

1 *Type of the Paper (Article)*

## 2 **Comparative distal limb anatomy reveals a primitive** 3 **trait in 2 breeds of *Equus caballus*.**

4 **Sharon May-Davis**<sup>1, \*</sup>, **Zefanja Vermeulen**<sup>2</sup>, and **Wendy Y. Brown**<sup>1</sup>

5 <sup>1</sup> Canine and Equine Research Group, University of New England, Armidale, NSW 2351,  
6 Australia; wbrown@une.edu.au (W.Y.B.)

7 <sup>2</sup> Equine Studies, Asch, The Netherlands; info@equinestudies.nl

8 \* Correspondence: maydavis@bigpond.com

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11 **Simple Summary:** Understanding the complexities and evolutionary links between extinct and  
12 extant equids has been vital to genetic conservation and preservation of primitive traits. As  
13 domestication of the equid expanded, the loss of primitive traits that ensured survival in a wild  
14 environment have not been documented. In this study, the presence of functional 2<sup>nd</sup> and 4<sup>th</sup>  
15 interosseous muscles in the distal limb has been reported and yet, its existence could only be  
16 confirmed in relatives and two closely bred descendants of the extinct Tarpan. The morphology  
17 described was ligamentous in structure displaying clear longitudinal fibres with a skeletal origin  
18 and soft tissue insertion into the medial and lateral branches of the 3<sup>rd</sup> interosseous muscle dorsal  
19 to the sesamoids, similar in orientation to the inferior check ligament. Hence, providing a functional  
20 medial and lateral stability to the fetlock joint, which equates to one of the functions of the medial  
21 and lateral digits in the *Mesohippus* and *Merychippus*. The comparable anatomic links between  
22 species of the same family that experienced geographical isolation yet display structural conformity  
23 appears to be in response to a specific environment. Surmising this potential remnant of functional  
24 evolution is a primitive trait and not a breed anomaly.

25 **Abstract:** The 55-million-year history of equine phylogeny has been well documented from the  
26 skeletal record, however not the soft tissue structures that are now vestigial in modern horse. A  
27 recent study reported 2 ligamentous structures resembling functional 3<sup>rd</sup> and 4<sup>th</sup> interosseous  
28 muscles were evident in Dutch Konik horses. The current study investigates this finding and  
29 compares it to members of the genus *Equus* to identify either a breed anomaly or functional  
30 primitive trait. Distal limbs (n=571) were dissected from 4 species of *Equus*; *E. caballus*, *E. asinus*, *E.*  
31 *przewalskii* and *E. burchelli beohmi*. Breed representatives of *E. caballus* (n=18) included the primitive  
32 Dutch Konik. The 2<sup>nd</sup> and or 4<sup>th</sup> interosseous muscle was evident in all 4 species, but only 2 breeds  
33 of *E. caballus* expressed this trait - the Dutch Konik and Bosnian Mountain Horse. These 2 breeds  
34 were the only close descendants of the extinct Tarpan (*Equus ferus ferus*) represented in this study.  
35 In conclusion, the 2<sup>nd</sup> and 4<sup>th</sup> interosseous muscles originated from the distal nodule of respective  
36 splint bones and inserted into the corresponding branches of the 3<sup>rd</sup> interosseous muscle proximal  
37 to the sesamoids. Suggesting a functional role in medial and lateral joint stability and a primitive  
38 trait in modern equids.

39 **Keywords:** 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> Interosseous muscle; Dutch Konik; Bosnian Mountain Horse; Donkey;  
40 Przewalski; Zebra; Atavism.

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### 42 1. Introduction

43 Aristotle (384-322BC) is regarded by many as the founder of comparative anatomy because of  
44 his methodical and systematic approach to the study of animals [1]. His rigorous and comprehensive

45 methods provided the basis for numerous original theories, including Charles Darwin's 1859  
46 publication 'On the Origin of Species' [1,2]. Aristotle believed that 'form and function' were integral  
47 parts of the same science and his in-depth knowledge of bodily systems were likely derived from  
48 direct observations and dissections [1]. As medical practitioners began to embrace post mortem  
49 instruction, medieval practices were abandoned and the modernisation of medicine as we know it  
50 today was founded [3].

