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4 **Cryptic niche switching in a chemosymbiotic gastropod**

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20 **Running head**

21 Cryptometamorphosis

23 **Abstract**

24

25 Life stages of some animals, including amphibians and insects, are so different that they
26 have historically been seen as different species. ‘Metamorphosis’ broadly encompasses
27 major changes in organism bodies and, importantly, concomitant shifts in trophic
28 strategies. Many marine animals have a biphasic lifestyle, with small pelagic larvae
29 undergoing one or more metamorphic transformations before settling into a permanent,
30 adult morphology on the benthos. Post-settlement, the hydrothermal vent gastropod
31 *Gigantopelta chessoia* experiences a further, cryptic metamorphosis at body sizes around
32 5-7 mm. The terminal adult stage is entirely dependent on chemoautotrophic symbionts;
33 smaller individuals do not house symbionts, and presumably depend on grazing. Using
34 high resolution x-ray microtomography to reconstruct the internal organs in a growth
35 series, we show this sudden transition in small but sexually mature individuals
36 dramatically reconfigures the organs, but is in no way apparent from external morphology.
37 We introduce the term ‘cryptometamorphosis’ to identify this novel phenomenon of a
38 major body change and trophic shift, not related to sexual maturity, transforming only the
39 internal anatomy. Understanding energy flow in ecosystems depends on the feeding
40 ecology of species; the present study highlights the possibility for adult animals to make
41 profound shifts in biology that influence energy dynamics.

42

43

44 **Background**

45

46 Metamorphosis in animals involves profound physical transformation in the morphology
47 of an individual, linked to significant changes in ecology and behaviour. For example,
48 holometamorphic insects and amphibians have radically different body plans, niche, and
49 ecology in their larval and adult stages, often occupying distinct trophic levels. Marine
50 invertebrates commonly have a biphasic life history, with dispersing larval stages in the
51 plankton and a post-settlement stage following a terminal metamorphosis. Some
52 ontogenies are even more dramatic in that they have multiple metamorphoses, a series of
53 larval stages each with very different morphology. Many larval stages were historically
54 described as different genera from the adult animals, such as nauplius and zoea larval
55 stages of crustaceans [1, 2]. ‘Hypermetamorphosis’ has been used to describe animals
56 with multiple and drastically different larval phases, a term generally used for insects and
57 fish [3]. Although metamorphosis appears to occur synchronously in external
58 morphology and internal anatomy, the shift in external morphology may be de-coupled
59 from changes in the internal organs [4]. Anatomical transitions often require that animals
60 go through a non-feeding transitional stage, such as the insect pupa or the
61 metamorphosing larva in fish [5, 6].

62

63 A metamorphic transition usually alters the trophic interactions of an organism. In marine
64 invertebrates, larvae and adults of benthic species are spatially as well as morphologically
65 separated. In freshwater systems, larvae and adults can co-occur, so communities are
66 often assessed by feeding guild rather than taxonomy [7]. Modelling energy flow in all
67 food webs necessarily depends on simplifying assumptions, such as consistency in
68 metabolic scaling with biomass [8, 9]; however, general patterns could be occluded by
69 additional, unrecognised major trophic shifts within adult or larval life stages.
70 Reconstructing food webs in key marine ecosystems is difficult because they generally
71 have longer food chains than terrestrial ecosystems [10]. Deep-sea hydrothermal vent
72 ecosystems are highly productive and host a large biomass of comparable density to
73 tropical reefs, but have a relatively simple, reduced trophic complexity [11].
74 Hydrothermal vents thus offer an important model system to test ideas of trophic ecology
75 and energy flow, if we fully understand the trophic positions and niche switching
76 dynamics of their constituent species.

77

78 A large bodied gastropod endemic to hydrothermal vents in the Southern Ocean was
79 reported to undergo a substantial transition [12], from grazing to total dependency on

80 internal endosymbiotic bacteria, at an unusually late stage of post-settlement life.
81 *Gigantopelta chessoia* Chen *et al.*, 2015 is a large (body length ~50 mm) peltospirid
82 gastropod first discovered in 2012 [13, 14]. This gastropod is unusual in having a large,
83 specialised bacteria-housing organ or ‘trophosome’ inside the body, ontogenetically
84 derived from an oesophageal gland [12]. Dependency on microbes allows the animals to
85 reach comparably large body sizes [15]. Only two gastropod genera are known to have
86 this specialised organ, all other vent molluscs with internal chemosymbionts house the
87 microbes in their gill tissue [12].

