

1 **Short running page heading:** Ossicular chain of Cainotheriidae

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3 **Title:** The ossicular chain of Cainotheriidae (Mammalia, Artiodactyla)

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13

14 **Abstract:**

15

16 This work describes an unparalleled sample of isolated fossil auditory ossicles of cainotheriid
17 artiodactyls from the Paleogene karstic infillings of Dams (Tarn-et-Garonne, Quercy, France).
18 This collection comprises a total of 18 mallei, 28 incudes and three stapedes. It allows the
19 documentation of both intra- and interspecific variability of ossicular morphology within
20 Cainotheriidae. We show that despite considerable intraspecific variability, the malleus, the
21 incus, and the stapes appear to be taxonomically informative at the Cainotheriidae scale. This
22 work further provides the first description of a reconstructed ossicular chain of a terrestrial
23 Paleogene artiodactyl species, found in a basicranium of the late Oligocene cainotheriine
24 *Caenomeryx* cf. *procommunis* (Pech Desse locality).

25

26 **Key-Words:** Morphometry, Malleus, Incus, Stapes, Paleogene, Quercy

27

28

29 **Introduction**

30

31 The middle ear ossicles - the malleus, the incus, and the stapes – form a bony chain
32 contained within the air-filled middle ear cavity. Their presence is a hallmark of Mammalia
33 (e.g., Simpson, 1959; Luo et al. 2001). The three ossicles are the smallest bones of the
34 mammalian skeleton and they play a fundamental role in hearing process. Indeed, the
35 ossicular chain transmits the airborne sound waves from the tympanic membrane (at the
36 interface between the outer and middle ear) to the fluid-filled cochlea of the inner ear, while
37 performing an anatomical impedance match between this two media (e.g., Wever &
38 Lawrence, 1954; Dallos, 1973; Schubert, 1978; Killion & Dallos, 1979; Peake & Rosowski,
39 1991; Hemilä et al. 1995; Nummela & Thewissen, 2008; Mason, 2016). Morphology of the
40 ossicular chain and its specificity within different mammalian groups has been intensely
41 studied for systematic purposes (e.g., Doran, 1878; Fleischer, 1973; Schmelzle et al. 2005;
42 Wible & Spaulding, 2012; Mason, 2013; Stoessel et al. 2016; Maier & Ruf, 2016a, 2016b;
43 Kerber & Sánchez-Villagra, 2018; Loza et al. 2018), or functional aspects (e.g., Fleischer,
44 1978; Nummela, 1995; Nummela & Sánchez-Villagra, 2006; Puria & Steele, 2010). Most of
45 these works deal with extant taxa, and, because of their fragility and small size, ossicles are
46 rarely preserved - or at least retrieved - in the fossil records. Yet, when retrieved, they bring
47 useful observations for the systematics or ecology of taxa, or both [e.g., systematic position of
48 Pakicetidae (Thewissen & Hussain, 1993) and hearing mechanisms in early cetaceans
49 (Nummela et al. 2004; Nummela et al. 2007) among Artiodactyla]. Still, fossil ossicles are
50 rarely preserved all three together, and almost never found in anatomical connection.

51 Here, we describe a broad sample of isolated ossicles of Cainotheriidae from Paleogene
52 karstic infillings from Quercy (Tarn-et-Garonne, France). Cainotheriids are an extinct family
53 of small artiodactyls (even-toed ungulates) documented in the fossil record between the late
54 Eocene and the middle Miocene in Western Europe (Blondel, 2005). Because of their unique
55 dental morphology, the phylogenetic position of Cainotheriidae within artiodactyls is still
56 debated. They have been related to different European endemic families (Romer 1966; Webb
57 & Taylor, 1980; Gentry & Hooker, 1988) without reaching a consensus, or to modern groups
58 of artiodactyls such as ruminants (Geisler & Uhen, 2005; O'Leary & Gatesy, 2007; Lihoreau
59 et al. 2015) or tylopods (Geisler & Uhen, 2003; Geisler et al. 2007; Thewissen et al. 2007).
60 The recent phylogenetic study of Weppe et al. (2019) retrieved Cainotheriidae closely related
61 to the European endemic families Mixtotheriidae, Anoplotheriidae and Robiacinidae.

62 Cainotheriidae are particularly abundant in karstic localities from Quercy, southwestern
63 France. This family, which includes at least five genera within two sub-families ranges from
64 rabbit-sized species to size of a small ruminant (Erfurt & Métais, 2007; Theodor, 2010).
65 Contrary to many European endemic ungulates which went extinct at the end of the Eocene
66 (Sudre & Legendre, 1992; Blondel, 2001), cainotheriids made it through the
67 Eocene/Oligocene transition and they are one of the very few artiodactyl groups to diversify
68 during Oligocene times (Blondel, 2005). The ossicles we describe here originate from two
69 loci from the karstic network of Dams, discovered in 2016. This karstic network was emptied
70 during the extensive phosphate exploitation that took place in Quercy during the late 19th
71 century. The network, however, still houses a great quantity of clay infillings including two
72 channels that yielded a great quantity of cainotheriid remains, namely DAM1 and DAM3.
73 These two infillings within Dams network bracket the Eocene-Oligocene transition (Weppe et
74 al. 2019) and they document a period that corresponds to a major faunal turnover in Western
75 Europe linked to climatic, geographic and oceanic circulation changes (Legendre, 1987;
76 Berggren & Prothero, 1992). Based on the unprecedented sample from Dams, including a
77 total of 18 mallei, 28 incudes and three stapedes, and on an in-situ ossicular chain from Pech
78 Desse (Quercy, France, late Oligocene), we discuss the intra- and interspecific variability of
79 ossicle morphology within Cainotheriidae and describe for the first time a reconstructed
80 ossicular chain for a Paleogene terrestrial artiodactyl species.

81

82 **Material and methods**

83

84 **Material**

85

86 Most of the specimens included in this analysis come from the Dams karstic network located
87 near Caylus (Tarn-et-Garonne) in Quercy (SW France). All specimens studied are curated at
88 the University of Montpellier (UM). The Dams material was collected after screenwashing of
89 40kg from DAM1 and 30kg from DAM3 in 2016. Raw fossil material was first concentrated
90 by wet screening of the red clays collected in Dams locality, (0.7-mm mesh size), and then
91 picked up with smooth tweezers, under a stereomicroscope. The material consists of 16
92 mallei, 16 incudes, and two stapedes from DAM1 (late Eocene, Mammalian Paleogene
93 reference level 19 [MP19]; Weppe, 2018) and 12 incudes (among which two are in
94 connection with the malleus head), and one stapes from DAM3 (early Oligocene, MP22;

95 Weppe, 2018). The taxonomic identification of the isolated ossicles from Dams relies on a
96 strong corpus of evidences: 1) the relative abundance of mammalian fossil remains; 90% of
97 the remains (cranial, dental and postcranial) from DAM1 belong to the small cainotheriid
98 artiodactyl *Paroxacron valdense*. Therefore, all the mallei and 16 incudes upon 18, that can be
99 referred to the same morph, likely correspond, based on the relative abundance criterion, to
100 *Paroxacron valdense*; 2) Artiodactyla hallmark; the incus presents a processus longum
101 slightly smaller than the processus brevis which is a characteristic of Artiodactyla (Doran,
102 1878; Wilkie, 1936; Thewissen & Hussain, 1993; Thewissen, 1994; Milinkovitch &
103 Thewissen, 1997); 3) incudo-malleal joint association; the association between the malleus
104 and the incus, besides general size compatibility and match between the articular surfaces, is
105 based on fused incudo-malleal complexes found in DAM3.

106

107 Based on these criteria, the ossicles from DAM1 are all assigned to *Paroxacron valdense*,
108 the only cainotheriid species retrieved in this channel (Weppe, 2018). In contrast, five
109 different cainotheriid species co-occur in DAM3, making specific attribution of the isolated
110 ossicles impossible (in the current state of our knowledge). A list of the included material is
111 provided in supplementary information Table S1. Other incudes of similar size have been
112 found in both levels (DAM1 and DAM3), but they are not included in this study due to their
113 markedly different morphology that would point to rodents or chiropterans instead of
114 artiodactyls. In addition to the ossicles from Dams localities, we reconstruct in this work the
115 in-situ location of the cainotheriid ossicular chain based on a basicranium (UM PDS 3353)
116 from the late Oligocene locality of Pech Desse (MP28, Quercy; Hugueney, 1997) that
117 preserves the ossicles trapped in the middle ear cavity. Two cainotheriine species are retrieved
118 in Pech Desse, namely *Plesiomeryx* cf. *cadurcensis* and *Caenomeryx* cf. *procommunis* (Remy
119 et al. 1987). Based on the overall larger dimensions of the specimen, it is here referred to as
120 *Caenomeryx* cf. *procommunis*.

