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Limb loading of thrinaxodon liorhinus: postural inference of non-mammaliaform cynodonts

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RE: PhD FINAL SUBMISSION with Response to Examiners

Dear Prof. Roger Gibson and Graduate Committee

I would like to thank all three examiners for their advice and helpful comments on my PhD submission. A revised version is attached. The examiners' suggested corrections of minor errors were adopted as requested. I revised the entire document for clarity by rewording certain sentences and following suggestions for text additions to clarify and/or justify statements, particularly within the literature review portion of the document. Due to these changes, pagination changed. For your convenience, I provide the original page number in bold font followed by the new page number in brackets. Please see the detailed check-list below for an item-by-item accounting of revisions and corrections. I also provide a summary of how I responded to each of the comments and suggestions of the external examiners.

Yours sincerely,



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Thesis Title: **Limb loading of *Thrinaxodon liorhinus*: postural inference of non-mammaliaform cynodonts.**

General Comments:

- All editorial corrections adopted as requested.
- Revision, addition, and clarification of sentences were completed.
- New figures were added in order to clarify and bolster support for statements.

Response to Examiner one:

I thank the examiner for his/her valuable comments.

- Phylogenetic relationships
    - **Page 12, 1st paragraph**:
        - (Page 14): Additional clarity of the phylogenetic relationship among the taxa studied. Also, added a cladogram.
    - **Page 12, 1st paragraph**:
        - (Page 13, paragraph 2, line 2): Replaced ‘hypothetical ancestor’ with ‘representing an intermediate stage’.
    - **Page 18, 1st paragraph**:
        - (Page 22): Clarity of relationships by referencing the added cladogram.
    - **Page 79, 1st paragraph, line 6-12**:
        - (Page 92, paragraph 1, line 14): Added text for clarity
            - ‘This suggests that the underlying changes evident in the evolutionary sequence may have been driven by selection pressures for cost-effective locomotion, with additional

autopomorphic features reflecting specialized behaviours superimposed on the more general structural (and postural) changes (Reilly *et al*. 2007; Riskin *et al*. 2016)'.

- Defining postures and gaits
    - **Page 12, throughout the thesis**: Defined and illustrated the different postures tested.
        - (Page 13, paragraph 2, line 6): Clarified author citation from:
            - To: The initial postcranial description of *Thrinaxodon* was published in a monograph sixty-two years ago (Brink 1956), which remains influential on more recent assessments of the postcranium of cynodonts (such as Jenkins 1971).
        - (Page 16): Replaced Figure illustrating posture of *Thrinaxodon*.
        - (Page 16): Replaced Kemp 1983 with Brink 1956 in figure caption.
        - (Page 16, paragraph 1): Added text: 'Functional gait and limb posture are defined by the degree of adduction and abduction of the humerus. Thus, a sprawled posture is represented by the humerus being abducted and positioned approximately in a horizontal plane during the stance phase, while in the semi-sprawled posture the humerus is only semi-abducted (i.e., oriented at an angle of 45˚ from the sagittal plane). Parasagittal posture includes the propodial of the limb positioned in an orientation relatively more beneath the body (Blob 2001), i.e., adducted into an approximately vertical plane'.
- Reconstruction of the limb musculature and estimates of their forces
    - **Page 26 through to table 2.3 and figure 2.2**:
        - (Page 37): Justification of using Greyhound, mole and opossum as a basis for comparative analysis of cynodont muscle attachment sites.
            - Added text: 'These loading data (i.e., muscle force magnitude - Williams *et al*. 2008, 2008; Gosnell 2010; Rose *et al*. 2013; Kawano *et al*. 2016) were obtained from direct observations of the extant taxa behaviour and their structural peculiarities were inferred to the extinct taxa under investigation (Oliveira and Schultz 2015). These inferences were based on the anatomy of the humerus and femur as well as the muscle attachments to the bone'.

