1   **Dental topographic analysis of feeding tools allows dietary inference in diverse animals**

2   Christopher Stockey[1], Neil F. Adams[1], Thomas H.P. Harvey[1], Philip C.J. Donoghue[2] & Mark A. Purnell[1].

3   [1] Centre for Palaeobiology Research, School of Geography, Geology and the Environment, University of

4   Leicester, University Road, Leicester, LE1 7RH, UK.

5   [2] School of Earth Sciences, University of Bristol, Life Sciences Building, Tyndall Avenue, Bristol, BS8 1TQ, UK.

6   Corresponding author:

7   Christopher Stockey (css25@le.ac.uk)

8   **Abstract**

9   1.   Understanding the relationship between form, function and diet in feeding structures is critical to

10   constraining the roles of organisms in their ecosystem and adaptive responses to food resources. Yet

11   analysis of this relationship in invertebrates has been hampered by a reliance on descriptive and

12   qualitative characterization of the shapes of feeding structures. This has led to a lack of robust

13   statistical analyses and overreliance on analogy and plausibility, especially for extinct taxa and animals

14   that are hard to observe feeding.

15   2.   Here we test the efficacy of dental topographic metrics in quantification of form in invertebrate

16   mandibles and assess their relationship with diet. Specifically, we analysed the mandibles of 45

17   species of extant orthopterans. Orthopterans' well constrained diets make them an ideal model

18   system for our study.

19   3.   We find that topographic metrics applied to Orthoptera successfully recover the same relationship

20   between dietary intractability and dental tool morphology as they do in mammals, and that

21   combination of individual metrics in multivariate analysis most strongly captures this relationship.

22   Furthermore, multivariate topographic metrics calibrated to the food consumed by mammals

23   accurately predict dietary differences between orthopterans (82.2% taxa correctly assigned).

24   4.   Our results demonstrate that these metrics can be used in quantitative analysis and comparison of

25   non-homologous mouthparts to reliably investigate the relationship between diet, form and function

26   of feeding tools across Bilateria. We anticipate that this will facilitate more rigorous ecological

27   analysis of fossil and historical material, providing new methods to investigate adaptive responses

28   and community level interactions through time.

29   **Key Words**

30   Dental morphology; Dietary ecology; Ecomorphology; Invertebrate; Macroevolution; Orthoptera;

31   Palaeoecology; Topographic Metrics.

32   **Introduction**

33   Analysis of the relationship between form and function of feeding structures provides a powerful tool for

34   investigating the role of organisms in their ecosystem and the nature of adaptive responses to changes in food

35   resources. This is as true for invertebrates as it is for vertebrates, yet analysis of the relationship between form,

36   function, and diet in invertebrate feeding structures has been hampered for decades because of a general reliance

37   on descriptive and qualitative characterization of the shapes of feeding structures. This descriptive approach has

38   made it difficult to undertake robust statistical testing of hypotheses, leading to an overreliance on analogy and

39   plausibility when attempting to assess the function of feeding structures in extinct taxa or those that are hard to

40   observe feeding. Despite many calls to address this issue across a range of disparate phyla (Hickman 1980;

41   Patterson 1984; Bernays 1991; Padilla 2004; Blanke *et al.* 2017; Clemo & Dorgan 2017; Krings *et al.* 2020),

42   quantitative analysis has remained largely unexplored (Patterson 1984) but see ref Blanke *et al.* (2017) as a notable

43   exception. The few quantitative analyses of invertebrate feeding structures performed to date offer significant

44   improvements over qualitative classifications of morphology. These include mollusc radulae, polychaete jaws,

45   mandibles of dragonflies and beetles, and marine arthropods, but all such studies are limited in scope. Some

46   capture a small selection of shape variables taken to have functional significance (Patterson 1984; Clemo & Dorgan

47   2017), while the handful of recent applications of Finite Element Analyses to invertebrate feeding structures

48   (Hörnschemeyer, Bond & Young 2013; Blanke *et al.* 2017; Bicknell *et al.* 2018; Krings *et al.* 2020) are focused on

49   small numbers of taxa and individuals because of the computational demands and time consuming nature of robust

50   FEA.

51   Here we adopt a different approach, offering a potentially powerful and widely applicable solution to quantification

52   of the morphology of feeding structures in invertebrates. Dental topographic analysis (M'kirera & Ungar 2003;

53   Evans *et al.* 2007; Karme 2008; Pineda-Munoz *et al.* 2017; Ungar *et al.* 2018) was developed primarily for analysis of

54   mammal teeth, but its applicability outside of gnathostome vertebrates has never been tested (apart from a

55   preliminary proof of concept study (Purnell & Evans 2009)). The methods are not computationally demanding and

56   capture a number of functionally significant attributes, potentially from any kind of tooth-like structure i.e. any

57   anatomical component involved in the processing of food items immediately prior to ingestion. Dental topographic

58   analysis lends itself to statistical testing and allows quantitative comparison of non-homologous structures.

59   Our approach addresses the long-standing call for quantification of shape and its relationship with diet in

60   invertebrate feeding structures, exploring a range of hypotheses designed to test the relationship between

61   dental topographic metrics and diet. Establishing this relationship will allow dental topographic measures to

62   serve as dietary eco-metrics, and extend the potential applications of this relatively simple and efficient

63   approach to a broad range of homologous and non-homologous feeding structures in disparate animals.

64   **Materials and Methods**

65   To test whether dental topographic metrics, developed and validated on mammals, can be generalized to

66   other groups with non-homologous dental tools we applied them to invertebrates with well constrained diets.

67   Our overarching null hypothesis is that dental topographic metrics do not reflect differences between dietary

68   categories in invertebrates. Rejection of this hypothesis leads to questions of the degree to which the dental

69   topographic metrics track diet and the degree to which results from non-homologous feeding structures are

70   comparable. We addressed this by testing two further hypotheses: first, that dental topographic metrics are

71   correlated with diet in invertebrates; second, that the absolute values of dental topographic metrics are

72   comparable between invertebrates and vertebrates that have similar diets but non-homologous food-

73   processing structures. If this second hypothesis finds support, it follows that multivariate topographic metrics

74   can be calibrated to the food consumed by one group of animals, and then used to infer diet in a distant group

75   with non-homologous feeding structures. We tested this using multivariate analysis to compare feeding

76   structures and diet in vertebrates and invertebrates.

77   Our analysis uses a number of topographic metrics (Fig. 1). Orientation Patch Count (OPCR) measures the

78   complexity of a functional food processing surface (Evans *et al.* 2007; Evans & Jernvall 2009). Other

79   topographic metrics – mean slope, relief index, angularity, and sharpness – measure the angle, height,

80   serratedness and pointedness of the tooth surface (M'kirera & Ungar 2003; Karme 2008; Ungar *et al.* 2018).

3

81 When used individually each of these metrics captures only a single aspect of tooth morphology. Multivariate

82 analysis, on the other hand, combines metrics to provide a more holistic evaluation of the shape of dental

83 tools. In mammals this approach, sometimes referred to as MPDMA (multi proxy dental morphological analysis

84 (Pineda-Munoz *et al.* 2017)), allows for stronger inferences of diet from tooth shape (Pineda-Munoz *et al.*

85 2017). We follow this approach here (Fig. 1).