88

89 In small individuals of *Gigantopelta chessoia*, where the gonad is apparently completely
90 developed, the oesophageal gland is very small and not enlarged into a ‘trophosome’ [12].
91 We hypothesised that this unusual metamorphic shift, the later enlargement of the
92 ‘trophosome’, is associated with a change in diet from grazing to total dependency on
93 internal symbionts, with the inoculation of chemosymbionts occurring during this
94 transition. Surprisingly, the small individuals (without a developed ‘trophosome’) and
95 larger individuals, are otherwise identical in external morphology. In order to examine
96 the arrangement of the internal organs non-invasively, we used synchrotron x-ray
97 computed tomographic (CT) imaging of the soft parts across post-settlement ontogeny. In
98 the present study, our aims were to determine whether this transition was a gradual
99 isometric growth of particular organ, or a more transformative change at a specific stage
100 of growth that could be considered a cryptic, internal metamorphosis.

101

102

103 **Methods**

104

105 Specimens of *G. chessoia* were collected from chimney surfaces using a suction sampler
106 on the remotely operated vehicle (ROV) *Isis* during the RRS *James Cook* expedition JC80
107 from the hydrothermal vent field at 2,644 m deep on segment E2 of the East Scotia Ridge
108 [12, 13]. Six post-settlement specimens representing post-settlement ontogeny (body
109 lengths: 2.2 mm, 3.5 mm, 5.1 mm, 7.8 mm, 12.2 mm, 23.0 mm) were selected from
110 specimens fixed and stored in 10% buffered formalin, and then post-stained with 1%
111 iodine solution for five days prior scanning by synchrotron hard x-ray micro computed
112 tomography (micro-CT).

113

114 Specimens were scanned at one of two different synchrotron facilities, to take advantage
115 of differences in the optimised fields of view of optics. Scans of small specimens (below

116 7 mm body length) were completed at beamline 8.3.2 at the Advanced Light Source
117 (ALS), Lawrence Berkeley National Laboratory (Berkeley, USA). Larger specimens
118 (above 7 mm) were visualised in hutch #3 of the beamline BL20B2 at the SPring-8
119 (Hyogo, Japan). At the ALS, scans were done using monochromatic X-rays at 23 keV,
120 with a home-built lens system with Mitutoyo 5x or 2x lenses and a LuAG:Ce 150 or 500
121 micron thick scintillator. A PCO.edge sCMOS camera was used, yielding effective pixel
122 sizes of 1.72 μm for the 5x lens or 3.44 μm for the 2x lens. Samples were rotated through
123 180 degrees while 2049 images were collected. Reconstruction was performed with
124 Tomopy and Xi-cam (alpha release, 2017, <http://www.camera.lbl.gov/xi-cam-interface>).
125 At the SPring-8, similar scans were performed at 25 keV [16] using a Hamamatsu
126 Photonics K.K. CCD camera [17], yielding 1860 images with effective pixel sizes of 6.5
127 μm or 13.16 μm .

128

129 The resulting images were processed in Adobe Photoshop CC for contrast enhancement
130 and then imported into Amira v5.3.3 (FEI Visualisation Sciences Group), aligned into a
131 single stack and highlighting the materials of interest. Post-processing including surface
132 rendering and smoothing to generate the final tomographic model following previously
133 published methods [18, 19].

134

135 For transmission electron microscopy (TEM), a portion of the oesophageal gland and gill
136 leaflet from small (2.6 mm body length) and large (25.8 mm body length) *Gigantopelta*
137 specimens fixed in 10% buffered formalin were first sliced to approximately 0.2-0.4 mm
138 thick with a razor blade after washing with filtered sea water. In order to best preserve
139 the ultrastructure, we employed an ice-free high-pressure freezing/freeze substitution
140 (HPF/FS) technique [20] (Leica EM-PACT2). Frozen samples were post-fixed with 2%
141 OsO_4 in acetone for 3-4 days at -80°C , and then gradually brought to room temperature,
142 rinsed with acetone, and embedded in epoxy resin (TAAB, Aldermaston, U.K.). An
143 ultramicrotome (Leica EM-UC7) was used to make ultrathin sections (70 nm) which were
144 then post-stained with 2% uranyl acetate and lead solution (0.3% lead nitrate and 0.3%
145 lead acetate, Sigma-Aldrich). Transmission electron microscopy (TEM) was performed
146 using a Tecnai 20 TEM (FEI) at an acceleration voltage of 120 kV.