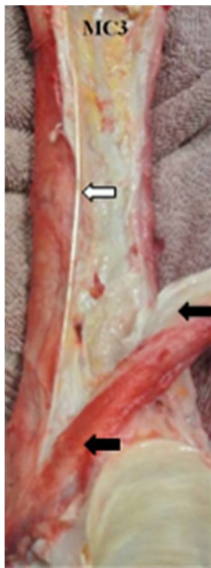
51 By the 1800's, this new anatomic enlightenment inspired many scholars and scientists, whilst  
52 simultaneously invoking strong academic debate and controversy [4,5]. Nonetheless, the science of  
53 comparative anatomy prevailed and shaped the fundamental principles of taxonomy in extinct and  
54 extant species [6]. Even when geographically isolated, the similarity between species was undeniable  
55 and furthermore, it became evident that structural conformity was in direct response to the  
56 environment [2,6]. These distinctions were developed through the study of comparative anatomy  
57 and formed the basis of sound phylogenetic interpretation and evolutionary taxonomy [6].

58 It was this methodical approach to comparative observation that palaeontologist O. C. Marsh  
59 utilised when he correlated fossil evidence and established the 55-million-year history of equine  
60 phylogeny [7]. His research deciphered the skeletal transformation from polydactyl to monodactyl,  
61 including connective soft tissue structures operating the distal digits [7,8]. These skeletal archives  
62 presented a convoluted, but connected history, originating with the polydactyl *Eohippus*, until the  
63 present-day monodactyl *E. caballus* [7-9]. The adaptations of the distal digits through the millennia  
64 corresponded to a period in history when the climate became progressively drier and open plains  
65 expanded at the expense of forests [8].

66 For the polydactyl horse, living in an open environment exposed it to greater stressors, namely  
67 foraging competition and predatory pressures; hence speed and endurance became a vital trait in  
68 order to survive. However, as its distal limb was designed for stability and not speed, the mass of the  
69 extra digits became an energetic cost that hindered locomotive efficiency [8,10,11]. Consequently, the  
70 distal limb required new adaptations that favoured efficient fore and aft linear movement with less  
71 flexibility [8,11]. So, in direct response to its environment, the polydactyls distal limb evolved by  
72 favouring the reduction of the medial and lateral digits, whilst elongating the middle; thus, becoming  
73 the tight jointed, rigid hoofed monodactyl that we encounter today [7-11].

74 In modern horse, polydactyl atavism or primitive characteristics have been reported in the  
75 appendicular skeleton and although considered rare, so have reports of atavistic or vestigial soft  
76 tissue structures in the distal limb. [12-15]. Evidently, these soft tissue structures were once strong  
77 and functional in the polydactyl, but now the morphology is regarded as rudimentary and ineffectual  
78 (Figure 1) [12,15]. However, two atavistic soft tissue structures reported in the forelegs; the medial  
79 interosseous muscle (IM2) and lateral interosseous muscle (IM4) are of interest to this study. Both are  
80 vestigial remnants of medial and lateral limb reduction, but in the modern horse, there is only one  
81 interosseous muscle considered functional – the middle or 3<sup>rd</sup> interosseous muscle (IM3) [12-16]. This  
82 muscle (IM3) evolved even further and now contains a large number of strong ligamentous fibres  
83 with elastic properties that functionally support the fetlock joint when the limb is either standing, or  
84 during locomotion [15,16].

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88 **Figure 1.** The white arrow denotes the atavistic soft tissue structure located along the medial  
89 palmar border of the 2<sup>nd</sup> metacarpal in a 17-year-old Thoroughbred horse. The black arrow denotes  
90 the resected 3<sup>rd</sup> interosseous muscle.

91  
92 When present in the modern horse, the IM2 and IM4, have been described as thin, pale and  
93 fleshy ligamentous structures originating from the distal nodule of the 2<sup>nd</sup> and or 4<sup>th</sup> metacarpal that  
94 ends inconspicuously near the fetlock joint (3<sup>rd</sup> metacarpal, 1<sup>st</sup> phalanx and sesamoids) [12,15]. In  
95 contrast, a recent study revealed strong chord-like bands in primitive Dutch Konik horses originating  
96 from the distal nodules of the 2<sup>nd</sup> and 4<sup>th</sup> metacarpals, and 2<sup>nd</sup> and 4<sup>th</sup> metatarsals [17].