121

122 **Micro CT scanning and virtual reconstruction**

123

124 The ossicles were scanned, using the high-resolution micro CT-scanner EasyTom of the
125 technical facility of the Montpellier Rio Imaging platform, at a high voltage (150 kV) using a
126 copper filter and small sample holders (2 and 4 cm diameters), allowing to be close to the X-
127 ray source and therefore, to reach a voxel size of 11.89 μm for isolated specimens, and 23.81
128 μm for the partial cranium UM PDS 3353. They were reconstructed virtually in 3D using the

129 threshold tool of Avizo 9.5 (VSG-FEI) software. The specimens partly encrusted with matrix
130 were cleaned using the manual segmentation tool of the same software. The virtual
131 reconstruction of the in-situ location of the ossicular chain of cainotheriid from Pech Desse
132 was realized using the freeware MorphoDig (Lebrun, 2018). Anatomical terminology used in
133 this study mainly follows that of Wible and Spaulding (2012); orientations are based on the
134 reconstruction of the in-situ ossicle chain of the cranium UM PDS 3353.

135

136 **Geometric morphometric analysis**

137

138 Nine mallei and nine incudes from DAM1 and five incudes from DAM3 are included in the
139 geometric morphometric analyses, other specimens being discarded due to their fragmentary
140 condition. In addition, the incus and malleus from the Pech Desse specimen were also added
141 to the analyses. Only three stapedes were unearthed in Dams localities, which does not allow
142 a proper discussion of the morphometrical variability of this ossicle. To quantify the malleus
143 and incus shape variations, we digitized a set of 3D landmarks using MorphoDig software
144 (MorphoDig 0.8) (Fig. 1). Nine landmarks were placed on the malleus including five on the
145 articular area. The first one was placed at the highest point of the curve along the medial
146 margin of the articular area. The second one occupies the same position on the distal margin
147 of the articular area. The third landmark is located at the center of the ridge of the articular
148 area. The fourth and the fifth landmarks were placed on the deepest points along the dorsal
149 and ventral borders of the articular facet, respectively. The sixth landmark was placed at the
150 extremity of the muscular process and the seventh one is located on the notch between the
151 muscular process and the manubrium. The eighth is positioned at the deepest point of the
152 angulation formed by the neck of the malleus with the manubrium. The last one was placed at
153 the extremity of the anterior process. On the incus, we positioned nine landmarks, including
154 five on the articular area. The first one was placed on the deepest part on the lateral border of
155 the articular area and the second one at an equivalent location on the medial border.
156 Landmarks three and four were placed on the most salient points of the articular surface i.e.,
157 on the dorsal and ventral edge, respectively. The fifth landmark is located at the central
158 position of the bulge formed by the intersection of the two articular facets. Landmarks six and
159 seven are positioned on the incudal body. The sixth one is at the middle of the swelling along
160 the upper border of the incudal body and the seventh was placed at the middle of the curve
161 joining the short and the long process posteroventrally. The eighth landmark was placed
162 internally to the long process where it becomes thinner, while the ninth one was positioned at

163 the extremity of the short process. The treatment of the raw dataset was performed using R
164 software version 3.4.4 (R Core Team, 2018). All 10 replicates were scaled to unit centroid
165 size (i.e. “the square root of the sum of squared distances from each landmark to the centroid
166 of the configuration” Claude, 2008:139), translated, rotated, and superimposed through the
167 Generalized partial Procrustes Analysis (pGPA) following Claude (2008, 2013; see also
168 Bookstein, 1990; Rohlf, 1990; Dryden & Mardia, 1998). Then, to apprehend the shape
169 variability of the malleus and incus, we performed a Principal Component Analysis (PCA;
170 Pearson type) on the Procrustes coordinates (resulting from the pGPA). Error measurement
171 follows Yezerinac *et al.* (1992; see also Claude *et al.* 2003; Claude, 2008: 65-66, 2013). Data
172 and script are available in supplementary material dataset 1.

173

174 **Data availability statement**

175 All specimens studied are curated at the University of Montpellier (UM) and can be freely
176 consulted upon request. The 3D models of the middle ear reconstruction of *Caenomeryx* cf.
177 *procommunis* is available in open access on MorphoMuseum (<https://morphomuseum.com/> ;
178 Assemat, in press). The scripts used to perform the morphometric analyses are provided in
179 supplementary information.

180

181 **Description**

182

183 **Malleus (Figs. 2-3)**

184

185 The description of the cainotheriid malleus (Fig. 2) primarily relies on material from
186 the DAM1 locality as DAM3 only yielded malleus articular surfaces fossilized in anatomical
187 connection with the incus. The malleus is the most lateral part of the ossicular chain. In life, it
188 contacts the tympanic membrane by a long, flat-shaped manubrium and a short and angular
189 lateral process. The malleo-incudal complex is unfused for all documented specimens from
190 DAM1. The globose head of the malleus bears the articular surface for the incus. The latter is
191 divided into two facets separate by a deep and wide groove; the superior articular facet for the
192 incus lies in the dorsal aspect, while the inferior articular facet, of about the same size, lies at
193 ca. 35° angle to it (Fig. 2A). The general shape of the articular surface is saddle-like and is
194 asymmetrical related to the fact that the groove is wider and shallower on the dorsomedial

195 part. On the anterior surface, the head comes to a small, rounded point that we identify as a
196 blunt capitular spine (Wible & Spaulding, 2012). A thin and sharp bony crest joins the basis
197 of this spine to the anterior process, the outer lamella (Henson, 1961). Lateral to the capitular
198 spine, the surface of the head is carved by a small pit. The neck of the malleus is relatively
199 straight and it forms with the head an obtuse angle which confers to the malleus a general
200 sigmoidal shape. It is relatively broad and lines the osseous lamina on the posteromedial edge.
201 The osseous lamina consists of a particularly thin portion of bone bearing a depression on the
202 lateroventral aspect of the malleus (Fig. 2D); there is no clear demarcation with the basis of
203 the manubrium. The specimens of our sample seem to display a short blunt anterior process,
204 also known as processus gracilis, or prearticular, and mentioned in living artiodactyls (Wible
205 & Spaulding, 2012; Maier & Ruf, 2016b). It might have displayed a much thinner terminal
206 part but it would have been broken away during the fossilization process and left no trace. As
207 illustrated for carnivorans by Wible & Spaulding (2012), the base of the manubrium
208 corresponds to the confluence of the neck, lateral process, and ventral margin of the osseous
209 lamina. The manubrium is long with a flat and thin tympanic surface. The latter is much wider
210 than the lateral edge which bears a ridge becoming narrower at the manubrium's extremity.
211 Unfortunately, the manubrium is partly broken on all isolated specimens. It displays a well-
212 developed lateral process at the posterior margin of its base. The medial margin of the bone
213 bears a strong and conical muscular process forming a 40° angle with the manubrium. The
214 course of the chorda tympani nerve is marked on the ventral aspect of the muscular process.

215

216 **Comparison.** The small and flat head of the malleus of the cainotheriids from Dams is closer
217 in proportion and shape to Ruminantia as illustrated in *Capreolus* and *Giraffa* by Fleischer
218 (1973) than to *Sus*, *Hippopotamus*, and *Camelus*. Like ruminants, they also display a long
219 neck and a wide osseous lamina. In suids, hippos, and camelids, the neck is shorter and the
220 osseous lamina remains smaller and closely appressed to the head. Compared to hippos and
221 suids, the cainotheriid malleus also displays a shorter but stockier muscular process which is
222 closer to the manubrium. The muscular process global shape in cainotheriids is relatively
223 similar to that of some living ruminants (e.g. *Bos taurus*, *Ovis aries*, and *Cervus elaphus*).

