydrogen-rich minerals in the two molars (Fig. 1B,D) locally produces some noise (clearly visible in the histogram as an anomalous peak at the very end of the color map). However, it does not affect contrast in the EDJ, enabling the manual segmentation of enamel and dentine, and therefore a correct identification of the former (Fig. 3).

248

249 **4 | DISCUSSION**

Our results indicate that n- μ CT provides a higher anatomical resolution for two specimens, whose internal anatomy could not be adequately segmented by means of X-ray μ CT scans, due to several problems: an extreme similarity between the chemical composition of the matrix and that of the fossilized tissues (in the cranium of *Pliobates*); a high similarity between the chemical composition of different fossilized tissues (in the molars of *Barberapithecus*); and the presence of high-density elements (in the cranium of *Pliobates*). Standard X-ray and synchrotron radiation μ CT are preferable because of the greater sharpness of the images, the lack of activation, and the availability of the facilities in the case of the former. However, n- μ CT provides a better contrast of different isotopes of the

259 same element and more easily penetrates metals than X-ray μ CT. These advantages
260 provide a better definition of the bone/sediment boundary and between different dental
261 tissues, thereby enabling a better segmentation of the images stack in fossils that are
262 problematic with standard μ CT. The complementarity of the contrasts obtained by n- μ CT
263 and its enhanced penetration power are particularly indicated for thick and heavy-element
264 rich fossil material—two conditions that are commonly found in paleontological specimens.
265 We therefore conclude that neutron radiation represents an accessible and successful
266 alternative to X-rays for the μ CT of fossil specimens when the latter fail to obtain the
267 desired outcome.

268

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280

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TABLE 1 List of the studied specimens, including dimensions and scanning parameters.

Specimen	ML	MW	VxS-X	VxS-N	FoV
IPS58443.1	66.0	49.9	68.22	19.895524	42.97 x 47.75
Maxilla zoom				14.215323	30.71 x 35.79
Inner ear zoom				14.215323	30.71 x 35.34
IPS1724n	6.31	5.15	7.56	17.381885	20.04 x 38.64
IPS1724o	6.99	4.94	7.56	17.381885	20.04 x 38.64

Abbreviations: FoV-X, field of view (mm x mm) of n- μ CT; ML, maximum length (mm); MW, maximum width (mm); VxS-N, n- μ CT voxel size (μ m); VxS-X, X-ray μ CT voxel size (μ m).

389

390 FIGURE 1 Selected images of X-ray and neutron microcomputed tomography (μ CT) of two
 391 *Barberapithecus* molars (IPS1724n,o) and the *Pliobates* cranium (IPS58443.1). (A-B)
 392 Cross-section of IPS1724n through the metaconid and hypoconulid, based on X-ray (A)
 393 and neutron (B) μ CT. (C-D) Cross-section of IPS1724o through the protoconid and
 394 hypoconulid, based on X-ray (C) and neutron (D) μ CT. (E-F) Cross-section of IPS58443.1
 395 through the petrosal bone, based on X-ray (E) μ CT and neutron (F) μ CT. Note: the
 396 sections compared differ slightly due to different slice thickness.

397

398

399 FIGURE 2 Whole images stack histograms from different sources: (A) X-ray

400 microcomputed tomography (μ CT) of IPS1724n; (B) neutron μ CT of IPS1724n; (C) X-ray

401 μ CT of IPS1724o; (D) neutron μ CT of IPS1724o; (E) X-ray μ CT of IPS58443.1; (F) neutron

402 μ CT of IPS58443.1.

403

404

405 FIGURE 3 Virtually reconstructed right M₂ of *Barberapithecus huerzeleri* (IPS1724n)

406 based on neutron microcomputed tomography (μCT) scans, in oblique (semiocclusal and

407 mesiobuccal) view: (A) external morphology (enamel surface); (B) inner morphology

408 (enamel-dentine junction, EDJ); (C) enamel surface superimposed in semitransparency to

409 the EDJ.

410