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

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Introduction

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

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

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

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

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

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 mucoid degeneration, myxoid degeneration,
289 chondroid 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 chondroid 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). Chondroid 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

Acknowledgements

432

References

434 Baker AJ, Haddrath O, McPherson JD, and Cloutier A. 2014. Genomic support for a moa-
435   tinamou clade and adaptive morphological convergence in flightless ratites.
436   *Molecular Biology and Evolution*.
437 Beale G. 1985. A radiological study of the kiwi (*Apteryx australis mantelli*). *Journal of the*
438   *Royal Society of New Zealand* 15:187-200.
439 Beale G. 1991. The maturation of the skeleton of a kiwi (*Apteryx australis mantelli*) – a ten
440   year radiological study. *Journal of the Royal Society of New Zealand* 21:219-220.
441 Benjamin M, Toumi H, Ralphs JR, Bydder G, Best TM, and Milz S. 2006. Where tendons
442   and ligaments meet bone: attachment sites ('entheses') in relation to exercise and/or
443   mechanical load. *Journal of Anatomy* 208:471-490.
444 Biggs JR. 2013. Captive management guidelines for the southern cassowary *Casuarius*
445   *casuarius johnsonii*. Cairns Tropical Zoo, Cairns. Available at
446   www.aszk.org.au/docs/Cassowaryhusbandryjamesbiggs2013.pdf (accessed 29 May
447   2014).
448 Bland YS, and Ashhurst DE. 1997. Fetal and postnatal development of the patella, patellar
449   tendon and suprapatella in the rabbit; changes in the distribution of the fibrillar
450   collagens. *Journal of Anatomy* 190:327-342.
451 Bourdon E, De Ricqles A, and Cubo J. 2009. A new Transantarctic relationship:
452   morphological evidence for a Rheidae–Dromaiidae–Casuariidae clade (Aves,
453   Palaeognathae, Ratitae). *Zoological Journal of the Linnean Society* 156:641-663.
454 Brinkmann J. 2010. Vergleichende untersuchungen an der hinterextremität palaeognather
455   vögel im hinblick auf deren phylogenetische systematik. Mit überlegungen zum
456   grundplan und zur basalen aufspaltung der rezenten vögel (Neornithes). Eberhard
457   Karls Universität Tübingen.
458 Buck FM, Grehn H, Hilbe M, Pfirrmann CW, Manzanell S, and Hodler J. 2009. Degeneration
459   of the long biceps tendon: comparison of MRI with gross anatomy and histology.
460   *American Journal of Roentgenology* 193:1367-1375.
461 Clarke JA, and Norell MA. 2002. The morphology and phylogenetic position of *Apsaravis*
462   *ukhaana* from the Late Cretaceous of Mongolia. *American Museum Novitates* 3387:1-
463   46.

Clarke JA, Zhou Z, and Zhang F. 2006. Insight into the evolution of avian flight from a new clade of Early Cretaceous ornithurines from China and the morphology of *Yixianornis grabaui*. *Journal of Anatomy* 208:287-308.

Cooper A, Lalueza-Fox C, Anderson S, Rambaut A, Austin J, and Ward R. 2001. Complete mitochondrial genome sequences of two extinct moas clarify ratite evolution. *Nature* 409:704-707.

Cracraft J, Barker FK, Braun M, Harshman J, Dyke GJ, Feinstein J, Stanley S, Cibois A, Schikle P, Beresford P, García-Moreno J, Sorenson MD, Yuri T, and Mindell DP. 2004. Phylogenetic relationships among modern birds (Neornithes): towards an avian tree of life In: Cracraft J, and Donoghue MJ, eds. *Assembling the Tree of Life*. Oxford, GB: Oxford University Press, 468-489.

de Vriese B. 1909. Recherches sur l'anatomie comparée de la rotule. *Bulletin de L'Academie Royale de Medecine de Belgique*.

Deeming DC. 1999. *The Ostrich: Biology, Production and Health*: CABI.

Dye SF. 1987. An evolutionary perspective of the knee. *The Journal of Bone & Joint Surgery* 69:976-983.

Fowler ME. 1991. Comparative clinical anatomy of ratites. *Journal of Zoo and Wildlife Medicine* 22:204-227.

Gadow H. 1880. *Zur vergleichenden anatomie: der muskulatur des beckens und der hinteren gliedmasse der ratiten* G. Fischer.

Gadow H. 1885. On the anatomical differences in the three species of rhea. *Proceedings of the Zoological Society of London* 53:308-322.

Gagliano N, Menon A, Martinelli C, Pettinari L, Panou A, Milzani A, Dalle-Donne I, and Portinaro NM. 2013. Tendon structure and extracellular matrix components are affected by spasticity in cerebral palsy patients. *Muscles Ligaments Tendons J* 3:42-50.

Gangl D, Weissengruber GE, Egerbacher M, and Forstenpointner G. 2004. Anatomical description of the muscles of the pelvic limb in the ostrich (*Struthio camelus*). *Anatomia Histologia Embryologica* 33:100-114.

Hackett SJ, Kimball RT, Reddy S, Bowie RC, Braun EL, Braun MJ, Chojnowski JL, Cox WA, Han K-L, and Harshman J. 2008. A phylogenomic study of birds reveals their evolutionary history. *Science* 320:1763-1768.