86 Our analysis is based on 45 species of extant orthopterans. Orthopterans are ideal for testing our hypotheses

87 because diets of many taxa are well categorized on the basis of in situ field observations, differential feeding

88 experiments, and crop and faecal analysis (Gangwere 1967; Gangwere, Evans & Nelson 1976; Gangwere &

89 Spiller 1995; Gangwere *et al.* 1998; Kang, Gan & Li 1999; ElEla, ElSayed & Nakamura 2010). Our analysis is

90 based on the entire gnathal edge of the left mandibles removed from dry museum specimens (Fig. 1), selecting

91 the best preserved of the available specimens representing the final instar.
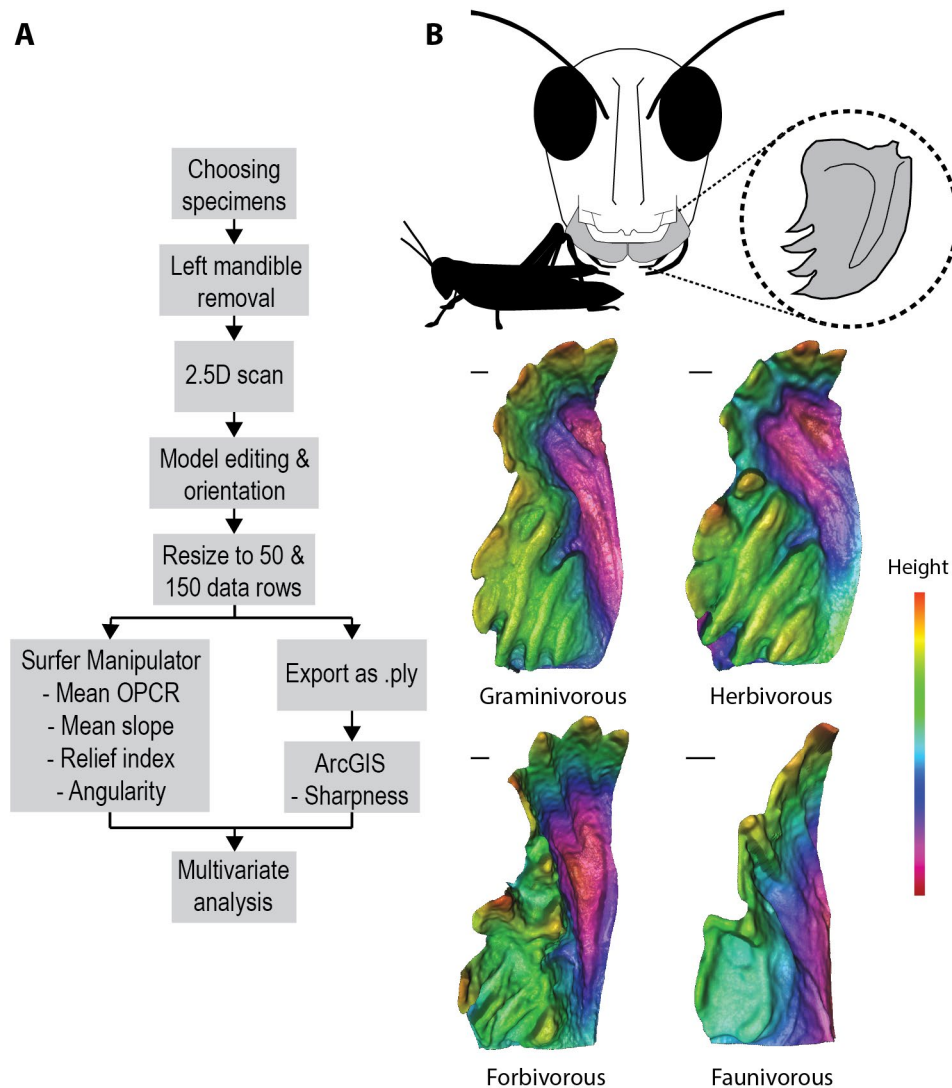
A

B

Figure 1 A) Workflow showing the generation of topographic metrics for orthopteran mandibles B) The functional regions of orthopteran left mandibles representative of each dietary category. Scale bars = 100 μm. Graminivorous, Aiolopus strepens, NHMUK 012504759. Herbivorous, Chorthippus dubius, NHMUK 013803298. Forbivorous, Angaracris barabensis, NHMUK 013803273. Faunivorous, Velarifictorus micado, NHMUK 012504733.

Dietary classification of orthopteran mandibles

Species were classified as faunivorous, forbivorous, herbivorous, or graminivorous based on the relative proportion of foodstuffs in their diet (Supplementary Table 1). Diets were classified based on both quantitative and qualitative descriptions. Faunivores preferentially eat other insects and oligochaetes. Forbivores

102   predominantly eat forbs, c. >75% (herbaceous flowering plants that are not grasses). Herbivores eat forbs and

103   grasses in roughly equal proportions. Graminivores predominantly eat grasses, c. >75%.

104   For analysis, diets were ranked in order of relative intractability based on the material properties of

105   constituent foodstuffs. In order of increasing intractability: 1, faunivory; 2, forbivory; 3, herbivory; 4,

106   graminivory. Intractability describes the structural strength of a foodstuff and how much processing it

107   requires(Evans & Sanson 2005). Animal muscle and soft tissues require less processing than chitinous

108   exoskeletons, and plant material is more intractable than both (Evans *et al.* 2007; Ennos 2011). C3

109   photosynthesising herbaceous plants are less tough than C4 photosynthesising grasses (Bernays 1991;

110   Cotterell 2010).

111   *Model generation and orientation of orthopteran mandibles*

112   2.5D models of mandibles were generated using focus variation optical microscopy. To ensure metrics were

113   generated consistently across all samples, orientation was standardised both mechanically during data

114   acquisition and digitally after model production (see supplement for additional details). Specimens were

115   oriented to maximise data capture during model generation. To orient the molar region orthogonal to the Z-

116   axis, model orientation was standardised digitally using an 'automatic robust' method in MeasureSuite 5.1

117   (Alicona Imaging, GmbH, Graz, Austria). Models were cropped to their functional surface and undercuts were

118   removed using MeasureSuite and Rhinoceros 6 (Robert McNeel & Associates, Seattle, USA). For consistency,

119   the X-axis was arbitrarily set in Rhinoceros as being parallel to the oral boundary of the model.