147

148

149 **Results & Discussion**

150

151 We reconstructed the whole digestive systems of six *Gigantopelta* specimens across a

152 size range spanning an order of magnitude, from 2.2 mm to 23.0 mm (Figure 1). It is clear
153 from the reconstructions that during ontogeny the oesophageal gland suddenly expands
154 and takes over the visceral mass between body sizes of 5.1 mm to 7.8 mm, a small
155 difference compared to the normal adult body size of ~ 50 mm. From quantification of
156 organ volumes (Figure 2), the overall relative volume of the digestive system within the
157 total body does not change significantly during growth, and increases isometrically as
158 approximately 20% of the body volume. Meanwhile, the proportion of the digestive
159 system occupied by oesophageal gland dramatically increases at the transition stage and
160 then grows isometrically with body size (Figure 2).

161

162 In early post-settlement stages, the oesophageal gland largely contains open space and
163 lacks endosymbionts (Figure 2a), whereas after the transition the same tissue expands to
164 form the ‘trophosome’ and it is filled with bacteriocytes (Figure 2b). Although previous
165 workers speculated that the gill epidermis may also house endosymbionts [21], we also
166 examined the gill epidermis via TEM and found that the gill lacks any endosymbionts
167 both before and after the transition stage. Taken together, this is evidence that a
168 transformation in anatomy is accompanied by a transition in feeding ecology. We interpret
169 this post-settlement transition in *Gigantopelta* as a metamorphosis, which drastically
170 alters its internal anatomy in changing the entire digestive system to enable a niche
171 switching to a terminal adult stage with total dependency on intracellular
172 chemosymbionts.

173

174 We consider the term ‘cryptometamorphosis’ to be a suitable descriptor for this
175 phenomenon. There is some disagreement about the definition of what is considered
176 ‘metamorphosis’, but broad consensus is that it involves both a change in morphology
177 and life history, which shifts the ‘adaptive landscape’ in that the selective pressures acting
178 at the individual level or group level are altered [22]. In this sense, although the
179 morphological changes in are internal rather than external, the post-settlement transition
180 in *Gigantopelta* qualifies as a true metamorphosis. There are cryptic aspects to any
181 metamorphosis, with anatomical transitions happening prior to external changes, such as
182 in butterflies prior to their emergence from a chrysalis [23]; however, in all other known
183 cases, metamorphoses are associated with profound and correlated changes in both
184 external and internal morphology, even if internal and external reconfigurations may be
185 decoupled [4]. In *Gigantopelta*, there is no external evidence in either the shell or the
186 body showing the change to the terminal form has been completed, yet the internal
187 reconfiguration changes the animal’s autecology.

188

189 Differential allometric rates of growth among organs can be induced by life history in
190 permanent or transient change; however, differences in proportions alone are not
191 metamorphosis. A metamorphosis, such as we see in *Gigantopelta*, includes the
192 transformation in organ structure and function as well as size. It is not uncommon for
193 animals to experience changes in trophic niche or target prey size with increasing size or
194 allometric change over ontogeny. In the yellowfin tuna *Thunnus albacares* (Bonnaterre,
195 1788) subadults change diets significantly at sizes around 45-50 cm forklength, from
196 feeding on plankton to feeding primarily on other teleosts [24]. This type of trophic shift,
197 however, has no metamorphic quality in terms of either internal or external morphological
198 transition. Metamorphosis is also conceptually and functionally different to changes
199 associated with phenology or sexual maturity, which are not necessarily linked with shifts
200 in niche occupation. Small-sized *Gigantopelta*, before the transformation, already have a
201 well-developed gonad and should therefore be regarded as sexually mature [12], their
202 cryptometamorphosis is thus not apparently related to sexual maturity.