Harshman J, Braun EL, Braun MJ, Huddleston CJ, Bowie RCK, Chojnowski JL, Hackett SJ, Han K-L, Kimball RT, Marks BD, Miglia KJ, Moore WS, Reddy S, Sheldon FH, Steadman DW, Steppan SJ, Witt CC, and Yuri T. 2008. Phylogenomic evidence for multiple losses of flight in ratite birds. *Proceedings of the National Academy of Sciences* 105: 13462-13467.

Hashimoto T, Nobuhara K, and Hamada T. 2002. Pathologic evidence of degeneration as a primary cause of rotator cuff tear. *Clincial Orthopaedics and Related Research* 415:111-120.

Haughton S. 1864. Notes on Animal Mechanics: No. 3. On the muscular mechanism of the leg of the ostrich. *Proceedings of the Royal Irish Academy (1836-1869)* 9:50-61.

Hogg D. 1980. A re-investigation of the centres of ossification in the avian skeleton at and after hatching. *Journal of Anatomy* 130:725.

Hou L. 1997. Mesozoic birds of China. *Phoenix Valley Bird Park, Lugu Hsiang, Taiwan*:1-137.

Houde P, and Haubold H. 1987. *Palaeotis weigelti* restudied: a small middle Eocene ostrich (Aves: Struthionithiformes). *Palaeovertebrata* 17:27-42.

512 Hutchinson JR. 2002. The evolution of hindlimb tendons and muscles on the line to crown-
513   group birds. *Comparative Biochemistry and Physiology Part A: Molecular &*
514   *Integrative Physiology* 133:1051-1086.
515 Järvinen M, Józsa L, Kannus P, Järvinen TL, Kvist M, and Leadbetter W. 1997.
516   Histopathological findings in chronic tendon disorders. *Scandinavian Journal of*
517   *Medicine & Science in Sports* 7:86-95.
518 Johnston P. 2011. New morphological evidence supports congruent phylogenies and
519   Gondwana vicariance for palaeognathous birds. *Zoological Journal of the Linnean*
520   *Society* 163:959-982.
521 Józsa L, Réffy A, and Bálint J. 1984. The pathogenesis of tendolipomatosis; an electron
522   microscopical study. *International Orthopaedics* 7:251-255.
523 Kaiser GW. 2007. *The Inner Bird: Anatomy and Evolution.* UBC Press.
524 Khan KM, Cook JL, Bonar F, Harcourt P, and Åstrom M. 1999. Histopathology of common
525   tendinopathies: Updates and implications for clinical management. *Sports Medicine*
526   27:393-408.
527 Lansdown A. 1970. A study of the normal development of the leg skeleton in the quail
528   (*Coturnix coturnix japonica*). *Journal of Anatomy* 106:147.
529 Lee K, Feinstein J, and Cracraft J. 1997. The phylogeny of ratite birds: resolving conflicts
530   between molecular and morphological data sets. *Avian molecular evolution and*
531   *systematics*:173-211.
532 Livezey BC, and Zusi RL. 2006. Higher-order phylogeny of modern birds (Theropoda, Aves:
533   Neornithes) based on comparative anatomy: I. – Methods and characters. *Bulletin of*
534   *the Carnegie Museum of Natural History* 37:1-556.
535 Livezey BC, and Zusi RL. 2007. Higher-order phylogeny of modern birds (Theropoda, Aves:
536   Neornithes) based on comparative anatomy. II. Analysis and discussion. *Zoological*
537   *Journal of the Linnean Society* 149:1-95.
538 Macalister A. 1864. On the anatomy of the ostrich (*Struthio camelus*). Proceedings of the
539   Royal Irish Academy: JSTOR. p 1-24.
540 Maddison WP, and Maddison DR. 2011. Mesquite: a modular system for evolutionary
541   analysis. Version 2.75 http://mesquiteproject.org
542 Maffulli N, Renstrom P, and Leadbetter WB. 2005. *Tendon Injuries: Basic Science and*
543   *Clinical Medicine*: Springer.
544 Maisano J. 2002. The potential utility of postnatalskeletal developmental patterns in
545   squamate phylogenetics. *Zoological Journal of the Linnean Society* 136:277-313.
546 Maxwell EE, and Larsson HCE. 2009. Comparative ossification sequence and skeletal
547   development of the postcranium of palaeognathous birds (Aves: Palaeognathae).
548   *Zoological Journal of the Linnean Society* 157:169-196.
549 McGowan C. 1979. The hind limb musculature of the brown kiwi, *Apteryx australis mantelli*.
550   *Journal of Morphology* 160:33-73.
551 Mitchell KJ, Llamas B, Soubrier J, Rawlence NJ, Worthy TH, Wood J, Lee MS, and Cooper
552   A. 2014. Ancient DNA reveals elephant birds and kiwi are sister taxa and clarifies
553   ratite bird evolution. *Science* 344:898-900.
554 Owen P. 1839. On the anatomy of the southern Apteryx (*Apteryx australis*, Shaw). *The*
555   *Transactions of the Zoological Society of London* 2:257-301.
556 Owen P. 1883. On Dinornis (Part XXIII.): containing a description of the skeleton of
557   *Dinornis parvus*, Owen. *The Transactions of the Zoological Society of London*
558   11:233-256.
559 Parker WK. 1864. V. On the osteology of gallinaceous birds and tinamous. *The Transactions*
560   *of the Zoological Society of London* 5:149-241.