120   *Topographic metric generation and analysis of orthopteran mandibles*

121   Models were processed using SurferManipulator (Alistair Evans, 2008; version 20110921) and Surfer® 14

122   (Golden Software, LLC, Golden, USA). To maximise comparability with previous work, models were reduced to

123   50 and 150 data rows (50 row data are comparable with previous analyses of single teeth; 150 with a tooth

124   row (Evans *et al.* 2007; Smits & Evans 2012; Winchester *et al.* 2014; Pineda-Munoz *et al.* 2017; Rannikko *et al.*

125   2020)). In OPCR analysis, the minimum patch size was set to 3 and models were rotated stepwise eight times

126   (Evans & Jernvall 2009). Mean slope was calculated by dividing the slope into 9 categories. Angularity is the

127   second derivation of slope. Relief index was calculated as the ratio of the 3D surface area to its projected 2D

128   area. Sharpness index was calculated in ArcMap™ 10.8 (Esri® Inc., Redlands, USA) using the natural breaks

129   method classification (Jenks' optimization) with three classes (Karme 2008; Ungar *et al.* 2018). The surface

130   slopes were divided into three regions with the smallest possible variation within each and maximum possible

131   variation between them. Sharpness was calculated by dividing the size of the steepest region of the slope by

132   the combined size of the other two shallower regions. Multivariate analysis was performed through a PCA of

133   the five individual topographic metrics. The resulting five principal components were saved. All statistical

134   analyses were performed in JMP® 14 (SAS Institute, Cary, USA) unless otherwise stated. All analyses presented

135   here were performed on models reduced to 50 data rows. Univariate metrics of models reduced to 150 data

136   rows are included in the supplement.

137   To test the discriminatory power of individual topographic metrics and multivariate analysis of orthopterans,

138   we performed ANOVA of each topographic metric and MANOVA of the first four principal components

139   combined. To reduce the risk of false positive results from ANOVA due to violations in the assumption of equal

140   variances, when variances were found to be unequal by any statistical test, Welch's ANOVA was performed. All

141   topographic metrics and principal component values in each dietary category were normally distributed

142   (Shapiro-Wilks, α = 0.05) except for mean OPCR in forbivores and PC1 and PC2 in graminivores. Tukey HSD

143   tests were used to assess pairwise differences between dietary categories. The hypothesis that pairs of diets

144   differing more in rank of relative intractability had higher tallies of significant differences in metrics and

145   principal components was tested using Spearman's rank correlations. To test the hypothesis that multivariate

146   metrics (principal components) can be used to accurately predict diet in invertebrates an LDA of principal

147   components 1-4 was performed. The relationship between dietary intractability and topographic metrics was

148   tested using Spearman's Rank correlations of ordinal ranks of dietary intractability with individual metrics and

149   with principal components. In instances where multiple comparisons were made these were controlled for

150   using a Benjamini-Hochberg procedure with a false discovery rate set at 0.05 (Benjamini & Hochberg 1995).

151   *Controlling for phylogenetic effects in orthopterans*

152   Our comparison of metrics between orthopterans and mammals allows us to test the hypothesis that dental

153   topographic metrics capture dietary signals that span broad phylogenetic distances. Nonetheless, we tested

154   the hypothesis that the relationship between metric values and diet within orthopterans is more a reflection of

155   morphological similarities corresponding to closeness of evolutionary relationships than it is a measure of

156   ecological similarity. This requires a hypothesis of relationships within Orthoptera, but no phylogenetic tree

157 containing all of our 45 taxa has been published and there is insufficient sequence data to produce one. We

158 produced an informal supertree of all 45 taxa from published literature (see supplement), constructed on the

159 basis of well supported nodes reflecting character state changes and genetic divergence (Rowell & Flook 1998;

160 Fries, Chapco & Contreras 2007; Chapco & Contreras 2011; Zhang *et al.* 2013; Chen *et al.* 2018; Mugleston *et*

161 *al.* 2018; Song *et al.* 2018). Polytomies were used where nodes separating taxa in published literature were

162 poorly supported (e.g. low bootstrap value).

163 Phylogenetic closeness was calculated as the pairwise distances of summed branch lengths between taxa,

164 treating all individual branch lengths as equal to 1. Mantel tests analysed correlations of phylogenetic

165 closeness with pairwise distances of topographic metrics and principal component values, using the R package

166 ade4 (Dray & Dufour 2007). Orthoptera is made up of two monophyletic suborders, Ensifera and Caelifera

167 (Flook & Rowell 1997). All of the faunivorous taxa in our study are members of Ensifera and make up 9 of 12

168 ensiferan species represented. To account for any potential bias arising from this correlations of phylogenetic

169 closeness with pairwise differences in topographic metric and principal component values were tested across

170 Orthoptera and Caelifera separately. A Benjamini-Hochberg procedure with a false discovery rate set at 0.05

171 was used to control for multiple comparisons.

172 *Comparison of non-homologous feeding structures: orthopterans and mammals*

173 Values for mean OPCR, mean slope and relief index of molars downsampled to 50 data rows were obtained

174 from the literature for 93 mammal taxa representative of the orders Artiodactyla, Carnivora, Diprotodontia,

175 Rodentia, and Primates (Pineda-Munoz *et al.* 2017; Rannikko *et al.* 2020).

176 Plant-based diets in mammals were reclassified as 'herbivorous' according to our dietary scheme due to a lack

177 of published data that allows unequivocal comparisons between mammals and orthopterans at a finer scale of

178 dietary resolution (the only exception being *Phacochoerus africanus* which was reclassified as a graminivore)

179 (Pineda-Munoz *et al.* 2017; Rannikko *et al.* 2020)*.* Carnivorous and insectivorous mammals retained their

180 dietary categories (Pineda-Munoz *et al.* 2017) for comparison with faunivorous orthopterans. Multivariate

181 analysis was performed through a PCA combining orthopterans and mammals, based on the three individual

182 topographic metrics available for all. The resulting three principal components were saved. We were unable to

183 reject the null hypothesis of normality for topographic metrics and principal components for each dietary

184 category except for mean OPCR for carnivorous Carnivora, relief index and principal component 2 for

185 herbivorous Diprotodontia, and relief index for insectivorous Rodentia (Shapiro-Wilks, α = 0.05). ANOVA of

186 principal component 1 was used to test for differences between dietary categories for orthopterans and

187 mammal orders; post-hoc Tukey HSD tests were used to assess pairwise differences.

188 The most stringent test of comparability of multivariate topographic metric values across non-homologous

189 feeding structures was provided through LDA of the first three principle components of the combined mammal

190 and orthopteran analysis, using the resulting mammalian multivariate metric (PC1) values to predict dietary

191 categories in orthopterans. All taxa were classified as either 'herbivorous' (diet made up of >50% or

192 preferentially eat plants) or 'carnivorous' (diet made up of >50% or preferentially eats other animals) in order

