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AN ALLOMETRIC ANALYSIS OF ONTOGENETIC CHANGES (VARIATION) IN THE CRANIAL MORPHOLOGY OF LARVAE OF *HYDATICUS BIMARGINATUS* (SAY) (COLEOPTERA: DYTISCIDAE: DYTISCINAE)

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ABSTRACT

Distortion coordinates (Cartesian Transformations) are used to compare the ontogenetic allometry in cranial morphology of first, second, and third instars of *Hydaticus bimarginatus* (Say). The most significant difference in the dorsoventral view is the expansion of the posterior lateral margins. Cranial expansion is likely due to an increase in the mass of the adductor muscles which are responsible for closing the mandibles. The ontogenetic shift in head orientation to a more subprognathic position evident in the analysis of lateral silhouettes indicates that second and third instars may be adapted to feeding on substrate associated prey. These differences are thought to reflect possible changes in prey regimes and habitat preference occurring during larval development.

Key words: *Hydaticus bimarginatus*; larva; crania; allometry; ontogeny, prey.

INTRODUCTION

Extra oral digestion (EOD) is a common feeding method employed by a variety of arthropod taxa (1), including a majority of dytiscid larvae (2). In this feeding method, the mandibles are utilized almost exclusively for prey capture, manipulation and consumption. This feeding strategy allows for the exploitation of larger prey than through "piece meal" consumption (1). Dytiscid larvae are obligatory and opportunistic predators, with prey selection limited principally by the size and shape of the prey (2; 3). This conclusion has been reinforced by observations of larval predation *ex situ*, in which larvae can be propagated into adults by consuming prey that they may never encounter in nature. Although Tate and Hershey (4) attempted to mimic *in situ* prey regimes, their electrophoretic analysis of gut contents of various field caught dytiscid larvae never the less differed from those feeding exclusively in laboratory systems. Thus, prey consumed in laboratory environments may not accurately reflect prey exploited by larvae in natural systems.

Natural habitats of various dytiscid species may include prey components that are unique to their specific microhabitats and these differences in prey regimes are reflected in the variation in larval cranial morphology (5, 6). Due to the small, complex nature of dytiscid reproductive habitats and the small size of both the predator and prey, direct observations of predation in the field are difficult. However, based on the relative importance of mandibles in prey capture and manipulation, it is our belief that natural selection will shape cranial morphology and mandibular geometry to optimize the efficiency of the exploitation of food resources most often encountered and utilized in nature. Thus, observable differences in cranial morphology between species or instars may be interpreted as indications of differential exploitation of *in situ* prey regimes (5, 6).

Numerous descriptive studies have shown that significant inter- and intraspecific variation in cranial (7, 8, 9, 10) and mandibular (11) morphology is present in Dytiscidae. There have been comparatively few studies dealing with allometric change in larval insects and only one (6) was focused on a dytiscid species. In this study significant ontogenetic changes in dorsoventral cranial morphology were shown for larvae of *Agabus disintegratus* (Crotch) by using distortion grid transformation analysis. These variations were interpreted biomechanically as adaptations that would allow instars to exploit different prey regimes. However, this study focused exclusively on the dorsoventral morphological transformations. Few studies describing lateral head morphology in dytiscid larvae have been attempted (*e.g.*, 12, 13) and none has compared shifts in lateral cranial architecture among instars.

Hydaticus bimarginatus (Say) is determined to be more advanced phylogenetically (14) than *A. disintegratus* which was evaluated by Brannen *et al.* (6). Thus, the objectives of this study were to: (1) utilize distortion grid analysis to describe and compare dorsoventral and lateral ontogenetic cranial morphology of first, second and third instar larvae of *H. bimarginatus*; (2) evaluate the biomechanical implications of modifications in cranial architecture to infer differences in prey regime composition.

MATERIALS AND METHODS

Morphological observations were performed on larvae collected between 30 June and 15 September 2004 from an ephemeral habitat in Bibb County (N 32° 52.033', W 83° 47.999'), Georgia, USA and identified as *H. bimarginatus* by culture into adults by Jackson *et al.* (15). First and second instars were identified by an association with these mature larvae.

Heads of first (n=6), second (n=10), and third (n=9) instars of *H. bimarginatus* were measured dorsally along the coronal suture from the posterior margin of the cranium to the distal margin of the frontoclypeus, excluding the frontoclypeal sensilla to determine lengths (HL). Head widths (HW) were measured dorsally at the widest point. The mandibular length was calculated by measuring ventrally from the center of the articulation to the mandibular apex (11). Head length ratios were determined by dividing head length (HL) by head width (HW). Gape was measured ventrally from the center of each mandibular ball that articulates with the cranium. Intermandibular ratios were as calculated by dividing intermandibular distances (ID) by head lengths (HL) and widths (HW). Brooks-Dyar indices (16) of dimensional change between first and second instars and second and third instars of *H. bimarginatus* were computed for cranial lengths, widths, and intermandibular distances.