97 Therefore, the aim of this study is to investigate the strong chord-like bands reported in the  
98 primitive Dutch Konik horse and compare them to domestic horse breeds along with other available  
99 species in the genus *Equus*. We describe the morphology, anatomic origin and insertion, and postulate  
100 the function of the bands, with the objective to provide a better understanding of whether they are a  
101 rare finding, a breed anomaly or a primitive trait. We conclude the presence of an IM2 and or IM4 in  
102 its current morphological form as noted in this study, relates to a primitive trait and furthermore, one  
103 that is possibly, a functional remnant of limb reduction in response to an undulating environment.

## 104 105 2. Materials and Methods

### 106 2.1 Ethical statement

107 No horses were euthanized for the purpose of this study and all observations were obtained post  
108 mortem.

### 109 110 2.2. Animal Details

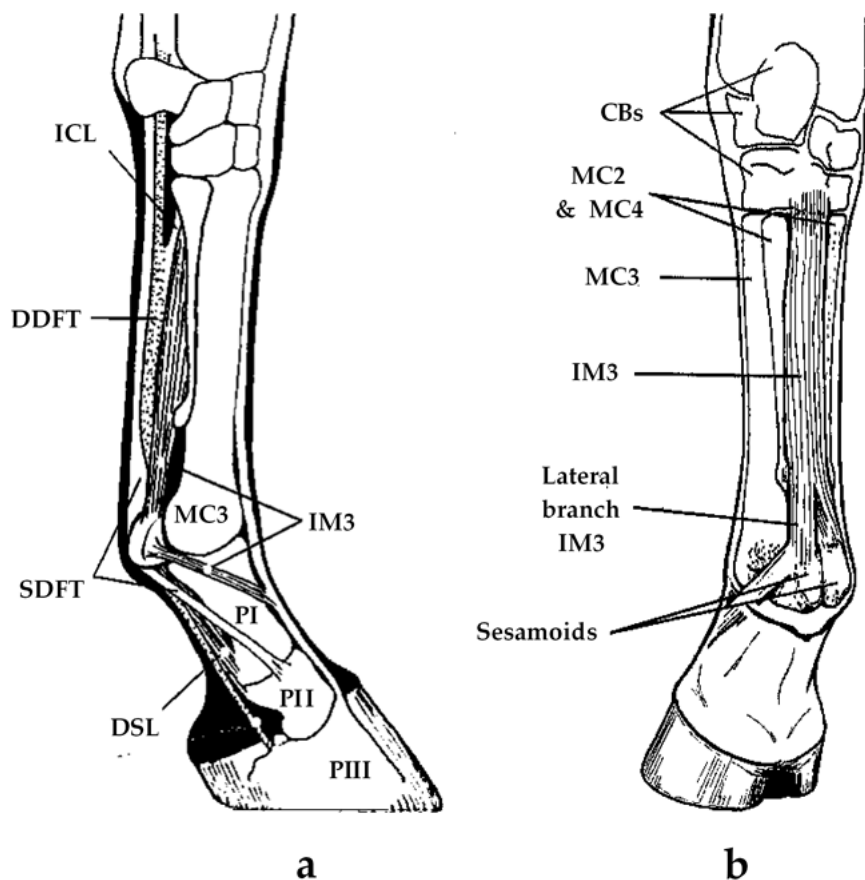
111 Dissections were performed on 571 distal limbs (DL) from 150 individual animals from the genus  
112 *Equus*; 484 DL were sourced from 121 domestic horses (*E. caballus*); 57 DL from 16 primitive Dutch  
113 Konik horses (*E. caballus*); 15 DL from 9 Przewalskis (*E. przewalskii*); 11 DL from 3 Donkeys (*E. asinus*)  
114 and 4 DL from 1 Grant's Zebra (*E. burchelli boehmi*). Animals ranged in age from 6 months to 30 +  
115 years.

116 The 484 DL from domestic horses were sourced in Australia (308); The Netherlands (60); Japan  
117 (40); New Zealand (36); United Kingdom (32); Sweden (4) and Slovenia (4). The 57 DL from Dutch  
118 Konik horses (DK) were sourced from The Netherlands; the 15 DL from Przewalskis from Hungary;  
119 the 11 DL from Donkeys (feral) from Australia and the 4 DL from the Grant's Zebra from Emmen  
120 Zoo in The Netherlands. There were 17 domestic breeds represented: Thoroughbred (208 DL);  
121 Crossbreds (56 DL); Warmbloods (56 DL); Australian Stock Horse (52 DL); Standardbred (16 DL);  
122 Quarter Horse (16 DL); Welsh Mountain Pony (12 DL); Arabian (8 DL); Irish Sport Horse (8 DL);  
123 Appaloosa (8 DL); Hunter (8 DL); Hackney (8 DL); Exmoor Pony (8 DL); Fjord (8 DL); Icelandic (4

