A Flexor Muscle (*Musculus Semimembranosus*) Shows ~~the~~ a New Criteria ~~of~~to measure the Genicular Joint Angle When ~~the~~ Terrestrial Mammals Walk

Con formato: Izquierda: 2,5 cm, Derecha: 2,5 cm, Arriba: 2,5 cm, Abajo: 2,5 cm, Numeración: Continua

Fumihiro Mizuno[1] and Naoki Khono[1,2]

[1]Graduate school of Life and Environmental Science, University of Tsukuba, Ibaraki, Japan,
[2]National Museum of Nature and Science, Ibaraki, Japan
Correspondence: Fumihiro Mizuno
Email: fmizuno86@geol.tsukuba.ac.jp

Abstract

Background. The genicular or knee joint angles of terrestrial mammals are kept constant ~~when they are~~while the stance phase of their walking, but the angles differ among ~~the species~~taxa. It is known that the knee joint angle correlates with taxa and body masses among extant mammals, but several extinct mammals such as desmostylians do not have closely related descendants. Furthermore, fossils have lost its soft tissues by the time they are unearthed, therefore, estimating its body mass is a hard problem. ~~This~~These factors cause~~s~~ a huge problem in reconstructing the proper posture of extinct mammals. ~~The knee joint under pressure to flex by gravity, tries to extend against it by the *musculus quadriceps femoris*. However, it is impossible to extract only the agonist elements from this muscle complex.~~A muscle reaction called co-contraction is known ~~that~~to increase~~ing the~~ joint stiffness~~. B~~; both, agonist and antagonist muscles ~~applying~~working ~~to~~on the same joint at the same time~~work when co-contraction occurs~~. The ~~antagonist muscle against the extensor muscle that extends the knee joint is the *m.*~~*musculus semimembranosus* flexes the knee joint and acts a~~i~~s an antagonist muscle to those which extend the keen joint. Therefore, ~~T~~the angle between the *m. semimembranosus* and the tibia would be kept constant because of the generation of co-contraction~~ of those muscles during walking~~, and consequently the constant joint angles are estimated from ~~the antagonist~~this muscle.

Methods. Twenty-one species of terrestrial mammals were examined to find the elements that ~~have a relationship between~~constitute the angle ~~made with~~between the m. semimembranosus and the tibia based on the period between the hindlimb touched down and taken off the ground~~, which~~. Measurements were captured from ~~the~~ videos with high-speed mode (420 fps), ~~picked~~ picking 13 pictures from the first 75 % of each movie wh~~en they~~ile animals were walking~~, and t~~. The angles between the main force line~~s~~ of the m. semimembranosus and the tibia, which were defined as θ_{sm-t} in ~~this~~ our study, were measured.

Results. More than ~~80~~85 % of target animals~~, which was~~ (17 out of ~~21~~20 species)~~,~~ had the difference between the maximum and minimum angles between the *m. semimembranosus* and the tibia (θ_{sm-t}) of stance instance (SI), which were each picked pictured used and defined in ~~this~~our study, during the stance (SI-1 to SI-13) within ± 10 degrees from the middle. The difference between each SI next to the next had a slight difference, therefore, the θ_{sm-t} transition was smooth. According to the results of the total stance differences among the target animals, the θ_{sm-t} was kept constant during a stance; therefore, the average of the θ_{sm-t} (θ_{ave}) could represent each animal.~~ The correlation coefficient was 0.26 between the angle of θ_{ave} and the body mass without Suricata which has a unique behavior when compared with other target animals. The low correlation coefficient indicated few relationships between the body mass and θ_{ave}.~~ The statistically differences were not detected between the and θ_{ave} variables (taxon, ambulatory style, and body mass); therefore, it could not say the θ_{ave} correlates these variables in our study.

Con formato: Fuente: Cursiva

Con formato: Fuente: Cursiva

Comentado [Rev1]: See comments in review

Con formato: Fuente: Cursiva

Comentado [Rev2]: Mean?

Comentado [Rev3]: Actually, it is not constant, but have a 20 degree variation, what could be considered relatively constant. Also, you do not have statistic confirmation, but only description, so I suggest rephasing so that is clear.

Conclusion. The θ_{ave} was 100 ± ~~21~~ 10 degrees ~~even if the species had any~~regardless taxon, body mass, ~~limb posture,~~ or ~~gait~~ ambulatory style. It is simply necessary to measure only three points on skeletons to determine the $\theta_{\text{~~sm~~ ~~t~~ave}}$ and thus, this new approximation to understand the hindlimb posture could be applied to the study of the hindlimbs~~reconstruct the proper posture~~ of ~~the~~ extinct mammals ~~that do not have descendants could also be possible~~with no closely related extant descendants.

Con formato: Fuente: Negrita

Introduction

The hindlimbs act as a ~~the~~ propulsive devices in terrestrial locomotion (Demes et al., 1994). ~~Walk, run, and jump are the behaviors that are commonly done among the terrestrial mammals on land.~~ These common terrestrial behaviors require limbs to support body mass against the gravity. This means that ~~the~~ terrestrial mammals must resist collapsing joints against the gravity; therefore, on land, active movements require to keep ~~extending~~ extended ~~the~~ joints. Although ~~the~~ limbs have same roles that support body mass, the joint angles are different between species (Biewener, 1983, 2005; Inuzuka, 1996; Dutto et al., 2006; Polly, 2007; Fujiwara, 2009; Dick & Clemente, 2017). For example, the angle at the knee joint in Asian elephants ~~had~~was around 160 degrees (Ren et al., 2008), chacma baboons had 137 degrees (Patel et al., 2013), domestic cats had 115 degrees, lion had 124 degrees (Day & Jayne, 2007). The limb joint angle is unique in each species, but the joint has wider rotatable range than the angle kept by each species during standing or walking. This causes the problem to reconstruct skeletal specimens in accurate posture when they were alive. In particular, ~~the~~ extinct taxa have some high wall to reconstruct their accurate postures, because ~~they cannot be observed~~ the ~~actual~~ angle when they were alive cannot be observed. For example, desmostylian mammals, which do not have any closely related living descendants, have been reconstructed in several different postures even though almost complete skeletons of the same species have been unearthed ~~almost complete skeletons~~ (Domning, 2002; Inuzuka, Sawamura & Watabe, 2006; Fujiwra, 2009). ~~Even~~Furthermore, the earlier~~ly~~ diverging cetaceans such as pakicetids and ambulocetids had functional hindlimbs, the extant cetaceans had completely ~~had~~ lost hindlimbs though (Thewissen, Madar, & Hussain, 1998; Gingerich, 2001; Thewissen et al., 2001; Madar, 2007; Gingerich et al., 2009; Gingerich et al., 2017). These extinct mammals have no extant mammals to use as references for ~~the~~ skeleton reconstruction. Therefore, the knowledge on the hindlimb postures in terrestrial mammals on land is important to understand the transition of locomotive ability through the mammalian evolution even if it ~~directs~~adapts their life from land to sea. ~~To resolve this problem, it is important to reveal the relationships between the joint angle and skeletons.~~