224

225 **Inter- and intraspecific variation.** The morphology of the 16 mallei from DAM1 shows a
226 noticeable variability mainly affecting the length of the malleus neck, the angulation of the
227 lateral process, the shape, depth and orientation of the articular surface, and also the location,
228 length and shape of the muscular process (Fig. 2G-H; Fig. S1A-H). In addition, in comparison

229 with the specimens from DAM1, the malleus from Pech Desse displays a different
230 morphology of its articular surface. In order to quantify this inter- and intraspecific shape
231 variation of the malleus, we performed two pGPAs, one with all available mallei (interspecific
232 analysis; see Material and methods) and one without the Pech Desse specimen (intraspecific
233 analysis). Due to small breakages on the latter specimen, interspecific shape variation has
234 been quantified by taking into account only the first seven landmarks. For the interspecific
235 analysis, measurement error is 6.92 % for centroid size and 16.88 % for shape. Inter-specimen
236 size and shape variations are stronger than the intra-specimen ones (specimen factor is
237 significant in both ANOVA ($F = 135.4$; $p < 0.001$) and Procrustes ANOVA ($F = 50.23$; $p =$
238 0.001); see also Claude, 2013). The Fig. 3A presents the projection of the specimens on the
239 first factorial plane. PC1 (52.96 % of the variance) clearly separates the Pech Desse specimen
240 from the other mallei and highlights deep morphological difference in term of shape and
241 orientation of the articular facets of the two species. The mallei from DAM1 present
242 intraspecific variation mainly on PC2 (19.72 % of the variance) that corresponds with small
243 size variation of the articular surface and slight variations of orientation of the body of the
244 malleus in regards to its head. More precisely, projection of individuals on the first factorial
245 plane (48.82 % of the variance) of the intraspecific analysis (Fig. 3D; where measurement
246 error for centroid size is 5.08 % with specimen factor significant ($F = 187.7$; $p < 0.001$) and
247 measurement error for shape is 23.41% with specimen factor significant ($F = 33.72$; $p =$
248 0.001)), underlines these variations. Indeed specimens from DAM1 are distributed more or
249 less randomly along the first two PCs that both stand for size variation of the articular surface.
250 Furthermore, PC1 also presents variation of orientation of the anterior process and PC2
251 highlights variation of orientation of the muscular process and of the main body in regards to
252 the malleus head. This disparity of orientation of the malleus body can also be observed
253 directly by superimposing all the mallei while keeping the same position for the articular
254 surface (Figs. 2G-H, 3E-F).

255

256 **Incus (Figs. 4-5)**

257

258 The incus is the middle ossicle joining the malleus and the stapes. It is conical and
259 stocky in shape (Fig. 4 A-B). The wide articular area of the incudomalleal joint is composed
260 of two asymmetrical facets. It displays the same asymmetry as previously described for the
261 malleus. A salient edge, of different size depending on the specimen, separates the articular
262 surface from the incudal body (Fig. 4D). The dorsal aspect of the incus is variably convex,

263 from nearly flat to strongly domed. The cainotheriid incus displays two processes of similar
264 length, set apart by a wide angle; the processus brevis and the processus longum.
265 The processus brevis prolongs posteriorly the incudal body. The processus longum, which
266 connects the stapes by the lenticular apophysis, is located on the ventral edge of the incus,
267 posterior to the articular surface. The distal extremity of the processus longum of all incudes
268 of our sample is broken so that no lenticular apophysis is documented. A groove starts at the
269 base of the processus longum and runs all along of it. The separation between the two
270 processes is strengthened by the length of the body of the incus. The processus brevis of the
271 cainotheriid incus displays a thinning at its extremity while the processus longum is thicker.
272 The extremity of the processus longum ends in a small spike that corresponds to the
273 attachment of the broken lenticular apophysis.

274

275 **Comparison.** The cainotheriid incus is morphologically similar to that of other artiodactyls
276 with two processes of about the same length, whereas in most other mammals, the processus
277 longum is longer than the processus brevis (Doran, 1878). Cainotheriid incudes from Dams
278 differ from those of *Camelus* in having further apart processes, separated by a wider angle.
279 The articular area of cainotheriid incudes appears to be shallower than in camels, but deeper
280 than in bovines (Doran, 1878, Pl. 61). The body of studied incudes is close in shape to that of
281 *Sus scrofa*. It is more massive in the hippos and the llama which also display a more
282 cylindrical shape. Ruminants display a wide range of incudal body morphologies (Doran,
283 1878; Wilkie, 1925; Wilkie, 1936). The scarcity of illustrations available in the literature
284 prevents a broader comparison among artiodactyls.

285

286 **Inter- and intraspecific variation.** The incudes from DAM1 and DAM3 show a noticeable
287 variability of the depth of the articular facet and of the angulation between the processus
288 brevis and the processus longum. They also display a variation of their dorsal part bulge (Fig.
289 4F, Fig. 5A-B, Fig. S1I-M). In order to quantify inter- and intraspecific shape variations, we
290 performed a pGPA on a dataset of nine three-dimensional landmarks (Fig. 1B; see Material
291 and methods section, Geometric morphometric analysis) for a sample of 15 incudes from
292 DAM1, DAM3 and Pech Desse. For this analysis, measurement error is 3.14 % for centroid
293 size, 18.82 % for shape and inter-specimen size ($F = 309.4$; $p < 0.001$) and shape ($F = 44.13$;
294 $p = 0.001$) variations are significantly stronger than the intra-specimen ones. The two first
295 PCs explain 56.32 % of the variance (Fig. 5A). The main shape variation contributions of PC1
296 (39.12 % of the variance) correspond with an elongation of the processus brevis, correlated

297 with a smoother and thinner dorsal bulge toward the positive values (Fig. 5 B). PC1 also
298 displays shape and orientation variations of the articular facets following the same trends
299 observed for the interspecific variation of the malleus (see above). PC2 (17.20 % of the
300 variance) mainly highlights the variation of angulation between the two processes (Fig. 5 C).
301 The projection of the specimens on the first factorial plane results in two slightly overlapping
302 groups corresponding to DAM1 and DAM3 localities. DAM3's morphospace, with a surface
303 that covers 41 % of the whole sample's morphospace, is nearly five times more expanded
304 than that of DAM1 (that covers 8.6 %). Only one specimen from DAM3 lies within DAM1's
305 morphospace. Incudes from DAM1 present intraspecific variability along PC1 and PC2,
306 highlighting mostly variation of the angulation between the two processes and also, small size
307 variation of the articular surface. PC1 also clearly isolates the Pech Desse specimen from the
308 incudes retrieved in Dams. Indeed, in terms of Mahalanobis distance on the first five PCs, the
309 specimens from DAM3 are always retrieved at least twice as far from the Pech Desse
310 specimen as from the centroid of DAM1 (see Tab. S2).

311

312 **Stapes (Fig. 6)**

313

314 The stapes is the most proximal element of the ossicular chain. Located
315 medioposteriorly relative to the other ossicles, it is composed of a head, two crura (anterior
316 and posterior), and a footplate. Regarding its connectivity, in life, the head articulates with the
317 lenticular apophysis of the incus while the footplate sits on the fenestra vestibuli, retained by
318 the annular ligament. The isolated nature of the stapes and the high resolution of μ CT-scan
319 acquisition performed here permit us to describe the structure of this ossicle in cainotheriids
320 in more detail than was possible for Orliac and Billet (2016).

321 The stapedes from DAM1 present a global trapezoidal shape with two long crura
322 separated by a wide foramen intercrurale (Fig. 6 A-B). The latter has the same size on the
323 medial and lateral surface and it extends from the head to the large footplate. The two DAM1
324 stapedes are very similar in terms of size and shape of the foramen intercrurale. Their
325 footplate are roughly oval in shape and concavo-convex, with a prominent umbo. In the
326 specimen from DAM3, the footplate is bean-shaped (Fig. 6 C vs M). In the DAM1 stapes, the
327 rim is thick along its whole length, unlike that of DAM3, the rim of which is thicker
328 posteriorly and much thinner anteriorly. On the stapes head, the articular facet for the
329 lenticular apophysis of the processus longum is narrow and oval, slightly convex

330 anteroposteriorly and slightly concave mediolaterally (Fig. 6 A, D). The processus muscularis
331 stapedis, located on the top of the posterior crus, appears to be larger in the DAM1 stapedes
332 than in that from DAM3. The general shape of the bone is asymmetrical in specimens from
333 both DAM1 and DAM3, with the anterior crus longer and slenderer than the posterior one.
334 The posterior crus is slightly straighter in DAM1 stapedes.