124 DL), Morgan (4 DL) and Bosnian Mountain Horse (BHM) (4 DL). There were 289 forelegs and 282  
 125 hindlegs with each DL displaying 2 splint bones; the 2nd and 4th metacarpals in the forelegs (MC2  
 126 and MC4); the 2nd and 4th metatarsals in the hindlegs (MT2 and MT4). In total, 1142 splint bones  
 127 were examined and associated soft tissues (interosseous muscles).  
 128

### 129 2.3 Dissections

130 The forelegs and hindlegs were skinned from the proximal carpus and tarsus to the coronet  
 131 band. Disarticulation of the distal limbs occurred between the 1st and 2nd row of carpals in the  
 132 foreleg, and the tibial talus joint in the hindleg. This exposed the superficial fascia of the DL, and the  
 133 extensor and flexor tendons. Resection of the flexor tendons, the inferior check ligament, nerves and  
 134 various vessels from the palmar aspect of the 3rd metacarpal (MC3) and 1st phalanx was next;  
 135 exposing the greater part of MC2 and MC4 including the IM3 (Figure 2).  
 136  
 137



**Key:** CBs – Carpal bones; DDFT – Deep digital flexor tendon; DSL – Distal sesamoidean ligament; ICL – Inferior check ligament; IM3 – 3<sup>rd</sup> Interosseous muscle; MC2 – 2<sup>nd</sup> Metacarpal; MC3 – 3<sup>rd</sup> Metacarpal; MC4 – 4<sup>th</sup> Metacarpal; PI – 1<sup>st</sup> phalanx; PII – 2<sup>nd</sup> phalanx; PIII – 3<sup>rd</sup> phalanx; SDFT – Superficial digital flexor tendon.

138  
 139 **Figure 2.** (a) Lateral view of the distal forelimb revealing the flexor and extensor structures of  
 140 the MC3 and phalanges. (b) Caudal view of the distal forelimb revealing the IM3 on the palmar  
 141 surface of MC3. Adapted from Stashak [18].  
 142

143 Careful resection of the IM3 began at its origin located on the proximal palmar surface of MC3  
 144 and traversed distally to reveal, if present, the IM2 and IM4 originating from the distal nodules of  
 145 MC2 and MC4. When not present the resection of the IM3 and its medial and lateral branches