561   Patak AE, and Baldwin J. 1998. Pelvic limb musculature in the emu *Dromaius*
562   *novaehollandiae* (Aves: Struthioniformes: Dromaiidae): adaptations to high-speed
563   running. *Journal of Morphology* 238:23-37.
564   Pearson K, and Davin A. 1921a. On the sesamoids of the knee-joint. Part I: Man. *Biometrika*
565   13:133-175.
566   Phillips MJ, Gibb GC, Crimp EA, and Penny D. 2010. Tinamous and moa flock together:
567   mitochondrial genome sequence analysis reveals independent losses of flight among
568   ratites. *Systematic Biology* 59:90-107.
569   Picasso MB. 2010. The hindlimb muscles of *Rhea americana* (Aves, Palaeognathae,
570   Rheidae). *Anatomia Histologia Embryologia* 39:462-472.
571   Pourlis A, and Antonopoulos J. 2013. The ossification of the pelvic girdle and leg skeleton of
572   the quail (*Coturnix coturnix japonica*). *Anatomia Histologia Embryologia*.
573   Reese S, Pfuderer UR, Bragulla H, Loeffler K, and Budras KD. 2001. Topography, structure
574   and function of the patella and the patelloid in marsupials. *Anatomia Histologia*
575   *Embryologia* 30:289-294.
576   Sales J. 2005. The endangered kiwi: a review. *Folia Zoologica* 54:1.
577   Sánchez-Villagra MR. 2002. Comparative patterns of postcranial ontogeny in therian
578   Mammals: An analysis of relative timing of ossification events. *Journal of*
579   *Experimental Zoology* 294:264-273.
580   Sarin VK, Erickson GM, Giori NJ, Bergman AG, and Carter DR. 1999. Coincident
581   development of sesamoid bones and clues to their evolution. *The Anatomical Record*
582   257:174-180.
583   Shanthi LM, Chandra Sekhara RTS, Jagapathi RP, and Ravindrareddy Y. 2007. Gross
584   anatomical studies on the os coxae and femur of emu (*Dromaius novaehollandiae*).
585   *Indian Journal of Poultry Science* 42:61-63.
586   Sharma P, and Maffulli N. 2005. Tendon injury and tendinopathy: healing and repair. *Journal*
587   *of Bone and Joint Surgery American Edition* 87:187-202.
588   Smith JV, Braun EL, and Kimball RT. 2013. Ratite nonmonophyly: independent evidence
589   from 40 novel Loci. *Systematic Biology* 62:35-49.
590   Stewart J. 1994. Ratites. In: Ritchie BW, Harrison GJ, and Harrison LR, eds. *Avian*
591   *Medicine: Principles and Application*. Florida: Wingers Publishing, Inc., 1284-1326.
592   Thompson DAW. 1890. On the systematic position of *Hesperornis*. *University College,*
593   *Dundee, Studies from the Museum of Zoology* 10:1-15.
594   Turvey ST, and Holdaway RN. 2005. Postnatal ontogeny, population structure, and extinction
595   of the giant moa *Dinornis*. *Journal of Morphology* 265:70-86.
596   Vickaryous MK, and Olson WM. 2007. Sesamoids and Ossicles in the Appendicular
597   Skeleton. In: Hall BK, ed. *Fins into Limbs: Evolution, Development and*
598   *Transformation*. Chicago 60637: University of Chicago Press, 323-341.
599   Vigorita VJ. 1999. *Orthopaedic Pathology*: Lippincott Williams & Wilkins.
600   Vogel K, and Peters J. 2005. Histochemistry defines a proteoglycan-rich layer in bovine
601   flexor tendon subjected to bending. *Journal of Musculoskeletal and Neuronal*
602   *Interactions* 5:64-69.
603   von Kossa J. 1901. Ueber die im organismus kunstlich erzeugbaren verkalkungen. *Beiträge*
604   *für Pathologische Anatomie* 29.
605   Wang X-r, Ji Q, Teng F-f, and Jin K-m. 2013. A new species of *Yanornis* (Aves: Ornithurae)
606   from the Lower Cretaceous strata of Yixian, Liaoning Province. *Geological Bulletin*
607   *of China* 32.
608   Zhou Z, Clarke J, and Zhang F. 2008. Insight into diversity, body size and morphological
609   evolution from the largest Early Cretaceous enantiornithine bird. *Journal of Anatomy*
610   212:565-577.

