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Horn polyphenism and related head shape variation in a single-horned dung beetle: *Onthophagus (Palaeonthophagus) fracticornis* (Coleoptera: Scarabaeidae)

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1 **Institute**

2 Dipartimento di Biologia Animale e dell'Uomo, Università degli Studi di Torino, Via
3 Accademia Albertina 13, 10123 Torino, Italy.

4
5 **Title**

6 Horn polyphenism and related head shape variation in a single-horned dung beetle:
7 *Onthophagus (Palaeonthophagus) fracticornis* (Preyssler, 1790) (Coleoptera:
8 Scarabaeidae).

9
10 **Short title**

11 Horn and head shape polyphenism in *Onthophagus fracticornis*.

12
13 **Authors**

14 Anna L. M. Macagno, Astrid Pizzo, Angela Roggero, Antonio Rolando, Claudia
15 Palestrini

16
17 **Corresponding author**

18 Anna L. M. Macagno, Dipartimento di Biologia Animale e dell'Uomo, Università
19 degli Studi di Torino, via Accademia Albertina 13, 10123 Torino, Italy.

20 Fax: +39 011 6704508

21 E-mail: anna.macagno@unito.it

22

23

24 **Abstract**

25 Horns of *Onthophagus* beetles are typical examples of phenotypically plastic
26 traits: they are expressed as a function of environmental (nutritional) stimuli, and
27 their reaction norm (i.e. the full set of horn lengths expressed as a response to
28 different degrees of nutritional states) can be either linear or threshold-dependent.
29 Horned males of *Onthophagus* (*Palaeonthophagus*) *fracticornis* (Preyssler, 1790)
30 bear a single triangular cephalic protrusion of vertex carina, which has received
31 phylogenetic support as the most primitive horn shape in the genus. Inter- and intra-
32 sexual patterns of horn expression were studied in *O. fracticornis* by means of static
33 allometries while associated variations in head shape were assessed using geometric
34 morphometric techniques. The relation between log-transformed measurements of
35 body size and vertex carina supported an isometric scaling in females. On the
36 contrary, a sigmoidal model described better the horn length-body size allometry in
37 males, with a switch point between alternative morphs at a pronotum width of 3.88
38 mm. Sigmoidal static allometries of horns in *Onthophagus* populations arise from a
39 threshold-dependent developmental process of horn growth. This process underlies
40 the expression of both plesiomorphic and apomorphic horn shapes in the genus. Given
41 that the single-horn model has been identified as primitive, we propose that such a
42 developmental process giving rise to it may be evolutionarily ancient as well. Horn
43 expression was accompanied by a deformation of the head which makes *minor* and
44 *major* morphs appear even more different. Therefore, in this species both horn and
45 head shape expression contribute to male dimorphism.

46

47 **Key words**

48 Developmental switch, geometric morphometrics, phenotypic plasticity,
49 polyphenism, static allometry.

50

51 **Introduction**

52 Among horned beetles, *Onthophagus* are emerging as model systems for the
53 study of the origin and differentiation of exaggerated sexually selected structures.
54 This highly diversified and species-rich genus is one of the focal taxa for studies on
55 the evolution and development of morphological diversity (Emlen 2000). Whenever
56 expressed, horns differ greatly among species with regard to shape, size, and physical
57 location; they may extend from the base, centre, or front of the head and from several
58 locations on the thorax (Emlen et al. 2005). The genus *Onthophagus* includes a large
59 number of species whose males develop large and conspicuous horns, while females
60 display very reduced ornaments or none at all.

61 Males are commonly dimorphic with respect to horn expression, with large
62 males expressing disproportionately long horns whereas smaller males remain
63 hornless or express greatly reduced horns (Balthasar 1963; Simmons et al. 1999).
64 Although the contemporary presence of a wide range of horn lengths partly depends
65 on genetic differences among males (West-Eberhard 2003), horn size is mainly
66 controlled by environmental (nutritional) stimuli, being mostly determined by
67 differential proliferation of imaginal discs that respond to hormonal signals
68 depending on feeding conditions (Emlen and Nijhout 1999, 2001). On the whole, this
69 process is a typical case of phenotypic plasticity, defined as the ability of a single
70 genotype to give rise to a range of different phenotypes in response to environmental
71 factors (West-Eberhard 2003).

72 When an individual phenotype varies as a continuous function of some
73 environmental variable, its reaction norm is the full set of phenotypic responses to
74 that variable (West-Eberhard 2003). In *Onthophagus* beetles, horn expression is
75 related to body growth, which is influenced in turn by environmental conditions.
76 Hence, the horn length-body size scaling relationship depicts the horn reaction norm
77 (Emlen and Nijhout 2000), which can be represented by means of a static allometry
78 based on measurements of conspecific individuals at the same life stage (Shingleton