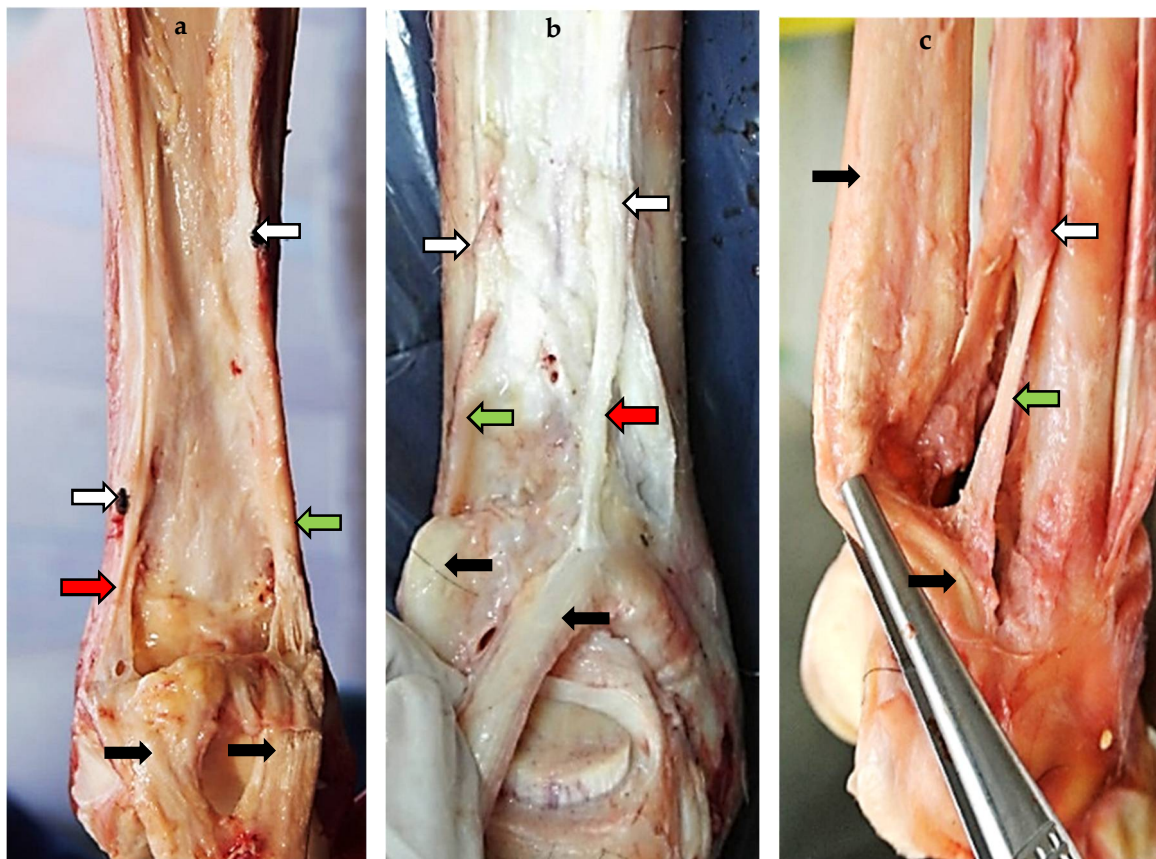


146 continued distally until insertion onto the respective medial and lateral sesamoids. However, when  
 147 the IM2 and IM4 were present the resection required slow and precise strokes so not to compromise  
 148 the origin or insertion of the IM2 and IM4. Any connective fascia was then carefully removed  
 149 revealing the IM2 and IM4 in its entirety. The same process of resection was repeated for the  
 150 metatarsals in the hindleg from the plantar surface.

### 151 3. Results

152 All *Equus* expressed the IM3, however the IM2 and IM4 were noted only in Dutch Konik horses,  
 153 Przewalskis, donkeys and Bosnian Mountain horse. The Grant's zebra expressed the IM4 only in the  
 154 forelegs (Table1).

155 Anatomically, the IM2 and IM4 originated from the nodules of the MC2 and MC4, and the MT2  
 156 and MT4, respectively. The point of insertion for the IM2 was the medial branch of the IM3; the point  
 157 of insertion for the IM4 was the lateral branch of the IM3. All points of insertion were dorsal to the  
 158 sesamoids. The thickness of the IM2 and IM4 appeared to remain constant from origin to insertion  
 159 (Figure 3). At the point of insertion, the fibres of the IM2 and IM4 appeared to interconnect with those  
 160 of the IM3 (Figure 4). These fibres were longitudinal in arrangement from origin to insertion and  
 161 appeared consistent with a collagenous protein with elastic properties, such as that found in tendons  
 162 or special ligamentous structures such as the IM3.

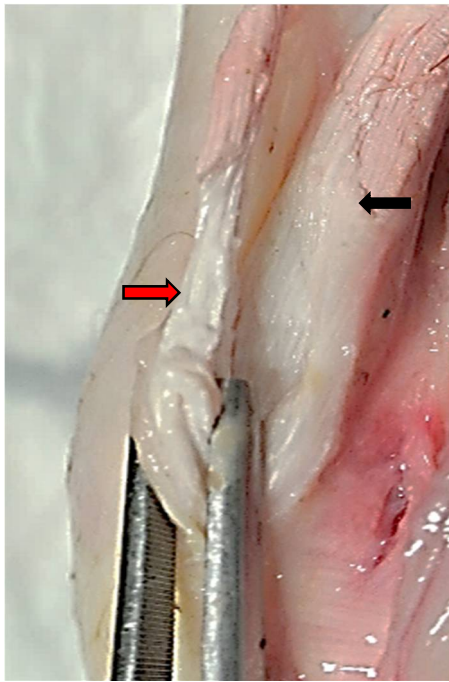


178 **Figure 3.** Physical appearance of dissected interosseous muscles IM2 and IM4 in the: (a)  
 179 Dutch Konik (primitive horse); (b) Przewalski; and (c) Grant's zebra. Arrows indicate: the  
 180 distal nodule of the splint bone (white); IM2 (red); IM4 (green); and IM3 ligament (black).

