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# Structure, ontogeny and evolution of the patellar tendon in emus (*Dromaius novaehollandiae*) and other palaeognath birds

The patella (kneecap) exhibits multiple evolutionary origins in birds, mammals, and lizards, and is thought to increase the mechanical advantage of the knee extensor muscles. Despite appreciable interest in the specialized anatomy and locomotion of palaeognathous birds (ratites and relatives), the structure, ontogeny and evolution of the patella in these species remains poorly characterized. Within Palaeognathae, the patella has been reported to be either present, absent, or fused with other bones, but it is unclear how much of this variation is real, erroneous or ontogenetic. Clarification of the patella's form in palaeognaths would provide insight into the early evolution of the patella in birds, in addition to the specialized locomotion of these species. Findings would also provide new character data of use in resolving the controversial evolutionary relationships of palaeognaths. In this study, we examined the gross and histological anatomy of the emu patellar tendon across several age groups from five weeks to 18 months. We combined these results with our observations and those of others regarding the patella in palaeognaths and their outgroups (both extant and extinct), to reconstruct the evolution of the patella in birds. We found no evidence of an ossified patella in emus, but noted its tendon to have a highly unusual morphology comprising large volumes of adipose tissue contained within a collagenous meshwork. The emu patellar tendon also included increasing amounts of a cartilage-like tissue throughout ontogeny. We speculate that the unusual morphology of the patellar tendon in emus results from assimilation of a peri-articular fat pad, and metaplastic formation of cartilage, both potentially as adaptations to increasing tendon load. We corroborate previous observations of a 'double patella' in ostriches, but in contrast to some assertions, we find independent (i.e. unfused) ossified patellae in kiwis and tinamous. Our reconstructions suggest a single evolutionary origin of the patella in birds and that the ancestral patella is likely to have been a composite structure comprising a small ossified portion, lost by some species (e.g. emus,

moa) but expanded in others (e.g. ostriches).

1 Title: Structure, ontogeny and evolution of the patellar tendon in emus (*Dromaius*  
2 *novaehollandiae*) and other palaeognath birds

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## 16 Introduction

17 The patella (kneecap) is a sesamoid bone which develops within the main extensor tendon of  
18 the knee. Although the avian patella evolved independently from those of both lizards and  
19 mammals (Dye 1987; Sarin et al. 1999), it is unclear when and in what form the avian patella  
20 first evolved, and whether it might also have evolved repeatedly within avian species. The  
21 phylogenetic position of Palaeognathae (ratites and their relatives; emus, ostriches, tinamous,  
22 kiwis and kin) as a relatively basal clade of birds – sister group to the enormously diverse  
23 Neognathae, comprising all other crown clade birds – means that they are an important  
24 outgroup for exploring the early evolution of traits, such as the patella, in birds.

25 The configuration of the main knee extensor tendon in palaeognathous birds is a subject of  
26 some ambiguity. Some species are reported to have a patella (Deeming 1999; Picasso 2010),  
27 whereas in others the equivalent structure is said to be absent (Shanathi et al. 2007); available  
28 data and literature indicate several contradictions. The patella's potential to alter limb  
29 mechanics means that elucidation of this tendon's configuration could provide insight into the  
30 evolution of cursoriality and large body size in ratites, and whether flight has been lost  
31 multiple times in palaeognaths. Skeletal development has long been used as a source of data  
32 for phylogenetic analyses (Maisano 2002; Sánchez-Villagra 2002; Maxwell & Larsson 2009),  
33 and additionally the little-studied patella may provide another morphological characters of  
34 use in resolving the unclear and highly controversial evolutionary relationships and history of  
35 palaeognathous birds (Cracraft et al. 2004; Harshman et al. 2008; Bourdon et al. 2009;  
36 Johnston 2011; Smith et al. 2013; Mitchell et al. 2014).

37 The incomplete understanding and imprecision regarding form, function, ontogeny and  
38 phylogeny of patellae in palaeognaths is typical of most, if not all, sesamoid bones. The  
39 frequent perception of patellar structures as unimportant (Pearson & Davin 1921a; Kaiser  
40 2007; Vickaryous & Olson 2007) is probably the reason that few studies have examined this  
41 anatomical region in detail, particularly in birds. As such, the configuration of the patella  
42 (and its tendon) in palaeognaths is a confused issue. Within the clade, ratites have been  
43 described to lack the patella by some (Thompson 1890; Fowler 1991; Stewart 1994). In  
44 contrast, others note the prominence of the patella in these species (de Vriese 1909), and an  
45 extensive review of bird anatomical characteristics compiled by Livezey and Zusi (2006) lists  
46 it as present in all ratites.

47 Gadow (1880, 1885) writes (though does not illustrate) that ratites do in fact have patellae,  
48 but that they give the impression of absence through fusion of the patella to the tibial crest.  
49 Superficially this seems supported by other studies, which note fusion of the patella in kiwis  
50 and extinct moa (Beale 1985; Beale 1991; Sales 2005; Turvey & Holdaway 2005). However,  
51 Beale (1985) expressed doubt over his identification of the patella in kiwis, noting that such  
52 fusion would be unusual. Indeed, Beale's published radiographs confirm his doubts by  
53 showing that the form and position of the 'patella' structure is more consistent with that of  
54 the cranial cnemial crest of the tibia (also called the crista cnemialis cranialis, proximal tibial  
55 ossification centre, or tibial epiphysis).

56 The cranial cnemial crest is a “traction epiphysis” (Hogg 1980; Hutchinson 2002) – an  
57 initially separate intratendinous ossification that fuses with the tibia – and so can easily be  
58 misidentified as a patellar sesamoid. The true patella remains independent throughout life in  
59 most birds and is normally located well proximal to the cranial cnemial crest, within the  
60 patellar sulcus of the femur. In contrast, the development of the cranial cnemial crest and its  
61 fusion to the tibia (remaining entirely separate from the patella) is well documented in  
62 embryological/ontogenetic studies (Hogg 1980; Pourlis & Antonopoulos 2013). It seems that  
63 kiwis do possess a true independent patella in the expected location, as illustrated in a  
64 myological study of adult kiwis (McGowan 1979), but misidentification seems to be a  
65 prominent problem, with several other studies likewise mistakenly identifying the cranial  
66 cnemial crest as the patella in birds.

67 The potential for misidentification of the patella (or cranial cnemial crest) makes it difficult to  
68 evaluate statements when there are no clear descriptions or accompanying evidence.

69 Ostriches (*Struthio*) are well documented to have both a true (unfused) patella and a second  
70 more distal ossification, distinct from the first; a so-called double patella (Haughton 1864;  
71 Macalister 1864; Thompson 1890; de Vriese 1909; Deeming 1999; Gangl et al. 2004). For  
72 the other Palaeognathae, however, there are fewer definitive data explicitly exploring patella  
73 presence and form, and where they do exist these data lack clear illustrations. For example,  
74 some authors refer to a patella in greater rheas (*Rhea americana*) (Brinkmann 2010; Picasso  
75 2010), whilst other earlier literature surprisingly suggests that this species possesses a double  
76 patella as in ostriches (de Vriese 1909). A patella is also referred to in dwarf cassowaries  
77 (*Casuarius bennetti*) (Brinkmann 2010) but has not been found in a limited study of southern  
78 cassowaries (*Casuarius casuarius*) (Biggs & Zoo 2013). Volant tinamous are said to have  
79 only a fibrocartilaginous structure in lieu of an ossified patella (Parker 1864). In extinct  
80 elephant birds (Aepyornithidae), patellar presence has been inferred from muscle scars on  
81 the cnemial crest (Livezey & Zusi 2006). However, the latter evidence is inconclusive  
82 because the patella itself would not articulate to such scars, but rather do so via a patellar  
83 tendon, and so the scars cannot by themselves establish the presence or absence of a patellar  
84 sesamoid bone. An independent patella was mentioned in just one specimen of moa  
85 (*Dinornithiformes*) described by Owen (1883). Thus confusion, misidentification and  
86 specimen rarity all combine to make ascertaining the status of the patella in palaeognath  
87 species difficult.