Dorsoventral (Fig. 1A) and lateral silhouettes (Fig. 1 B) were drawn for crania of first, second, and third instars of *H. bimarginatus* using images from a WILD M5A dissecting microscope equipped with a Camera Lucida and digital images were taken with a Canon D60 digital camera attached to a Meiji RZ trinocular scope. For placement of dorsal and ventral landmarks, specimens were observed in depression slides up to 400X magnification. Lateral measurements and landmarks were determined by placing specimens in a depression slide cradled in a wax channel for increased stability and to control precision of positioning. In addition to the cranial outlines, dorsoventral landmarks included: cervical sensilla, origin of the coronal suture, egg bursters, dorsal mandibular articulations, origin of occipital suture, corneal lenses (17) one, two and three (18), anteromaxillary margins, cervical notches, and tentorial pits on the venter. The lateral landmarks included: origin of occipital suture, mandibular articulations, temporal spines, and corneal lenses (18). Dorsal and ventral cranial structures were combined in each dorsoventral silhouette. The cranial positional angle (CPA) of all three instars was calculated from lateral silhouettes. Independent line segments were drawn through the mandibular articulations and tangent to the cervical region. The angle where these two segments intersected was measured (Fig. 1C).

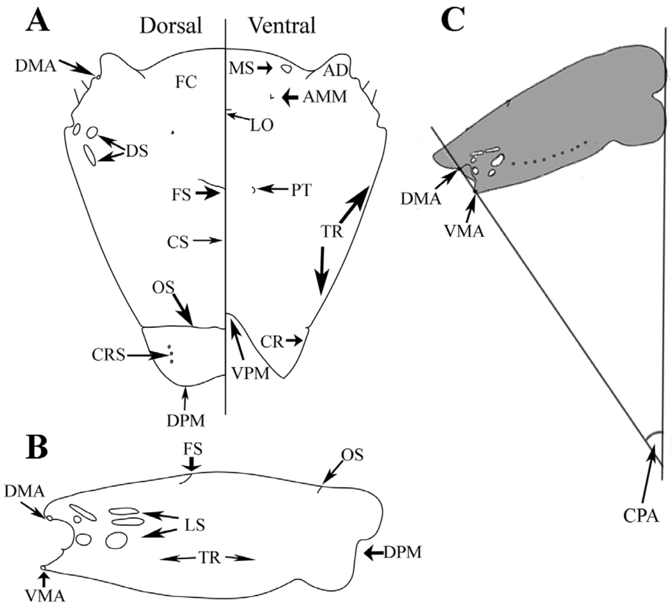

Figure 1. Dorsoventral (A) and lateral (B) views of a Hydaticus bimarginatus Say first instar showing internal landmarks used in distortion grid analyses. Legend: AD, adnasale; AMM, anterior maxillary margin; CS, coronal suture; CRS, cervical sensilla; CR, cervical region; DMA, dorsal mandibular articulation; DPM, dorsum of posterior margin; DS, dorsal corneal lenses; FS, origin of frontoclypeal suture; LO, labial margin origin; LS, lateral corneal lenses; MS, mandibular scar; OS, occipital suture; PT, posterior tentorial pit; TR, temporal region; VMA, ventral mandibular articulation; and VPM, ventral posterior margin. Technique (C) for computation of cranial positional angle (CPA, see materials and methods).

Thompson (19, first published in 1917) established the distortion grid method used for this study. This procedure employs a grid system over-laying either the assumed basal taxon (evolutionary allometry) or a preceding instar of the same species (ontogenetic allometry). A grid with lines of constant length and distance in both vertical and horizontal planes was superimposed over the dorsal and lateral illustration of the first instar head using Adobe Illustrator 10. Positioning of a grid over cranial illustrations of subsequent instars required distortion of the lines in order to maintain their relative position with respect to specific morphological landmarks present in the previous instar. Interpretation of these distortion grids was used to assess ontogenetic changes in cranial architecture occurring during larval development.

RESULTS

There were no significant differences between head length-width ratios (HL/HW) of first ($\bar{x} = 1.10 \pm 0.05$) and second ($\bar{x} = 1.12 \pm 0.02$) or third instars ($\bar{x} = 1.13 \pm 0.03$) of *H. bimarginatus* ($p > 0.05$). HL/HW ratios between the second and third also showed no significant differences ($p > 0.05$). Both proportional intermandibular distances computed against head width (ID/HW) and head length (ID/HL) appear to decline during the transition between the first and second stadia (Table I). However, these differences were not significant ($p > 0.05$). Brooks-Dyar indices (Table I) computed for increases in intermandibular distance (ID_1/ID_2 and ID_2/ID_3) were 0.65 from the first to the second and 0.69 from the second to the third. Comparable to indices computed for width ($HW_1 \rightarrow HW_2 = 0.65$; $HW_2 \rightarrow HW_3 = 0.69$) but not for length, which showed a small decrease in length ($HL_1 \rightarrow HL_2 = 0.70$; $HL_2 \rightarrow HL_3 = 0.69$).