~~The hindlimbs act as the propulsive devices in terrestrial locomotion (Demes et al., 1994). All the known terrestrial mammals have hindlimbs. Even the early diverging cetaceans such as pakicetids and ambulocetids had functional hindlimbs, the extant cetaceans completely had lost hindlimbs though (Thewissen, Madar, & Hussain, 1998; Gingerich, 2001; Thewissen et al., 2001; Madar, 2007; Gingerich et al., 2009; Gingerich et al., 2017). Therefore, the knowledge on the hindlimb postures in terrestrial mammals on land is important to understand the transition of locomotive ability through the mammalian evolution even if it directs from land to sea. In particular, the knee joint has a role to control limb motions (Pandy et al., 1988). However, to observe the knee joint angle with live specimen without equipment is hard, because the femur is completely covered by the *musculus quadriceps femoris* and the *m. biceps femoris*. Furthermore, its flexion must accompany a slip~~

Con formato: Sangría: Primera línea: 1,77 car.

Con formato: Color de fuente: Rojo oscuro

~~motion between the femur and the tibia; therefore, the rotation is not circle, and the rotation center is not continued on the center (Castaing, J., and Jean-Jacques Santini, 1986; Yin et al., 2015).~~

Several previous studies explore the relationship between the limb posture and variables~~,~~ such as taxa, body mass~~es~~, and skeletal morphologies among extant mammals (Biewener, 1983, 1989, 1990, 2005; Day & Jayne, 2007; Fujiwara, 2009; Fujiwara & Hutchinson, 2012: Dick & Clemente, 2017). These previous studies indicated there is a tendency, ~~that the larger body mass~~ mammals with the larger body mass ha~~s~~ve the mo~~re~~st upright limb posture. However, there are several exceptions of th~~e~~is relationship between ~~the~~ limb posture and ~~the~~ body mass (Fujiwara, 2009). Furthermore, there is a huge problem with the estimati~~o~~n~~g~~ of body mass ~~of~~in extinct mammals because fossils have already lost soft tissues when unearthed. To resolve these problems, it is important to discover some criteria which are not affected by other factors as possible.

~~The *musculus quadriceps femoris* and the *m. semimembranosus* are known as the knee joint extensor and flexor muscles, respectively. These muscles are agonist and antagonist muscles to each other. There is an action that both agonist and antagonist muscles contract simultaneously called co-activation or co-contraction (Smith, 1981; Le et al., 2017; Latash, 2018).~~ The quadrupedal mammals maintain their joint angles in limited range while standing or walking (Manter, 1938; Gray, 1944; Goslow, Reinking & Stuart, 1973; Goslow et al., 1981; Alexander & Jayes, 1983; Inuzuka, 1996; Fischer et al., 2002; McGowan, Baudinette & Biewener, 2005). If the joint angles are constant, the distance between the ground and the center of mass is also constant. Therefore, limbs move as a ~~like~~ pendulum while walking (Cavagna, Heglund & Taylor, 1977; Griffin, Main & Farley, 2004). When a joint angle is locked against the force to change the angle via gravity, muscles work not only the agonist muscle but also the antagonist muscle. This action ~~is confirmed that it~~ increases joint stiffness in humans (Olmstead et al., 1986; Louie & Mote, 1987; Nielsen et al., 1994; Riemann & Lephart, 2002; Knarr, Zeni & Higginson, 2012). Some electromyographic studies of quadrupedal mammals showed that both~~,~~ agonist and antagonist muscles ~~stimulated~~ act ~~in~~ at the same time during the stance phase which is ~~a~~the period ~~a~~in which the hindlimb supports ~~its~~the body mass (Engberg & Lundberg, 1969; Tokuriki, 1973; Deban, Schilling & Carrier, 2012; Araújo et al., 2016). While ~~the~~ walking of quadrupedal mammals, the joint angles are maintained in limited range, and both agonist and antagonist muscles are stimulated; therefore, co-contraction would ~~be~~ occur~~e~~ ~~ccurred~~ at that time. The *m. semimembranosus* is known as the knee joint flexor muscle, which is an antagonist muscle of the *m. quadriceps femoris* when the joint extends.

The *musculus semimembranosus* attaches on the ischial tuberosity and the interior-proximal end of the tibia (Fig. 1) (Böhmer. et al., 2020). These attachment positions do not move~~,~~ and involved parts of the skeletons do not change its shape greatly among taxa; therefore, the positional relationship between muscle and these parts of the skeleton also

Con formato: Sangría: Primera línea: 1,77 car.

Comentado [Rev4]: Please see comment in review

Comentado [Rev5]: Please see comment in review

Comentado [Rev6]: Please re-write, there are too many repetid concepts.

Comentado [Rev7]: I softened this sentence, because actually bones (pelvis, femur and tibia) do change their shapes among taxa, but what you are considering in this study can be considered quite "constant" shows the relationship ~~between~~ among ~~skeletons~~skeleton elements. ~~This~~Our study aims revealing the joint angle of terrestrial mammals between the *m. semimembranosus* and the tibia during walking ~~to show its~~ and explore its relationship~~s~~ with taxa, body masses, and ambulatory styles.