88 Like almost all members of its clade, the presence and configuration of the patella and its  
89 tendon in emus (*Dromaius novaehollandiae*) is also unclear. Myological studies have not  
90 explored the question (Patak & Baldwin 1998), and whilst one study lists patellar presence  
91 early in ontogeny (Maxwell & Larsson 2009), another has described the patella to be absent  
92 in adult emu specimens (Shanthi et al. 2007). The former finding, however, is again a cranial  
93 cnemial crest rather than a patella (Maxwell pers. comm, 2013). In this study, we first  
94 describe the gross and microscopic anatomy of the emu patellar tendon throughout ontogeny,  
95 in order to better understand the patellar phenotype in this species. We then put these data  
96 into the context of the evolution of Palaeognathae as a whole, using other published data and  
97 our own novel observations to infer the evolution of the patella in this clade. In doing so we

98 aim to clarify the status of the patella and its tendon amongst palaeognath species, infer how  
99 many times the patella has evolved and its temporal origin(s) within birds, and reconstruct  
100 patterns of evolutionary change (i.e. patellar loss or expansion) in this lineage.

101

## 102 **Materials and Methods**

103 A note on terminology: the triceps femoris muscle group is equivalent to the mammalian  
104 quadriceps femoris group, and in birds comprises Mm. iliotibialis (3 main heads),  
105 femorotibialis (3 heads), and ambiens (2 heads in ostriches; 1 in other palaeognaths). The  
106 common tendon of these extensor muscles is called the patellar tendon, aponeurosis, or  
107 sometimes ligament. Compositionally, this structure has been shown to be closer to tendon  
108 than ligament (Bland & Ashhurst 1997; Livezey & Zusi 2006), and it is homologous with the  
109 tendon that contains the patella in ratite and other birds, and so herein will be referred to as  
110 the patellar tendon, even if a patellar sesamoid ossification is absent.

111 We harvested the right patellar tendon from nine emu (*Dromaius novaehollandiae*) cadavers,  
112 which were euthanized as part of another study and stored frozen (-20°C). The emus were  
113 from a UK farm population (Leicestershire Emus and Rheas, Leicestershire). Four were five  
114 weeks old (mean bodyweight 4.41kg ± standard deviation of 0.94kg), one was six months  
115 (19.3kg), one was 12 months (30kg), and three were 18 months (mean 40.2kg ± 2.25kg). All  
116 lacked any obvious musculoskeletal or gait pathologies that might complicate our  
117 interpretations. We also collected the left patellar tendon and patellae from an adult (71.3kg)  
118 ostrich (*Struthio camelus*) and the right patellar tendon and patella from an adult (1.8kg)  
119 guinea fowl (*Numida meleagris*), for comparative purposes, and checked the gross  
120 morphology of these against other museum specimens and the literature to ensure they were  
121 representative of these two species.

122 The patellar tendons were dissected out of the thawed cadavers, fixed in 10% neutral buffered  
123 formalin and radiographed for evidence of gross mineralisation using a high powered ceiling  
124 mounted X-ray generator (Polydoros, Siemens Medical, Erlangen, Germany) in combination  
125 with a computerised radiography system (FCR Profect CS, Fujifilm, Bedfordshire, UK),  
126 using settings of 60kV and 32mAs.

127 All specimens were sectioned longitudinally along the midline and, size permitting, most  
128 were further sectioned in longitudinal and transverse directions. The tendon sections were  
129 dehydrated and embedded in paraffin wax blocks for histological examination under light  
130 microscopy. Microtome sections were cut between 4-6µm, and stained with routine  
131 Haematoxylin and Eosin as well as SafraninO/Fast green stain for cartilage and von Kossa for  
132 calcium salts (von Kossa 1901). Ostrich sections were additionally stained with Masson's  
133 trichrome to highlight features of bone micro-anatomy.

134 We reconstructed evolutionary patterns in Mesquite software (Maddison & Maddison 2011),  
135 using the phylogeny of Livezey and Zusi (Livezey & Zusi 2007) for the main topology of  
136 Ornithurae (crown group birds and their closest fossil outgroups). However, we adopted the

137 alternative phylogeny for crown group birds (Neornithes/Aves) proposed by others (Hackett  
138 et al. 2008; Harshman et al. 2008; Phillips et al. 2010; Smith et al. 2013; Baker et al. 2014;  
139 Mitchell et al. 2014) which places tinamous within ratites and ostriches as occupying the  
140 most basal phylogenetic position within Palaeognathae. Alternate topologies were also  
141 examined in a sensitivity analysis of our conclusions, by comparing the evolutionary patterns  
142 of the patella resulting from the enforcement of different phylogenetic frameworks. We  
143 specifically examined the topologies of Lee et al. (1997), Cooper et al. (2001), Bourdon et al.  
144 (2009) and Johnston (2011).

145 On this basis, we scored the patella as: absent (0), a small flake of bone (1), a rounded nodule  
146 (2), a subrectangular block with strong articular surfaces for the patellar sulcus of the femur  
147 (3) or a craniocaudally expanded triangular crest, rising high above the patellar sulcus (4).  
148 These five graded scores were assumed to be ordered because they form a continuous series  
149 (Table 1). Due to easy confusion between the patella and cranial cnemial crest, we ensured  
150 that the patella was only counted as present when there was documented visual evidence of it,  
151 rather than if its presence was solely supported by in-text statements (unless these were  
152 extremely clear and detailed). This meta-analysis would ideally be from multiple specimens,  
153 and an absent score in juvenile specimens was treated more as uncertainty, rather than as  
154 definitive evidence of absence.

155 To gain further data for this meta-analysis, we also examined avian osteological collections  
156 held by the Natural History Museum at Tring (NHMUK), University Museum of Zoology  
157 Cambridge (UMZC), and the Grant Museum at the University College of London (LDUCZ).

158

## 159 **Results**

### 160 *Patella tendon morphology in ageing emus reveals the lack of an ossified patella*

161 Proximally, the emu patellar tendon in all ages (5 weeks to 18 months) is attached to the  
162 triceps femoris muscles via an abrupt and particularly distinctive junction (Fig. 1A). It is off-  
163 white and fairly firm, particularly proximally, close to its muscle junction. Other muscles  
164 overlie and are attached to the patellar tendon superficially, but are not ideally positioned, or  
165 sufficiently strongly connected, to contribute as much force along their line of action, as is  
166 the triceps femoris. The patellar tendon sits within the patellar groove on the distal femur,  
167 bordered by the lateral (larger) and medial (smaller) femoral condyles. At a position  
168 approximately one-third along its length, whilst it is still within the patellar groove, the  
169 tendon partially splits to form superficial and deep portions (Fig. 1A and B). The superficial  
170 portion bridges the femorotibial joint and inserts onto the lateral and cranial cnemial crests of  
171 the tibiotarsus, whilst the deep portion blends with a grossly fatty structure within the patellar  
172 sulcus of the femur, and is also attached to the cartilaginous femorotibial menisci (Fig. 1B);  
173 mostly to the medial meniscus, but also to cranial and medial parts of the lateral meniscus.  
174 Examination of transverse cross-sections of the patella in emus of all ages (five weeks to 18  
175 months) revealed that splitting of the distal patellar tendon creates a superficial roughly  
176 triangular portion and deep, rounded portion (Fig. 1C).



177 Radiography revealed no evidence of any gross mineralisation in the patellar tendons of 18  
178 month old emus; there was no patellar sesamoid (Fig. 1D-G). This lack of any discernible  
179 mineralisation/ossification was confirmed microscopically, by the absence of calcium salts  
180 using von Kossa staining in birds of this age (data not shown).