79 et al. 2007). Three major types of such horn static allometries have been highlighted:
80 a positive one, arising from differential (but continuous) horn vs. body size growth
81 rates (Moczek et al. 2004; Emlen et al. 2007); a broken one (Nijhout and Wheeler
82 1996, Emlen and Nijhout 2000, Moczek and Nijhout 2002, Moczek 2008) and a
83 sigmoidal one, both characterized by a discontinuity and derived from a size
84 dependent re-programming of horn growth during development (Nijhout and Wheeler
85 1996). More specifically, the sigmoidal allometry is underlain by a re-programming
86 model of growth operating through a nutrition-dependent developmental switch
87 whereby males smaller than a certain critical body size develop small or no horns and
88 males larger than the threshold size express fully developed horns (Emlen and
89 Nijhout 2000). The expression of linear vs. sigmoid scaling relationships is thought to
90 require fundamentally different genetic and developmental control mechanisms
91 (Emlen and Nijhout 2000; Nijhout and Grunert 2002). Recent studies have shown
92 that in species where males develop a pair of cephalic horns, the latter type of
93 allometry is the most frequent (Emlen 1994, 1996; Moczek and Emlen 1999; Moczek
94 and Nijhout 2002; Emlen et al. 2007) and the underlying ontogenetic processes begin
95 being investigated (Emlen and Nijhout 1999, 2001; Moczek 2006).

96 Emlen and colleagues (2005) conducted the most comprehensive analysis of
97 *Onthophagus* horn radiation so far, although a mere 2% of species in the genus were
98 included. They highlighted multiple horn gains and losses as well as morphological
99 modifications along the tree branches, suggesting that relatively little genetic distance
100 is needed to develop strikingly different horn shapes. In spite of a likely
101 underestimation of the evolutionary events responsible for their current stunning
102 diversification, the study of Emlen and colleagues (2005) revealed that a single
103 triangular horn extending from the vertex carina was likely ancestral to this genus and
104 resulted from a single initial evolutionary gain. Subsequent modifications involved
105 the splitting of this original head horn into a pair of horns or, less frequently, into a
106 row of three horns. Sexual dimorphism in horn development, with females dispensing

107 with horn production, is also believed to be ancestral to horn expression (Emlen et al.
108 2005).

109 Horned males of *Onthophagus (Palaeonthophagus) fracticornis* (Preysslner,
110 1790) bear a single triangular cephalic horn that protrudes from the centre of the
111 vertex carina and largely reflects the ancestral shape from which horn diversification
112 started (Emlen et al. 2005). In this species, individuals with long to very short horns
113 coexist in the same populations. Medium sized horns protrude straight from the
114 vertex carina, while long horns become S-shaped distally. Females never express
115 horns. Instead, they bear a conspicuous vertex carina. In the present study, we provide
116 a detailed description in terms of allometric relationships of male horn and its female
117 homologue, the vertex carina, to determine inter-sexual differences in type and
118 magnitude of responses to nutritional stimuli. Horn expression results from
119 irreducible interactions between genetic, developmental and environmental factors,
120 each of them possibly having multiple and eventually significant effects over other
121 morphological traits. Recent studies show that costs associated with weapon
122 development can influence the neighbouring morphological structures and that this
123 trade-off is of evolutionary importance (Emlen 2001). In this framework, we tested
124 whether horn development was associated with head shape variation. An integrated
125 analytical approach, combining traditional and geometric morphometrics, was used to
126 characterize *O. fracticornis* horn static allometry and morphological modifications in
127 head shape.

128

129 **Materials and Methods**

130 **Fieldwork**

131 Between July and August 2006, about one hundred individuals (54 males, 48
132 females) were collected from two pastures located in Ferret Valley (Aosta Valley
133 region), North-West Italian Alps (*Montitaz Desot*, 1559 m a.s.l., ED50 UTM E
134 343119 N 5077148; *Praz-Sec*, 1652 m a.s.l., ED50 UTM E 345793 N 5079235). Cow

135 dung pads with typical colonization signs (tunnel entrances on the top, excavated
136 material all around) and 20 cm of soil below dung pads were carefully inspected for
137 beetles.

138 **Morphological measurements and statistical analyses**

139 In the laboratory, individuals were cleaned in boiling distilled 140 water for
140 10 min and dissected; heads and pronotums were then separately fixed on plasticine
141 supports, taking care to align the edges of each structure on the same horizontal plane
142 by completely plunging their convex ventral portion in horizontally-levelled
143 plasticine. All morphological measurements were taken using a two-dimensional
144 image analysis equipment, including a Leica Z16Apo stereoscope, a Leica DFC320
145 digital camera (Leica Microsystems AG, Wetzler, Germany), and the software LAS v
146 2.5.0 (Leica Application Suite).

147 After photographing the lateral profile of the head, measurements were taken
148 following its outer edge from the frontal carina (i.e., the most clearly identifiable
149 homologous point between females and males of different morphs) up to the top of
150 the vertex carina (or the protruding horn). Pronotum width was measured as a proxy
151 for body size (Emlen 1994; Moczek 1998). Kolmogorov-Smirnoff tests were used to
152 test for normality of natural variation in pronotum width and horn length (Sugiura et
153 al. 2007).

154 Horned beetle morphology is commonly described in terms of static
155 allometries, i.e. through scaling relationships where the horn length of individuals of
156 different body size is graphed as a function of body size (Eberhard and Gutiérrez
157 1991; Emlen and Nijhout 2000; Moczek and Nijhout 2003). In *O. fracticornis*, such
158 scaling relationships were determined by fitting to the data a simple linear (
159 $y = y_0 + b \cdot x$) and a four-parameter non-linear regression in the form

160
$$y = y_0 + \frac{a \cdot x^b}{c^b + x^b} \text{ (Moczek et al. 2004)}$$

161 where x is body size, y is horn length, y_0 specifies minimum horn length, a defines the
162 horn length range in the sample, b is a slope coefficient and c is body size at the point
163 of inflection of the sigmoid curve. The residuals from the two regressions were
164 compared by means of an Extra Sum of Squares F -test to find out what regression
165 better fitted the data set (Moczek et al. 2004).