335

336 **Comparisons.** The morphology of the cainotheriid stapedes from Dams differs from those of
337 the cow and llama which present a more rectangular general shape in lateral view, due to
338 more symmetrical crura and smaller footplates (Doran, 1878; Fleisher, 1973; Costeur et al.
339 2016). The cainotheriid stapedes from Dams, like in hippopotamids, display a wide foramen
340 intercrurale extending to footplate; hippopotamids however differ in having a smaller head
341 and a more elliptic (i.e. not asymmetrical) footplate (Fleisher, 1973, fig. 41; Orliac & Billet,
342 2016, fig. 2C-D). Asymmetrical crura as observed in cainotheriids are also observed in
343 camelids (*Camelus bactrianus*; Bai et al. 2009), suoids (*Tayassu tajacu* and *Microstonyx*
344 *erymanthius*; Orliac & Billet, 2016) and ruminants (e.g. *Giraffa camelopardalis*; Doran,
345 1878).

346

347 **Reconstruction of the ossicular chain of the cainotheriid *Caenomeryx filholi***

348

349 The marked intra-specific variability of ossicle shape makes it difficult to reconstruct
350 an articulated ossicular chain based on composite material. We therefore performed a
351 reconstruction of the ossicular chain based on in-situ ossicles preserved in the middle ear
352 cavity of the basicranium UM PDS 3353 from Pech Desse (MP 28, Quercy). The malleus and
353 the stapes were preserved within the left bullar space, while the right side preserved the
354 malleus and the incus. Due to postmortem soft-tissue decay, the ossicles were no longer in
355 connection. A complete middle ear was therefore virtually reconstructed using the left bulla,
356 petrosal and incus and mirror-3D models of the right malleus and stapes. Based on the relative
357 position of the tympanic ring of the bulla and of the fenestra vestibuli of the petrosal, we
358 propose a reconstruction of the three-dimensional orientation of the ossicles within the middle
359 ear cavity (Fig. 7A-D). The malleus is positioned so that it closes anterodorsally the tympanic
360 ring with its anterior process, and that the manubrium contacts the tympanic membrane with
361 its flat part oriented ventrally. Unfortunately, the anterior process of the malleus is broken
362 away on both sides, and the connectivity with the bulla could not be fully assessed. The incus
363 contacts the malleus via the articular area, while it connects the petrosal by soft tissues fixed

364 on the processus brevis. The contact with the stapes is realized by the lenticular apophysis, a
365 very fragile structure that is not preserved here. Nevertheless, according to the location of the
366 fenestra vestibuli (determining the position of the stapes within the middle ear cavity), this
367 process seems to have been orthogonal to the processus longum of the incus. The 3D model of
368 the middle ear reconstruction is available on MorphoMuseuM (Assemat, in press).

369 Compared to the mallei from DAM1, the articular surface of the malleus from Pech
370 Desse presents a wider angle between the superior and inferior articular facets (nearly 90° for
371 Pech Desse). In addition, the shape of their articular surfaces are clearly distinct; while the
372 articular surface of the mallei of DAM1 (in posterior view) clearly disrupt from the neck of
373 the malleus with a subrectangular shape that extends ventrodorsally, the head of the malleus
374 of Pech Desse is much slender ventrodorsally and somehow extends continuously the neck of
375 the malleus medially (Fig. 2A-B vs Fig. 7I; see also Fig. 3A-C). The orientation of the
376 muscular process is also more medial. The malleus is generally more gracile in the specimen
377 from Pech Desse. It preserves the delicate structure of the manubrium's extremity which is
378 spatulated (Fig. 7C-D), for the DAM1 material, it is difficult to assess if the manubrium is not
379 spatulated or if this feature was broken away on specimens. The shape of the incus from Pech
380 Dess reflects the differences observed at the level of the malleus articular surface. The stapes
381 from Pech Desse (Fig. 7M-P) also exhibits a quite different morphology from that of DAM1,
382 with a general profile slenderer anteroposteriorly, a smaller head (might be due to in situ
383 partial preservation), slight differences in crura width and orientation, and footplate outlines
384 that are oval instead of being bean-shaped. However, the morphology of the stapedial
385 footplate is very similar, being elongated, concavo-convex in dorsal view, and bearing a wide
386 stapedial footplate rim (Fig. 7M-P). The observation of the contact between the petrosal and
387 the bulla (Fig. 7Q) using μ CT-scan data have not permitted us to confirm the presence of the
388 processus internus praearticularis (pipa; Maier & Ruf, 2016b), not visible in intracranial view
389 between the tegmen tympani and the basisphenoid bone because of tight contact between the
390 two structures.

391

392 **Discussion**

393

394 Inter- and intraspecific variability of the shape of the ossicles has been described for a
395 few group of mammals such as the African mole-rats (Bathyergidae, Lange et al. 2007), the
396 hominoid primates (Stoessel et al. 2016) and the golden moles (Chrysochloridae, Mason et al.

397 2018). The sample from DAM1 composed of 16 mallei and 16 incudes, all referred to
398 *Paroxacron valdense*, allows the consideration of intraspecific variability of these ossicles in
399 this small extinct artiodactyl. The malleus shows a wide shape range; variations mainly affect
400 the size of the articular facet and the rotation of malleus head relative to the body (Fig. 2 G-
401 H). This considerable variability of the shape might result in non-negligible variations in the
402 orientation of the ossicle chain in the auditory area or, in turn, in a similar variation range at
403 the incus level. Indeed, just as for the malleus, the incudes from DAM1, also all referred to
404 *Paroxacron valdense*, show a remarkable variability of shape (Fig. 4E-F; Fig. 5), mostly
405 concerning the length of the processus brevis, the angle between the two processes, the dorsal
406 bulging of the body, and the width of the articular facet.

407 Despite relatively significant shape variation, the morphospace of the incudes
408 specimens from DAM1 - representing one single species - is smaller than that of those from
409 DAM3, and rather well separated from it, except for one DAM3 specimen that lies within
410 DAM1 morphospace. This individual from DAM3 could be a representative of the genus
411 *Paroxacron* and could either document the species *Paroxacron bergeri* or *Paroxacron* sp.
412 retrieved in DAM3 (Weppe, 2018). Indeed, the genus *Paroxacron* crosses the
413 Eocene/Oligocene transition and is found both in DAM1 (with the single oxacronine
414 cainotheriid species *Paroxacron valdense*) and DAM3 fossiliferous levels. The larger
415 morphospace covered by the specimens from DAM3 compared to DAM1 could be explained
416 by the co-occurrence in DAM3 of three different cainotheriid genera (Oxacroninae:
417 *Paroxacron*; Cainotheriinae: *Plesiomeryx* and *Caenomeryx*), comprising five different
418 species. The morphology of the younger ossicles from Pech Desse, assigned to *Caenomeryx*
419 cf. *procommunis*, is also markedly different from those described from DAM1 and DAM3,
420 confirming the potential systematic and phylogenetic interest of ossicular morphology, as
421 shown by the wide array of morphologies observed within and between higher rank mammal
422 groups (e.g., Doran, 1878; Fleischer, 1973; Nummela, 1995; Schmelzle et al. 2005; Mason,
423 2013; Solntseva, 2013). The morphology of the stapes has, for example, been proposed as a
424 hallmark for major divisions among Placentalia (Novacek & Wyss, 1986). The variation of
425 shape observed among cainotheriid incudes between DAM1 and DAM3 supports the potential
426 interest of ossicles at the generic level. Yet, establishing a morphotype reference based on
427 ossicles for each cainotheriid genus seems most unlikely given the usual scarcity of these
428 smallest bones among the fossil material collected in localities. At a wider scale, ossicles are
429 likely to provide interesting signal at the Artiodactyla level and documentation of the
430 morphology of the cainotheriid ossicle chain could be of interest to address the phylogenetic

431 relationships of this extinct family. Actually, cainotheriids have been proposed to be closely
432 related to tylopods (e.g., Gentry & Hooker, 1988; Thewissen et al. 2007), or closer to
433 ruminants (e.g., Geisler & Uhen, 2005; O’Leary & Gatesy, 2007). Recent results based on
434 dental evidence placed them together with Anoplotheriidae and Mixtotheriidae, close to
435 Ruminantia (Weppe, 2018). One stapes has been described for Anoplotheriidae (*Diplobune*
436 *minor*; Orliac et al. 2017, fig. 3); it is morphologically very close to that described here for
437 *Paroxacron valdense*, with asymmetrical crura, a large slender head, a wide foramen
438 intercrurale, and a somewhat convex footplate. However, the general morphological signal
439 carried by the ossicular chain is difficult to interpret at the Artiodactyla level yet, because
440 modern groups also display a wide range of morphologies and specializations. The
441 morphology of the malleus of Dams cainotheriids is close in proportion and shape to that of
442 Ruminantia with a long neck, a wide osseous lamina, and a similar global shape of the
443 muscular process. Compared to camelids and hippos, the manubrium of *Caenomeryx* is much
444 more spatulated and shows a morphology close to rodents (e.g., *Rattus Microtus*, Fleischer
445 1973:fig.29, 31), carnivorans (e.g. *Mustela*, Fleischer 1973:fig.53) or primates (e.g., *Galago*,
446 *Macaca*, Fleischer 1973:fig.18-19); the spatulated aspect strongly recalls that of golden moles
447 (Willi et al., 2006). The meaning of spatulated manubrium in terms of sound transmission
448 remains unclear as it is found in a wide array of mammalian species.