Materials & Methods

The angles between *m. semimembranosus* and tibia *in vivo* were collected from ~~21~~ 20 extant species among ~~21~~20 genera, ~~14~~13 families within seven orders~~: i.e., *Elephas maximus* (Asian elephant), *Cercopithecus neglectus* (De Brazza's monkey), *Chlorocebus aethiops* (grivet monkey), *Macaca fuscata* (Japanese macaque), *Dolichotis patagonum* (Patagonian mara), *Ammotragus lervia* (Barbary sheep), *Capra hircus* (goat), *Cervus nippon* (Japanese deer), *Giraffa camelopardalis* (giraffe), *Rangifer tarandus* (reindeer), *Chrysocyon brachyurus* (maned wolf), *Canis lupus* (eastern wolf), *Felis catus* (cat), *Panthera leo* (lion), *Suricata suricatta* (meerkat), *Helarctos malayanus* (sun bear), *Ursus thibetanus* (Asian black bear), *Equus caballus* (Kiso horse (Japanese local horse)), *Diceros bicornis* (black rhinoceros), *Tapirus terrestris* (Brazilian tapir), *Macropus giganteus* (eastern gray kangaroo), which employ the sagittal posture~~ (Table 1). These species were selected to be as various as possible to cover the superorder and order of mammals (Afrotheria, Proboscidea; Euarchontoglires, Primates, Rodentia; Laurasiatheria, Artiodactyla, Carnivora, Perissodactyla; and Marsupialia, Diprotodontia), wide range of body mass ~~from 0.73 kg (i.e., *S. suricatta*)~~(i.e., from 4.5 kg of *Cercopithecus neglectus* to 4060 kg ~~(i.e.,~~of *Elephas*~~.~~ *maximus*), and three ~~walking patterns~~ambulatory styles (plantigrade, digitigrade, and unguligrade), and live on land without limitations ~~of height~~ to extend ~~its~~their limb joints~~:~~, i.e., they do not live in ~~the~~ tunnels ~~and~~ or under ~~the~~ ground (Table 1). All the ~~studied species~~target animals were kept in zoos where Higashi Park Zoological Gardens (Okazaki, Japan), Higashiyama Zoo and Botanical Garden (Aichi, Japan), Hitachi Kaminé Zoo (Ibaraki, Japan), Toyohashi Zoo and Botanical Park (Aichi, Japan), and Ueno Zoological Gardens (Tokyo, Japan), and all observations on living individuals were operated under official permission. Significant pathologies and/or malformations were not detected in ~~all~~any of the ~~targets~~specimens.

All the target animals were subjected to videos by a digital movie camera (EX-FH20, Casio, Japan) with high-speed mode (420 fps). The camera was mounted on a tripod on the visitors route. Therefore, the distances from each target were dependent on each exhibition/cage. All videos were taken from the lateral side and the nearly same level of the ~~of walking when a~~ target animal when they walk~~sed~~ across vertically and completely (without stopping, turning, ~~and~~ or changing speed) the camera ~~with more than three steps~~ on a flat ground. We waited until each target walked across the camera voluntary because we had not applied any treatments on them; therefore, it had taken several weeks of months to take movies.~~These taken videos were treated the following processes to collect data. First, preparing pictures to measure the angles; pictures were captured from each video in every~~

Con formato: Sangría: Primera línea: 1,77 car.

Con formato: Fuente: Cursiva

~~frame between a target animal's foot had touched down and taken off~~ We chose three movies in each target species which walked with complete one cycle (touched down to next touched down), straight and vertically to the camera. Each movie was converted to still images in every frame when a period between touched down and took off with GOM Player (GOM & Company, South Korea).~~;~~ This period did not depend on time, it depended on the target's behavior. Each converted still images of the one period were cut off last 25%~~ of captured pictures were cut off~~, because "~~-~~the muscles that are anatomically positioned to produce limb retraction – the *gluteus superficialis* and *medius*, *semimembranosus* and cranial *biceps femoris* – were active in the second half of swing and approximately the first 50–75% of stance" (Deban, Schilling & Carrier, 2012).~~;~~ The sill images of each this period divided so that 13 pictures including the first and the last (Fig. 2A).~~were picked from the first 75% in equally intervals. Second, measuring the angle of each picked pictures~~ ~~Several d~~Drawings were applied on each of the 13 pictures to measure the angle: a line between the ankle joint and the proximal end of the tibia, ~~with~~ parallel to the Achilles tendon, and a line between the ischial tuberosity and the proximal end of the tibia were~~was~~ drawn~~; the angle between these two lines were measured~~ with Inkscape (Inkscape project) (Fig. 2B~~1~~).

~~A blue line represents a line between the ankle joint and the proximal end of the tibia with parallel to the Achilles tendon. A pink line represents a line between the ischial tuberosity and the proximal end of the tibia. An angle filled with yellow represents θ_{sm-t}. Each target animal has three stances, and each stance measured 13 angles.~~

~~This~~ Our study defined, one ~~picked~~ picture of the 13 pictures as step instant (SI) and numbered it SI-1 to SI-13~~;~~. ~~a~~A series of SI-1 to SI-13 was defined as one stance. ~~measured. Each species was taken three stances.~~ We measured the angle using drawn lines in each picture ~~which was drawn lines~~ for one stance and took three stances for each target species~~ with Inkscape (Inkscape project)~~. Then calculate average value of each SI and the value was defined as θ_{sm-t}. Body mass of each species came from previous studies (Table 1) or records taken by zoos. ~~This~~ Our study compared the transition of θ_{sm-t} in a stance ~~between~~ among species~~ or~~, ~~gait pattern~~ambulatory styles (unguligrade, digitigrade, and plantigrade) and the average of the θ_{sm-t} (i.e., θ_{ave}) versus body mass.

~~The maned wolf used for dissection in this study (Fig. 1B) was dead at the Ueno Zoological Garden on May 17th, 2018, and it was transferred to the National Museum of Nature and Science (Ibaraki, Japan) on July 5th, 2019, where it now bears the registration number is NSMT-M72566.~~

Results

Six taxa, i.e., *Elephas* (Proboscidea), *Cervus* and *Rangifer* (Artiodactyla), *Tapirus* (Perissodactyla), *Felis* and *Panthera* (Carnivora), had ~~the~~ a difference between the maximum and minimum angles during a stance less than 10 degrees, which means the θ_{sm-t} changed within ± 5 degrees from the middle. *Cervus* has the smallest difference during a stance, 5.80

Comentado [Rev8]: Please see comment in attached file

Con formato: Sangría: Primera línea: 1,77 car.