193 to avoid dietary categories unique either to mammals or orthopterans.
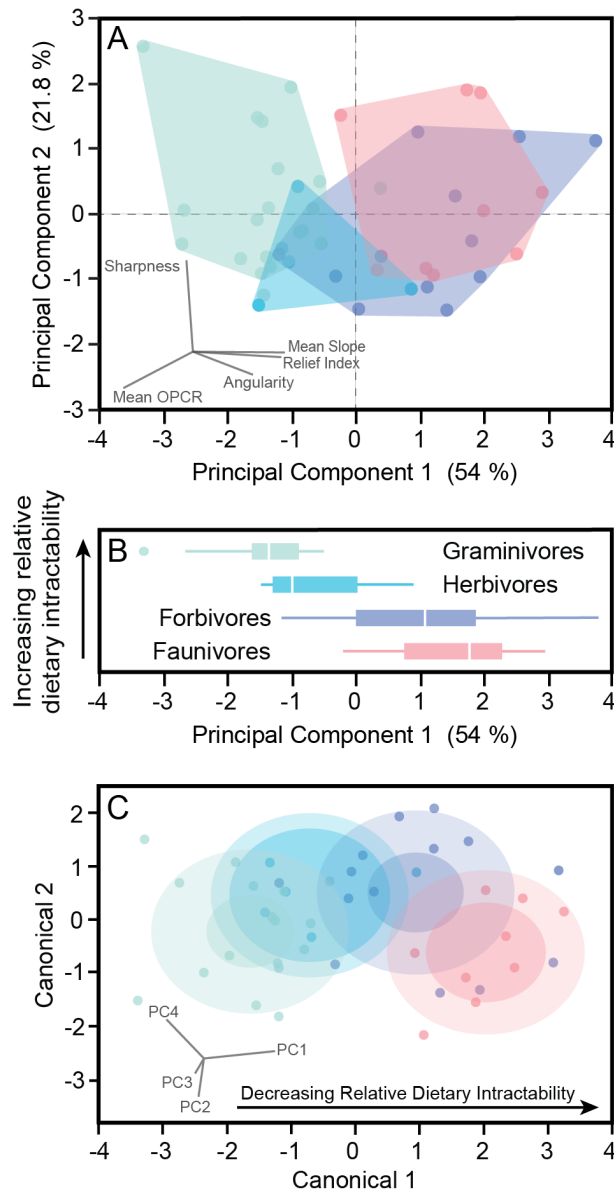
194 **Results**

Figure 2 Multivariate analyses of five topographic metrics (mean OPCR, mean slope, relief index, angularity and sharpness) for left mandibles of 45 orthopteran species. Colour coding of dietary categories follows B. A) Principle components analysis, showing the first two components. B) Boxplots of principal component 1; outliers are shown as individual points. C) Linear discriminant analysis of the first-four principal components. Outer, paler ellipses indicate the region estimated to contain 50% of the population for that group. Inner, darker ellipses indicate the 95% confidence region of the group estimated to contain the true mean of the group.

Our results allow us to unequivocally reject the null hypothesis that dental topographic metrics do not differ between dietary categories in the invertebrates analysed. Mean OPCR, mean slope, relief index and angularity all differ between dietary categories (Table 1, ANOVA, $p < 0.05$). Mean slope and mean OPCR each successfully

205 differentiate between diets in four pairs of categories, more than the other univariate metrics (Table 2, Tukey

206 HSD, $p < 0.05$).

207 The first four principal components (PC) of the PCA capture 99% of the variance. MANOVA of PC 1-4 shows

208 significant differences between dietary categories (Fig. 2a-b; MANOVA, df = 3, F = 4.056, $p = 0.013$). Taken

209 individually, PC 1 differs between dietary categories (Table 1, ANOVA, $p < 0.05$) and successfully differentiates

210 between four pairs of categories, the same as mean OPCR and mean slope (Table 2, Tukey HSD, $p < 0.05$).

211 Difference in intractability between pairs of diets correlates positively with the number of univariate

212 topographic metrics and principal components that find significant differences between them (Spearman's

213 rank, ρ = 0.926, $p = 0.008$).

214 Linear discriminant analysis of PC 1-4 classified 72.1% of orthopterans to the correct dietary category (Fig. 2c).

215 Taxa that were misclassified were commonly assigned to a dietary category reflecting consumption of the

216 same food but in differing relative proportions. For example, 1 graminivorous species was misclassified as a

217 forbivore, and 2 forbivorous species were misclassified as graminivores (see supplementary table 9).

218 *Table 1 ANOVA of topographic metrics and principal components, resulting from a multivariate analysis*

219 *combining them, between dietary categories in orthopterans. Superscript W denotes Welch's ANOVA was*

220 *performed as a result of unequal variances. Benjamini-Hochberg procedure uses a false discovery rate of 0.05.*

Topographic Metric	DF	F Ratio	Prob > F	p < B-H Critical Value
Mean OPCR	**3, 41**	**16.396**	**< 0.001**	**Yes**
Mean Slope	**3, 41**	**18.986**	**< 0.001**	**Yes**
Relief Index^w	**3, 13.702**	**11.344**	**< 0.001**	**Yes**
Angularity	**3, 41**	**4.307**	**0.010**	**Yes**
Sharpness	3, 41	1.636	0.196	-
Principal Component 1	**3, 41**	**23.421**	**< 0.001**	**Yes**
Principal Component 2	3, 41	1.517	0.224	-
Principal Component 3	3, 41	0.197	0.898	-

Principal Component 4[w]	3, 17.285	3.054	0.056	-
Principal Component 5[w]	3, 19.219	2.924	0.060	-

221

222 *Table 2 Significant pairwise differences (Tukey HSD) between dietary categories in orthopterans; lower left of*

223 *table tallies the variables that differ.*

	Faunivorous	Forbivorous	Herbivorous	Graminivorous
Faunivorous	-	mean OPCR	PC1, mean OPCR, mean slope	PC1, mean OPCR, mean slope, relief index, angularity
Forbivorous	1	-	PC1, mean slope	PC1, mean OPCR, mean slope, relief index
Herbivorous	3	2	-	
Graminivorous	5	4	0	-

224

225 Correlation tests provide further support for the strength of the relationship between topographic metrics and

226 dietary intractability. Mean OPCR, mean slope, relief index and angularity all correlate with dietary

227 intractability (Mean OPCR and mean slope particularly strongly; Table 3). PC 1 correlates more strongly with

228 dietary intractability than any univariate topographic metric (Table 3, ρ = -0.805, $p < 0.001$). No other principal