181 *Cartilage-like tissue develops within a predominantly adipose patella tendon during emu*  
182 *ontogeny*

183 Histological examination of the emu patellar tendon revealed a basic structure maintained  
184 across all the ages examined, with some variations throughout ontogeny. Histology  
185 confirmed the presence of an abrupt junction between the patellar tendon and the triceps  
186 femoris muscles proximally. In all ages, the collagen fibres here have a dense and  
187 predominantly transverse orientation, which become more longitudinally oriented as they  
188 penetrate into the tendon distally (Fig. 2A). Surprisingly, the body of the patella tendon is  
189 overwhelmingly comprised of adipocytes, with collagen fibre bundles running throughout in  
190 mixed orientations resulting in a meshwork-like appearance. In some sections, a blood vessel  
191 is seen in the proximal deep region of the tendon, within the adipocyte-rich tissue (Fig. 2B).  
192 The body of adipose/collagenous tissue is bounded (both on superficial and deep surfaces) by  
193 continuous, longitudinally-oriented and more classically tendon-like dense collagen bundles  
194 (Fig. 2C). Cells in the collagen fibre bundles are typically slender and fusiform with elongate  
195 nuclei, resembling tenocytes (Fig. 2D).

196 Throughout all observed ontogenetic stages, the tendon's surface is covered with flattened or  
197 polygonal cells in a layer 2-3 cells thick; likely synovial-lining cells. Blood vessels are visible  
198 in the synovial sub-intima and deeper fascia, particularly laterally (apparently away from the  
199 point of tendon contact with bone). Synovial villi are visible on the deep surface in some  
200 sections (Fig. 2E).

201 Where it splits into superficial and deep portions distally, the surfaces of the tendon have a  
202 lining layer of cells that oppose each other (Fig. 2F). The synovial layer is continuous over  
203 the tendon split but does not interdigitate with it.

204 Histological sections from the five week old emu patellar tendons are generally more cellular  
205 overall compared to the older (six, 12 and 18 month old) emus. A further difference between  
206 the age groups is the appearance of an apparent third tissue type, in addition to the adipose  
207 and collagenous tissues already described. In the five week old emu patellar tendons, this  
208 tissue is basophilic with H&E staining and highly vacuolated or vesicular (Fig. 3A). This  
209 material is most abundant in the deep, proximal region of the tendon and also close to the  
210 tendon split laterally. There are lesser amounts along the deep surface distally, and around the  
211 dense collagen bundles running along the proximal (triceps) and superficial muscle-tendon  
212 junctions. The substance is also present in minimal amounts within the body of the tendon,  
213 especially in the deepest third, where it seems to follow collagen bundles as they travel  
214 through the adipose tissue. The cells associated with this material have plump and sometimes  
215 finely stippled nuclei, in contrast to the compressed nuclei of adipocytes or the elongate  
216 nuclei of tenocytes.

217 In the patellar tendon of the six month old emu, the basophilic vesicular tissue is still present,  
218 distributed near to the proximal and superficial muscle junctions, near to the deep surface  
219 proximally and also laterally prior to the tendon split. None is visible in any appreciable  
220 quantity in the tendon distally. This material has taken up more H&E stain and is less  
221 vesicular and more homogenous than in the five week old emus. Certain collagen fibres near  
222 to this material also appear slightly more basophilic (Fig. 3B).

223 Much like the in the six month old emu, the vesicular material in the 12 month emu patellar  
224 tendon takes up more H&E stain and looks less vesicular and more homogenous. In addition,  
225 there are some areas where rounded cells sit within chondrocyte-like lacunae, giving the  
226 tissue a chondroid (cartilage-like) appearance. The tissue has a similar distribution as in the  
227 six month old emu.

228 In the 18 month old emus, the basophilic chondroid and vesicular tissue is most extensive.  
229 This chondroid tissue is abundant along the proximal muscle junction, interspersed between  
230 collagen bundles and often contains large clusters of adipocytes (Fig. 3C). Rounded,  
231 chondroid cells occupy lacunae within the basophilic tissue, and also within nearby collagen  
232 fibre bundles (Fig. 3D). In both the five week and 18 month old patellar tendons,  
233 SafraninO/fast green stains the vesicular and chondroid tissue intensely red, differentiating it  
234 from nearby collagen fibre bundles (Fig. 4A-4D).

235 Patellar tendon sections from the ostrich and adult guineafowl are, by comparison, more  
236 conventional with the patellar tendon comprising predominantly dense collagenous bundles  
237 (5A) and containing ossified patellar sesamoids (one in guineafowl and two in ostriches) (Fig.  
238 5B-D). Similar to the emu, the patellar tendon of the ostrich includes regions of cartilage-like  
239 tissue, especially between and deep to the ossified sesamoids. It also contains areas with  
240 apparently discrete strata of adipose tissue, unlike the diffuse and abundant incorporation of  
241 fat within the emu patellar tendons. Both the proximal and distal ostrich patellae are almost  
242 entirely trabecular bone with fatty and cellular marrow, with regions of well differentiated  
243 cortical bone, mostly superficially, containing secondary osteons running along the bones'  
244 longitudinal axes. There are small areas of calcified cartilage at some edges of the bones, but  
245 no articular cartilage lining the deep surface as in the guineafowl. The guineafowl patella has  
246 an outer compact bone cortex with some longitudinal secondary osteons, inner trabecular  
247 structure with mostly fatty marrow, and a thick articular cartilage pad lining its deep surface.

#### 248 *The patella morphology in other palaeognaths and outgroups*

249 Based upon many extremely well-preserved complete skeletons, particularly from Cretaceous  
250 fossil deposits in China and Mongolia, a patella is clearly absent in extinct birds that are basal  
251 to Hesperornithiformes (Table 1) (Hou 1997; Clarke & Norell 2002; Clarke et al. 2006; Zhou  
252 et al. 2008; Wang et al. 2013). Although a patella may have originated earlier than in the  
253 common ancestor of Hesperornithiformes and Neornithes (see below), the fossil record of  
254 many ornithurine taxa argues strongly against this possibility. Hou (1997) speculated that a  
255 sliver of bone in a *Confuciusornis* specimen was a patella, but its disarticulated position  
256 renders its identification uncertain, and the absence of a patella in dozens of other

257 Confuciusornithidae casts additional doubt on this identification, which could be pathology or  
258 another unusual ossification.

259 In our studies of museum specimens, we have observed a true osseous patella in multiple  
260 tinamou and kiwi specimens. Where present in these taxa, the patella is a small flake-like  
261 bone enclosed in the patellar tendon, situated in the patellar groove of the femur (Fig. 6). We  
262 also observed the double patella in ostriches evident in over 12 specimens from diverse  
263 collections and well documented in the literature (Haughton 1864; Macalister 1864;  
264 Thompson 1890; de Vriese 1909; Deeming 1999; Gangl et al. 2004). In contrast, we did not  
265 find evidence of patellae in other palaeognath specimens (emus, rheas, cassowaries, moa, and  
266 elephant birds).

267 Our reconstructions of the evolution of the patella in birds show it to have evolved only once  
268 in this lineage, in the common ancestor of Neornithes and Hesperornithiformes (Fig. 7).  
269 Other trees (not shown) using variations on the two main topologies presented give similar  
270 results. No matter how the relationships within Palaeognathae are organised (based on the  
271 most detailed recent analyses), our phylogenetic optimizations always show a small flake of  
272 bone as plesiomorphic for Neornithes (ancestral for the Hesperornithiformes + Neornithes  
273 clade); a condition shared by the common ancestor of Neognathae. However, patterns of  
274 patellar evolution within Palaeognathae itself can vary considerably depending on tree  
275 topology.

276

## 277 **Discussion**

278 The patellar tendon of emus has a highly unusual morphology, and lacks any evidence of a  
279 patellar sesamoid ossification. Unlike the typical tendon of most tetrapod vertebrates, in  
280 which the microanatomy consists predominantly of dense, parallel collagenous bundles  
281 (Khan et al. 1999; see also Fig. 5A), the patellar tendon of emus is composed almost entirely  
282 of adipocytes contained in a collagen bundle meshwork. Additionally, in older emus there are  
283 patchy regions of cartilage-like tissue that are most prominent near the proximal muscle-  
284 tendon junction, which corresponds to the location of the patellar sesamoid in most other  
285 birds, including the proximal patella in ostriches. This appears to represent a vestigial, totally  
286 unossified sesamoid remnant.