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194 **Figure 4.** Physical appearance at the point of insertion of a dissected 2<sup>nd</sup> interosseous  
195 muscles (IM2) into the medial branch of the 3<sup>rd</sup> interosseous muscle (IM3) in the left hindleg  
196 of a Dutch Konik horse. Arrows indicate: IM2 (red); and IM3 ligament (black).

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**Table 1.** The documented occurrence of 2<sup>nd</sup> and 4<sup>th</sup> interosseous muscles (IM2 and IM4) in  
the distal limbs (n = 571) of four *Equus* species, dissected post mortem.

Species		Distal limbs (n) <sup>1</sup>		IM2 (n)		IM4 (n)	
		Left	Right	Left	Right	Left	Right
<i>Equus caballus</i> : Domestic horse (17 breeds, n=121)	Fore	121	121	1*	1*	1*	1*
	Hind	121	121	1*	1*	1*	1*
<i>Equus caballus</i> : Primitive horse (Dutch Konik, n=16)	Fore	14	13	12	11	12	11
	Hind	14	16	14	14	14	15
<i>Equus przewalskii</i> (Przewalski, n=9)	Fore	9	2	11	2	11	2
	Hind	2	2	2	2	2	2
<i>Equus asinus</i> (Donkey, n=3)	Fore	3	3	3	3	3	3
	Hind	3	2	3	2	2	2
<i>Equus burchelli boehmi</i> (Grant's zebra, n=1)	Fore	1	1	0	0	1	1
	Hind	1	1	0	0	0	0

200 <sup>1</sup> Note: All 4 limbs were not available for some animals. \* Denotes Bosnian Mountain Horse

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#### 202 4. Discussion

203 In this study, the presence of a strong cord-like band resembling a tendon or ligament in the  
204 distal limb was identified and described in four species of the genus *Equus*. The bands originated  
205 from the distal nodules of the splint bones, matching the previously reported description of the  
206 atavistic IM2 and IM4 [12,15]. However, in contrast to a thin and feeble ligamentous structure,  
207 morphological variations in size and insertion were noted, with conspicuous cord-like bands

208 inserting into corresponding branches of the IM3 (Figures 3 and 4). The described bands were  
209 observed in 1 domestic breed (BMH) from 17; 1 primitive breed (DK); donkeys; Przewalskis and 1  
210 Grant's zebra.

211 Of the 18 breeds representing *E. caballus*, only the close descendants of the extinct Tarpan (*E.*  
212 *ferus ferus*), the DK and BMH expressed the IM2 and IM4 [19,20]. This coincides with a previous study,  
213 where the DK and BMH were the only 2 from 20 breeds of *E.caballus* that exhibited a full nuchal  
214 ligament lamellae [21]. Thus, implying the ligamentous structures noted in the current and previous  
215 study, may well be attributed to the Tarpan as a heritable characteristic [21].

216 The strong cord-like bands described in this study were not vestigial, but displayed clear  
217 demarcations associated with origins and insertions as would be expected of a functional structure.  
218 Somewhat like the inferior check ligament that originates from the palmar surface of the distal row  
219 of carpals and inserts into the deep digital flexor tendon providing stability and support (Figure 3)  
220 [12,15]. In fact, both structures have proximal skeletal origins with distal soft tissue insertions and  
221 identical orientation, implying similar function (Figures 3 and 4). Furthermore, the morphology of  
222 the strong bands described in this study, present surprising similarities within members of the genus  
223 *Equus* that are geographically isolated, suggesting heritability from a common ancestor.

224 As previously described, vestigial or atavistic soft tissue structures in the distal limb are reported  
225 as rudimentary and non-functional. Yet, identifying new soft tissue structures that are functional in  
226 an equid's distal limb, is not unprecedented [12,15,22,23]. A recent study in a miniature donkey  
227 (*E.asinus*), introduced a new ligament on the palmar surface of the 3<sup>rd</sup> metacarpal with functional  
228 capabilities of stabilising the superficial digital flexor tendon [22]. An earlier study reported fascial  
229 bundles in 5 horses descending distally from the nodule of the splint bone and attaching to the 3<sup>rd</sup>  
230 metacarpal, securing the smaller metacarpal to the larger one [23]. Although reports of soft tissue  
231 structures like those described in this study have not been found for extant members of *Equus*, this  
232 does not apply to families closely related to *Equidae* or polydactyls. In fact, all 3 interosseous muscles  
233 have been reported in extant and extinct polydactyls [24-29].