166 Geometric Morphometric techniques (Bookstein 1991; Rohlf and Marcus
167 1993; Marcus et al. 1996; Adams et al. 2004) are a powerful means to describe shape
168 variation, providing a useful tool for addressing a variety of biological problems.
169 Here, a landmark-based approach was adopted to quantify intraspecific modifications
170 of head morphology and their association with horn development. Eight landmarks on
171 the frontal side of the head (Fig. 1) were digitized by using *TpsDig* 2.10 (Rohlf
172 2006): they were all positioned on the left hand side to prevent bias possibly caused
173 by asymmetry (Pizzo et al. 2006a, 2006b, 2008) and chosen for their relative ease of
174 identification, homology between the two sexes and the morphs, and ability to
175 capture the general head shape, excluding the portion from which the horn originates.
176 To evaluate the confidence of the landmark configuration, a repeatability test was
177 conducted by digitalizing the same set of landmarks on the same specimen 10 times:
178 the landmark configuration was accepted only if the ratio between variance on the
179 same specimen and variance of the total sample [variance = $\Sigma(\text{Procrustes}$
180 $\text{distances})^2 / (N-1)$, where N is the number of objects considered in each set of
181 measures] was less than or equal to 0.05 (Pizzo et al. 2006a, 2006b, 2008). The
182 landmarks of each specimen were optimally aligned using a Generalized Procrustes
183 Analysis (GPA) to remove the non-shape effects of translation, rotation, and scale
184 (Rohlf 1990; Rohlf and Slice 1990). The thin-plate spline (TPS) method (Bookstein
185 1989) was used to allow a multivariate description of shape variables in an Euclidean
186 space tangential to the reference point (the mean shape) in Kendall's shape space
187 (Kendall 1981, 1984). The effect of horn expression on head shape was evaluated in
188 two ways.

189 1) To reveal differences between *major* and *minor* males, individuals which
190 could not be unambiguously assigned to one of the two morphs ($c \pm S.E.$) were
191 excluded from analyses and the remaining males were contrasted to a comparable
192 number of randomly chosen females. Generalized procrustean superimposition (Rohlf
193 1990; Rohlf and Slice 1990), partial warps, relative warps and visualization of
194 deformation grids, which allowed a description of shape variation, were performed
195 using *tpsRelw* 1.45 (Rohlf 2007). Discriminant analysis was carried out on relative
196 warp scores to obtain a classification matrix based on head shape variation. We used
197 the percentages of correct classification to evaluate whether *major* and *minor* morphs
198 could be described as two distinct groups on the basis of head shape variation.

199 2) To assess the effect of horn expression on male head morphology, the
200 entire sample of males was considered and a multivariate test for a general linear
201 model predicting male head shape variation (captured by partial warps and uniform
202 shape component: Bookstein 1991) as a function of horn length was computed. A
203 series of bivariate regressions of each partial warp and uniform component (as
204 dependent variables) onto horn length (as independent variable) were carried out and
205 the overall significance of multivariate regression was assessed by *Wilks' Lambda*
206 and *Goodall F-test* (Goodall 1991). All tests as well as the visualization of the
207 deformation grid predicting head shape as a function of horn length were performed
208 in *tpsRegr* 1.28 (Rohlf 2003).

209

210 **Results**

211 **Horn allometry**

212 Male pronotum width was normally distributed (*Kolmogorov-Smirnoff* test:
213 $d=0.05$, $P>0.20$), whereas horn length was not (*Kolmogorov-Smirnoff* test: $d=0.21$,
214 $P<0.01$), the plot suggesting a bimodal distribution instead (Fig. 2).

215 Both a linear and a sigmoidal regression fitted male horn length-body size
216 data. After removing 2 outliers (studentized residual values: > 3.00 and < -3.00), a

217 simple linear regression model explained over 98% of horn length variation
 218 ($r^2=0.9783$, $y = -3.1 + 1.17x$); a sigmoid regression model led to a moderate, but
 219 nonetheless significantly better fit ($r^2=0.9897$, $y = 0.73 + \frac{1.41 \cdot x^{25.57}}{3.88^{25.57} + x^{25.57}}$; Extra
 220 Sum of squares F -test: $F=26.89$, $P<0.001$), suggesting a model of growth where
 221 males smaller than a certain critical body size develop small or no horns and males
 222 larger than the threshold size express fully developed horns was more appropriate to
 223 describe horn development than a linear reaction norm (Fig. 2). The same analyses
 224 performed on log-transformed data gave comparable results (linear regression:
 225 $r^2=0.8754$, $y = -4.31 + 3.41x$; sigmoid regression: $r^2=0.9254$,
 226 $y = -0.36 + \frac{1.19 \cdot x^{26.91}}{1.34^{26.91} + x^{26.91}}$; Extra Sum of squares F -test: $F=16.10$, $P<0.001$).