- Background introductory material
    - **Page 11, 1st paragraph, line**:
        - (Page 12): Extension of the opening paragraph.
            - Added text: 'The Early Triassic, between 252.2 and 247.2 million years ago, represents an age of deep transformation on Earth. The Permian-Triassic extinction event killed nearly 95% of all marine genera and 70% of land species (Sues and Fraser 2010). Therapsids ('mammal-like reptiles') represented the majority of the large-bodied terrestrial vertebrate fauna during the Permian, and were severely affected by the extinction event such that only a few lineages were able to persist'.
        - (Page 13, paragraph 1, line 9): Extension of the paragraph.
            - Added text: 'Many therapsids across independent lineages adopted fossorialism at this time of ecosystem upheaval, and researchers have hypothesized that it was an excellent survival strategy to cope with unfavourable habitat conditions. As burrowing is common today, even in the most primitive mammals (e.g., platypus), it seems likely that the early mammalian condition evolved from at least a semi-fossorial therapsid ancestor. Thus, studying the morphofunctional aspects of taxa associated with this transition can address this hypothesis'.
- Poor expressions, queries, grammatical infelicities and typos
    - **Page 1, 2nd paragraph, line 5**: Replace 'i.e.' with 'e.g.'
        - (Page 13, paragraph 1, line 4): Replaced as suggested.
    - **Page 12, 1st paragraph, line 4 up**: Clarified the sentence. Replace 'immense' with extensive'.
        - (Page 14, paragraph 1, line 1): Replaced as suggested.
    - **Page 12, 2nd paragraph, line 3**:
        - (Page 14, paragraph 1, line 1): Removed 'in particular'.
    - **Page 13, 1st paragraph, line 1**: Clarified the sentence.
        - (Page 17, paragraph 1, line 5up): Added 'muscle' angles.
    - **Page 13, 2nd paragraph, line 5**: Replaced 'in' with 'of'

- (Page 15, paragraph 1, line 2up): Replaced as suggested.

- **Page 13, 2nd paragraph, line 2up:**
  - (Page 17): Clarified the sentence by adding text: Conversely, parasagittal gaits decrease mediolateral stability and raise the centre of gravity, which increases the mechanical efficiency of the limb that allows for powerful force generation during an increase in velocity and longer distance travel, and decreases bending moments of the humerus and femur compared to those typically experienced during sprawled gaits (Biewener 1989; Christiansen and Paul 2001).
- **Page 14, 1st paragraph, line 1**: Clarified the sentence.
  - (Page 17, paragraph 2, line 1): A study on burrow dimensions led previous researchers (e.g., Damiani *et al.* 2003) to infer a more parasagittal posture in *Thrinaxodon*'s hind limb.
- **Page 14, 1st paragraph, line 4**: Replaced 'maintaining' with 'transmitting'.
  - (Page 17, paragraph 2, line 4): Replaced as suggested.
- **Page 15, 2nd paragraph, line 3**: Replaced 'scale' with 'scales'
  - (Page 18, paragraph 3, line 1): Replaced as suggested.
- **Page 15, 2nd paragraph, line 2**: Replaced 'too' with 'to'
  - (Page 19, paragraph 1, line 1-2): Replaced as suggested. Added text: 'The structure of vertebrate bones is complex and adapts to the loading conditions to which it is subjected (Martin 1991)'.
- **Page 15, 3rd paragraph, line 4**: Replaced 'reflects' with 'reflect'
  - (Page 19, paragraph 1, line 4): Replaced as suggested.
- **Page 15, 3rd paragraph, line 5**: Replaced 'modified' with 'met'
  - (Page 19, paragraph 1, line 4): Added text: 'By this modification in limb bone morphology, the locomotor signal can be inferred'.
- **Page 15, 3rd paragraph, line 6**: Replaced 'conducted' with 'investigated'
  - (Page 19, paragraph 1, line 8): Replaced as suggested.