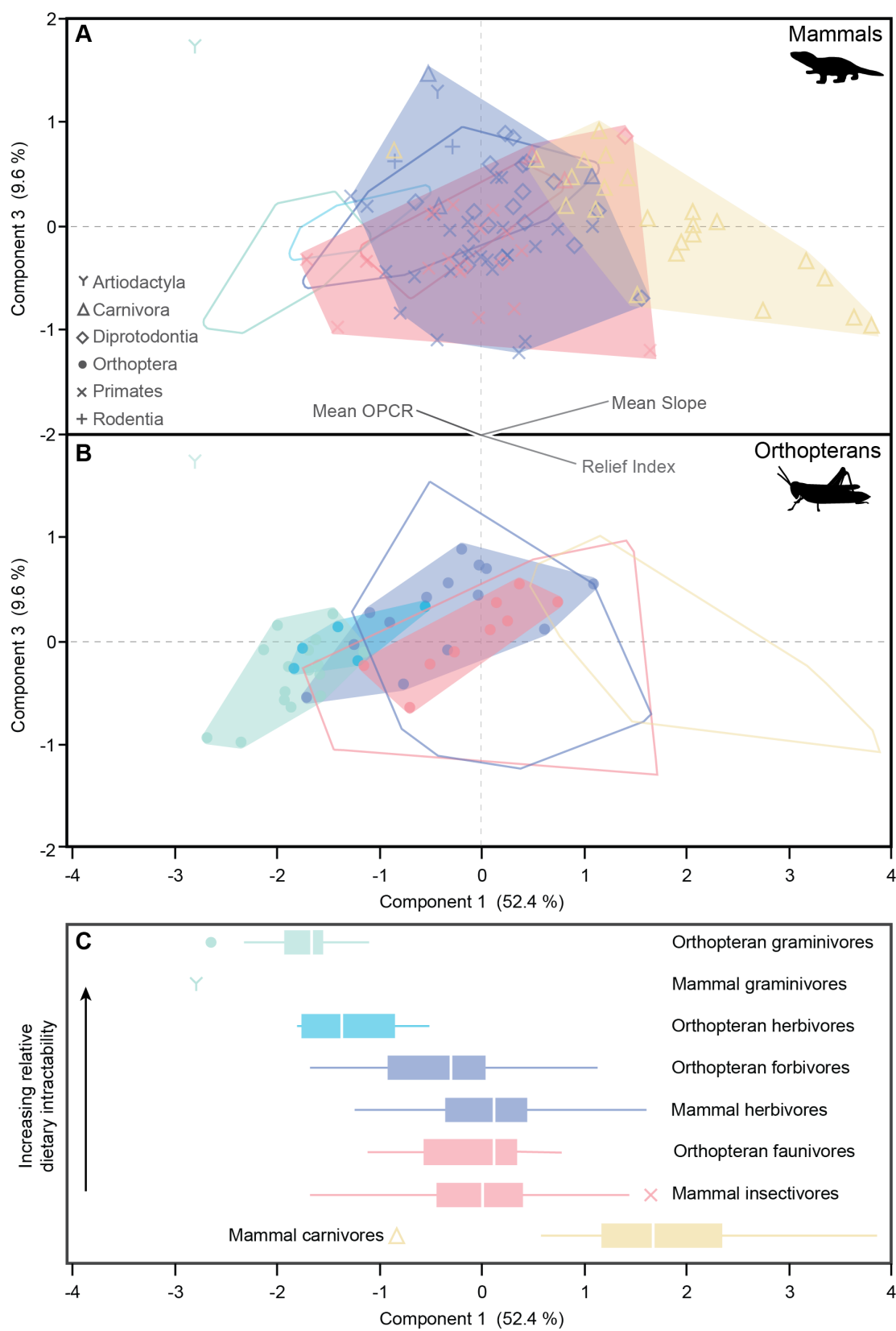229 components correlate with dietary intractability (Table 3).

230 *Table 3 Results of Spearman's rank correlation tests between topographic metrics calculated for orthopteran*

231 *mandibles and the relative dietary intractability. Benjamini-Hochberg procedure uses a false discovery rate of*

232 *0.05.*

Topographic Metric	Spearman ρ	*p* value	*p* < B-H Critical Value
Mean OPCR	0.701	< 0.001	Yes
Mean Slope	-0.743	< 0.001	Yes

Relief Index	**-0.655**	**< 0.001**	**Yes**
Angularity	**-0.441**	**0.002**	**Yes**
Sharpness	0.178	0.241	-
Principal Component 1	**-0.805**	**< 0.001**	**Yes**
Principal Component 2	0.091	0.551	-
Principal Component 3	0.079	0.607	-
Principal Component 4	0.242	0.110	-

233

234 Our results indicate rejection of the hypothesis that the relationship between metric values and diet in

235 orthopterans is predominantly a reflection of phylogenetic rather than ecological similarity. The correlations of

236 phylogenetic closeness and topographic metric values across the order Orthoptera and suborder Caelifera are

237 significant but weaker than correlations between metric values and dietary intractability detailed above (e.g.

238 for PC1, which is strongly correlated with diet: Mantel test across Orthoptera, R = 0.396, p = 0.001; within

239 suborder Caelifera, R = 0.175, p = 0.006; See supplementary Table 5 for details).

Figure 3 Plot of principal components 1 and 3 from analysis of three topographic metrics (mean OPCR, mean

243 *slope, relief index) for 45 orthopteran and 93 mammalian species with comparable diets(Pineda-Munoz et al.*

244 *2017; Rannikko et al. 2020). For clarity, the two panels focus on visualising results for mammalian (A) and*

245 *orthopteran taxa (B), but the axes and convex hulls are identical. PC 2, not shown, primarily reflects the*

246 *separation of orthopterans from mammals. Ursus maritimus, a hypercarnivore, is omitted from the vertebrate*

247 *carnivorous convex hull because its dentition largely reflects its ancestral herbivorous state due to its recent*

248 *speciation(Evans et al. 2007). C. Boxplots indicating the distribution of principal component 1. Outliers are*

249 *shown as individual points.*

250

251 *Table 5 ANOVA of principal component 1 values for comparable dietary categories between orthopterans and*

252 *taxonomic orders of mammals.*

Orthopteran Dietary Category	Mammal Dietary Category	DF	F Ratio	Prob > F
Faunivory	Insectivory	3, 26	1.564	0.222
Herbivory	**Herbivory**	**5, 47**	**7.456**	**< 0.001**
Forbivory	**Herbivory**	**5, 56**	**2.719**	**0.029**

253

254 Comparison of dental topographic metrics for orthopterans with mammals indicates dietary similarities, in

255 spite of the phylogenetic distance between them. This comparability extends to the absolute values of the

256 dietary metrics, not just their relative values in the overall range for their taxon. PCA of orthopteran and

257 mammalian taxa reveals that PC 1 primarily reflects variation between taxa with respect to diet (Fig. 3). Dietary

258 categories separate along PC 1 in order of dietary intractability (Fig. 3), and orthopteran dietary categories

259 separate in the same order as in the orthopteran only analysis (Fig. 2-3). In mammals, PC 1 scores of

260 insectivores, which have a more intractable diet, are lower than carnivores (Fig. 3). Faunivorous orthopterans'

261 PC 1 scores are more similar to insectivorous mammals than they are to carnivorous mammals (Fig. 3), and

262 ANOVA of PC 1 values comparing faunivorous Orthoptera and insectivorous mammal orders (Diprotodontia,

263 Carnivora and Primates) fails to reject the null hypothesis that they are not different (Fig. 3; Table 5; ANOVA, *p*

264 = 0.222). PC 1 values differ between faunivorous Orthoptera and carnivorous mammals (Carnivora; Welch's T

265 test, DF = 26.374, t = -6.276, *p* < 0.001). PC 1 values of herbivorous Orthoptera overlap with herbivorous

266 mammals (Fig. 3), but ANOVA comparing herbivorous Orthoptera with herbivorous mammals (Artiodactyla,

267 Carnivora, Diprotodontia, Rodentia, and Primates) finds some significant differences (Table 5; ANOVA, $p <$

268 0.001; pairwise differences (Tukey HSD, $p < 0.05$), Orthoptera differ from Carnivora, Diprotodontia, and

269 Rodentia, but not from Artiodactyla and Primates). PC 1 values of forbivorous orthopterans overlap more with

270 herbivorous mammals than herbivorous orthopterans do (Fig. 2), and while ANOVA finds a significant overall

271 difference between forbivorous Orthoptera and herbivorous mammal orders, there is only one pairwise

272 difference (Table 5; ANOVA, $p = 0.029;$ Diprotodontia differ from Orthoptera, Tukey HSD, Diff = 0.729, $p < 0.05$;

273 Artiodactyla, Carnivora, Primates and Rodentia do not, Tukey HSD, $p > 0.05$).

274 Our results also support the hypothesis that the relationship between multivariate topographic metrics and

275 diet in one group can provide meaningful information on dietary differences in another group with non-

276 homologous feeding structures. Linear discriminant analysis of the three principal components from the

277 combined PCA, using the known diets of the mammalian taxa as a training dataset, correctly predicts

278 orthopteran diets as herbivorous or carnivorous 82.2% of the time (the LDA performs better than for

279 mammalian taxa, which were correctly classified only 62.3% of the time).