227 Female vertex carina scaled largely isometrically with respect to pronotum
 228 width (linear regression, untransformed data: $r^2=0.9985$, $y = 0.10 + 0.20x$; Fig. 2;
 229 linear regression, log-transformed data: $r^2=0.9323$, $y = -1.36 + 0.91x$).

230 **Geometric Morphometrics**

231 Given the point of inflection of the sigmoid curve \pm its S.E. (3.88 ± 0.03 mm),
 232 males with pronotum width higher than 3.91 mm and lower than 3.85 mm were
 233 respectively classified as *major* (N=19) and *minor* (N=23) and compared with 22
 234 randomly chosen females. Values of the first two relative warp scores obtained from
 235 TPS analysis of the head shape were plotted on an axis system. On the whole, relative
 236 warp 1 and 2 described more than 50% of total head shape variation. In the
 237 morphospace, *major* males were grouped in a distinct cluster along the relative warp
 238 1, thus being clearly differentiated from both *minor* males and females, which were in
 239 turn only partially differentiated along relative warp 2 (Fig. 3). Discriminant analysis
 240 performed on male head relative warp scores showed that the two-class grouping fits
 241 the data adequately (*Wilks' Lambda*=0.22, $\chi^2=52.17$, $P<0.001$) and that 100% of
 242 *major* and 91.3% of *minor* males were correctly classified.

243 When all males were considered, regression between horn length and head
244 shape as described by partial warps gave statistically significant results (*Wilks'*
245 *Lambda*=0.21, $F_{12,39}=12.461$, $P<0.001$; *Generalized Goodall F-test*: $F_{12,600}=16.16$,
246 $P<0.001$). It is noteworthy that the deformation predicting head shape as a function of
247 horn length followed closely the one described by relative warp 1 in Fig. 3. The
248 increase in horn length matches with a stretching of clypeus and a convergence of
249 landmarks defining genae and eyes towards the medial axis of the head: as a result,
250 the heads of males developing long horns are more elongated in the fore-hind
251 direction, less enlarged at the genal level and, on the whole, more pointed at the level
252 of the clypeus than those of males expressing short to no horns. Conversely, no
253 correlation was found between female vertex carina height and head shape (*Wilks'*
254 *Lambda*=0.62, $F_{12,35}=1.77$, N.S.; *Generalized Goodall F-test*: $F_{12,552}=1.43$, N.S.).
255

256 **Discussion**

257 The sigmoidal shape of the outlined horn length-body size allometry reflects a
258 bimodal male frequency distribution which results in the separation between small
259 hornless (*minor*) and big horned (*major*) male morphs. Therefore, in *Onthophagus*
260 *fracticornis*, male horn expression is likely to occur through a size-specific
261 reprogramming model of growth whereby a nutrition-dependent developmental
262 switch causes horn growth to be suppressed in small males and exponential growth to
263 occur only in large males (Nijhout and Wheeler 1996). The ontogenetic mechanisms
264 of this polyphenic development have been extensively studied in the congeneric *O.*
265 *taurus*, for which both horn length and body size have been shown not to be
266 genetically inherited, but rather expressed under environmental controls, primarily
267 depending on larval feeding conditions (Moczek and Emlen 1999). The sigmoid
268 scaling relation is the macroscopic effect of developmental processes that regulate
269 horn growth in response to circulating titres of Juvenile Hormone (JH). Horns
270 originate from imaginal discs that explosively proliferate during a brief sensitive

271 period within the prepupal stage (Moczek and Nijhout 2004); under the assumption
272 that JH is secreted as a function of body size, only large males expressing JH titres
273 above a threshold during this sensitive period will moult into horned adults; smaller
274 males will develop rudimental horns or remain hornless (Emlen and Nijhout 1999). In
275 the static allometry, the switch point between the two morphs is identified by the
276 point of inflection of the sigmoid curve; in its proximity, horn length varies
277 conspicuously within a relative narrow range of body sizes. Morph determination
278 occurs relatively late in larval development: the developmental switch determining
279 the fate of a male larva occurs approximatively three days before pupation and thus
280 when larval growth is almost completed (Moczek and Nijhout 2002). As a
281 consequence, although the scaling relationship between horn length and body size is
282 highly non-linear due to a switch in developmental trajectories, male morphologies
283 remain typically continuous and with broad overlap between alternative morphs
284 (Moczek 2008). Contrary to males, in females the relation between log-transformed
285 measurements of body size and vertex carina supported an isometric scaling.

286 The sexual dimorphism exhibited by this species has been proposed to be
287 plesiomorphic in the genus *Onthophagus*, with females never developing horns
288 (Emlen et al. 2005). The presence of a single horn has been proposed as ancestral in
289 the genus *Onthophagus* (Emlen et al. 2005). We therefore suggest that horn
290 polyphenism operating through the described reprogramming model of growth might
291 be evolutionarily ancient as well. Even though *O. fracticornis* was not included in the
292 phylogeny reconstructed by Emlen et al. (2005), the same primitive horn shape was
293 represented, among others, by *O. nuchicornis*. This species belongs to the same
294 subgenus as *O. fracticornis* (i.e., *Palaeonthophagus*) and bears a single laminar
295 polyphenic head horn, with *major* and *minor* males coexisting in the same
296 populations as well (Martin-Piera and López-Colón, 2000). On the basis of Zunino's
297 (1979) considerations on the monophyly of the subgenus *Palaeonthophagus*, the
298 position of *O. fracticornis* in the phylogeny proposed by Emlen et al. (2005) can be
299 equated to that of *O. nuchicornis*. Nonetheless, since some reversals are assumed to