- **Page 15, 3rd paragraph, last line**:
    - (Page 19, paragraph 1, line 4up): Added text: ‘data for’
- **Page 17, 1st paragraph, line 3-4**:
    - (Page 21, paragraph 1, line 4up): Added text for clarity: ‘The von Mises yield criterion calculates the yield of ductile materials by conjecture that tension and compression are equal in strength’.
- **Page 18, 1st paragraph, last two sentences**:
    - (Page 23, paragraph 1, line 5): Replaced text to correct meaning of the sentence: ‘homologous’ with ‘analogous’.
- **Page 19, Aim number 4**:
    - (Page 25): Added text: ‘of other cynodonts’
- **Page 19, Aim number 5**:
    - (Page 95, paragraph 2, line 2): Added text to compare *Tachyglossus* to the fossil taxa.
    - ‘The femoral vastus muscles would be an EES factor in the diaphysis of *Tetracynodon darti* (BP/1/4335), *Galesaurus* (BP/1/4506), *Thrinaxodon* (BP/1/1730 and BP/1/1693) and *Tachyglossus* (Figures 3.18-3.32: 3)’.
    - ‘in both the extinct and extant species examined’
    - ‘T*achyglossus*, in comparison to the fossil taxa, exhibited the lowest torsional rigidity (Table 3.1).’
- **Page 20, 2nd paragraph, line 5-4up**: Replaced ‘ancestral mammals’ with ‘intermediate grade non-mammalian therapsids’
    - (Page 26, paragraph 3, line 5up): Replaced as suggested.
- **Page 24**: Clarified meaning in the Body Mass paragraph by adding text.
    - (Page 32): One of the essential biological properties that are associated with the physiological and ecological aspect of an individual is Body Mass (BM). These relationships may aid in understanding the palaeobiology of fossil species that is inferred by estimating BM. As BM increases in an evolving lineage, anatomical and physiological dimensions change (Biewener

1983). A different rate of change in a parameter compared to the rate of change in BM is called allometry. A higher rate of change in the parameter is defined as positive allometry (Schmidt-Nielsen 1975; Casinos *et al*. 1993). Conversely, a lower rate of change in the parameter is defined as negative allometry (Casinos *et al*. 1993; Dial *et al.* 2008). If the rate of change is the same in parameter and BM, it is defined as isometry.

- **Page 31, line 3up**:
  - (Page 40): Added text to clarify the statistical analysis paragraph. Please see Examiner 2 list of corrections.
- **Page 31, line 3up**: Bottom line changed to "generated as the anatomically anterior view".
  - (Page 41): Added text. 'generated as the anatomically anterior view'.
- **Page 79, line 8-9**:
  - (Page 92): Added text for clarity: 'This suggests that the underlying changes evident in the evolutionary sequence may have been driven by selection pressures for cost-effective locomotion, with additional autopomorphic features reflecting specialized behaviours superimposed on the more general structural (and postural) changes (Reilly *et al*. 2007; Riskin *et al*. 2016)'.
- **Page 80, 2nd paragraph, last line**:
  - (Page 94): Added text for clarity: 'Differences in muscle mass and the angle at which the muscle generates a moment on the limb bone, therefore, also play a role in the overall stress and strain that the bone experiences (Remes 2008)'.
- **Page 80-81, line 5**:
  - (Page 39): Added text referring to Figure 2.2.
- **Page 83, 1st paragraph, line 1**: Replaced 'species' with 'taxa'
  - (Page 96, paragraph 2, line 4): Replaced as suggested.
- **Page 83, 1st paragraph, line 2**:
  - (Page 96, paragraph 2, line 3): Added text: 'Humeri of Therocephalia, one of the oldest taxa under investigation, exhibited the highest torsional rigidity (Figure 3.4), suggesting a

sprawled posture in the forelimb that was able to withstand the stress and strain experienced by the limb'.

- **Page 83, 1st paragraph, line 10-11**:
  - (Page 97, paragraph 1, line 1-2): Clarified text: The stress experienced on a sprawled limb posture increases when the bone loads are exposed to parasagittal gait biomechanics (Blob and Biewener 2001).
- **Page 83, 2nd paragraph, line 2-3**:
  - (Page 97, paragraph 2, line 2): Clarified text: Due to the angle in which the humerus and femur attach to the pectoral and pelvic girdle, respectively, the location of the min. EES and ES has a larger area along the bone.
- **Page 83, line 3up**: Replaced 'i.e.' to 'e.g.'
  - (Page 97, paragraph 2, line 3up): Replaced as suggested.
- **Page 85, 2nd paragraph, last line**: Removed.
- **Page 85, last paragraph, lines 2-1up**: Clarified with additional text.
  - (Page 100, paragraph 1, line 4): However, cross-sectional properties produce a reasonable amount of knowledge on the distribution of material (e.g., estimating resistance to strain) and it would be intriguing to explore properties at different percentages of diaphyseal length (i.e., cross-sections at 25% and 75%), especially if there are ridges and processes present at the 50% cross-section, e.g., elongation of the deltopectoral crest along the diaphyseal length.
- **Page 86, Conclusion paragraph**:
  - (Page 101): Proof read and corrected typos and grammatical errors
- **Page 87, Reference list:** Correct sequence and spelling of Padian
  - (Page 102): Corrected sequence and the spelling of Padian.