449 The cainotheriid incus brings little information and is morphologically similar to that
450 of other artiodactyls with two processes of about the same length (Doran, 1878). Finally, the
451 stapedial morphology seems to be closer to that of Anoplotheriidae than to any modern
452 representatives of Artiodactyla illustrated in the literature. However, knowledge of early
453 members of Ruminantia and Camelidae is necessary before a proper discussion is engaged on
454 morphological proximity of Cainotheriidae with one modern group or the other.

455 The present cainotheriid sample provides a very first glimpse into Paleogene artiodactyl
456 ossicle evolution and variability. Compared to the ossicles from DAM1 (dated at ca. 35 Ma),
457 and DAM3 (ca. 32 Ma), the in-situ ossicular chain from Pech Desse (ca. 25 Ma) exhibits a
458 quite different morphology, with more gracile elements. These morphological changes could
459 echo a shift in cainotheriid ecological habits between the late Eocene and the late Oligocene.
460 This shift may relate with deep environmental changes, such as the opening of the vegetation
461 cover that occurred after the Grande Coupure in Western Europe (Collinson, 1992;
462 Cavagnetto & Anadón, 1996). However, documentation of the ossicular morphology of other
463 Oligocene–Miocene cainotheriid genera (i.e., *Plesiomeryx* and *Cainotherium*) is necessary to

464 properly address this question in the light of the phylogenetical signal, as revealed by cranio-
465 dental morphology.

466

467 **Conclusions**

468

469 The unprecedented fossil ossicles sample from the karstic network of Dams, including
470 a total of 18 mallei, 28 incudes and three stapedes, allows the documentation of intra- and
471 interspecific variability of auditory ossicle morphology within Cainotheriidae. This
472 descriptive work constitutes the first description of a reconstructed ossicular chain of a
473 terrestrial Paleogene artiodactyl species. Despite considerable intraspecific variability, the
474 malleus, the incus, and the stapes appear to be taxonomically informative at the
475 Cainotheriidae scale. This highlights the interest of picking these tiny bones (~1-2 mm long)
476 when sorting out the sediments. Internal investigation of fossils by μ CT-scan imaging will
477 certainly also allow for completing our knowledge of Paleogene artiodactyl ossicles, thereby
478 widening our observations and conclusions, notably from a phylogenetic perspective.

479

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481

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493

494 **Author contributions**

495 A. Assemat performed the morphological study, the statistical analyses, and wrote the paper.

496 M. J. Mourlam supervised the statistical analyses and corrected different versions of the
497 manuscript.

498 R. Weppe and J. Mougoust collected the material in the field, sorted out the specimens, and
499 launched morphological analysis.

500 P.-O. Antoine collected the material in the field, supervised the field training and sediment
501 screenwashing, and corrected the manuscript.

502 M. J. Orliac designed research, sorted out the material, prepared the figures, and wrote the
503 manuscript.

504

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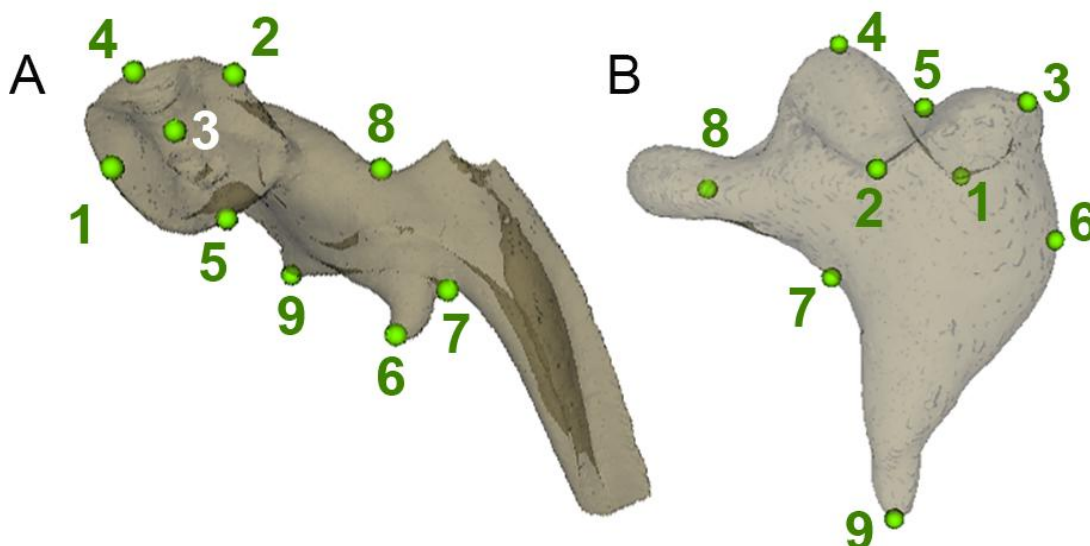
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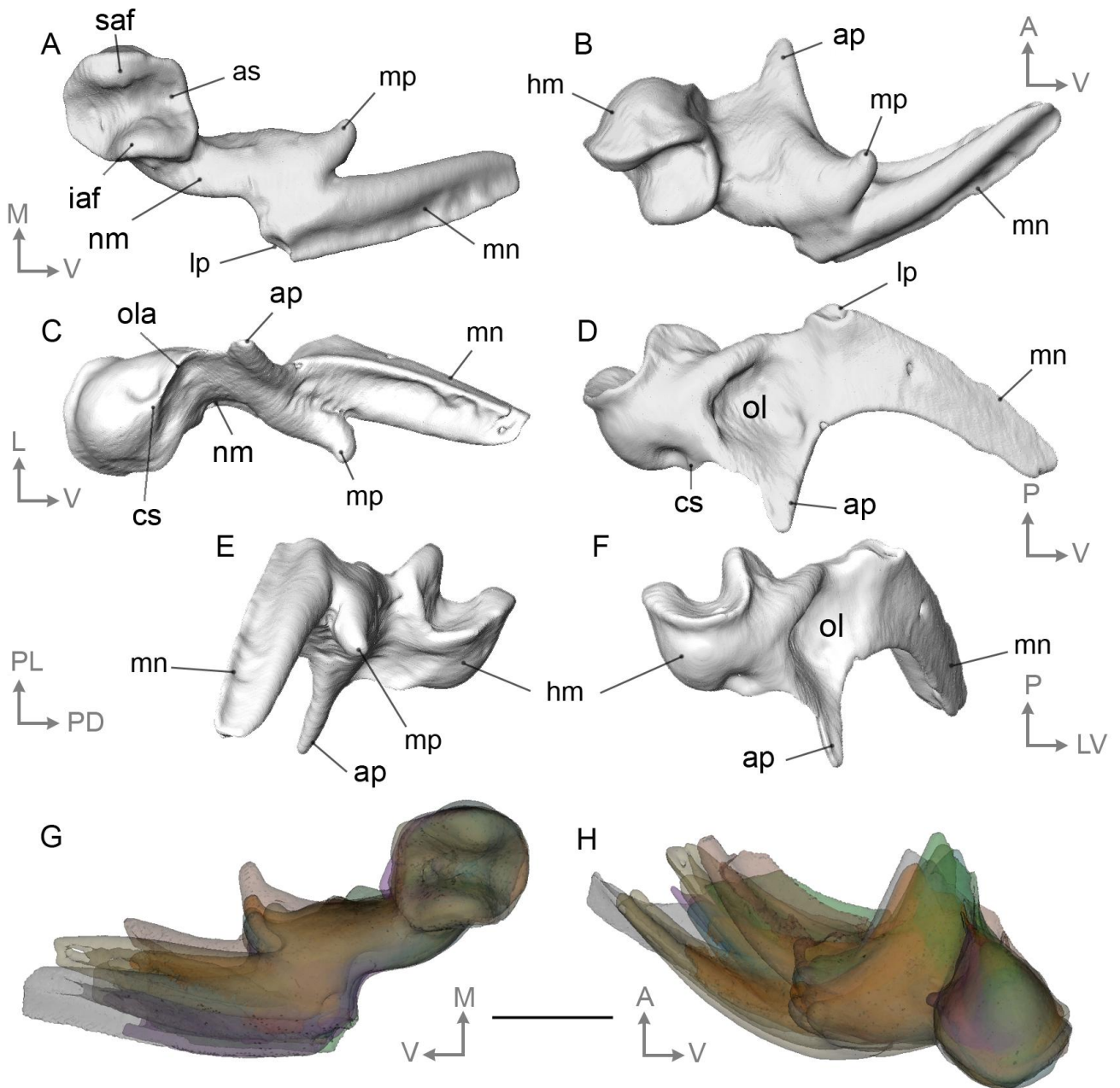
677 **Figure captions**