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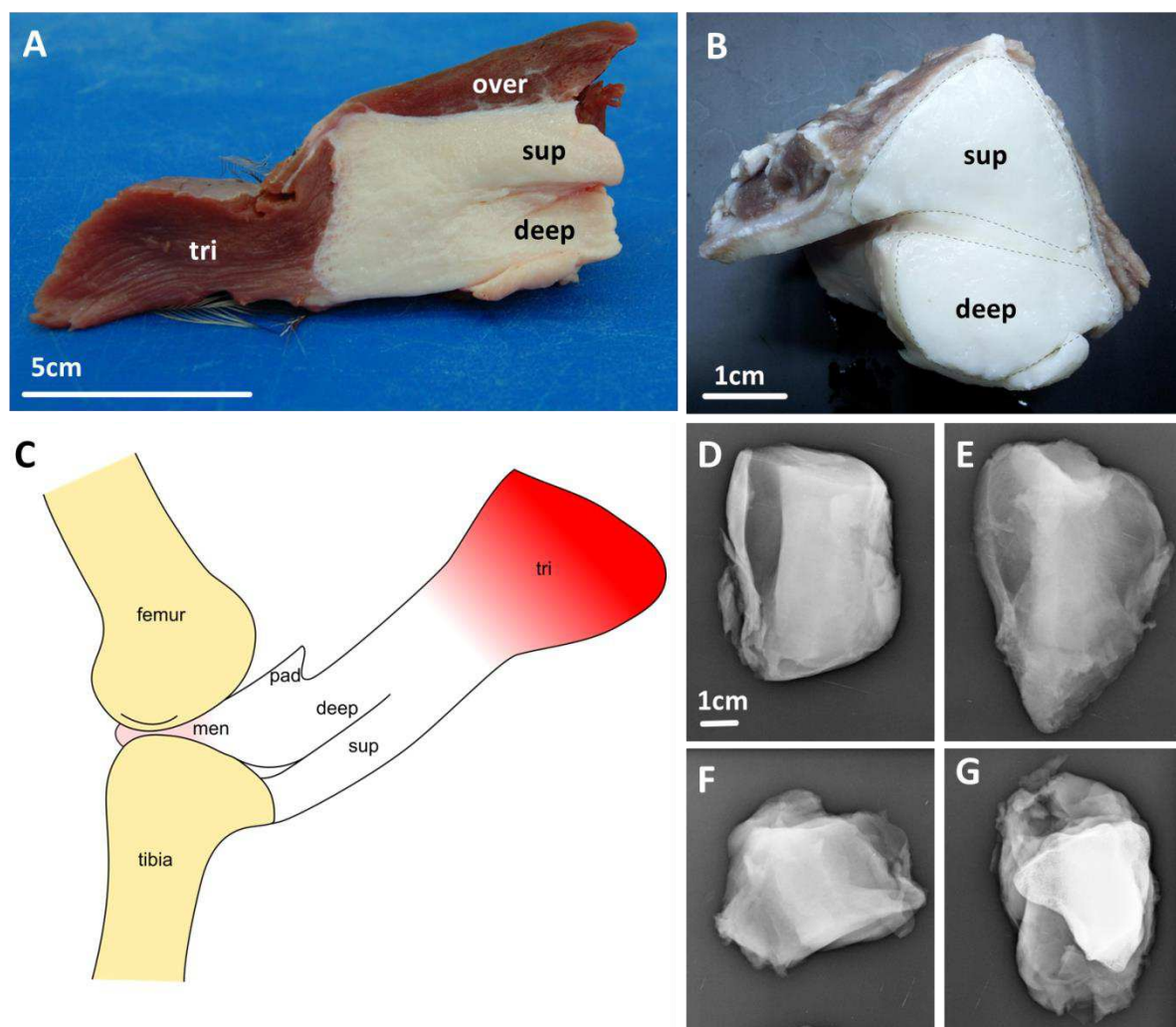
Figure 1: Gross patellar morphology. A; excised patellar tendon of a six month old emu in longitudinal cross section, showing the triceps femoris mucle group attaching proximally (tri), overlying muscles superficially (over), and the tendon partially splitting distally to form superficial (sup) and deep (deep) portions. Left of image = proximal. B; transverse cross section of the distal patellar tendon from an 18 month old emu, showing the triangular superficial portion (sup) and rounded deep portion (deep). Left of image = lateral. C; schematic drawing of the emu patellar tendon reflected from the femur. Distally the tendon splits into superficial (sup) and deep (deep) portions, with the former inserting onto the cranial tibial crest while the latter blends with a triangular fatty structure (pad) that attaches to the menisci (men). D-F; patellar tendon radiographs of the three oldest emus (18 months), showing no evidence of any mineralisation. Top of image = proximal. G; patellar tendon radiograph of a reindeer with similar dimensions as an adult emu tendon, demonstrating the appearance of a mineralised patellar sesamoid. Top of image = proximal.
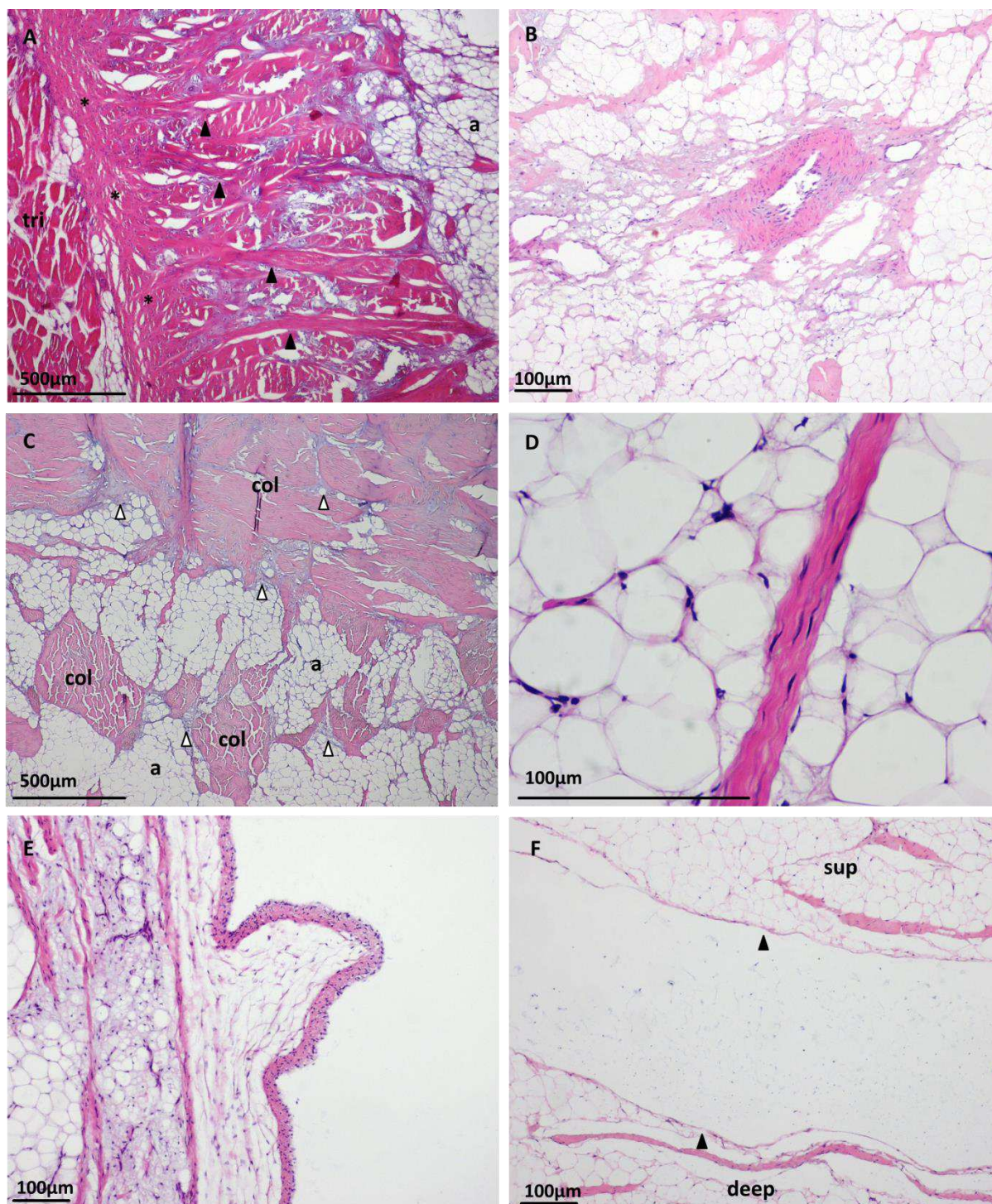
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Figure 2: Histology of emu patellar tendon. A; the patellar tendon junction with the triceps muscles (tri) in an 18 month old emu. Collagen bundles appear to run transversely along the muscle junction (asterisks), before turning longitudinally (filled arrows) and penetrating into the adipocyte rich (a) body of the tendon. H&E. B; the patellar tendon of a 12 month old emu, with a blood vessel within the tendon body seen in the proximal deep region. H&E. C; the patellar tendon of an 18 month old emu. Collagen bundles (col) run along the superficial surface (top right of image), interspersed with basophilic vesicular and chondroid tissue (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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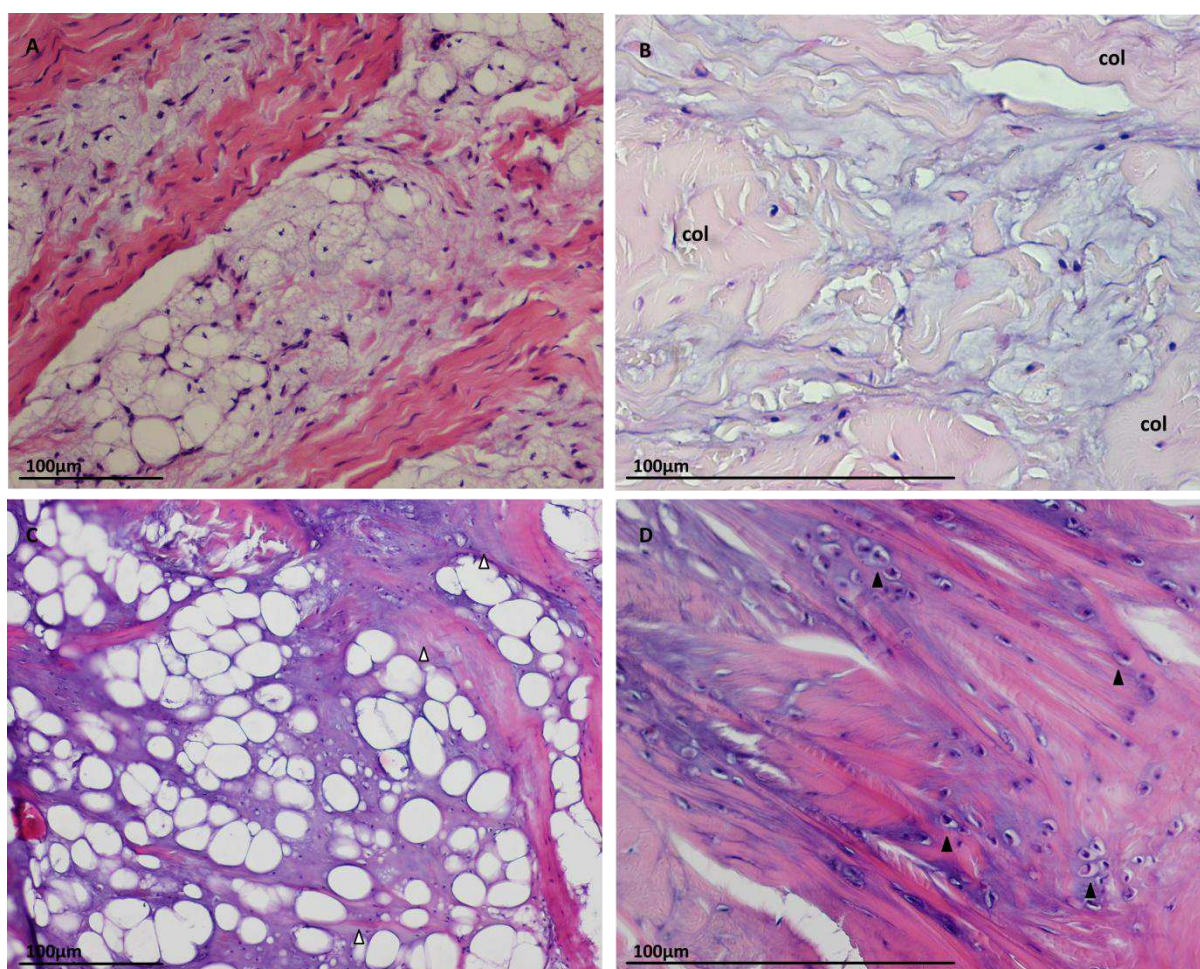