287 As it first appears, the basophilic tissue seen in the tendons resembles tendon/ligament  
288 changes described elsewhere in the literature as mucoïd degeneration, myxoïd degeneration,  
289 chondroïd metaplasia, and/or fibrocartilage metaplasia (Khan et al. 1999; Vigorita 1999;  
290 Hashimoto et al. 2002; Buck et al. 2009). Terminology varies, but characteristically the  
291 tendon cells become rounded and chondroïd in appearance and produce a stainable,  
292 sometimes granulomatous matrix (Järvinen et al. 1997; Buck et al. 2009). This type of tendon  
293 change is usually reported in the context of tendon pathology, particularly in cases where the  
294 duration of symptoms is longstanding (Hashimoto et al. 2002). Chondroïd cells can also be  
295 seen at tendon/ligament junctions with bone (Vigorita 1999).

296 Similarly, the presence of fat within tendon is associated with age-related degeneration  
297 (Hashimoto et al. 2002). Accumulation of adipocytes in a tendon, variably called  
298 tendolipomatosis, lipoid degeneration, or fatty infiltration, is well documented (Hashimoto et  
299 al. 2002; Gagliano et al. 2013), though the aetiology is unclear. The adipocytes occupy spaces  
300 between the collagen fibres and may amass to such a point that they weaken and disrupt the  
301 fibres (Józsa et al. 1984; Hashimoto et al. 2002). However, it seems unlikely that the  
302 appearance of the patellar tendon in these emus is due to an underlying pathology. The  
303 appearance is present from a young age and consistent between individuals, suggesting that it  
304 represents a 'normal' phenotype for this tendon in emus. Tendinopathies also predispose the  
305 tendon to rupture (Sharma & Maffulli 2005), and there are no reports of patellar tendon  
306 rupture in emus. Rather than tendinopathy, this emu patellar tendon composition is more  
307 likely indication of a limited ability for tendon remodelling (in this case physiologically  
308 rather than pathologically) (Khan et al. 1999). It may also reflect the strange evolutionary  
309 history of emus (and other ratites) which seemingly involves loss of the patella, possibly  
310 during the convergent evolution of flightlessness (e.g., Harshman et al. 2008; Johnston 2011;  
311 Smith et al. 2013; Baker et al. 2014).

312 Tendon is known to modify its structure in response to external mechanical load, and this is a  
313 more plausible explanation for the unusual appearance of the emu patella tendon. The  
314 presence of a cartilage-like tissue in the adult emus is consistent with fibrocartilage formation  
315 observed in tendons subject to bending or compression (Vogel & Peters 2005). This  
316 hypothesis is supported by the apparent ageing-related differentiation of this tissue. In the  
317 youngest emus, corresponding regions exhibit basophilic vesicular material and rounded cells  
318 with plump nuclei; Safranin O-positive staining supports the presence of high proteoglycan  
319 content in this tissue. The evidence suggests that the basophilic vesicular material may be the  
320 extra-fibrillar matrix of the tendon which contains proteoglycan and has a vacuolated  
321 appearance when newly formed (Maffulli et al. 2005). The vesicular appearance might also  
322 result if this tissue derives from metaplastic adipocytes.

323 In older emus, this apparent extra-fibrillar matrix becomes more basophilic and less vesicular.  
324 Some of the rounded cells are surrounded by a lacunar space, giving the impression of  
325 chondrocytes. Nearby collagen bundles are also more basophilic-staining and contain  
326 chondrocyte-like cells, suggesting they are either transforming or being replaced by this  
327 tissue. In the oldest emus, this tissue is highly reminiscent of cartilage and Safranin O  
328 staining indicates it has high proteoglycan content. Regardless, this tissue is far from ossified,  
329 and the absence of a documented patellar ossification in the literature on emu morphology at  
330 least circumstantially supports the inference that this absence represents the general condition  
331 for emus.

332 The appearance of so much fat tissue within what is supposed to be a tendon is less  
333 straightforward to understand. A commonly presumed function of sesamoids is protective;  
334 reinforcing the tendon from high compressive forces as it bends around bone. Fat pads near  
335 joints are hypothesised to have a similar function in cushioning tendon and stress dissipation  
336 (Benjamin et al. 2006). We speculate that emu patellar tendon morphology might be the  
337 result of assimilation of a peri-articular fat pad. Through comparative dissections, we have

338 observed that the deep portion of the emu patellar tendon corresponds in its location and  
339 attachments to the infrapatellar fat pad of other birds, lending some support to our fat pad  
340 assimilation hypothesis. If this hypothesis were correct, it would represent a novel solution  
341 for dealing with tendon compression. Other animals lacking ossified patellae have patellar  
342 tendon modifications (e.g. a fibrocartilaginous patelloid structure in marsupials (Reese et al.  
343 2001)) which are thought to play a similar protective role. However, so far emus appear  
344 unique in possessing diffuse cartilaginous changes and incorporation of fat within the patellar  
345 tendon.

346 In attempting to complete our character matrix, which had ambiguous entries due to a lack of  
347 published data, we have observed osseous patellae in several tinamou and kiwi specimens.  
348 Some previous studies note the patella to be a cartilage (or fibrocartilage) structure in kiwis  
349 and tinamous (Owen 1839; Parker 1864), which has led to it being considered absent as a  
350 skeletal feature by others (e.g. for tinamous in Livezey and Zusi, 2006). In other birds, the  
351 patella begins as a cartilaginous nodule and ossifies late relative to the other bones  
352 (Lansdown 1970; Hogg 1980; Pourlis & Antonopoulos 2013), and considering our studies of  
353 museum specimens it seems likely that this is also the case for (at least some species of)  
354 kiwis and tinamous. It is also possible that in these species, the patella does not ossify in  
355 every mature individual (e.g., the apparent absence of a patella in Beale's (1985, 1991)  
356 studies of kiwis). Other sesamoid bones can exhibit variable presence between individuals  
357 (e.g. dorsal carpal sesamoid (Hogg 1980)); however this would be unusual for the patella  
358 which, as far as research to date has shown, is a relatively constant feature in the species  
359 possessing it. Much larger sample sizes of mature individuals would be needed to answer this  
360 question.

361 Unfortunately, the lack of patellae in other palaeognath specimens cannot reliably be used to  
362 infer its absence. As a small, late-ossifying bone buried in soft tissue, accidental loss would  
363 also be a very plausible explanation for the lack of patellae in osteological museum  
364 specimens. For the species where patellar presence and form remains unclear, further study of  
365 tissues *in situ* is necessary, or substantial evidence from well-preserved museum or fossil  
366 specimens with an unambiguous ossified patella *in situ*.

367 The situation with moa specimens poses an excellent example. Although Owen (1883)  
368 figured and described what he thought to be a patella in one *Dinornis* specimen, we consider  
369 this identification dubious. The absence of a patella in so many other moa skeletons  
370 examined in the NHMUK collection and in the literature, despite the preservation of gut  
371 contents, scleral ossicles, tracheal rings and other fine details in the same specimens renders  
372 the argument that the patella simply was not preserved or found to be implausible.  
373 Unfortunately, Owen's single "patella" specimen has gone missing in the NHMUK  
374 collections (S. Chapman, pers. comm., 2014). It is likely to have been a tarsal sesamoid or  
375 other bone rather than a patella, as the tarsal sesamoids are grossly similar in size and shape  
376 to a patella, are commonly present in moa skeletons (our observations numerous NHMUK  
377 specimens of *Dinornis* and *Pachyornis*) and yet Owen did not describe a tarsal sesamoid.  
378 Regardless, future studies of the anatomy of diverse dinornithiform (and other Palaeognathae,  
379 including the key stem taxa called lithornithids) species should remain alert for the possibility



380 of the presence of a patella in some species or individuals, to test our assumption and Owen's  
381 identification and to refine understanding of the evolution of the patellar sesamoid in  
382 Palaeognathae.