Response to Examiner two:

I thank the examiner for his/her valuable comments.

- Introduction paragraph (please see Examiner 1 and 3 list of corrections)
    - **(Introduction paragraph)**: Added text:
        - (Page 20): **A) '**defined material properties i.e., Young's modulus (elasticity of the material), Poisson's ratio and density, as well as loads and forces that are applied to the nodes of the element, produce a deformation calculated in the solution phase that in turn results in an estimate of the stress and strain of the material (Rayfield 2007; Kupczik 2008)'**.**
            - (Page 20): 'The elastic modulus, Young's modulus, illustrates the strain a structure experiences in response to the stress loading axially onto it, while the Poisson's ratio describes the tension or compression the structure is subjected to (Blake 1990; Tamvada 2014). Material properties of bone differ depending upon the direction of assessment, permitting bone to be tested as anisotropic or orthotropic in elasticity (Rayfield 2007). Directly testing the elasticity of bone, however, allows for the FEM to be processed as isotropic. A more comprehensive study has yet to be conducted on the effects of modelling more complex bone elasticity parameters on the resulting FEMs'.
        - (Page 19**): B) '**The application of FEA in biological sciences was established after the technique was accepted in engineering (Brekelmans *et al*. 1972). As the practical technique of FEA proved to be a success in orthopaedic biomechanics, theoretical research was cultivated in assessing the role of skeletal stress and development (Huiskes and Hollister 1993; Beaupre and Carter 1992)'.
            - (Page 20): 'This technique allows for a broad-scale evolutionary assessment, functional and adaptive significance of skeletal morphology (Brassey 2014) and comparative analysis with extant taxa in palaeontology (Kupczik 2008; Hohn-Schulte 2010; Tamvada 2014)'.

- (Page 20): The FEM must reflect complexity of the actual element but still be simple enough for appropriate digital manipulation (Rayfield 2007). In the pre-processing phase, complexity of the models and the computing time required to produce the most accurate results has to be considered.

- **Page 28, Chapter 2:** Replaced 'morphospace' with 'virtual space'.
  - (Page 21, Chapter 2): Replaced as suggested.
- **(Page 28, Pre-Process: Boundary Conditions paragraph)**: Added text:
  - (Page 37): 'These loading data (i.e., muscle force magnitude - Williams *et al*. 2008, 2008; Gosnell 2010; Rose *et al*. 2013; Kawano *et al*. 2016) were obtained from direct observations of the extant taxa behaviour and their structural peculiarities were inferred to the extinct taxa under investigation (Oliveira and Schultz 2015). These inferences were based on the anatomy of the humerus and femur as well as the muscle attachments to the bone. Each muscle force was distributed over an area of 3 mm2 at the specific origin/insertion point of the muscles'.
- ***Cynognathus* humerus (Please see Examiner 3 list of corrections)**
  - Suggestion adopted: The humerus was digitally restored and the analysis was repeated. The new data and results were added to the final thesis document. (Page 44, 50, 56 for new results).
- **Figures of the FEA results in Chapter 3**: Suggestion adopted:
  - A) The contour plots were scaled to the same upper limits and the summary figures were added to Chapter 3.
  - B) Scale Bars were corrected
  - C) The additional data (i.e., time and date) were removed from the figures.
  - D) The blue gradient backgrounds were adjusted to standard black backgrounds.
- **Variation within a taxon (please see Examiner 3 list of corrections):**
  - (Page 94, paragraph 3, line 1): Added text: 'Variation within species was most evident among specimens of *Thrinaxodon* (specifically BPI/1/1730 - Figure 3.2) and may have been size- and structurally dependent'.