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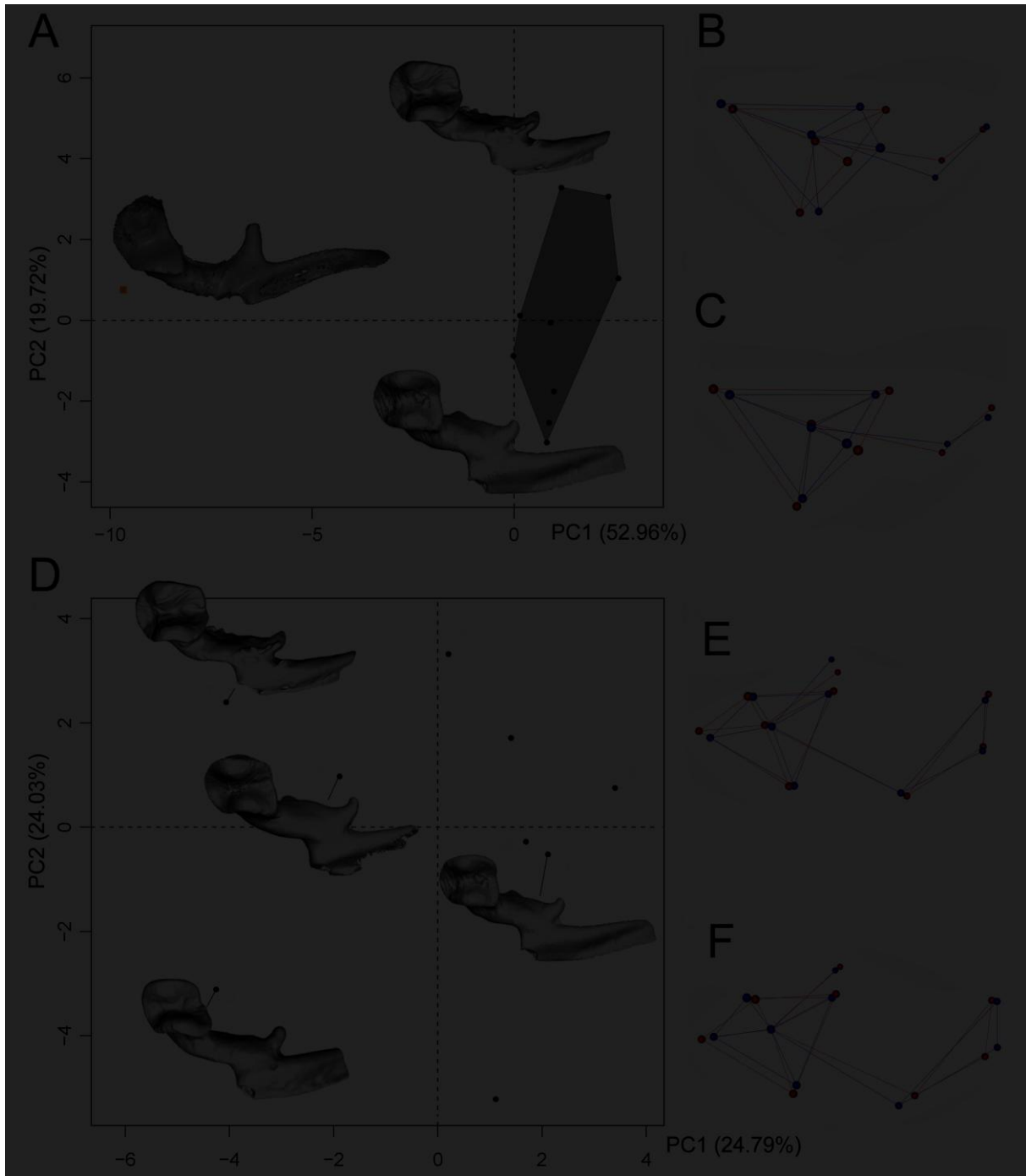
679 **Figure 1.** Position of landmarks used in the Generalized partial Procrustes analysis (pGPA)
 680 and principal component analysis (PCA) on the malleus (A) and the incus (B) of late Eocene
 681 and early Oligocene Cainotheriidae from Dams localities (DAM1 and DAM3, Phosphorites of
 682 Quercy, SW France).

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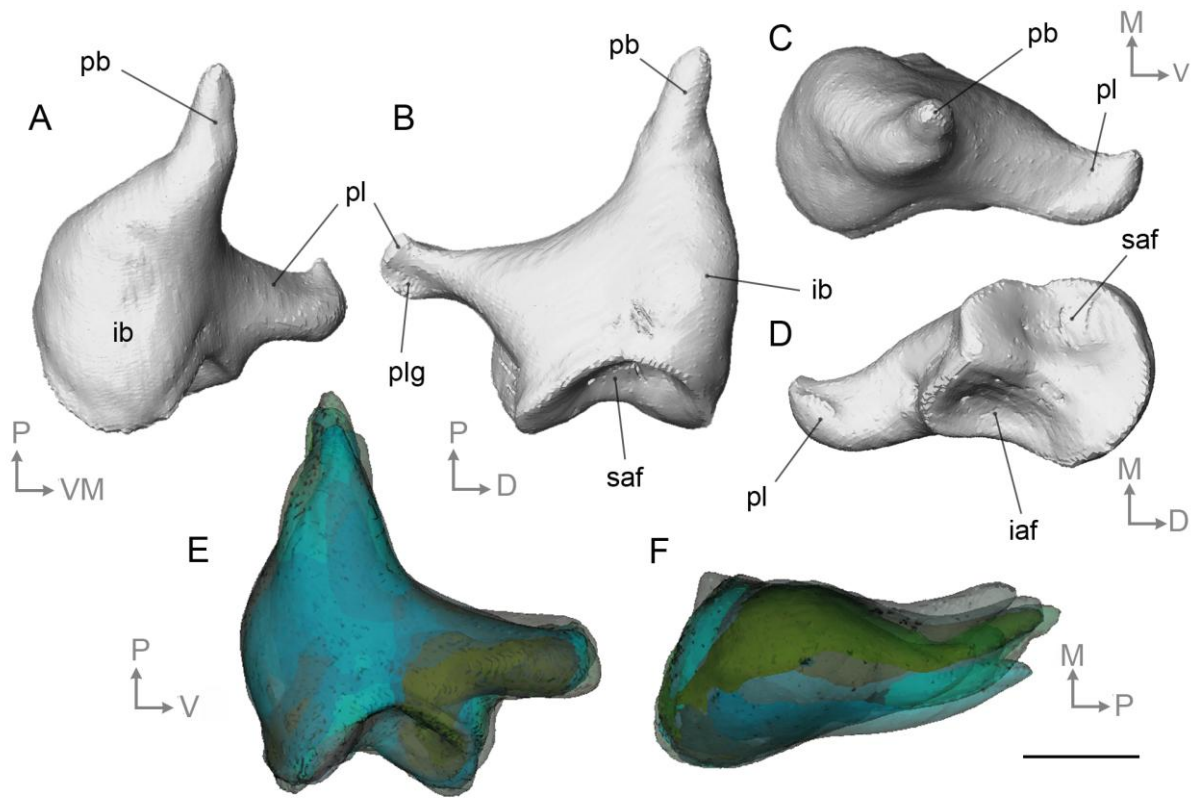
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686 **Figure 2.** Left cainotheriid malleus from Dams DAM1 (DAM1 330) in (A) posterior, (B)
687 medial, (C) anterior, (D) lateral, (E) medioventral, and (F) dorsolateral views. G-H,
688 Illustration of morphological variability as observed in (G) posterior and (H) dorsomedial
689 views of a right malleus). Abbreviations: ap, anterior process; as, articular surface; cs,
690 capitular spine; hm, head of malleus; iaf, inferior articular facet; lp, lateral process; mn,
691 manubrium; mp, muscular process; nm, neck of malleus; ol, osseous lamina; ola, outer
692 lamella; saf, superior articular facet. Orientations: A, anterior; D, dorsal; L, lateral; M,
693 medial; P, posterior; V, ventral. Scale bar = 1 mm.
694