383 Sesamoid structures, such as the patella, are thought to increase the mechanical advantage of  
384 the muscles by increasing the tendon's moment arm around a joint. Sesamoids are also  
385 thought to protect the tendon from the considerable compressive forces it experiences  
386 bending against bony surfaces over joints. These two functions are the most frequently cited  
387 as to why sesamoids like the patella might have (repeatedly) evolved and been retained. The  
388 patella may confer further functional advantages, or develop for other reasons however,  
389 especially if the morphology of the patella when first evolved differs from its current one (i.e.  
390 it may have been exapted into new roles).

391 As the patella may have played an important role in the locomotor adaptation of  
392 palaeognaths, elucidation and comparison of its configuration may also prove informative in  
393 the question of whether ratite flightlessness was inherited from a single flightless ancestor  
394 (synapomorphic) or whether flight has been convergently lost on multiple occasions in ratites  
395 (e.g., Harshman et al. 2008; Johnston 2011; Smith et al. 2013; Baker et al. 2014; Mitchell et  
396 al. 2014). Though a conclusion cannot be made on the basis of this one piece of evidence,  
397 significant differences in ratite knee anatomy and patellar presence/absence are more  
398 plausible in a scenario where there are repeated losses of flight and convergent evolution of  
399 cursorial specializations.

400 Curiously, the loss(es) of an ossified patella in palaeognaths seems paralleled by other  
401 reductions of accessory ossifications in the limbs: the tarsal sesamoid seems lost in most taxa  
402 except some moa (see above; present in numerous NHMUK specimens of *Pachyornis* and  
403 *Dinornis*), and ossifications of the lower limb tendons are likewise absent in almost all taxa—  
404 although we have noticed them in some tinamou specimens and Houde and Haubold (1987)  
405 described them in the stem ratite *Palaeotis*. Both were probably ancestrally present in the  
406 most recent common ancestor of crown group birds (e.g., Hogg 1980; Hutchinson 2002),  
407 although even less is known about the tarsal sesamoid than the patella in birds. The  
408 coincidence of these reductions of limb ossifications with the evolution of cursorial  
409 morphology, flightlessness and large body size in Palaeognathae remains an intriguing  
410 mystery.

411 Our reconstructions of patellar evolution suggests that the patella has a single origin in the  
412 distant ancestor of modern birds (the clade containing Hesperornithiformes + Neornithes),  
413 rather than independent evolutions in Palaeognathae and Neognathae. Given our results, the  
414 form of the ossified patella in their common ancestor is most likely to have been a small,  
415 flattened flake of bone rather than a large lever-like structure as in later birds. As might be  
416 expected, the different tree topologies proposed for Palaeognathae result in different patterns  
417 of patellar evolution within this clade. However, we infer that within palaeognaths, the  
418 osseous patella has been lost at least once by emus and likely in moa too, possibly separately.  
419 In contrast, an ossified patella has been retained by some other palaeognaths and even  
420 evolved into a larger structure (e.g. ostriches). Data from other species (cassowaries, rheas,

421 and especially fossil taxa such as *Lithornis*, stem ostriches or hesperornithiforms, or less well  
422 preserved taxa such as *Ichthyornis*) as well as a robust palaeognath phylogeny would be of  
423 great value in more clearly tracing the evolution of the patella in this clade.

424

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432

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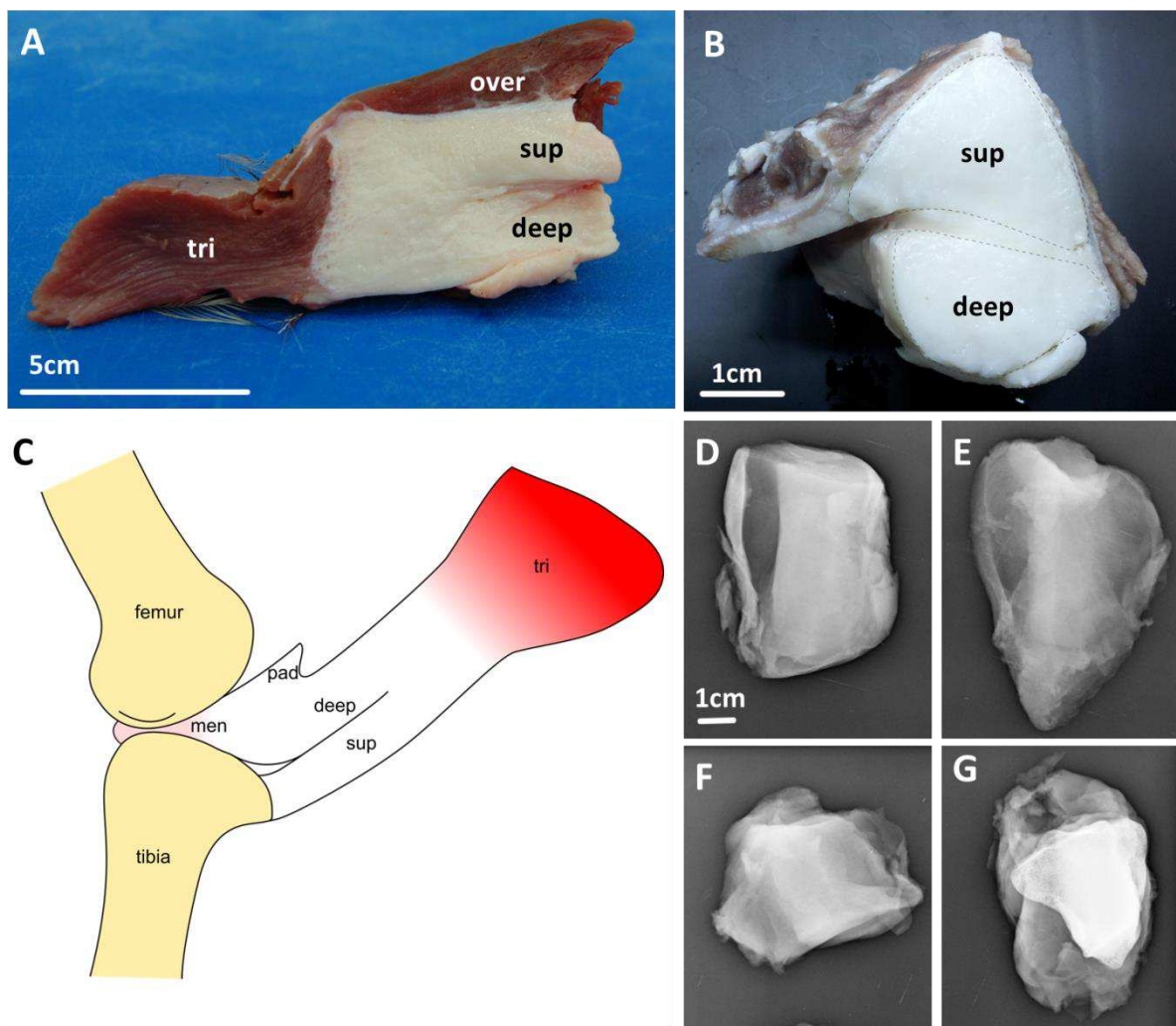
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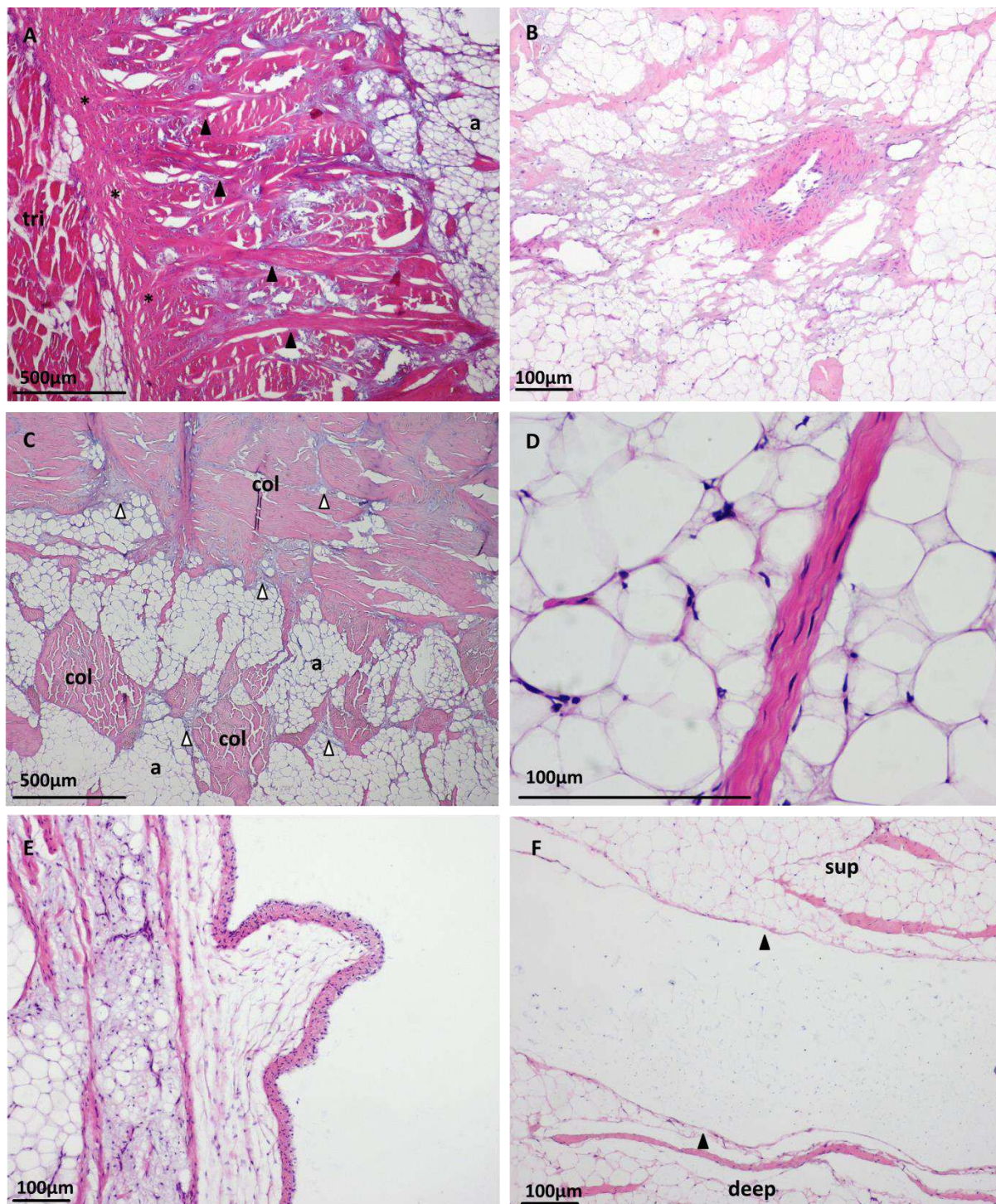
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612