Comentado [Rev9]: This has been mentioned above, you may choose where to leave it, but in just one place is enough

Con formato: Sangría: Primera línea: 1,77 car.

degrees. ~~Eleven~~Ten taxa, i.e., *Chlorocebus* and *Macaca* (Primates), *Dolichotis* (Rodentia), *Ammotragus*, *Capra*, and *Giraffa* (Artiodactyla), *Canis*, *Chrysocyon*~~, *Suricata*,~~ and *Helarctos* (Carnivora), and *Equus* (Perissodactyla), had the difference between the maximum and the minimum angles during a stance ~~less than~~between 10 and 20 degrees, which means the $\theta_{sm\text{-}t}$ changed, as a maximum, within ± 10 degrees from the middle. Three taxa, i.e., *Diceros* (Perissodactyla), *Ursus* (Carnivora), and *Ceropithecus* (Primates) had ~~the~~ a difference between the maximum angle and minimum angle during a stance ~~less than~~between 20 and 30 degrees, which means the $\theta_{sm\text{-}t}$ changed, as a maximum, within ± 15 degrees from the middle. Even though *Macropus* (Diprotodontia) had the largest difference between the maximum and minimum angles during a stance, 31.72 degrees, which means the $\theta_{sm\text{-}t}$ changed within ± 16 degrees from the middle. *Panthera* had the smallest standard deviation, 1.73. *Macropus* had the largest standard deviation, 11.5 (Fig. ~~2~~3 and Table 2).

Among all studied species, ~~The~~ the smallest difference of each SI ~~among the all target species~~ was SI-1 with ~~41.44~~31.97 degrees ~~as the smallest and~~, while the largest was SI-13 with ~~54.82~~39.65 degrees ~~and 12.81 of standard deviation as the biggest~~. However, the smallest ~~statistically~~ standard deviation was SI-~~7~~11 which is ~~10.22~~13.64. The ~~biggest~~ largest ~~statistically~~ standard deviation was ~~same as degree,~~ SI-13, 17.63~~.~~ ~~Primates examined had the smallest difference of degree at SI-6, 2.86, and the biggest difference of degree at SI-13, 13.74. However, the smallest standard deviation was at SI-5, 1.61, and the biggest standard deviation was at SI-13, 6.87~~ (~~Fig. 3 and~~ Table 2).

Taxonomically, primates had the smallest difference of degree at SI-7~~,~~ (2.86), and the ~~bigg~~largest difference of degree at SI-13~~,~~ (13.74). Statistically, the smallest standard deviation was at SI-5~~,~~ (1.61), and the ~~big~~largest standard deviation was at SI-13~~,~~ (6.87). ~~a~~Artiodactyls ~~examined~~ had the smallest difference of degree at SI-6, 11.29, and the ~~biggest~~ largest difference of degree at SI-1~~,~~ (21.15). ~~Both~~ Statistically, the smallest and the ~~biggest~~ largest standard deviation were at same SI~~, as degree,~~with 4.92 and 9.4~~8~~7 degrees respectively. Carnivorans ~~examined~~ had the smallest difference of degree at SI-~~7~~9~~,~~ (~~37.42~~29.39), and the largest~~biggest~~ difference of degree at SI-13~~,~~ (~~54.82~~16.51). ~~Statistically,Both~~ Both, the smallest and the largest ~~biggest~~ standard deviation were at same SI ~~as degree~~, with ~~13.02~~6.08 and ~~17.93~~11.74 degrees respectively. Perissodactyls ~~examined~~ had the smallest difference of degree at SI-3~~,~~ (6.98), and the largest ~~biggest~~ difference of degree at SI-13~~,~~ (13.27). ~~BothStatistically, t~~The smallest and the largest~~biggest~~ standard deviation were at same SI ~~as degree~~, with 3.51 and 7.39 degrees, respectively (Fig. 3 and Table 2).

Ambulatory, ~~digitigrade such as *Dolichotis* (Rodentia) and Carnivora except Ursidae had the smallest difference of degree at SI-3, 28.38, and the biggest difference of degree at SI-13, 50.14. However, the smallest standard deviation was at SI-2, 11.06, and the biggest standard deviation was at SI-13, 18.29. U~~unguligrades, such as Perissodactyla and Artiodactyla, had the smallest difference of degree at SI-~~6~~11, ~~28.54~~14.11, and the biggest difference of degree at SI-~~5~~1, ~~33.25~~21.15. However, the SI of statistically the smallest standard deviation differed

Comentado [Rev10]: Please see suggested changes in the paragraph just above.

from the angle, it was at SI-7, 8.745.45, and the biggest standard deviation was at SI-2, 10.54. Digitigrades such as *Elephas* (Proboscidea), *Dolichotis* (Rodentia) and Carnivora (except Ursidae) had the smallest difference of degree at SI-1, 27.08, and this SI had the smallest statistically standard deviation, 11.22. The biggest difference of degree at SI-9, 39.95. However, statistically the biggest standard deviation was at SI-13, 13.82. Plantigrade such as Ursidae and Primates had the smallest difference of degree at SI-7, 11.76, and the biggest difference of degree at SI-13, 23.3. However, the smallest standard deviation was at SI-6, 4.89, and the biggest standard deviation was at SI-13, 9.83 (Fig. 43 and Table 23).

All the target species except *Elephas* and *Macropus* had positive values of the difference between θ_{sm-t} of SI-2 subtracted from SI-1., while Tthe subtracted values of SI-3 – SI-2 were positive among the target species except *Cervus* and *Rangifer*, indicating they start the stance phase by flexing their knee joint. The number of species having negative value increasinged in the following steps, but the values inverted to positive soon. The subtracted values of adjacent SIs were repeatedly positive and negative with-in short span up to SI-9 and almost targetmost –species hadpresented the negative values after SI-10, showing extension of the knee joint when finishing the stance phase. There were no species that changed more than 10 degrees between adjacent SIs (Table 4).