300 have occurred in the evolution of *Onthophagus* horn shape, it cannot be excluded that
301 the horn shape observed in *Palaeonthophagus* could be derived from other types,
302 even though it resembles the putative plesiomorphic shape in the genus. This scenario
303 is further complicated by the fact that there are four principal trajectories of
304 *Onthophagus* horn evolution, related to changes in horn location, shape, allometry
305 and sexual dimorphism (Emlen et al. 2007). While the evolutionary patterns of
306 *Onthophagus* horn location, shape and dimorphism have been extensively analysed
307 (Emlen et al. 2005), the evolution of horn length / body size allometry deserves more
308 detailed phylogenetic analyses. However, although static allometries have been
309 described only in a few subgenera of *Onthophagus*, in *Onthophagus sensu stricto* the
310 sigmoidal trend seems to be widespread (Eberhard and Gutiérrez 1991, Emlen 1996,
311 Moczek and Nijhout 2003, Simmons et al. 2007, Emlen et al. 2007). The presence of
312 a threshold-dependent male horn polyphenism in both *O. (Palaeonthophagus)*
313 *nuchicornis* (Simmons et al. 2007) and *O. (Palaeonthophagus) fracticornis* (this
314 paper) suggests that this process is widespread across subgenera and might indicate
315 an ancient origin of its underlying developmental patterns, at least within the genus
316 *Onthophagus*.

317 The contemporary inspection of the relative warp plot and deformation grids
318 describing head shape variations provided a detailed characterization of differences
319 between sexes and morphs. *Major* males formed a distinct group along the relative
320 warp representing the stretching of clypeus and a convergence of landmarks defining
321 genae and eyes towards the medial axis of the head. As a result, *major* males have
322 heads which are more elongated in the fore-hind direction, less enlarged at the level
323 of the genae and, on the whole, more pointed at the level of the clypeus than those of
324 *minor* males and females. Furthermore, an explicit correlation emerged between horn
325 length and head shape. Horn growth matched with a pattern of head shape variation
326 which followed closely the one described by relative warp 1 within the shift from
327 *major* to *minor* morphs. It is therefore assumed that horn development and head
328 morphology are associated. Although landmarks were not placed in the

329 morphological region from which the horn protrudes, the correlation between horn
330 expression and head shape was nonetheless apparent. Our results thus revealed that in
331 *Onthophagus fracticornis*, the horn allometry mediated by a developmental switch
332 causes large males not just to be enlarged versions of their smaller conspecifics
333 (Emlen and Nijhout 2000), but to also develop a fundamentally different morphology.
334 In particular, since horn expression is accompanied by a modification of head shape,
335 male dimorphism is delineated by both horn presence and head morphology. Such a
336 head modification (in particular, clypeus stretching in response to horn development)
337 might have evolved as a balancing static system compensating barycentre backing
338 due to horn protrusion in *major* males. Similar patterns of head shape modification in
339 response to horn expression are likely operating in other *Onthophagus* species: a
340 morph dependent variation of head shape was also highlighted in *Onthophagus*
341 *taurus* and *Onthophagus illyricus* (Pizzo et al. 2006a), where *major* males develop a
342 pair of cephalic horns. The developmental mechanisms underlying this morphological
343 rearrangement have never been investigated. However, it is possible to hypothesize
344 that, in males developing horns, other areas of the head such as the clypeus may
345 respond to nutrition-mediated chemical stimuli. As a matter of fact, recent studies
346 have shown that there must be some interaction between developmental pathways of
347 different morphological structures: the production of some horn types can influence
348 the size of neighbouring (Emlen 2001) or even distant (Moczek and Nijhout 2004;
349 Simmons and Emlen 2006) structures due to the costs associated with enlarged
350 weapon development.

351 Environment-mediated polyphenic development is the highest degree of
352 phenotypic plasticity, which is thought to be a major enhancer of differentiation,
353 responsible for the origin of evolutionary novelties (West-Eberhard 2003; Moczek
354 2008). In *Onthophagus* beetles allometries are particularly prone to rapid evolution:
355 even slight evolutionary changes in the mechanics of developmental switches, in
356 particular changes in the body size threshold which defines the transition between
357 *minor* and *major* morphs, can have significant consequences on phenotypic

358 expression. It has been demonstrated that polyphenic thresholds and consequently the
359 underlying developmental mechanisms can diverge in closely related species or even
360 in geographically isolated populations of the same species (Moczek 2003, Moczek
361 and Nijhout 2003). The present study represents the first necessary step towards the
362 analysis of differentiation patterns within the three species of the so called
363 ‘*fracticornis-opacicollis*’ complex [*viz.* *O. opacicollis* Reitter, 1892; *O. similis*
364 (Scriba 1790); *O. fracticornis* (Preyssler 1790)]. A controversy regarding the
365 taxonomic status of this complex has been going on for more than a century because
366 species divergence appears to be a function of geography (different divergence
367 patterns have been highlighted in allopatric and sympatric populations). It has been
368 suggested that reproductive isolation is not complete across the geographic range of
369 these species and speciation processes are still ongoing in some areas (Martín-Piera
370 and Boto 1999). The analysis of static allometries will provide an invaluable
371 opportunity for studying divergence patterns in this species complex from an Evo-
372 Devo perspective.