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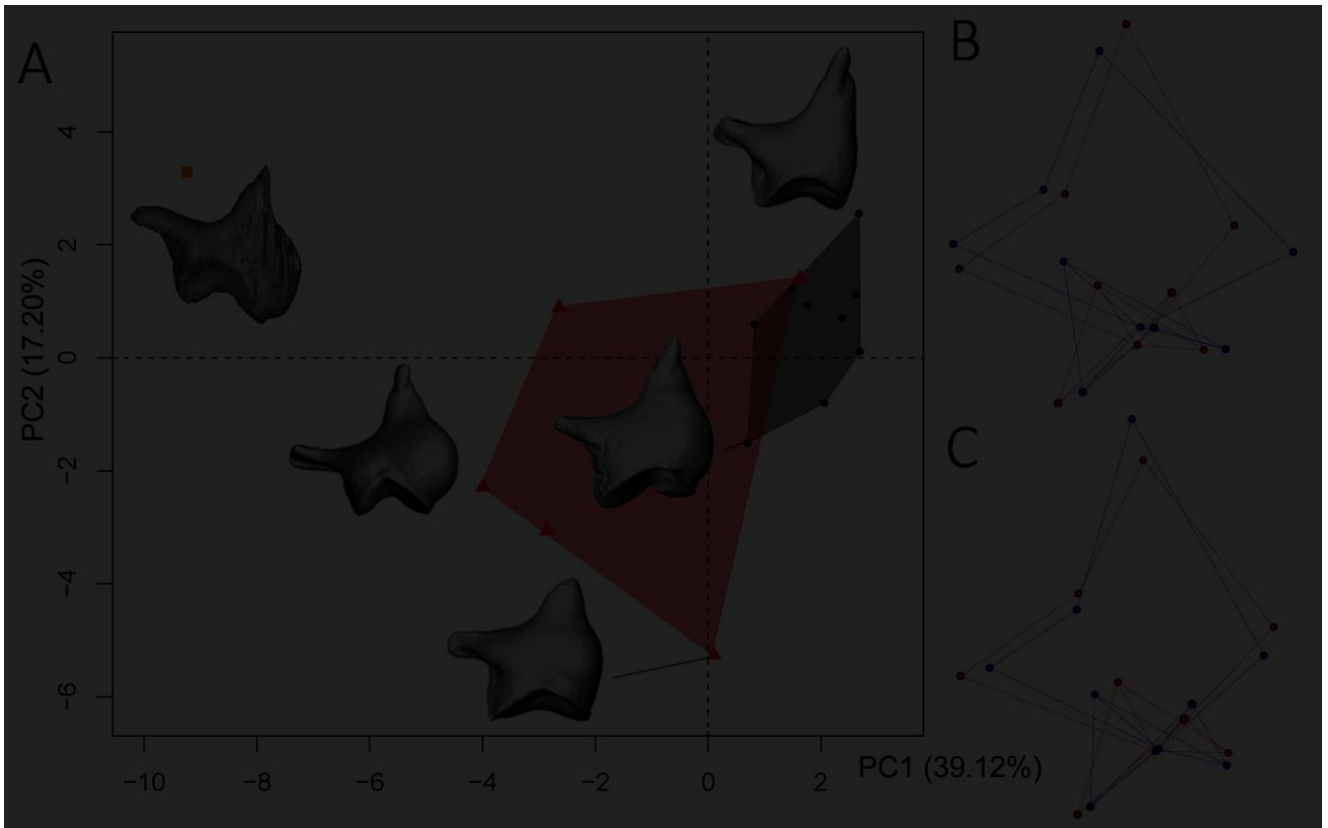
696 **Figure 3.** PCAs on Procrustes coordinates illustrating morphological variability of the
 697 cainotheriid malleus from DAM1 (late Eocene, Phosphorites of Quercy, SW France). (A)
 698 Inter- and intraspecific variation of the shape of the malleus based on 7 landmarks; projection
 699 of specimens from DAM1 (in black) and Pech Desse (in orange) on the first factorial plane;
 700 (B-C; E-F) Patterns of variation along PCs with maximal values in red and minimal in blue;
 701 (B) shape variation on PC1 of (A); (C) shape variation on PC2 of (A); (D) Intraspecific
 702 variation of the shape of the malleus based on 9 landmarks; projection of specimens from
 703 DAM1 on the first two PCs; (E) shape variation on PC1 of (D); (F) shape variation on PC2 of
 704 (D). See text for more details.



705

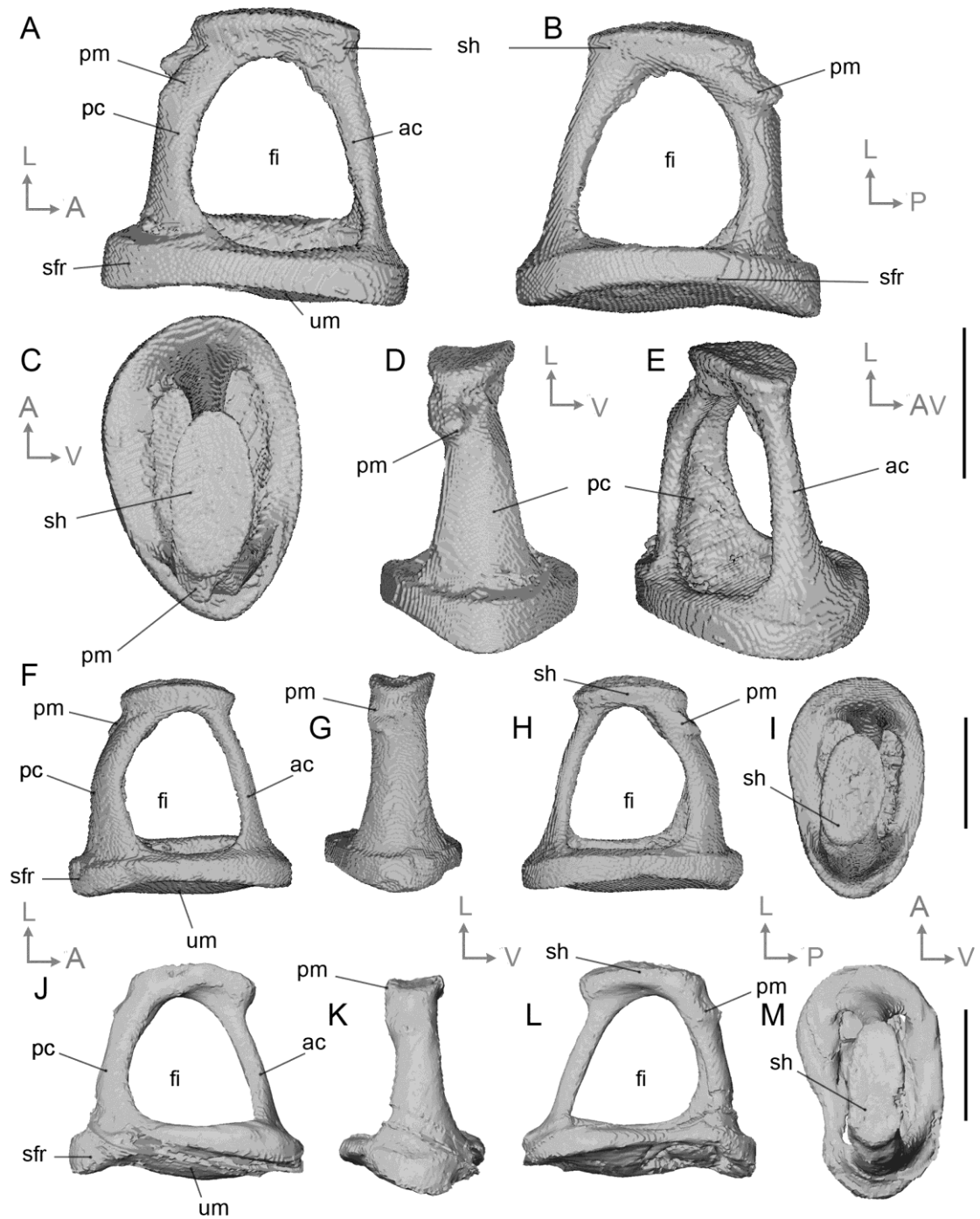
706 **Figure 4.** Left cainotheriid incus from Dams DAM1 (DAM 1 307) in (A) dorsomedial, (B)
 707 lateral, (C) posterior, (D) anterior, views. E-F, Illustration of morphological variability range
 708 as observed in (E) lateral and (F) dorsal views. Abbreviations: iaf, inferior articular facet; ib,
 709 incus bulge; pb, processus brevis; pl, processus longum; plg, processus longum groove; saf,
 710 superior articular facet. Orientations: A, anterior; D, dorsal; L, lateral; M, medial; P, posterior;
 711 V, ventral. Scale bar = 0.5 mm.