According to the results of the θ_{sm-t} transition, the whole target animalsevery studied species could be considered that they had relatively small differences between maximum and minimum ones during the stance phase (Fig. 23, and 34, and Table 2). The θ_{ave}average θ_{sm-t} of a stance (from SI-1 to SI-13) of all target animals was 96.85102.62 ± 23.8618.10 degrees. The smallest θ_{ave}this angle was presented by *SuricataDolichotis*, 72.9884.52 degrees, and the largest was *Elephas* presented the largest one, 120.71 degrees. More than 90 % of target animals (1918/2120) had this angle between 80 and 120 degrees, and more than 8085 % of targets (17/2120) had this angle between 90 and 110 degrees; the range is only 20 degrees. This showed that the total stance differences of the angles of the knee joint during the stance phase among the target animals were small; therefore, the average value of the θ_{ave} θ_{sm-t} (i.e., θ_{ave}) could represent each animal. Accordingly, we also analyzed the relationships between the θ_{ave} and the body mass. The correlation coefficient of all target animals was 0.4626 with the p-value 0.0328. The R-squared value was 0.17 (Fig. 45). There were nNone of the analyzed variables that showed significant differences in correlation with body mass, either taxonomically or in ambulatory (Table 5). In other words, it cannot be said that there was a correlation between the θ_{ave} and body mass. This correlation coefficient value meant that the relationship between the θ_{ave} and the body mass was low, and therefore, the θ_{ave} was stable and almost independent from the body mass.

Discussion

The quadrupedal animals are though to use their limbs with inverted pendulum-like movements (Cavagna, Heglund & Taylor, 1977; Griffin, Main & Farley, 2004). This inverted

Con formato: Fuente: Cursiva

Comentado [Rev11]: This two paragraphs are kind of repeting the information that is available in the Table, I suggest adding some interpretation of the results, that is speaking about extension and flexion of the knee joint, or highlight those results you will discuss further, or simply indicate where the reader can see this information (Table). But, if you do decide to keep this paragraphs as they are, please considere suggestions meade in the text.
This goes also to the last paragraph.

Con formato: Fuente: Cursiva

Con formato: Fuente: Cursiva

Con formato: Sangría: Primera línea: 1,77 car.

Con formato: Fuente: Cursiva

Con formato: Fuente: Cursiva

Comentado [Rev12]: θ sm-t ? Or may be change to angles?

Con formato: Sangría: Primera línea: 1,77 car.

pendulum-like movements are defined ~~that~~ considering the point of touchdown as pivot point, and the arm length is equal to the length between the pivot and the animal´s center of mass. The arm length of the inverted pendulum is assumed ~~as maintaining in~~ constant in these previous studies. In this regard, limbs are the only structure to control the distance between the ground and the body trunk, therefore, the inverted pendulum arm length ~~is depended~~ depends on the joint angles. ~~Furthermore, the angle between the femur and the *m. semimembranosus* has a small variance range because both of them attached on the pelvis. In other words, the knee joint angle is mainly depended on the angle between the *m. semimembranosus* and the tibia (θ_{sm-t}). When compared the θ_{sm-t} transition of SIs next to each other, both joint extension and flexion occurred during a stance (Table 3). This joint extension and flexion occurred alternately in the first half of a stance.~~ The knee joint ~~received~~ receives forces to flex from several factors such as a collision at touch down, gravity, and rising the center of mass, therefore, extensor muscles react~~ed to~~ against the flexion immediately. Biomechanically, the inverted pendulum arm is preferred to keep its length, therefore, the joint angle would also be ~~preferred~~ expected to be keept ~~in~~ constant. To increase the joint stiffness, co-contraction would ~~be~~ occurred at this time (Hogan, 1984). In other words, the flexor muscles would be stimulated at that time. Both, the femur focused in previous studies and the ischial tuberosity used in our study locate on the pelvis, and the pelvis does not rotate drastically during walking; therefore, this logic is also applicable to the θ_{sm-t}. When looking at one stance of our study, extension and flexion periods were not separated completely as in the case of extension in the first half of a stance and the flexion in the last half, and the difference between the of the θ_{sm-t} adjacent SIs showed that they were repeated in a short span (Table 4). The alternative increasing and decreasing of the θ_{sm-t} ~~joint angles~~ between each SIs allows quadrupedal mammals to maintain its joint angles. In other words, the role of co-contraction during walking is not to fix the joint angles, but to maintain the joint angles within a certain range by making small increase and decrease of the θ_{sm-t} were occurred in broad taxa in our study (Table 4). Therefore, ~~made~~ the angle transitions of the θ_{sm-t} ~~whole~~during one stance were small among the target species (Fig. 3~~4~~). The results in ~~this~~our study showed that 17 out of ~~21~~20 ~~studied~~target species had a slight difference of the θ_{sm-t} change, which was less than ± 10 degrees from its middle value, even though the largest difference was ± 15.86 (Fig. 3~~2~~ and Table 2).

According to the results of the ~~average angle of θ_{sm-t} (i.e., θ_{ave})~~, most of the ~~target animals~~species (>~~90~~85 %) had ~~its θ_{ave} in~~ 100 ± ~~21~~10 degrees (Fig 4~~5~~). The target animals fell into this range included all three ~~gait types~~ambulatory styles (i.e., unguligrade, digitigrade, and plantigrade) among four super orders (Afrotheria, Euarchontoglires, Laurasiatheria, and Marspialia). In addition, they also had wide range of the body mass, from 4.8 kg of *Felis* to ~~4060~~ 1100 kg of ~~*Elephas*~~*Diceros* (Table 1 and 3). ~~(Alexander & Pond, 1992)~~

Effective mechanical advantage (EMA) is one of the directions to estimate the mammalian limb posture: larger EMA ratio indicates more upright posture (Biewener,

Comentado [Rev13]: Why?

Comentado [Rev14]: Here you are talking about energetic convenience, which has not been clearly mentioned in the introduction, there you emphasize the importance of co-contraction only. Please see comment in review

Con formato: Sangría: Primera línea: 1,77 car.