203

204 Nutritional dependency on endosymbionts is often correlated with dramatic increases in
205 size and growth rate of marine lineages, a trend which could apply to *Gigantopelta* [15].
206 Metabolic rates and growth in ectotherms are also strongly dependent on temperature,
207 and vent endemic animals mostly live in temperatures much higher than the surrounding
208 seawater [11], with thermal regimes comparable to the subtropics. Some chemosymbiotic
209 holobionts such as the giant vent tubeworm *Riftia* [25, 26] and the giant vesicomid clam
210 *Calypptogena* [27] are characterised by extremely high growth rates, faster than any other
211 marine invertebrates [28]. There is also an evident influence of hosting symbionts on
212 gigantism in other environments, such as photosymbiotic living (*Tridacna*) and fossil
213 (rudist) giant bivalves that represent some of the largest benthic marine invertebrates that
214 ever lived [29]. Although it is not universally true that all holobionts are necessarily giants
215 [30, 31], this trend toward increased body size attributable to endosymbionts is seen in a
216 variety of reducing environments. Recently the giant shipworm *Kuphus polythalamius*
217 (Linnaeus, 1767) which inhabits shallow water reducing muds was found to exhibit
218 thioautotrophic endosymbionts [32], and endosymbionts were likely also responsible for
219 gigantism in its fossil relatives that were originally mistakenly described as a dinosaur
220 [33].

221

222 Chemosymbiotic holobiont vent snails (*Gigantopelta*, *Chrysomallon*, *Alviniconcha*, and
223 *Ifremeria*) likewise grow much larger than their close relatives in their respective families

224 [12, 34]. The pre-cryptometamorphosis size of *Gigantopelta chessoia* is approximately
225 the average adult size of other members of its subclass Neomphalina, and most others in
226 the group feed by grazing or deposit-feeding [35]; change from a grazer to a holobiont at
227 a late stage likely allows *Gigantopelta* to reach a much larger size at the terminal adult
228 stage and might therefore increase reproductive output. This is evidence that symbiosis,
229 a key feature of vent ecosystems, is advantageous to the host lineages no matter at what
230 stage the symbiont acquisition occurs.

231

232 Deep-sea bathymodioline mussels, a family which includes species in multiple deep sea
233 reducing environments such as woodfalls and hydrothermal vents, may be another
234 potential example of cryptometamorphosis [36]. These mussels probably initially settle
235 as solely filter-feeding young, and speculatively transition later to reliance on
236 endosymbionts [37, 38]. Fully-grown adult mussels house endosymbiotic bacteria in their
237 gills, and it remains unclear at what point inoculation occurs, or whether it is accompanied
238 by any other changes to gills or the digestive organs. Giant vent tubeworms in the genus
239 *Riftia* initially settle as later-stage trochophores with a complete larval digestive system
240 feeding on microbes, and then are inoculated with endosymbionts through the skin during
241 metamorphosis to the adult benthic form; these bacteria migrate across several layers of
242 host tissue to infect mesodermal tissue which develops into the trophosome entirely
243 independent of the digestive tract [39]. In later juveniles, the trophosome has already
244 completed development, and therefore the *Riftia* endosymbiont inoculation process can
245 be considered part of its metamorphosis process from larva to juvenile. Unlike *Riftia*, the
246 ‘trophosome’ of *Gigantopelta* is clearly derived from oesophageal gland tissue, part of
247 the digestive system, and its development into a bacteria-housing organ happens as a
248 separate metamorphosis.

249

250 The trophosome-like structure that characterises *Gigantopelta* is only found in one other
251 mollusc genus, also in the family Peltospiridae: the Scaly-foot Gastropod *Chrysomallon*
252 *squamiferum* Chen *et al.*, 2015, from the Indian Ocean [40]. *Gigantopelta* and
253 *Chrysomallon* have superficially similar but evolutionarily convergent adaptations to
254 exploit chemosynthetic resources in hydrothermal vent ecosystems [12]. Juveniles of
255 *Chrysomallon* have a hypertrophied ‘trophosome’ already at 2 mm body length, at a point
256 after settlement but before any development of the gonads [35]. *Chrysomallon* also has a
257 single oesophageal gland, but it is symmetrical and may be derived from a single gland
258 or a fused pair [35]. Other smaller, non-symbiotic peltospirids such as *Peltospira* and
259 *Rhynchopelta* have paired oesophageal pouches that are not used to house bacteria [41].

260 *Gigantopelta* only possesses a single oesophageal gland that is asymmetrical in all growth
261 stages (Figure 1). The isometric growth and lack of cryptometamorphosis in
262 *Chrysomallon* reinforces the evidence that the ‘trophosomes’ in *Gigantopelta* and
263 *Chrysomallon* represent parallel adaptive evolution and not a feature of the family
264 Peltospiridae.