712



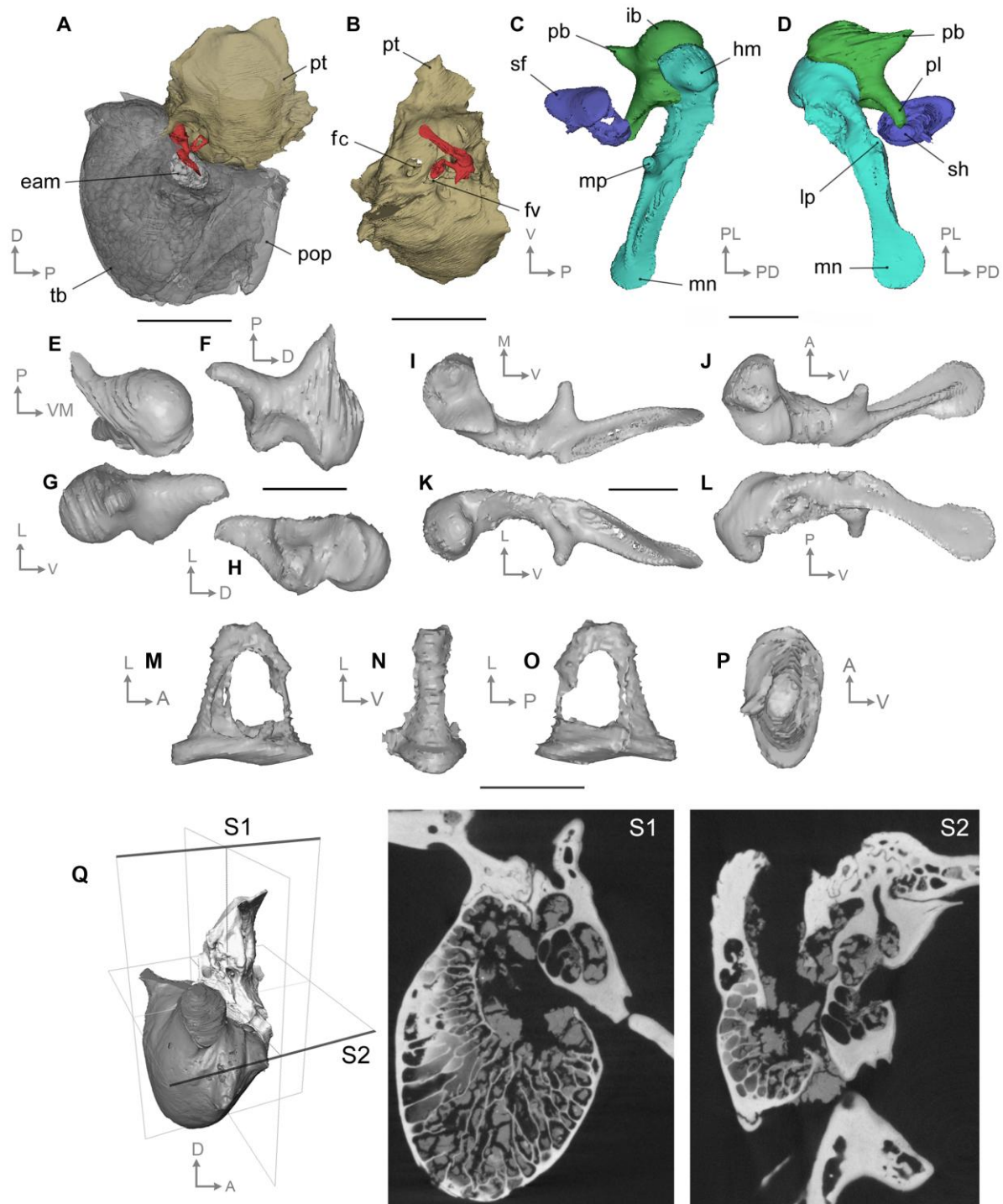
713

714 **Figure 5.** PCA on Procrustes coordinates illustrating morphological variability of the
 715 cainotheriid incus from the localities of DAM1 (grey surface; late Eocene), DAM3 (pink
 716 surface; early Oligocene) and Pech Desse (orange square; late Oligocene). (A) Projection of
 717 individuals on the first factorial plane; (B-C) Patterns of variation along PC1 (B) and PC2 (C)
 718 with maximal values in red and minimal in blue.



719

720 **Figure 6.** Right stapes of Paleogene Cainotheriidae from the Phosphorites of Quercy, SW
 721 France. (A-I) *Paroxacron valdense* from DAM1, late Eocene (A-E, DAM 1 316,) (F-I. DAM
 722 1 317) – A, ventral view; B, dorsal view; C, lateral view; D, posterior view; E, antero dorsal
 723 view. DAM 1 317 (F-I) – F, ventral view; G, posterior view; H, dorsal view; I, lateral view.
 724 (J-M) Cainotheriidae indet. from DAM3, early Oligocene (DAM 3 13) J, ventral view; K,
 725 posterior view; L, dorsal view; M, lateral view; Abbreviations: ac, anterior crus; fi, foramen
 726 intercrurale; pc, posterior crus; pm, processus muscularis; sfr, stapedial footplate rim; sh,
 727 stapes head; um, umbo. Orientations: A, anterior; D, dorsal; L, lateral; M, medial; P,
 728 posterior; V, ventral. Scale bars = 0.5 mm.



729

730 **Figure 7.** 3D reconstruction of the middle ear of *Caenomeryx* cf. *procommunis* from Pech
 731 Desse, late Oligocene, Phosphorites of Quercy, SW France (UM PDS 3353). A-B: Left
 732 middle ear with in-situ ossicles; C-D: virtually reassembled composite left ossicle chain in
 733 ventral (C) and dorsal (D) views; E-H: incus; I-L, malleus; M-P, stapes; Q, Petro-tympanic
 734 complex of UM PDS 3353 showing orthogonal slices (S1 and S2) at putative location of the
 735 processus internus prearticularis (pipa). Abbreviations: eam, external auditory meatus; fc,
 736 fenestra cochleae; fv, fenestra vestibuli; hm, head of malleus; ib, incus bulge; lp, lateral
 737 process of malleus; mn, manubrium; mp, muscular process of malleus; pb, processus brevis of
 738 the incus; pl, processus longum of the incus; pop, paroccipital process; pt, petrosal bone; sf,

739 stapedial footplate; sh, stapes head; tb, tympanic bulla. Orientations: A, anterior; D, dorsal; L,
740 lateral; M, medial; P, posterior; V, ventral. Scale bars, A-B = 5 mm; C-P = 1 mm.
741
742