1898,1990, 2005; Dick & Clemente, 2017). These previous studies showed larger species had the larger EMA. It is different from results of our study that the quadrupedal mammals had similar θ_{ave}. This was due to the difference in the measuring position where the angle measured in each study. The ischial tuberosity where the *m. semimembranosus* attaches places on near the posterior end of the pelvis. The orientation of the pelvis of which horizontally or vertically is related to the body mass, and the larger body mass has the more upright orientation (Polly, 2007). Therefore, the larger body mass has the larger differences between the angle of femur-tibia (traditional knee joint angle) and *m. semimembranosus*-tibia ($\theta_{sm\text{-}t}$ and θ_{ave}) as a previously standardized measurements: in other words, $\theta_{em\text{-}t}$ and θ_{ave} as our new measurements could show the small differences between the angle of large body mass species and small body mass species. Furthermore, EMA does not increase linearly more than 300 kg (Biewener, 1990, 2005; Dick & Clemente, 2017), and felids had crouched posture even with large body mass (Day & Jayne, 2007; Dick & Clemente, 2017). In contrast, θ_{ave} showed contrast value 100 ± 10 among every ambulatory style and wide body mass range (4.5 kg to 1100 kg) in our study (Table 1 and 3). ~~Among the studied species, *Suricata* (Meerkat, Carnivora), which does not fall into this range, has some unique characteristics when compared to the others. *Suricata* is considered as a digger, and its body is adjusted to live in tunnels (van Staaden, 1994). In this regard, our analyses also suggest that *Suricata* would reasonably be excluded from the walking style of the standard mammals. When the recalculate the correlation coefficient between the angle of θ_{mid} and the body mass without *Suricata*, it become 0.26. This value indicates there are few relationships between the body mass and θ_{ave}. Therefore, "standard" terrestrial mammals have the angle of θ_{ave}, 100 ± 21 degrees even if the species have any taxon, body mass, limb posture, or gait. Furthermore~~In addition, $\theta_{sm\text{-}t}$ was measured with three points on skeletons in ~~this~~our study: the ischial tuberosity, the interior-proximal end of the tibia and the distal end of the tibia (Fig. 1~~B~~). This indicates that the position of the ischial tuberosity and tibia can be fixed with 100 ± 10 degrees on the extant terrestrial quadrupedal mammals~~posture of the hind limbs of terrestrial mammals are able to reconstruct with only ischium and tibia, and thus the hind limbs of~~ and thus, this new approximation to understanding hindlimb postures could be applied to the study of the hindlimbs of extinct mammals ~~who~~ which ~~does~~ no~~t~~ ~~have phylogenetically~~ closely related extant descendants. If a femur exists or can be estimate its shape, the limb posture can be ~~are also able to~~ reconstructed with higher accuracy because both the caput femoris and the distal end of the femur can be put in the determined positions, where are the acetabulum and the proximal end of tibia, respectively. For example, the Desmostylia has been reconstructed in several different postures among researchers even it has been known from several fossils of ~~whole body~~whole-body skeletons (Shikama, 1966; Inuzuka, 1988; Domning, 2002; Inuzuka, Sawamura & Warabe, 2006). It because this extinct mammal has no extant closely related descendants and extremely bizarre tibia: the tibia strongly twisted interiorly (Shikama, 1966; Inuzuka, 1988). There are no extant mammals which have the tibia resemble to Desmostylia.

Con formato: Fuente: Cursiva

The θ_{ave}, which is 100 ± 10 degrees, is independent value from taxonomy, body mass, and ambulatory style, therefore, this degree can be applied on the Desmostylia.

Conclusion

The stimulation of agonist and antagonist muscles called co-contraction increases the joint stiffness. In the case of the knee joint angle, our result showed that the θ_{sm-t} transition had almost flat wave form. It indicated that the θ_{sm-t} ~~was~~ did not changed drastically during the first 75 % of stance phase and that the co-contraction represented by part of the *m. quadriceps femoris* ~~(as an agonist muscle)~~ and the *m. semimembranosus* ~~(as an antagonist muscle)~~ ~~was~~ can effectively support~~ed~~ the constant posture of ~~hind limb~~the knee joint in ~~all the~~most terrestrial mammals at least. That is more than ~~90~~ 85 % of target animals in ~~this~~our study had similar θ_{ave}, 100 ± ~~21~~ 10 degrees even if the animal is classified in any tax~~a~~on, and has any body mass, ~~limb posture,~~ or employs any ambulatory style~~gait~~. The θ_{sm-t} could be measured with three points on skeletons, ~~. t~~T~~herefore, θ_{ave} ~~shows the distinction of standard and non-standard mammals in regard to their gaits. In addition, the~~was independent from those variables. These features of θ_{ave} indicate that θ_{ave} becomes one of the criteria ~~possibility~~ for reconstruction of extinct mammals even if they have no extant close~~ly~~ related descendants ~~was also indicated~~.

Con formato: Sangría: Primera línea: 0 car.

Con formato: Sangría: Primera línea: 1,77 car.

Comentado [Rev15]: If you define quadriceps as the agonistic, you would be suggesting movement, in this case extension of the knee, saying co-contraction is enough to understand that these muscles are antagonistics between each other.

Acknowledgements

We thank Higashi Park Zoological Gardens, Higashiyama Zoo and Botanical Garden, Hitachi Kaminé Zoo, Toyohashi Zoo and Botanical Park, Ueno Zoological Garden, and cooperator for their permission to take movies of animals under their care. We also thank Yoko Tajima and Shin-ichiro Kawada (National Museum of Nature and Science) for providing access to the extant mammal collections under their care, and Naomi Wada (Yamaguchi Univ.) for lending the camera. We are grateful to Katsuo Sashida (then Univ. of Tsukuba, now Mahidol Univ., Thailand), Sachiko Agematsu (Univ. Tsukuba), Kohei Tanaka (Univ. Tsukuba), Ikuko Tanaka (Univ. Tsukuba) and Yasunari Shigeta (NMNS/Univ. Tsukuba) for providing useful advice, discussion, and generous encouragement during the course of ~~this~~our study.

Con formato: Sangría: Primera línea: 0 car.