613 Figure 1: Gross patellar morphology. A; excised patellar tendon of a six month old emu in  
 614 longitudinal cross section, showing the triceps femoris muscle group attaching proximally  
 615 (tri), overlying muscles superficially (over), and the tendon partially splitting distally to form  
 616 superficial (sup) and deep (deep) portions. Left of image = proximal. B; transverse cross  
 617 section of the distal patellar tendon from an 18 month old emu, showing the triangular  
 618 superficial portion (sup) and rounded deep portion (deep). Left of image = lateral. C;  
 619 schematic drawing of the emu patellar tendon reflected from the femur. Distally the tendon  
 620 splits into superficial (sup) and deep (deep) portions, with the former inserting onto the  
 621 cranial tibial crest while the latter blends with a triangular fatty structure (pad) that attaches to  
 622 the menisci (men). D-F; patellar tendon radiographs of the three oldest emus (18 months),  
 623 showing no evidence of any mineralisation. Top of image = proximal. G; patellar tendon  
 624 radiograph of a reindeer with similar dimensions as an adult emu tendon, demonstrating the  
 625 appearance of a mineralised patellar sesamoid. Top of image = proximal.





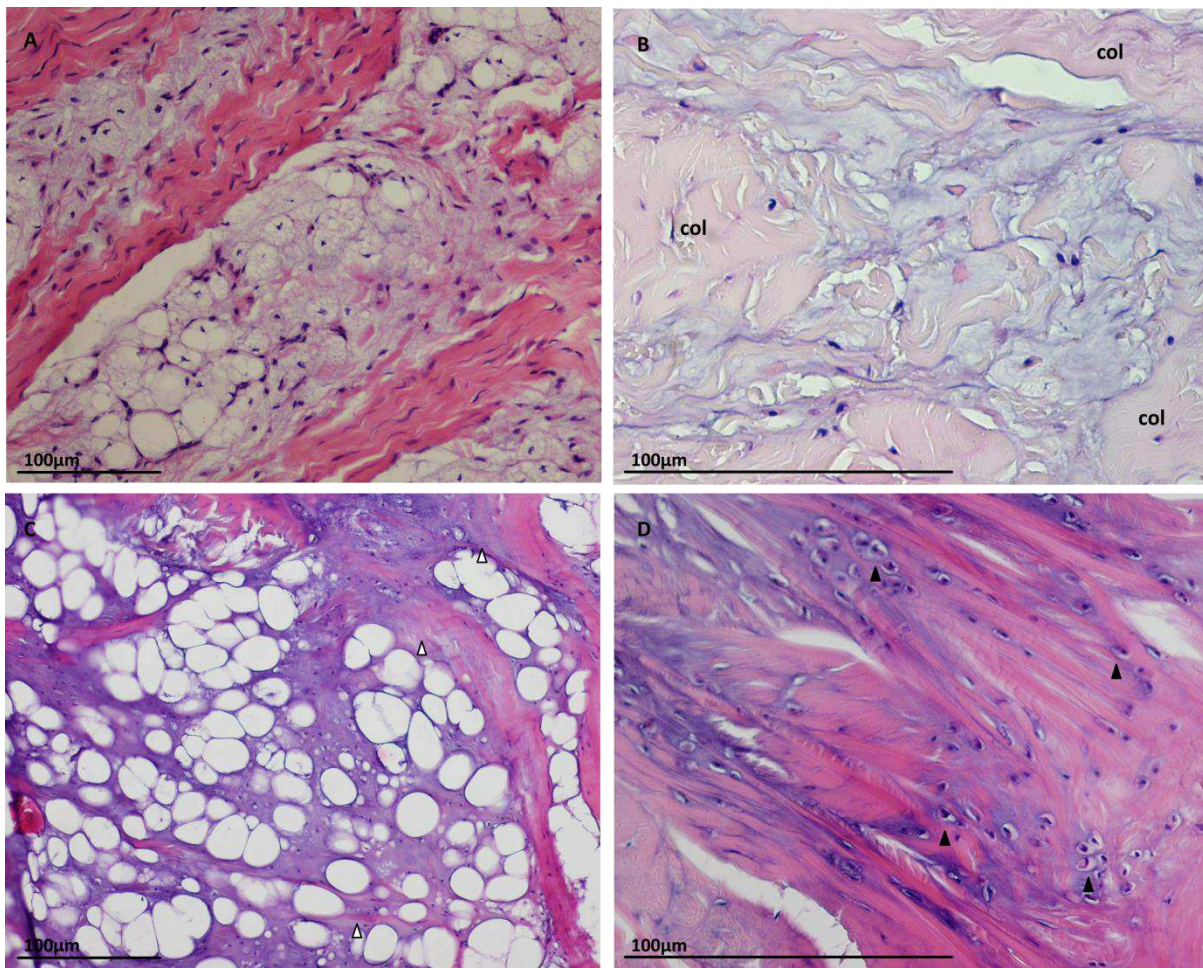
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627 Figure 2: Histology of emu patellar tendon. A; the patellar tendon junction with the triceps  
 628 muscles (tri) in an 18 month old emu. Collagen bundles appear to run transversely along the  
 629 muscle junction (asterisks), before turning longitudinally (filled arrows) and penetrating into  
 630 the adipocyte rich (a) body of the tendon. H&E. B; the patellar tendon of a 12 month old  
 631 emu, with a blood vessel within the tendon body seen in the proximal deep region. H&E. C;  
 632 the patellar tendon of an 18 month old emu. Collagen bundles (col) run along the superficial  
 633 surface (top right of image), interspersed with basophilic vesicular and chondroid tissue  
 634 (unfilled arrows). The body of the tendon is mostly composed of adipocytes (a) and collagen

635 bundles (c) in mixed orientations. Vesicular tissue, when present in the body of the tendon, is  
636 associated with collagen fibre bundles. H&E. D; slender tenocytes within the crimped  
637 collagen fibre bundles. E; patellar tendon of a five week old emu, displaying a synovial villus  
638 on the deep surface, close to the midline of the tendon. H&E. F; the patellar tendon of an 18  
639 month old emu. The edges of the superficial (sup) and deep (deep) portions of the distal  
640 tendon have a lining layer of cells (filled arrows). H&E.