Figure 3: Differentiation of tissue from a vesicular to cartilage-like appearance with age. A;
the patellar tendon of a five week old emu, showing the basophilic, vesicular material which
is often seen in association with collagen fibre bundles. H&E. B; the patellar tendon of a six
month old emu. The basophilic material has a more homogenous and less vesicular
appearance than in the five week old emus. The nearby collagen fibre bundles (col) are more
basophilic here than in other regions of the tendon. H&E. C; the patellar tendon from an 18
month old emu, near to the proximal muscle junction, showing the basophilic vesicular and
cartilage-like tissue containing clusters of adipocytes. This tissue has the appearance of
encroaching on nearby collagen fibre bundles (unfilled arrows). H&E. D; the patellar tendon
of the same 18 month old emu, from another region near the proximal muscle junction.
Chondroid cells (filled arrows) sit within the normally eosinophilic collagen fibre bundles,
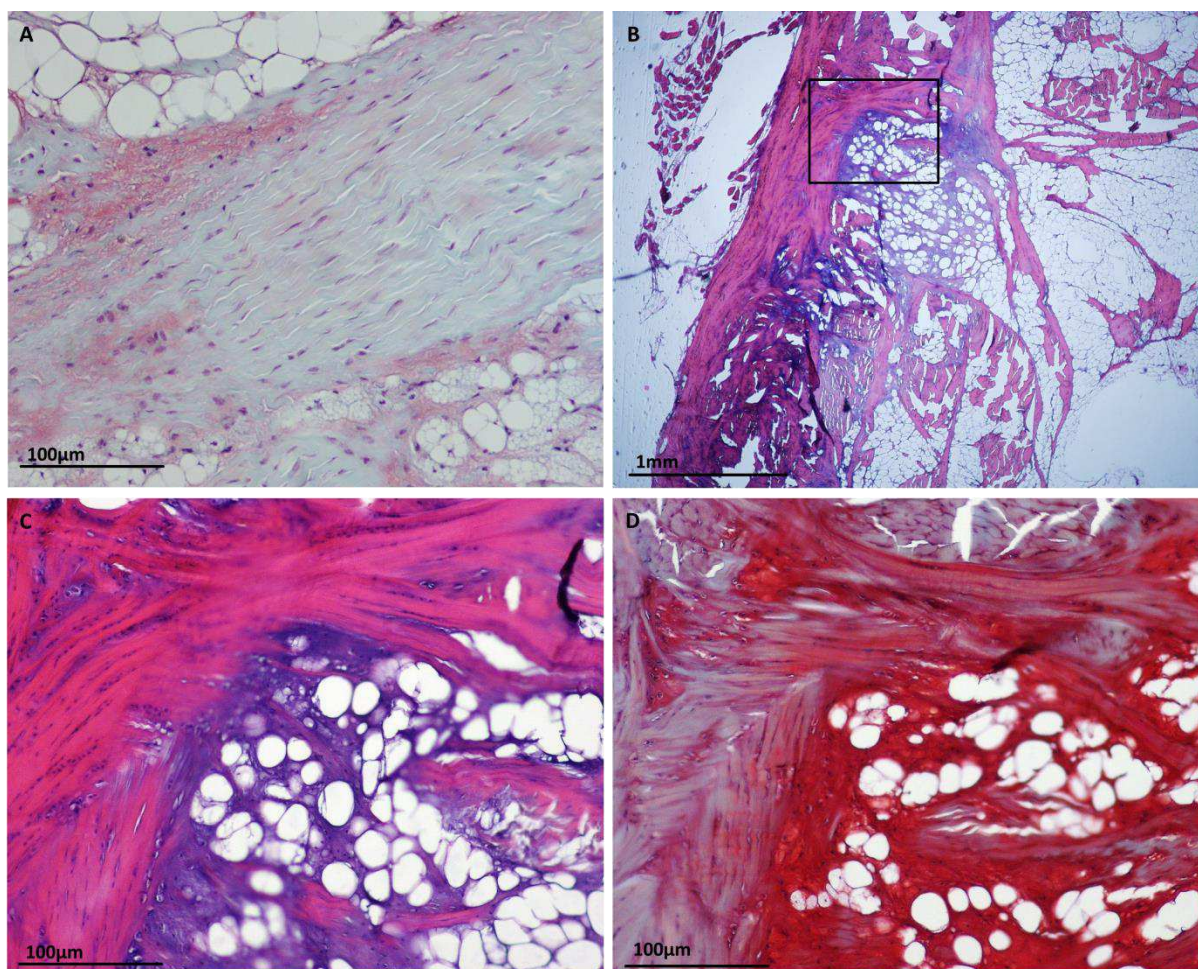creating an intense localised basophilia. H&E.