234 Studies have identified the presence of the IM2, IM3 and IM4 in the hippopotamus, tapir and  
235 canine, including marsupials such as the thylacine; of which, the origins and insertions displayed  
236 similar morphology in the hippopotamus and tapir to those found in this study [24,25,27]. The IM3  
237 and IM4 were noted in pigs, sheep and llamas, but not cattle or camels, where only the IM3 was  
238 mentioned per digit [24,28-32]. There were no definite anatomic origins or insertions noted for pigs  
239 or sheep, however, the llama's presentation corresponded anatomically to the current study  
240 [24,28,29].

241 Many of the species previously mentioned belong to the unguligrade mammals within the  
242 orders perissodactyl and cetartiodactyl [33]. The latter order provides evidence of skeletal entheses  
243 patterns in the distal limb, where the IM3 and IM4 attached in an extinct species of palaeomerycid, a  
244 deer like creature from the Miocene epoch [34,35]. During the same epoch, the perissodactyl forebears  
245 of *Equidae*, the tridactyl *Meshippus* and *Merychippus* had anatomic structures comparable to the tapir,  
246 including interosseous muscles [36]. The 3 digits in the tridactyl were referred to as II, III and IV  
247 (medial, middle and lateral respectively), with III being the largest, widest and dominant during  
248 weightbearing [36,37]. Each interosseous muscle corresponded to its metacarpal or metatarsal, hence  
249 the interosseous muscles in the *Meshippus* and *Merychippus* would be labelled IM2, IM3 and IM4  
250 from which they originated [12,15]. In current literature this anatomical arrangement remains  
251 constant between species, whether extinct or extant.

252 Furthermore, it has been suggested that functionally the 2<sup>nd</sup> and 4<sup>th</sup> digits in prehistoric tridactyls  
253 assisted in the prevention of lateral dislocation of the fetlock joint while increasing agility and  
254 maneuverability; whereas in *Merychippus*, these digits also helped increase traction over soft ground  
255 and savannas [36]. This concurs with the adaptive responses that feature in the camel's specialized  
256 distal limb to its sandy environment [30]; and applies to donkeys, where specific adaptations were in  
257 direct response to a specific environment, which has already been reported in ungulate morphology  
258 [2,6,38]. Unlike domestic horse, the distal limb in the donkey ends in a small boxy upright hoof with

259 thick outer walls that are extremely strong and pliable, permitting greater accuracy during placement  
260 in rocky and difficult mountainous terrain [38,39].

261 Looking into the phylogeny of *Equidae*, the genus *Equus* emerged from the hippomorphs 3.8  
262 million years ago (Mya); *Equus asinus* (donkey) diverged from its common ancestor with the caballine  
263 horses 2.1 Mya; *Equus quagga* – the zebras 1.2-1.6 Mya and *E. przewalskii* 50,000 years ago [34]. In this  
264 study, all three genera presented with the IM2 and or IM4, which suggests the strong cord-like bands  
265 have a functional role in their environment, as per the ancestral horse *Meshippus* and *Merychippus*  
266 [36]. This also equates to the DK, BMH, Przewalski, donkey and zebra that have evolutionary  
267 pathways involving mountainous terrain, soft pliable surfaces and more specifically, undulating  
268 environments where medial and lateral stability of the fetlock joint is necessary [19,20,34,40].  
269 Therefore, it could be postulated that a functional IM2 and IM4 are primitive traits in *E. caballus*.

270

## 271 5. Conclusions

272 The IM2 and IM4 in modern *Equus* have been described as a vestigial and non-functional  
273 remnant from tridactyl forebears; where it was postulated, they were structurally functional for  
274 traction and stability in the ancient equid. With distant relatives and 4 genera of *Equus* displaying the  
275 IM2 and IM4, we conclude the anatomic trait as described in this study, is not a breed anomaly, but  
276 a primitive trait found in 2 breeds of *E. caballus* known as the DK and BMH.

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278 S.M-D, Dissections, Resources Z.V., Supervision, review and editing W.Y.B.

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287 To those authors / editors/ publishers of those articles, journals and books cited in this manuscript.

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289 **Conflicts of Interest:** The authors declare no conflict of interest.

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