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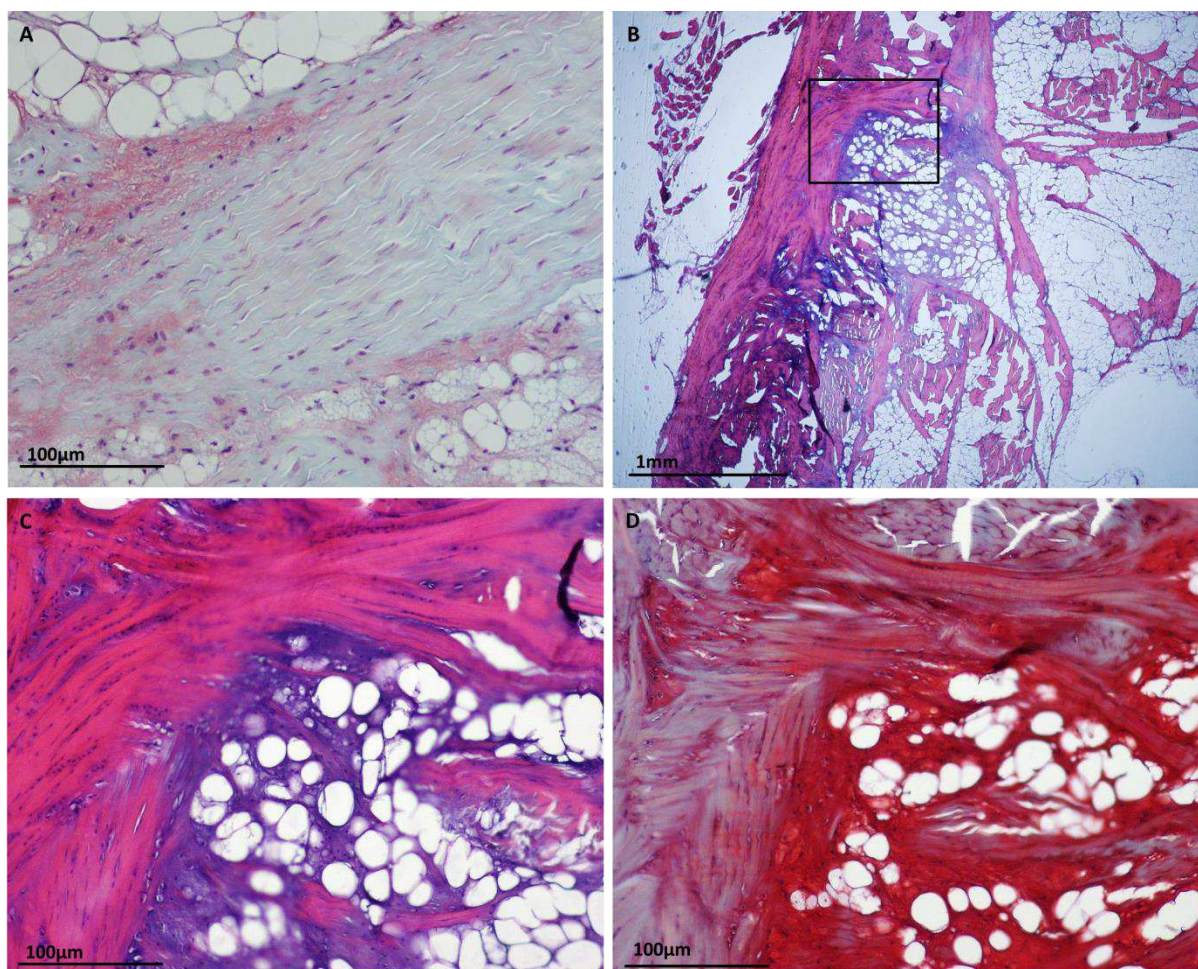




643

644 Figure 3: Differentiation of tissue from a vesicular to cartilage-like appearance with age. A;  
 645 the patellar tendon of a five week old emu, showing the basophilic, vesicular material which  
 646 is often seen in association with collagen fibre bundles. H&E. B; the patellar tendon of a six  
 647 month old emu. The basophilic material has a more homogenous and less vesicular  
 648 appearance than in the five week old emus. The nearby collagen fibre bundles (col) are more  
 649 basophilic here than in other regions of the tendon. H&E. C; the patellar tendon from an 18  
 650 month old emu, near to the proximal muscle junction, showing the basophilic vesicular and  
 651 cartilage-like tissue containing clusters of adipocytes. This tissue has the appearance of  
 652 encroaching on nearby collagen fibre bundles (unfilled arrows). H&E. D; the patellar tendon  
 653 of the same 18 month old emu, from another region near the proximal muscle junction.  
 654 Chondroid cells (filled arrows) sit within the normally eosinophilic collagen fibre bundles,  
 655 creating an intense localised basophilia. H&E.

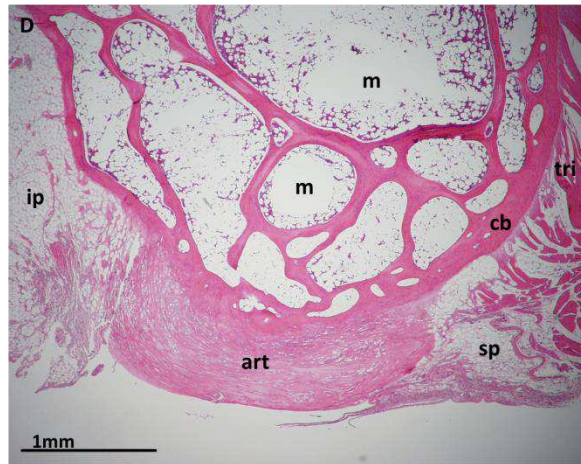
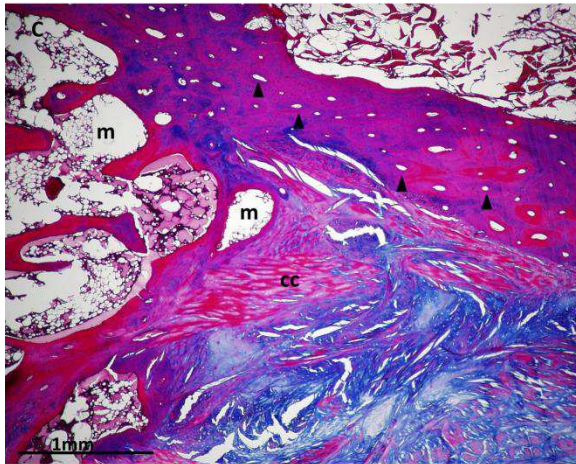
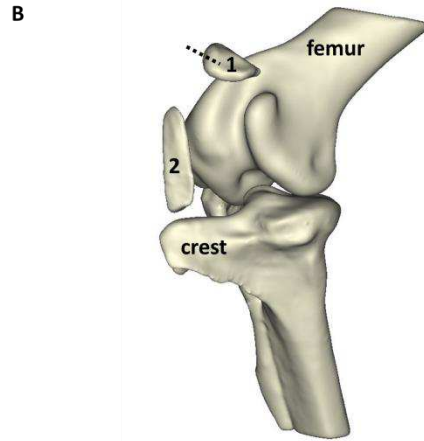
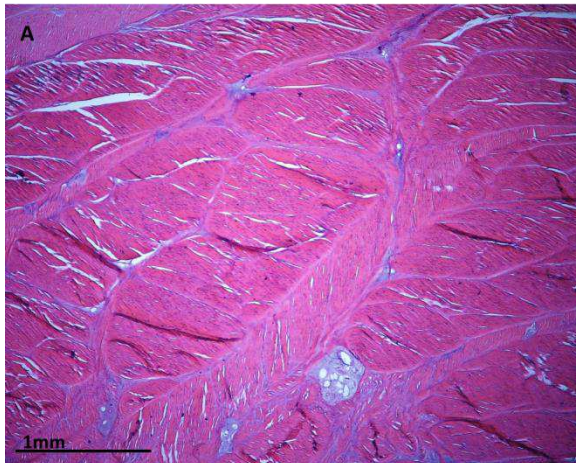