280 **Discussion**

281 Orthopterans and mammals shared a last common ancestor more than half a billion years ago (Dos Reis *et al.*

282 2015), and that ancestor lacked teeth, jaws or mandibles of any kind (Erwin & Davidson 2002). However, our

283 analysis of the essential topographic features of food-processing structures reveals a common underlying

284 signal of morphological adaptation, driven primarily by the intractability of their diets. Unequivocal rejection of

285 our null hypothesis and testing of the alternatives demonstrates that dental topographic measures track diet

286 and are comparable as dietary metrics between non-homologous structures in disparate groups of organisms.

287 Our analysis of orthopterans indicates that dental topographic metrics capture aspects of shape of feeding

288 structures that reflect adaptations for food processing and intractability of foodstuffs. Complexity (mean

289 OPCR), mean slope, relief index and angularity correlate with diet, and the number of pairwise differences

290 between metrics correlates with the distance between ranked dietary categories (Table 2; Table 3), i.e. the

291 most different diets exhibit the greatest number of differences. Complexity correlates positively with dietary

292 intractability (Table 3) while mean slope, relief index and angularity, each measuring a different aspect of the

293 pointedness or height of a feeding element, correlate negatively. All these relationships are the same as those

294   recovered in analyses of mammal teeth (M'kirera & Ungar 2003; Evans *et al.* 2007; Boyer 2008; Bunn & Ungar

295   2009; Evans & Janis 2014; Pineda-Munoz *et al.* 2017; Ungar *et al.* 2018; Rannikko *et al.* 2020).

296   Of the univariate metrics investigated, only sharpness fails to exhibit a significant pairwise difference between

297   diets in orthopterans. This is likely the result of the involvement of orthopteran mandibles in both food

298   prehension and food processing (Isely 1944). All mandibles must have sharp enough edges to cut and separate

299   consumable fragments from larger foodstuffs, reducing variation in absolute values between dietary

300   categories. Clearly, this is unlike the situation in organisms with functionally differentiated dentitions, such as

301   mammals. Alternatively, the absence of significant pairwise differences in sharpness between dietary

302   categories could be due to phylogenetically conserved developmental constraints on mandible morphology in

303   orthopterans (Smith *et al.* 1985). However, as we discuss below, the relationship between topographic metric

304   values and diet is primarily ecological.

305   We find that multivariate metrics more fully capture the ecological relationship between feeding element

306   morphology and diet than any univariate metric in invertebrates, as reported in vertebrates(Pineda-Munoz *et*

307   *al.* 2017). Multivariate metrics differentiate between and predict diet with a high degree of accuracy and

308   precision in orthopterans, with LDA achieving 72.1% correct assignment. Furthermore, PC 1 exhibits the

309   strongest relationship with dietary intractability of any metric (Table 3), whilst being impacted less by the

310   closeness of evolutionary relationships than similarly successful univariate metrics (Table 4). This corroborates

311   the hypothesis that more powerful dietary inferences can be made by combining dietary variables.

312   Our analyses confirm that the relationship between topographic metric values and diet in orthopterans is

313   predominantly a measure of ecological similarity rather than a reflection of closeness of evolutionary

314   relationships. The similarity of multivariate metrics between orthopterans and mammals with similar diets

315   discussed below demonstrates this further.

316   Multivariate analysis provides powerful confirmation that the relationship between form and function in

317   feeding structures holds true across phylogenetic distance: non-homologous feeding structures in distantly

318   related taxa are comparable. Combined analysis of orthopterans and mammals finds that PC 1 values are

319   similar between comparable dietary categories in mammals and orthopterans. Even more compelling are the

320   results of linear discriminant analysis: LDA based entirely on the scores for mammals from the combined

321   principle components analysis correctly differentiates between herbivorous and faunivorous orthopterans

322 with a level of accuracy that is marginally better than its ability to correctly categorise mammals (82.2% of

323 orthopterans correctly assigned to dietary category).

324 Faunivorous orthopterans are indistinguishable from insectivorous mammals grouped by taxonomic order

325 (ANOVA, F Ratio = 1.564, *p* = 0.222). This reflects similarity of diet, with orthopteran faunivores preferentially

326 eating chitinous insects rather than softer food, such as oligochaetes (Gangwere 1967; ElEla, ElSayed &

327 Nakamura 2010). In both invertebrates and vertebrates, graminivores have the lowest PC 1 scores of any

328 dietary category (Fig. 3). Forbivorous orthopterans are more similar to herbivorous mammals than are

329 herbivorous orthopterans (Fig. 3; Table 5). This may in part reflect the coarser classification of mammal diets,

330 which grouped browsers and grazers together as herbivores (Pineda-Munoz & Alroy 2014; Pineda-Munoz *et al.*

331 2017), potentially masking the distinction between diets dominated by forbs, by grasses and more generalized

332 herbivores. However, these and other significant differences between the absolute values of dental

333 topographic metrics from orthopterans and mammals with similar diets are likely the result of functional

334 constraints. As explained above, orthopteran mandibles must be sharp enough to function in food prehension

335 as well as processing, unlike the functionally differentiated dentitions of mammals, in which molars are not

336 involved in prehension. As a result, and particularly in mammals with less tractable diets, it is not uncommon

337 for molars to have reduced or a complete absence of sharp edges (M'kirera & Ungar 2003; Evans *et al.* 2007;

338 Boyer 2008; Bunn & Ungar 2009; Evans & Janis 2014; Pineda-Munoz *et al.* 2017; Ungar *et al.* 2018; Rannikko *et*

339 *al.* 2020). Therefore, it is unsurprising that the PC 1 scores of herbivorous orthopterans differ from the molars

340 of some mammal orders (ANOVA, F ratio= 7.456*, p* < 0.001; Tukey HSD, *p* < 0.01). Furthermore, these

341 differences are unlikely to be the result of phylogenetically conserved developmental constraints on dental

342 tool morphology because topographic metrics primarily recover an ecological signal in the relationship

343 between dental tool form and function in both invertebrates and vertebrates (Pineda-Munoz *et al.* 2017)

344 (Table 3; Table 4). This demonstrates that, whilst for the most part non-homologous food processing

345 structures from animals with similar diets are comparable, some consideration of functional constraints should

346 be made.