Figure 4: Appearance of the vesicular and cartilage-like tissue with Safranin O/fast green staining vs. H&E. A; the patellar tendon of a five week old emu, showing the vesicular material staining red with Safranin O/fast green, indicating proteoglycan content. Safranin O/fast green. B; the patellar tendon from an 18 month old emu, showing the deeply basophilic appearance of the chondroid tissue near the proximal muscle junction with the triceps tendon (left of image). The black box indicates the region magnified in C and D. H&E. C; magnified region of chondroid tissue in an 18 month old emu patellar tendon. H&E. D; the same magnified region showing the chondroid tissue staining intensely red. Safranin O/fast green.
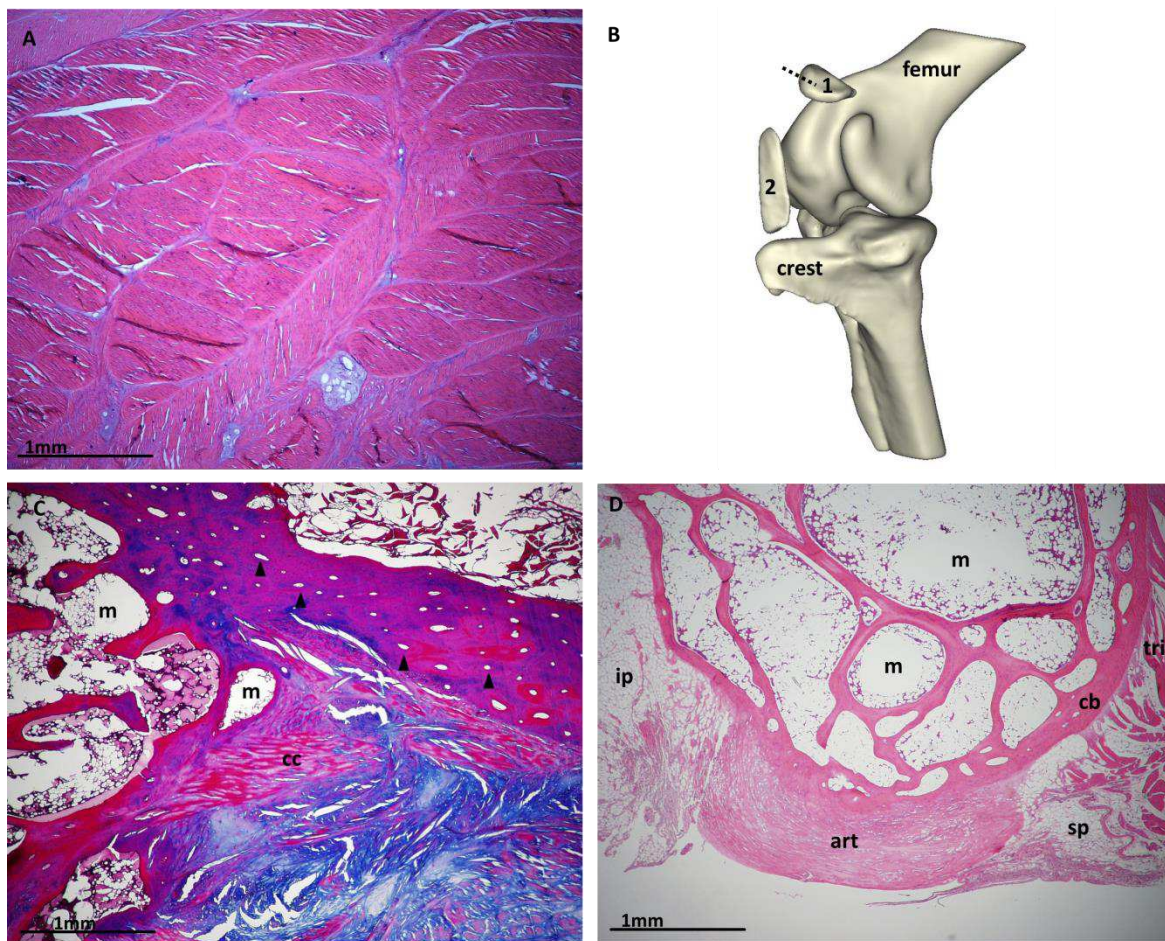
Figure 5: Configuration of ostrich and guineafowl patellae. A; part of a longitudinal section of the patellar tendon of an adult ostrich, predominantly composed of thick collagenous bundles with patchy regions of cartilage-like tissue and the occasional solitary adipocyte. H&E. B; 3D segmentation of computed tomography (CT) scan data from ostrich left knee, provided by Kyle Chadwick. The proximal patella (1) sits over the patellar sulcus of the femur, whilst the distal patella (2) overlies the large medial femoral condyle. The cranial cnemial crest (crest) is fused to the tibia in the adult. The dotted line shows the cut through the proximal patella seen in Fig C. C; transverse section of the proximal patella of an adult ostrich, showing osteons (black arrows) in the superficial aspect of the bone, trabeculae and medullary cavity occupying the majority of the patella (m), and calcified cartilage (cc). Superficial = top right of image, medial = right of image. Masson's trichrome. D; Longitudinal section of an adult guinea fowl patella, showing the triceps muscle attaching proximally (tri), suprapatellar (sp) and infrapatellar (ip) fat pads, outer cortical bone (cb), inner trabeculated structure, and deep articular cartilage lining (art). Proximal = right of image, deep = bottom of image. H&E.