373

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378

379 **Riassunto**

380 **Espressione polifenica del corno cefalico e conseguenti variazioni nella**
381 **morfologia del capo in *Onthophagus (Palaeonthophagus) fracticornis* (Preyssler,**
382 **1790) (Coleoptera: Scarabaeidae).**

383 Le strutture cefaliche degli Scarabeidi sono tipici esempi di tratti soggetti a
384 plasticità fenotipica: vengono espresse in funzione di stimoli ambientali e le norme di
385 reazione sottese al loro sviluppo, ovvero l'intero *range* di lunghezze delle corna

386 espresso in risposta a differenti condizioni nutrizionali della larva, può avere
387 andamento lineare o essere caratterizzate da un effetto soglia. In *Onthophagus*
388 (*Palaeonthophagus*) *fracticornis* (Preysslner, 1790) i maschi possono esprimere una
389 singola protrusione cefalica triangolare, emergente dalla carena del vertice ed estesa
390 in forma di lamina, un carattere considerato plesiomorfo all'interno del genere,
391 mentre le femmine esibiscono una carena del *vertex* pronunciata, senza tuttavia
392 sviluppare corna. I pattern inter- e intrasessuali di espressione del corno di *O.*
393 *fracticornis* sono stati analizzati in termini di relazioni di allometria statica; le
394 modificazioni della forma complessiva del capo che si evidenziano in concomitanza
395 con lo sviluppo del corno sono state indagate attraverso l'uso di tecniche di
396 morfometria geometrica. Nelle femmine, la regressione tra le misure,
397 logaritmicamente trasformate, della larghezza del pronoto e dell'altezza della carena
398 cefalica indica una relazione isometrica tra queste due variabili; al contrario, nei
399 maschi questa relazione risulta meglio descritta da un modello sigmoidale. La
400 transizione tra maschi *minor* e *major* avviene ad una larghezza del pronoto di 3.88
401 mm. Le allometrie statiche sigmoidali già descritte nelle popolazioni di *Onthophagus*
402 risultano da specifici processi di sviluppo che coinvolgono soglie di sensibilità alla
403 neotenina nei singoli individui. Si può ritenere che tali processi siano relativamente
404 antichi, in considerazione del fatto che sono alla base dello sviluppo polifenico in
405 specie che esprimono strutture sessuali secondarie plesiomorfe. Nella specie
406 analizzata, l'espressione del corno è accompagnata da una deformazione cefalica che
407 differenzia le morfe anche sulla base della morfologia generale del capo: di
408 conseguenza, sia il grado di espressione del corno sia la forma del capo concorrono a
409 determinare il dimorfismo maschile.

410

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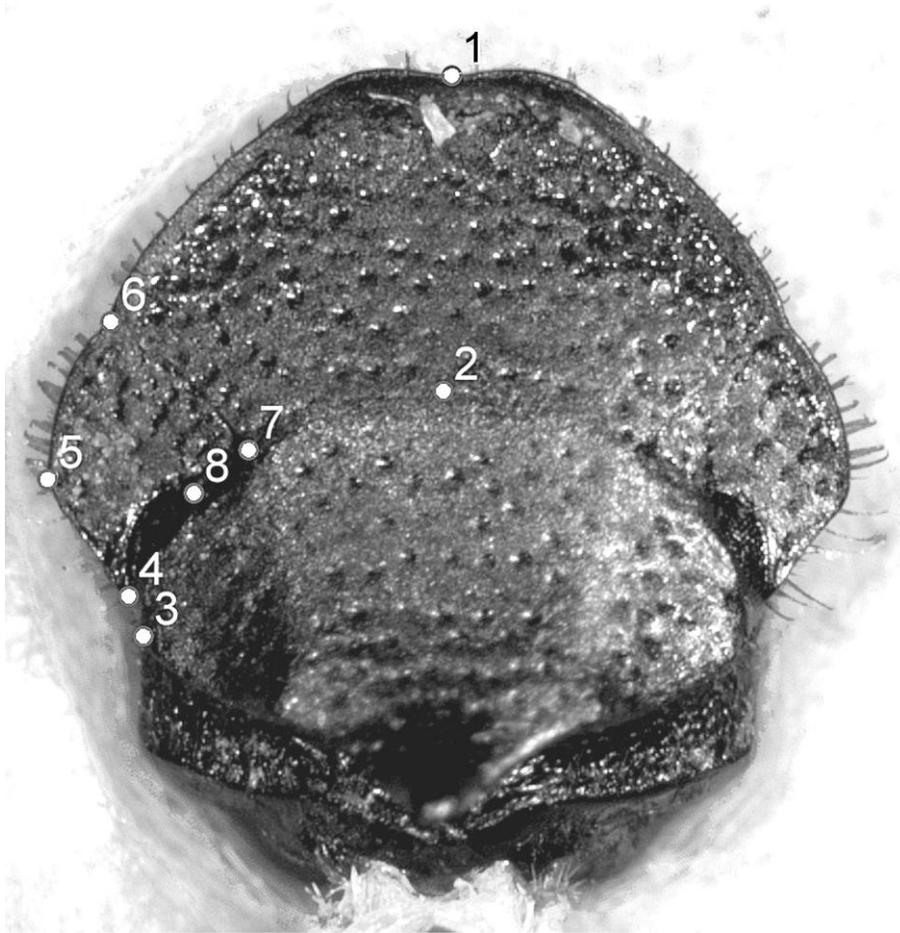
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528