- (Page 95, paragraph 2, line 5up): Added text: ‘There is variation within the three *Thrinaxodon* (specifically BP/1730 compared to BP/1/1693 and BP/1/7199) femora. The results obtained are dependent on the morphology of the limb bone that affects the bone’s ability to withstand the stress and strain it experiences, thus the clarification for opposite compressive and tensile surfaces within species’.

- **Paired t-test (Please see Examiner 3 list of corrections):**
  - Corrected to a Kruskal-Wallis test, as suggested.
- **Chapter 4: Typos and Grammatical errors**
  - Proof-read and adjusted appropriately.
- **Figures and Tables (Examiner 3):**
  - Added species names to the collection numbers, as suggested.

Response to Examiner Three:

I thank the examiner for his/her valuable comments.

- **Page 1**, **Title**: Replaced ‘validation’ with ‘inference’.
  - (Page 1), Modified text as requested.

- **Abstract**
  - (Page 11-12): Added text: ‘As *Thrinaxodon*’s posture is experimentally altered from sprawled to parasagittal, stress and strain their limb bones experience increases. This suggests that their posture is structurally optimised for a sprawled to semi-sprawled posture. Although, they certainly still have the capacity to dynamically adopt any of the three postures for a short period of time. Incorporating FEA into palaeontological research questions contributes to an explicit comparative investigation that

assesses how phenotypic changes reflect postural transitions at a critical time in the lineage leading to mammals'.

- **Fossil trackways**:
    - (Page 17, paragraph 1, line 5up): Added text: 'Research conducted by Kubo and Benton (2009), documents the change in posture from fossil trackways between the Permian and Triassic periods. Fossil trackways provide evidence of the size of footprints and the stride length that would allow for comparisons between sprawled vs. parasagittal posture (Wilson 2005), as well as offering insight into quadrupedal biomechanics related to changes in step widths'.
- **Variation in posture within an animal (please see Examiner 2 list of corrections):**
    - (Page 17, paragraph 1, line 6): Added text: 'There have been observations on postural variation within extant taxa dependent upon the speed and environment of the species (Reilly and Blob 2003). This postural variation among tetrapods, requires change in the limb muscle attachment angle (Reilly and Elias 1998; Reilly and Blob 2003)'.
    - (Page 97, paragraph 1, line 2): Added text: 'Research conducted by Reilly and Elias (1998) proved that tetrapod postural variation depends on speed, however, this was not considered in the present model since the results obtained used loading conditions applied to the bone during a standing (static) phase rather than the high walk (dynamic) phase as Reilly and Elias (1998) examined. Collectively, the results obtained in the present study together with the results from Reilly and Elias (1998) suggest that even if a species had a sprawled posture during standing (static) phase, it could still possess the dynamic ability to change their limb position more parasagittally during the walking phase'.
- **Limb bone morphology**:
    - (Page 19, paragraph 1, line 2): Corrected text: 'Functional adaptation of bone relies on its response to the external (e.g., ground reaction forces) and internal (e.g., muscle forces) loading regimes that affect morphology and in turn reflect function (Habib and Ruff 2008; Su 2011). By this modification in limb bone morphology, the locomotor signal can be inferred. Therefore, a relationship exists between bone morphology and the behaviour of species (Habib and Ruff 2008). This relationship is particularly useful in inferring behaviours to fossil taxa where behaviour can no longer be observed. Strain

regimes experienced by bone in response to *in vivo* loading have been investigated on extant species (Blob and Biewener 1999, 2001; Biewener and Taylor 1986; Gills and Biewener 2001)'.