656

657 Figure 4: Appearance of the vesicular and cartilage-like tissue with Safranin O/fast green  
658 staining vs. H&E. A; the patellar tendon of a five week old emu, showing the vesicular  
659 material staining red with Safranin O/fast green, indicating proteoglycan content. Safranin  
660 O/fast green. B; the patellar tendon from an 18 month old emu, showing the deeply  
661 basophilic appearance of the chondroid tissue near the proximal muscle junction with the  
662 triceps tendon (left of image). The black box indicates the region magnified in C and D.  
663 H&E. C; magnified region of chondroid tissue in an 18 month old emu patellar tendon. H&E.  
664 D; the same magnified region showing the chondroid tissue staining intensely red. Safranin  
665 O/fast green.





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667 Figure 5: Configuration of ostrich and guinea fowl patellae. A; part of a longitudinal section  
 668 of the patellar tendon of an adult ostrich, predominantly composed of thick collagenous  
 669 bundles with patchy regions of cartilage-like tissue and the occasional solitary adipocyte.  
 670 H&E. B; 3D segmentation of computed tomography (CT) scan data from ostrich left knee,  
 671 provided by Kyle Chadwick. The proximal patella (1) sits over the patellar sulcus of the  
 672 femur, whilst the distal patella (2) overlies the large medial femoral condyle. The cranial  
 673 cnemial crest (crest) is fused to the tibia in the adult. The dotted line shows the cut through  
 674 the proximal patella seen in Fig C. C; transverse section of the proximal patella of an adult  
 675 ostrich, showing osteons (black arrows) in the superficial aspect of the bone, trabeculae and  
 676 medullary cavity occupying the majority of the patella (m), and calcified cartilage (cc).  
 677 Superficial = top right of image, medial = right of image. Masson's trichrome. D;  
 678 Longitudinal section of an adult guinea fowl patella, showing the triceps muscle attaching  
 679 proximally (tri), suprapatellar (sp) and infrapatellar (ip) fat pads, outer cortical bone (cb),  
 680 inner trabeculated structure, and deep articular cartilage lining (art). Proximal = right of  
 681 image, deep = bottom of image. H&E.





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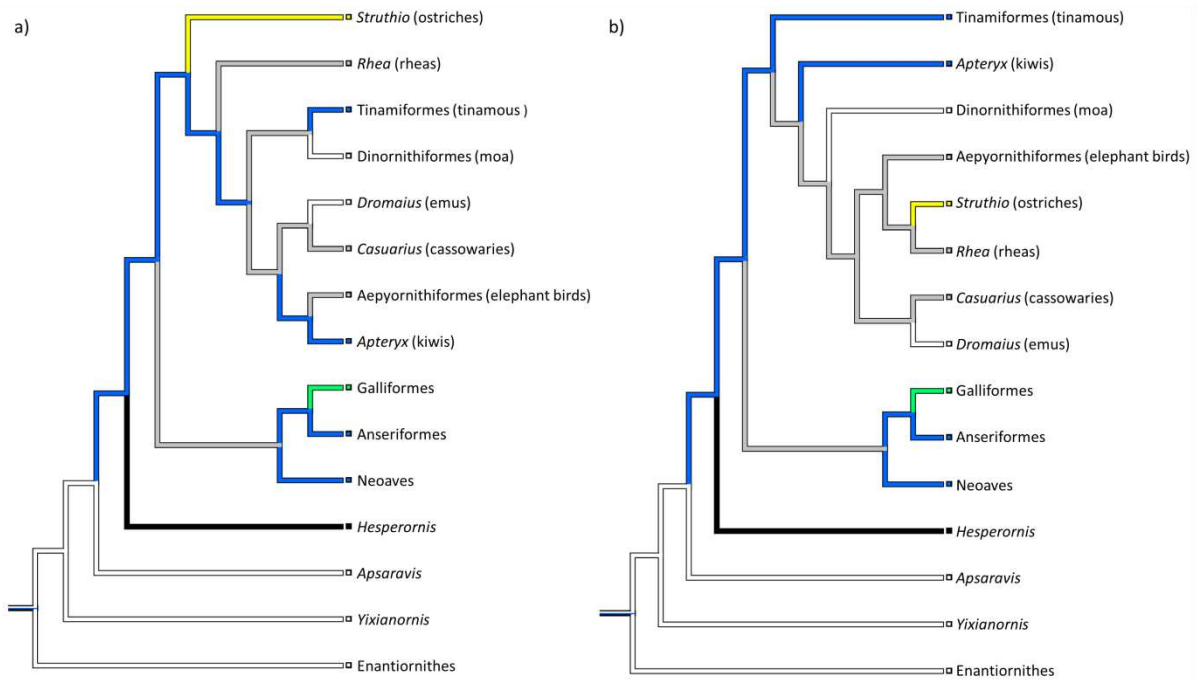
683 Figure 6: Limb bones of a tinamou (*Crypturellus obsoletus* specimen NHMUK S/1972.1.23).

684 Attached to the tibiotarsus (left) is a remnant of the patellar tendon containing a small

685 osseous patella. When articulated, the patella sits within the patellar sulcus of the femur

686 (right).

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Figure 7: Phylogenetic trees constructed from the data of a) Hackett et al 2008, Harshman et al 2008, Phillips 2010, Smith 2013, and Mitchell et al 2014 and b) Livezey and Zusi 2007. When the evolution of the patella is reconstructed over these trees and those of other authors, the patella shows a single origin in birds (blue). Key: grey = ?/no data or ambiguous data; white = 0/absent; blue = 1/single origin; green = 2/nodule of bone; yellow = 3/subrectangular block; black = 4/expanded triangular crest.

<b>Taxon</b>	<b>Patellar form (character state)</b>
Hesperornithiformes	Proximodistally elongate crest (4)
Neoaves	Small flake of bone (1)
Galloanserae	Rounded nodule (2)
<i>Struthio</i> (ostriches)	Large rectangular block with articular surfaces mediolaterally (3)
Rheiidae (rheas)	No data (?)
Tinamiformes (tinamous)	Small flake of bone (1)
<i>Casuarius</i> (cassowaries)	No data (?)
<i>Dromaius</i> (emus)	Absent (0)
Dinornithiformes (moas)	Absent (0)
<i>Apteryx</i> (kiwis)	Small flake of bone (1)
Aepyornithiformes (elephant birds)	No data (?)

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699 Table 1: Character state scores for patellar form in Palaeognathae, Neognathae and  
700 Hesperornithiformes, following the scores 0-4 described in the Methods. Basal outgroups to  
701 these taxa (e.g. *Apsaravis*, *Yixianornis*, *Yanornis*, Enantiornithes, other extinct birds) lack a  
702 patella (see text). Scores for taxa above the genus level were gauged by comparisons of  
703 multiple taxa within that clade and the polarity of characters within it (using published  
704 phylogenies as cited in the main text), where variation existed.

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