682

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
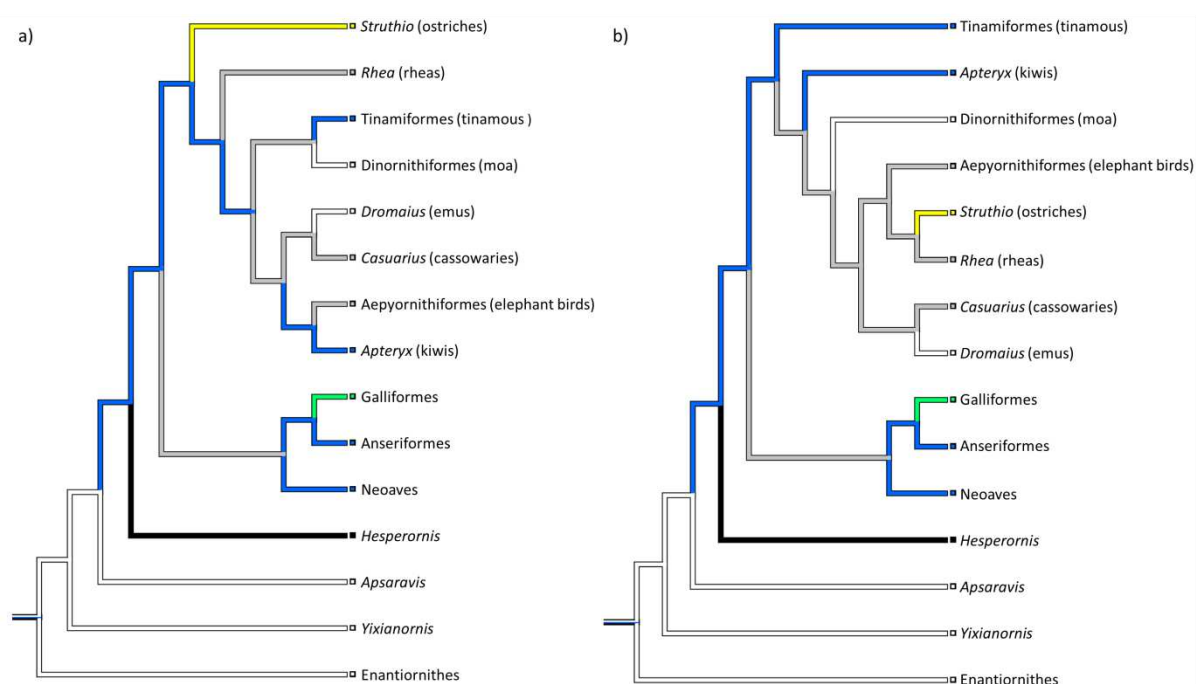686 (right).

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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/small flake of bone; green = 2/nodule of bone; yellow = 3/subrectangular block; black = 4/expanded triangular crest.

697 **Tables**

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

698

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.

705