529 **Figure captions**



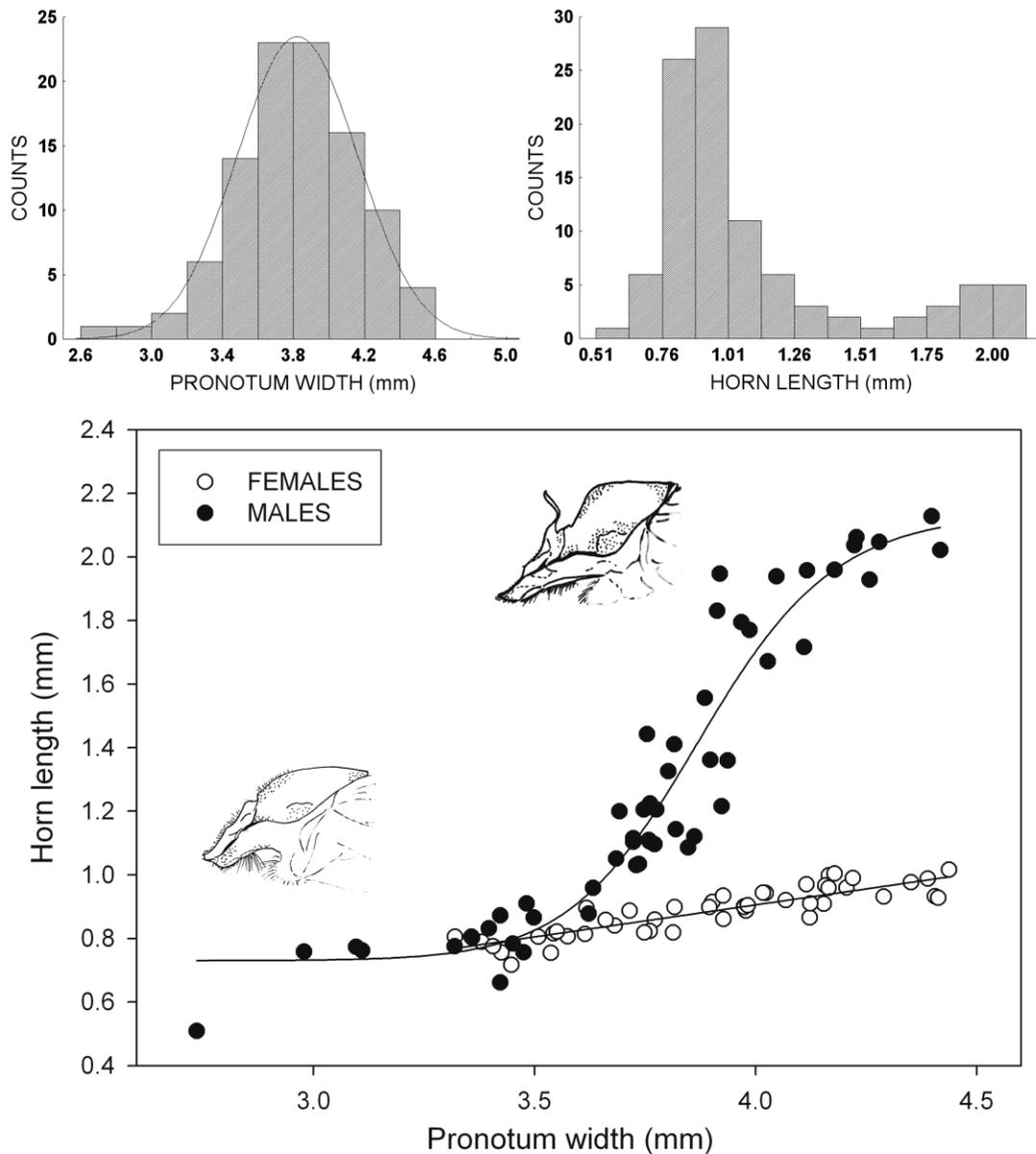
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532 **Figure 1.** Landmark configuration chosen to describe head shape. All
533 landmarks were set taking care to avoid positioning on the anatomical region from
534 which the horn originates.

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Figure 2. Static allometries describing the scaling relations between body size

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(measured as pronotum width) and horn length (or the corresponding trait in small

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males and females). Histograms of pronotum width and horn length frequencies are

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also shown. The described pattern results in the co-existence in the species of large

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horned (*major*) males and small (*minor*) ones bearing reduced horns or no horn. The