Con formato: Sangría: Primera línea: 1,77 car.

References

~~Alexander RMN, Pond CM. 1992. Locomotion and bone strength of the white rhinoceros, Ceratotherium simum. *Journal of Zoology* 227:63–69. DOI: 10.1111/j.1469-7998.1992.tb04344.x.~~

Araújo JF, Rodrigues FB, Abadia FG, Gervásio FM, Mendonça GBN, Damasceno AD, Vieira MF. 2016. Electromyographic analysis of the gait cycle phases of boxer dogs? *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia* 68:931–937. DOI: 10.1590/1678-4162-8770.

Con formato: Sangría: Primera línea: 0 car.

Con formato: Subrayado, Color de fuente: Rojo oscuro

Con formato: Portugués (Portugal)

Con formato: Portugués (Portugal)

Biewener AA. 1983. Allometry of quadrupedal locomotion: the scaling of duty factor, bone curvature and limb orientation to body size. *The Journal of Experimental Biology* 105:147–71.

Biewener AA. 1989. Scaling body support in mammals: limb posture and muscle mechanics. *Science* 245:45–48. DOI: 10.1126/science.2740914.

Biewener AA. 1990. Biomechanics of mammalian terrestrial locomotion. *Science* 250:1097–1103. DOI: 10.1126/science.2251499.

Biewener AA. 2005. Biomechanical consequences of scaling. *The Journal of Experimental Biology* 208:1665–1676. DOI: 10.1242/jeb.01520.

Böhmer, C., Theil, J. C., Fabre, A. C., & Herrel, A. 2020. *Atlas of terrestrial mammal limbs*. CRC Press.

Campos CM, Tognelli MF, Ojeda RA. 2001. Dolichotis patagonum. *Mammalian Species* 652:1–5. DOI: 10.1644/1545-1410(2001)652<0001:DP>2.0.CO;2.

Cassinello J. 1997. High levels of inbreeding in captive Ammotragus lervia (Bovidae, Artiodactyla): Effects on phenotypic variables. *Canadian Journal of Zoology* 75:1707–1713. DOI: 10.1139/z97-797.

Cavagna GA, Heglund NC, Taylor CR. 1977. Mechanical work in terrestrial locomotion: two basic mechanisms for minimizing energy expenditure. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 233:R243–R261. DOI: 10.1152/ajpregu.1977.233.5.R243.

Day LM, Jayne BC. 2007. Interspecific scaling of the morphology and posture of the limbs during the locomotion of cats (Felidae). *The Journal of Experimental Biology* 210:642–654. DOI: 10.1242/jeb.02703.

Deban SM, Schilling N, Carrier DR. 2012. Activity of extrinsic limb muscles in dogs at walk, trot and gallop. *The Journal of Experimental Biology* 215:287–300. DOI: 10.1242/jeb.063230.

Demes B, Larson SG, Stern JT, Jungers WL, Biknevicius AR, Schmitt D. 1994. The kinetics of primate quadrupedalism: "hindlimb drive" reconsidered. *Journal of Human Evolution* 26:353–374. DOI: 10.1006/jhev.1994.1023.

Dick TJM, Clemente CJ. 2017. Where Have All the Giants Gone? How animals deal with the problem of size. *PLOS Biology* 15:e2000473. DOI: 10.1371/journal.pbio.2000473.

Domning DP. 2002. The Terrestrial Posture of Desmostylians. *Smithsonian Contributions to Paleobiology*.

Dutto DJ, Hoyt DF, Clayton HM, Cogger EA, Wickler SJ. 2006. Joint work and power for both the forelimb and hindlimb during trotting in the horse. *The Journal of Experimental Biology* 209:3990–3999. DOI: 10.1242/jeb.02471.

Engberg I, Lundberg A. 1969. An electromyographic analysis of muscular activity in the hindlimb of the cat during unrestrained locomotion. *Acta Physiologica Scandinavica* 75:614–630. DOI: 10.1111/j.1748-1716.1969.tb04415.x.

Con formato: Fuente: Cursiva

Fa JE, Purvis A. 1997. Body Size, Diet and Population Density in Afrotropical Forest Mammals: A Comparison with Neotropical Species. *The Journal of Animal Ecology* 66:98. DOI: 10.2307/5968.

Fitzgerald CS, Krausman PR. 2002. Helarctos malayanus. *Mammalian Species* 696:1–5. DOI: 10.1644/1545-1410(2002)696<0001:HM>2.0.CO;2.

Fujiwara S. 2009. Olecranon orientation as an indicator of elbow joint angle in the stance phase, and estimation of forelimb posture in extinct quadruped animals. *Journal of Morphology* 270:1107–1121. DOI: 10.1002/jmor.10748.

Fujiwara S, Hutchinson JR. 2012. Elbow joint adductor moment arm as an indicator of forelimb posture in extinct quadrupedal tetrapods. *Proceedings of the Royal Society B: Biological Sciences* 279:2561–2570. DOI: 10.1098/rspb.2012.0190.

Garland T. 1983. The relation between maximal running speed and body mass in terrestruial mammals. *Journal of Zoology* 199:157–170.

Gingerich PD. 2001. Origin of whales from early artiodactyls : hands and feet of Eocene Protocetidae from Pakistan. *Science* 293:2239–2242. DOI: 10.1126/science.1063902.

Gingerich PD, Heissig K, Bebej RM, von Koenigswald W. 2017. Astragali of Pakicetidae and other early-to-middle Eocene archaeocetes (Mammalia, Cetacea) of Pakistan: locomotion and habitat in the initial stages of whale evolution. *PalZ* 91:601–627. DOI: 10.1007/s12542-017-0362-8.

Gingerich PD, Ul-Haq M, Von Koenigswald W, Sanders WJ, Smith BH, Zalmout IS. 2009. New protocetid whale from the middle Eocene of Pakistan: Birth on land, precocial development, and sexual dimorphism. *PLoS ONE* 4. DOI: 10.1371/journal.pone.0004366.

Griffin TM, Main RP, Farley CT. 2004. Biomechanics of quadrupedal walking: How do four-legged animals achieve inverted pendulum-like movements? *Journal of Experimental Biology* 207:3545–3558. DOI: 10.1242/jeb.01177.