- **Additional background information**:
    - (Page 24): Added time-calibrated phylogeny with silhouettes of species under investigation, as suggested.
- **Hypotheses**:
    - (Page 26): Added text: '*Thrinaxodon* humeral cross-sectional properties and biomechanical behaviour are optimised (most effective implementation of the posture under the measurable loading conditions) for semi-sprawled posture. Its hind limb posture is optimized for parasagittal postural loading'.
- **Chapter 2**:
    - Bone length and circumference measurements were added to Table2, as suggested.
- **Cross-sectional properties**:
    - (Page 100, paragraph 1, line 4): Added text to Discussion chapter 4: 'cross-sectional properties produce a reasonable amount of knowledge on the distribution of material (e.g., estimating resistance to strain) and it would be intriguing to explore properties at different percentages of diaphyseal length (i.e., cross-sections at 25% and 75%), especially if there are ridges and processes present at the 50% cross-section, e.g., elongation of the deltopectoral crest along the diaphyseal length'.
- **Figure of Cross-sections**:
    - (Page 119): Added a figure illustrating the cross-sectional shape at 50% diaphyseal length for all the specimens analysed, as suggested.
- **Orientation of slices for cross-sectional properties**:
    - (Page 31): Added text: 'The AP and ML axes on each cross-sectional image were oriented with established anatomical position of the humerus and femur being determined beforehand, thus allowing for meaningful comparisons of the Iy and Ix properties across individuals'.
- **Page 26**, Witmer (1995): Justification of Witmer (1995)
    - (Page 35): This relationship is based on the study by Witmer (1995) who stated that there must be phylogenetic characteristics that can be inferred from extant taxa to extinct taxa in addition to

correlation with the osteological structure (Erickson *et al*. 2002). Witmer (1995) referred to this inference as a phylogenetic bracket.

- **Isotropic material properties (please see Examiner 2 list of corrections):**
    - (Page 20-21): Added text: 'Material properties of bone differ depending upon the direction of assessment, permitting bone to be tested as anisotropic or orthotropic in elasticity (Rayfield 2007). Directly testing the elasticity of bone, however, allows for the FEM to be processed as isotropic. A more comprehensive study has yet to be conducted on the effects of modelling more complex bone elasticity parameters on the resulting FEM's.
    - (Page 35): Added text: 'Research based on directly testing the material properties of bone (Reilly and Burstein 1975) rendered it isotropic in the transverse plane. Isotropic material properties are assumed in a simplification of bone models since bone composition is nonhomogeneous, i.e., trabecular bone is anisotropic while cortical bone is transversely isotropic (Bankoff 2012). However, this simplification in the modelling of bone enables its application (Strait *et al*. 2005; Vignoli and Kenedi 2016; Sheng *et al*. 2017), thus, allowing for a general and broad interpretation of the results obtained'.
- **Table 2.2**:
    - Corrected the body mass to the nearest gram, as suggested.
- **Loading parameters**:
    - (Page 120): Added figures in the appendices to illustrate the loading parameters, as suggested.
- **Page 31, Statistical analysis**:
    - (Page 40) Added text: 'The statistical analyses were obtained on Microsoft Excel 2007 using the plugin, XLSTAT 2018 (Paris, France). A Kruskal-Wallis test was conducted on the fossil taxa to determine whether there were any significant difference between the three postures that were under investigation. The Kruskal-Wallis test was used when samples differed from a normal distribution. In the cases where the computed p-value was lower than the significant level ($p < 0.05$), resulting in rejection, and the null hypothesis (H0) would be rejected, one or more species is different from another for a given gait. To identify which of the gaits contributed to the H0 rejection, a multiple pair-

wise comparisons using Dunn's procedure was used. A post-hoc Bonferroni correction was performed to statistically assess the differences among the species for each gait'.

- **Page 35, Figure 3.3**:
    - Please see Examiner 2 list of corrections. The *Cynognathus* humerus was digitally adjusted and the analyses were repeated.
- **Page 51, t-test**:
    - (Page 40): Please see Examiner 2 list of corrections. The t-test was removed and a Kruskal-Wallis test was added to the statistical analysis.
- **Page 71, Cross-sectional properties**:
    - (Page 84): Added text: 'Note: all cross-sectional properties (i.e., Ix, Iy, Imax, and Imin) were size-standardized by taking the natural log of the variable divided by length to the fourth power'.
    - Units were removed.
    - Added text: 'size-standardised'.
- **Graphs (Examiner 2):**
    - Added species names to the collection numbers, as suggested.
- **Figure C2-C13**:
    - Figures removed and figure numbers adjusted accordingly, as suggested.
- **Appendix D**:
    - (Page 83): Figure moved to the main text, Results Chapter 3, as suggested.