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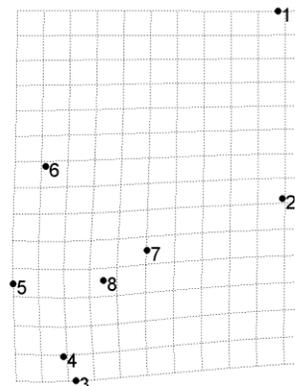
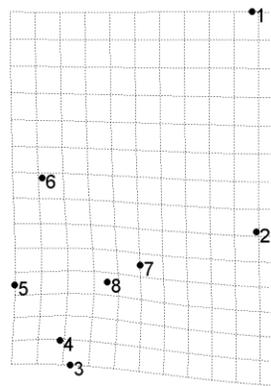
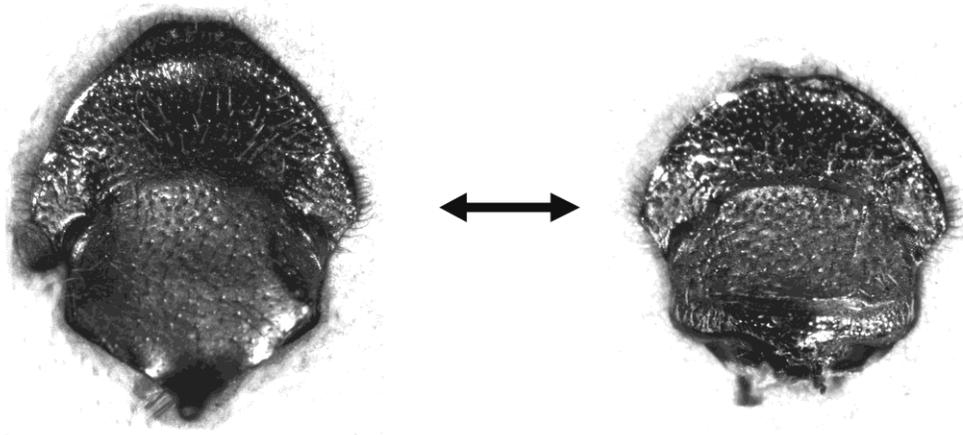
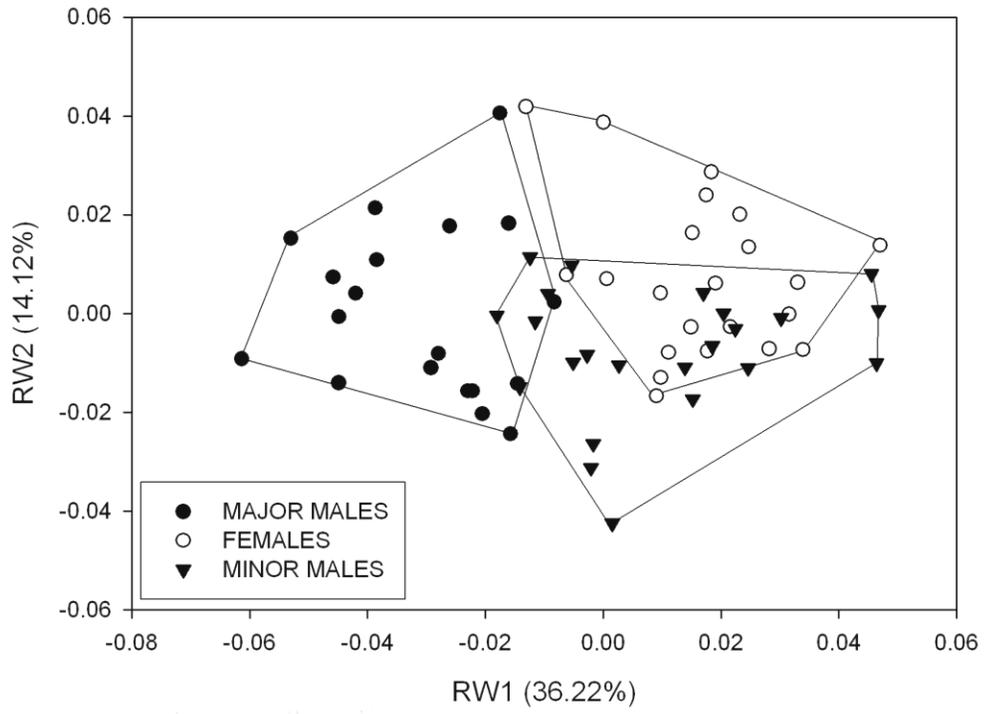
switch point between alternative morphs is set at a pronotum width of 3.88 ± 0.03

543 mm. By comparison, female vertex carina scales largely linearly with respect to body

544 size.

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548 **Figure 3.** Head shape differentiation between sexes and morphs. Scatterplots
549 of the two first relative warp scores obtained from the relative warp analyses are
550 reported. The amount of variation explained by each relative warp (axis) is expressed
551 in percentage terms. Thin-plate spline (TPS) transformation grids on each side of the
552 scatterplot refer to specimens plotted on the left (*major* males) and right (*minor*
553 males, females) extremity of the first relative warp axis; only the deformation along
554 the first relative warp (which best allows discrimination) is shown.
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560 **Contributing authors**

561

562 **E-mail addresses:**

563 Anna L. M. Macagno: anna.macagno@unito.it

564 Astrid Pizzo: astrid.pizzo@unito.it

565 Angela Roggero: angela.roggero@unito.it

566 Antonio Rolando: antonio.rolando@unito.it

567 Claudia Palestrini: claudia.palestrini@unito.it

568

569 **Mailing address (all authors):**

570 Dipartimento di Biologia Animale e dell'Uomo, Università degli Studi di Torino, Via

571 Accademia Albertina 13, 10123 Torino, Italy.