Haas SK, Hayssen V, Krausman PR. 2005. Panthera leo. *Mammalian Species* 762:1–11.

Hillman-Smith AKK, Groves CP. 1994. Diceros bicornis. *Mammalian Species* 455:1. DOI: 10.2307/3504292.

Hogan N. 1984. Adaptive Control of Mechanical Impedance by Coactivation of Antagonist Muscles. *IEEE Transactions on Automatic Control* 29:681–690. DOI: 10.1109/TAC.1984.1103644.

Inuzuka N. 1988. Reconstruction of Extinct Mammal, Desmostylus (In Japanese). *Biomechanisms* 9:7–19. DOI: 10.3951/biomechanisms.9.7.

Inuzuka N. 1996. Preriminary study on kinematic gait analysis in mammals. *Mammal Study* 21:43–57.

Inuzuka N, Sawamura H, Watabe H. 2006. Paleoparadoxia and the Nishikurosawa specimen from Oga (In Japanese), *Akita, northern Japan*. Akita, Japan.

Knarr BA, Zeni JA, Higginson JS. 2012. Comparison of electromyography and joint moment as indicators of co-contraction. *Journal of Electromyography and Kinesiology* 22:607–611. DOI: 10.1016/j.jelekin.2012.02.001.

Latash ML. 2018. Muscle coactivation: Definitions, mechanisms, and functions. *Journal of Neurophysiology* 120:88–104. DOI: 10.1152/jn.00084.2018.

Le P, Best TM, Khan SN, Mendel E, Marras WS. 2017. A review of methods to assess coactivation in the spine. *Journal of Electromyography and Kinesiology* 32:51–60. DOI: 10.1016/j.jelekin.2016.12.004.

Louie JK, Mote CD. 1987. Contribution of the musculature to rotatory laxity and torsional stiffness at the knee. *Journal of Biomechanics* 20:281–300. DOI: 10.1016/0021-9290(87)90295-8.

Madar SI. 2007. The postcranial skeleton of early Eocene pakicetid cetaceans. *Journal of Paleontology* 81:176–200. DOI: 10.1666/0022-3360(2007)81[176:TPSOEE]2.0.CO;2.

Mech LD. 1974. Canis Lupus. *Mammalian Species* 37:1–6. DOI: 10.2307/3503924.

Nielsen J, Sinkjær T, Toft E, Kagamihara Y. 1994. Segmental reflexes and ankle joint stiffness during co-contraction of antagonistic ankle muscles in man. *Experimental Brain Research* 102:350–358. DOI: 10.1007/BF00227521.

Obara H, Uramoto M, Hidetoshi O, Matsui M. 2000. Japanese macaque. In *Animal world helitage Red data animals* (Japanese). Tokyo: Kodansha Ltd, 139.

Olmstead TG, Wevers HW, Bryant JT, Gouw GJ. 1986. Effect of muscular activity on valgus/varus laxity and stiffness of the knee. *Journal of Biomechanics* 19:565–577. DOI: 10.1016/0021-9290(86)90162-4.

Padilla M, Dowler RC. 1994. Tapirus terrestris. *Mammalian Species* 481:1–8. DOI: 10.1007/978-1-4615-6434-8_37.

Pandy MG, Kumar V, Berme N, Waldron KJ. 1988. The dynamics of quadrupedal locomotion. *Journal of Biomechanical Engineering* 110:230–237. DOI: 10.1115/1.3108436.

Patel BA, Horner AM, Thompson NE, Barrett L, Henzi SP. 2013. Ontogenetic scaling of fore- and hind limb posture in wild chacma baboons (Papio hamadryas ursinus). *PLoS ONE* 8:1–8. DOI: 10.1371/journal.pone.0071020.

Polly PD. 2007. Limbs in mammalian evolution. In: Hall BK ed. *Fins into Limbs: Ecolution, Development, and Transformation*. Chicago: University of Chicago Press, 245–268.

Ren L, Butler M, Miller C, Paxton H, Schwerda D, Fischer MS, Hutchinson JR. 2008. The movements of limb segments and joints during locomotion in African and Asian elephants. *The Journal of Experimental Biology* 211:2735–2751. DOI: 10.1242/jeb.018820.

Riemann BL, Lephart SM. 2002. The sensorimotor system, Part II: The role of proprioception in motor control and functional joint stability. *Journal of Athletic Training* 37:80–84. DOI: 10.1016/j.jconhyd.2010.08.009.

Shikama T. 1966. Postcranial Skeltons of Japanese Desmostylia. *Paleontological Society of*

Con formato: Portugués (Portugal)

Japan Special Papers 12:1–241.

Shoshani J, Eisenberg JF. 1982. Elephas maximus. *Mammalian Species* 182:1–8.

~~Smith AM. 1981. The coactivation of antagonist muscles. *Canadian Journal of Physiology and Pharmacology* 59:733–747.~~

~~van Staaden MJ. 1994. Suricata suricatta. *Mammalian Species* 483:1. DOI: 10.2307/3504085.~~

Thewissen JGM, Illiams EM, Roe LJ, Hussain ST. 2001. Skeletons of terrestrial cetacean and the relationship of whales to ariodactyls. *Nature* 413:277–281.

Thewissen JGM, Madar SI, Hussain ST. 1998. Whale ankles and evolutionary relationships. *Nature* 395:452. DOI: 10.1038/26656.

Tokuriki M. 1973. Electoromyographic~~LECTROMYOGRAPHIC~~ and ~~AND~~ Joint-Mechanical~~JOINT-MECHANICAL~~ Studies~~STUDIES~~ ~~IN~~ in Quadrupedal ~~QUADRUPEDAL~~ Locomotion~~LOCOMOTION~~: I. WALK. *The Japanese Journal of Veterinary Science* 35:433-446_2. DOI: 10.1292/jvms1939.35.433.

~~Yin L, Chen K, Guo L, Cheng L, Wang F, Yang L. 2015. Identifying the functional flexion-extension axis of the knee: An in-vivo kinematics study. *PLoS ONE* 10:1–11. DOI: 10.1371/journal.pone.0128877.~~