347 By rejecting the null hypotheses that topographic metrics do not reflect dietary differences in invertebrates

348 our analyses confirm that they can provide a powerful quantitative analysis of feeding element morphology

349 and its relationship to diet and function in animals outside of jawed vertebrates. Multivariate topographic

350   metrics more fully capture the ecological relationship between feeding element form and function, convergent

351   across non-homologous structures. By doing so, topographic metrics of feeding elements can provide robust

352   and accurate predictions of diet in distantly related taxa. This highlights the potential of topographic metrics

353   for investigating dietary ecology in invertebrate taxa for which in situ feeding observations are not available,

354   including extinct taxa, for many of which diet is poorly constrained. For example, being able to interpret the

355   dietary significance of scolecodont morphology, which provides a fossil record of annelid jaws extending back

356   into the Cambrian Period, would unlock not only their potential to reveal the adaptive responses of

357   polychaetes over almost 500 million years of biosphere evolution, but also the functioning of sediment

358   infaunal communities through time (Clemo & Dorgan 2017). Furthermore, testing hypotheses of dietary

359   ecology in deep time provides a framework for analysis of predator-prey arms races as a driver of trophic

360   tiering in animal diversification e.g Peterson and Butterfield (2005); Sperling *et al.* (2013); Klug *et al.* (2017).

361   **Conclusion**

362   Dental topographic metrics provide a straightforward approach to analysis of the relationship between form

363   and function of feeding structures, and our work addresses the long standing call for quantitative approaches

364   to analysis of feeding structures in invertebrates. Our results indicate a high degree of comparability of dental

365   tools and dietary intractability in animals separated by vast phylogenetic distances. Further validation of the

366   relationship between dental topographic metrics and diet would be worthwhile, adding dietary specifics for

367   non-gnathostome groups in particular. But it is clear that multivariate dental topographic analysis can be

368   applied with confidence to a wide range of feeding structures with tooth-like functions, enabling quantitative

369   analysis and statistical hypothesis testing of the relationships between form, function and diet across much of

370   bilaterian phylogeny and through half a billion years of evolution.

376   **Conflict of Interest**

377   There are no conflicts of interest.

378   **Author Contributions**

379   **Conceptualization** M A Purnell, T H P Harvey; **Data Curation** C Stockey; **Formal Analysis** C Stockey, M A

380   Purnell; **Funding Acquisition** M A Purnell; **Investigation** C Stockey, M A Purnell; **Methodology** C Stockey, N F

381   Adams, M A Purnell, P C J Donoghue, Aileen O'Brien; **Project Administration** C Stockey, M A Purnell; **Resources**

382   C Stockey, M A Purnell, B Price (UK Natural History Museum); **Software** C Stockey, N F Adams; **Supervision** M A

383   Purnell, T H P Harvey, P C J Donoghue; **Validation** C Stockey; **Visualization** C Stockey, M A Purnell; **Writing –**

384   **Original Draft Preparation** C Stockey, M A Purnell; **Writing – Review & Editing** C Stockey, M A Purnell, P C J

385   Donoghue, N F Adams, T H P Harvey.

386   **Data Availability**

387   We intend to archive our data at the Leicester Research Archive.

388   **References**

389   Benjamini, Y. & Hochberg, Y. (1995) Controlling the false discovery rate: a practical and powerful approach to

390   multiple testing. *Journal of the Royal statistical society: series B (Methodological), 57*, 289-300.

391   Bernays, E.A. (1991) Evolution of insect morphology in relation to plants. *Philosophical Transactions of the*

392   *Royal Society of London Series B-Biological Sciences, 333*, 257-264.

393   Bicknell, R.D.C., Ledogar, J.A., Wroe, S., Gutzler, B.C., Watson, W.H., 3rd & Paterson, J.R. (2018) Computational

394   biomechanical analyses demonstrate similar shell-crushing abilities in modern and ancient

395   arthropods. *Proc Biol Sci, 285*.

396   Blanke, A., Schmitz, H., Patera, A., Dutel, H. & Fagan, M.J. (2017) Form-function relationships in dragonfly

397   mandibles under an evolutionary perspective. *J R Soc Interface, 14*.

398   Boyer, D.M. (2008) Relief index of second mandibular molars is a correlate of diet among prosimian primates

399   and other euarchontan mammals. *Journal of human evolution, 55*, 1118-1137.

400   Bunn, J.M. & Ungar, P.S. (2009) Dental topography and diets of four old world monkey species. *American*

401   *Journal of Primatology: Official Journal of the American Society of Primatologists, 71*, 466-477.

402   Chapco, W. & Contreras, D. (2011) Subfamilies Acridinae, Gomphocerinae and Oedipodinae are "fuzzy sets": a

403   proposal for a common African origin. *Journal of Orthoptera Research, 20*, 173-190.

404   Chen, L.-P., Chang, Y.-G., Li, J., Wang, J.-M., Liu, J.-L., Zhi, Y.-C. & Li, X.-J. (2018) Application of DNA Barcoding in

405   the Classification of Grasshoppers (Orthoptera: Acridoidea)—A Case Study of grasshoppers from

406   Hebei Province, China. *Zootaxa, 4497*, 99-110.

407   Clemo, W.C. & Dorgan, K.M. (2017) Functional Morphology of Eunicidan (Polychaeta) Jaws. *Biological Bulletin,*

408   *233*, 227-241.

409   Cotterell, B. (2010) *Fracture and life*. World Scientific.

410   Dos Reis, M., Thawornwattana, Y., Angelis, K., Telford, M.J., Donoghue, P.C. & Yang, Z. (2015) Uncertainty in

411   the timing of origin of animals and the limits of precision in molecular timescales. *Current biology, 25*,

412   2939-2950.

413   Dray, S. & Dufour, A.-B. (2007) The ade4 package: implementing the duality diagram for ecologists. *Journal of*

414   *statistical software, 22*, 1-20.

415   ElEla, S.A., ElSayed, W. & Nakamura, K. (2010) Mandibular structure, gut contents analysis and feeding group

416   of orthopteran species collected from different habitats of Satoyama area within Kanazawa City,

417   Japan. *Journal of Threatened Taxa, 2*, 849-857.

418   Ennos, R. (2011) *Solid biomechanics*. Princeton University Press.

419   Erwin, D.H. & Davidson, E.H. (2002) The last common bilaterian ancestor. *Development, 129*.

420   Evans, A.R. & Janis, C.M. (2014) The evolution of high dental complexity in the horse lineage. *Annales Zoologici*

421   *Fennici, 51*, 73-79.

422   Evans, A.R. & Jernvall, J. (2009) Patterns and constraints in carnivoran and rodent dental complexity and tooth

423   size. *J Vertebr Paleontol, 29*, 24A.

424   Evans, A.R. & Sanson, G.D. (2005) Biomechanical properties of insects in relation to insectivory: cuticle

425   thickness as an indicator of insect 'hardness' and 'intractability'. *Australian Journal of Zoology, 53*, 9-

426   19.

427   Evans, A.R., Wilson, G.P., Fortelius, M. & Jernvall, J. (2007) High-level similarity of dentitions in carnivorans and

428   rodents. *Nature, 445*, 78-81.