265

266 Both *Gigantopelta* and *Chrysomallon* have an elaborate circulatory system, including a
267 pumping muscular heart to transport hydrogen sulfide and oxygen to their endosymbionts
268 [12, 35]. The development of the ‘trophosome’ is fundamental to housing endosymbionts,
269 so other related anatomical adaptations in the circulatory system of *Gigantopelta* are
270 directly related to supporting its niche switching. It is unclear how different holobiont
271 lifestyles impact oxygen metabolism or metabolic scaling, as there is apparently little
272 difference between the metabolic rate of holobiont gastropods housing bacteria in a
273 ‘trophosome’, or within the gills [42]. What is important to consider in the case of
274 *Gigantopelta*, and possibly other species in especially chemosymbiotic ecosystems, is
275 that there is a substantial difference in energy flow between direct consumption of
276 nutrients through chemosymbiosis and food resources that originate outside the body.
277 Evolution of this lineage exploits the energy efficiency of symbiosis, but the potential
278 advantages are conferred at a surprisingly late stage in development that is unique among
279 vent holobionts.

280

281 Vent animals have many curious adaptations, but the limited species diversity and
282 relatively short interacting chains make vents a good model system for understanding
283 trophic dynamics [11], if we have multiple lines of evidence to understand the true energy
284 sources of the animals at different life stages in each ecosystem. The findings in this study
285 advance the understanding the ecology and evolution of chemosymbiotic organisms by
286 demonstrating that different holobiont lineages with similar symbiont-housing strategies
287 may have drastically different symbiont inoculation processes. Importantly, the newly
288 revealed phenomenon of cryptometamorphosis expands our concept and horizon of
289 animal metamorphosis and niche switching.

290

291

292 **Data, code and materials**

293 The datasets supporting the results of this article are included within the article and its
294 additional file, also available on Dryad [43].

295

296 **Ethics**

297 Study species were gastropod molluscs collected within the South Georgia and the South
298 Sandwich Island (SGSSI) Maritime Zone, with a scientific research permit from the
299 Government of SGSSI for the RRS *James Cook* expedition JC80. Additional permits
300 were granted by the Foreign and Commonwealth Office, U.K. under the Antarctic Act
301 1994 to undertake an expedition (Section 3, No. S3-03/2012) and for RRS *James Cook*
302 to enter Antarctica (Section 5, No. S5-05/2012). Animals collected were immediately
303 preserved in formalin after recovery and no live experiments were carried out.

304

305 **Authors' contributions**

306 CC conceived the study, participated in the design of the study, collected the specimens
307 used, carried out the lab work, participated in data analysis, and drafted the manuscript
308 with JDS; KL collected specimens and field data, and assisted with data interpretation;
309 KU carried out the TEM histology work and assisted with data interpretation; JDS
310 conceived the study, designed the study, contributed to lab work, participated in data
311 analyses, and drafted the manuscript with CC. All authors gave final approval for
312 submission and publication.

313

314 **Competing interests**

315 We have no competing interests.

316

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343

344 **Figure Legends**

345

346 Figure 1. 3D reconstructions of the digestive system in *Gigantopelta chessoia* across post-
347 settlement ontogeny, at body lengths (a) 2.2 mm, (b) 3.5 mm, (c) 5.1 mm, (d) 7.8 mm, (e)
348 12.2 mm, (f) 23.0 mm. The ‘trophosome’ is indicated by yellow, shades of blue indicate
349 other parts of the digestive system, including buccal mass, oesophagus, stomach,
350 digestive gland, and intestine. Scale bars = 1 mm for all parts.

351

352 Figure 2. Transmission electron micrographs of the ‘trophosome’ or oesophageal gland
353 in post-settlement *Gigantopelta chessoia* at body length (a) 2.6 mm and (b) 25.8 mm; as
354 well as (c) the relationship between body length and the relative percent body volume of
355 the whole digestive system combined (dashed grey line and triangles), the ‘trophosome’
356 (black line and filled circles), and the digestive system not including the ‘trophosome’
357 (light grey line and squares).

358

359

360 **Additional Files**

361

362 Video S1. A video illustrating a hypothesised scenario of isometric growth post-
363 settlement in *Gigantopelta chessoia* versus the observed cryptometamorphosis, showing
364 changes in the anatomy of the digestive system around the point of transition (3.5 mm to
365 12.2 mm body length). Simulated growth and rendering was done in Blender v2.79b.

366

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