429   Flook, P. & Rowell, C. (1997) The phylogeny of the Caelifera (Insecta, Orthoptera) as deduced from mtrRNA

430   gene sequences. *Molecular phylogenetics and evolution, 8*, 89-103.

431   Fries, M., Chapco, W. & Contreras, D. (2007) A molecular phylogenetic analysis of the Oedipodinae and their

432   intercontinental relationships. *Journal of Orthoptera Research, 16*, 115-125.

433   Gangwere, S. (1967) The feeding behavior of Atlanticus testaceus (Orthoptera: Tettigoniidae). *Annals of the*

434   *Entomological Society of America, 60*, 74-81.

435   Gangwere, S., Evans, F. & Nelson, M. (1976) The food-habits and biology of Acrididae in an old-field community

436   in southeastern Michigan. *The great lakes entomologist, 9*, 1.

437   Gangwere, S., McKinney, J., Ernemann, M. & Bland, R. (1998) Food selection and feeding behavior in selected

438   Acridoidea (Insecta: Orthoptera) of the Canary Islands, Spain. *Journal of Orthoptera Research*, 1-21.

439   Gangwere, S. & Spiller, D. (1995) Food selection and feeding behavior in selected Orthoptera sen. lat. of the

440   Balearic Islands, Spain. *Journal of Orthoptera Research*, 147-160.

441   Hickman, C.S. (1980) Gastropod radulae and the assessment of form in evolutionary paleontology.

442   *Paleobiology, 6*, 276-294.

443   Hörnschemeyer, T., Bond, J. & Young, P.G. (2013) Analysis of the functional morphology of mouthparts of the

444   beetle Priacma serrata, and a discussion of possible food sources. *Journal of Insect Science, 13*.

445   Isely, F.B. (1944) Correlation between mandibular morphology and food specificity in grasshoppers. *Annals of*

446   *the Entomological Society of America, 37*, 47-67.

447   Kang, L., Gan, Y. & Li, S. (1999) The structural adaptation of mandibles and food specificity in grasshoppers on

448   Inner Mongolian grasslands. *Journal of Orthoptera Research*, 257-269.

449   Karme, A. (2008) Diet analysis for bunodont omnivore groups Suina and Hominidae: extending, objectifying

450   and quantifying mesowear with 3D and GIS. Master's thesis, University of Helsinki.

451   Klug, C., Frey, L., Pohle, A., De Baets, K. & Korn, D. (2017) Palaeozoic evolution of animal mouthparts. *Bulletin*

452   *of Geosciences, 92*, 439-442.

453   Krings, W., Marce-Nogue, J., Karabacak, H., Glaubrecht, M. & Gorb, S.N. (2020) Finite element analysis of

454   individual taenioglossan radular teeth (Mollusca). *Acta Biomaterialia, 115*, 317-332.

455   M'kirera, F. & Ungar, P.S. (2003) Occlusal relief changes with molar wear in Pan troglodytes troglodytes and

456   Gorilla gorilla gorilla. *American Journal of Primatology: Official Journal of the American Society of*

457   *Primatologists, 60*, 31-41.

458   Mugleston, J.D., Naegle, M., Song, H. & Whiting, M.F. (2018) A comprehensive phylogeny of Tettigoniidae

459   (Orthoptera: Ensifera) reveals extensive ecomorph convergence and widespread taxonomic

460   incongruence. *Insect Systematics and Diversity, 2*, 5.

461   Padilla, D.K. (2004) Form and function of radular teeth of herbivorous molluscs: Focus on the future. *American*

462   *Malacological Bulletin, 18*, 163-168.

463   Patterson, B.D. (1984) Correlation between mandibular morphology and specific diet of some desert grassland

464   Acrididae (Orthoptera). *American Midland Naturalist, 111*, 296-303.

465   Peterson, K.J. & Butterfield, N.J. (2005) Origin of the Eumetazoa: testing ecological predictions of molecular

466   clocks against the Proterozoic fossil record. *Proceedings of the National Academy of Sciences, 102*,

467   9547-9552.

468   Pineda-Munoz, S. & Alroy, J. (2014) Dietary characterization of terrestrial mammals. *Proceedings of the Royal*

469   *Society B: Biological Sciences, 281*, 20141173.

470   Pineda-Munoz, S., Lazagabaster, I.A., Alroy, J. & Evans, A.R. (2017) Inferring diet from dental morphology in

471   terrestrial mammals. *Methods in Ecology and Evolution, 8*, 481-491.

472   Purnell, M.A. & Evans, A.R. (2009) Conodont tooth complexity: quantification, convergence with mammals,

473   and implications for dietary analysis. *Permophiles (Newsletter of the Subcommission on Permian*

474   *Stratigraphy), 53 Supplement 1*, 39-40.

475   Rannikko, J., Adhikari, H., Karme, A., Žliobaitė, I. & Fortelius, M. (2020) The case of the grass-eating suids in the

476   Plio-Pleistocene Turkana Basin: 3D dental topography in relation to diet in extant and fossil pigs.

477   *Journal of morphology, 281*, 348-364.

478   Rowell, C. & Flook, P. (1998) Phylogeny of the Caelifera and the Orthoptera as derived from ribosomal gene

479   sequences. *Journal of Orthoptera Research*, 147-156.

480   Smith, J.M., Burian, R., Kauffman, S., Alberch, P., Campbell, J., Goodwin, B., Lande, R., Raup, D. & Wolpert, L.

481   (1985) Developmental constraints and evolution: a perspective from the Mountain Lake conference

482   on development and evolution. *The Quarterly Review of Biology, 60*, 265-287.

483   Smits, P.D. & Evans, A.R. (2012) Functional constraints on tooth morphology in carnivorous mammals. *BMC*

484   *evolutionary biology, 12*, 1-11.

485   Song, H., Mariño-Pérez, R., Woller, D.A. & Cigliano, M.M. (2018) Evolution, diversification, and biogeography of

486   grasshoppers (Orthoptera: Acrididae). *Insect Systematics and Diversity, 2*, 3.

487   Sperling, E.A., Frieder, C.A., Raman, A.V., Girguis, P.R., Levin, L.A. & Knoll, A.H. (2013) Oxygen, ecology, and the

488   Cambrian radiation of animals. *Proceedings of the National Academy of Sciences, 110*, 13446-13451.

489   Ungar, P.S., Healy, C., Karme, A., Teaford, M. & Fortelius, M. (2018) Dental topography and diets of platyrrhine

490   primates. *Historical Biology, 30*, 64-75.

491   Winchester, J.M., Boyer, D.M., St. Clair, E.M., Gosselin-Ildari, A.D., Cooke, S.B. & Ledogar, J.A. (2014) Dental

492   topography of platyrrhines and prosimians: convergence and contrasts. *American Journal of Physical*

493   *Anthropology, 153*, 29-44.

494   Zhang, H.-L., Huang, Y., Lin, L.-L., Wang, X.-Y. & Zheng, Z.-M. (2013) The phylogeny of the Orthoptera (Insecta)

495   as deduced from mitogenomic gene sequences. *Zoological Studies, 52*, 1-13.