Table I. Selected dorsoventral cranial dimensions (in mm) and proportions for first, second and third instars of *Hydaticus bimarginatus* (Say).

Instar	**Head Length (HL)**	**Head Width (HW)**	**Mandible Length (ML)**	**HL/HW**	**Gape (GA)**	**ID/HL**	**ID/ HW**
First	1.23 ± 0.03	1.03 ± 0.02	0.63 ± 0.02	1.10 ± 0.05	0.83 ± 0.03	0.73 ± 0.03	0.81 ± 0.01
Second	1.77 ± 0.05	1.58 ± 0.05	0.95 ± 0.03	1.12 ± 0.02	1.27 ± 0.06	0.71 ± 0.03	0.80 ± 0.03
Third	2.58 ± 0.09	2.28 ± 0.08	1.33 ± 0.07	1.13 ± 0.03	1.83 ± 0.08	0.71 ± 0.03	0.80 ± 0.01

The dorsoventral cranial architecture of each of the three instars of *H. bimarginatus* varied with the most pronounced differences occurring in the posterior regions. The silhouette of the first instar is trapezoidal in shape with its maximum width between line segments b̲ and d̲ (Fig. 2A). A cervical region is present, delimited by a weakly developed constriction near line segment h̲. Corneal lens three is fully visible on the dorsum. The silhouette of the second instar differs significantly from the first (Fig. 2 A & B) in that the posterior-lateral margin between segment d̲ and f̲ is expanded and curved. The cervical region is compressed laterally between segments f̲ and h̲. There is expansion laterally along the midline (segment 5) and laterally in the frontoclypeal region. Corneal lens three has a more lateral position. The third instar cranium of *H. bimarginatus* continues the trends observed when comparing the first to the third (Fig. 2 C & D). However, the posterior-lateral margin expansion and constriction of the cervical region is more extensive. When comparing the second to the third instar, the horizontal variation between instars is less significant (Fig. 2 E & F). However, there is considerable vertical expansion of the lateral margin between segments c and g.

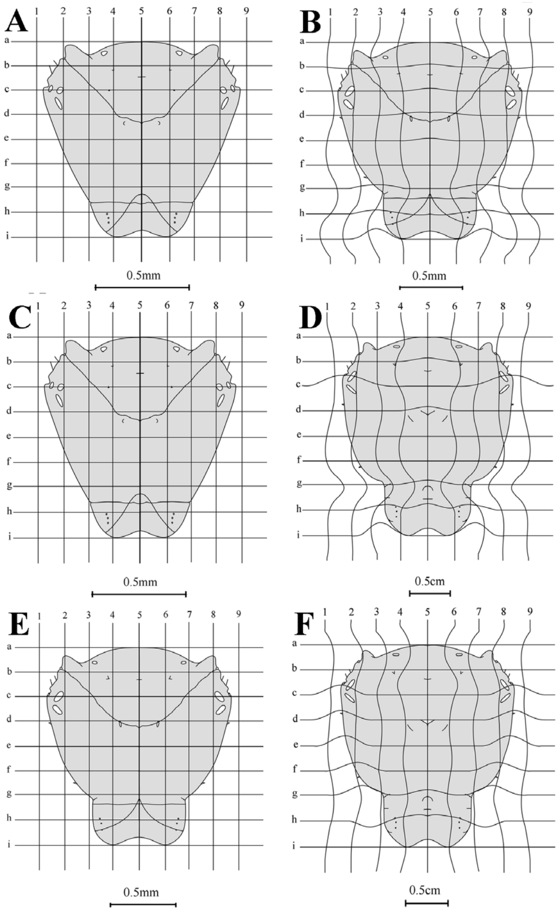

Figure 2. Distortion grids comparing dorsoventral silhouettes of *Hydaticus bimarginatus* (Say) first (A) and second instars (B); first (C) and third instars (D); and second (E) and third instars (F).

The lateral silhouettes (Fig. 3) also indicate significant changes in cranial architecture during development. The maximum depth of the first instar is located near the center of the cranium, at line segment 5. In the second and third instars the maximum depth has shifted posteriorly so that it is between segments 5 and 6. There has also been an increase in the depth of and changes in the orientation of the cervical region (Fig. 3; posterior to coordinates 7-a to 7-d). The ventral mandibular articulations are located slightly anterior to the dorsal articulation on both the first and second instars. However, the ventral articulations of the third instar have moved posteriorly; almost in vertical alignment with the dorsal articulation (Fig. 3). Cranial positional angles (CPA) computed (Fig. 1C) for first and second instars were 19° and 12° respectively far smaller than the 37° estimate for the mature larva. As with the dorsoventral analyses, when comparing the lateral view of second and third instars we do not see as significant a change as when comparing the first to second or first to third (Fig. 4).

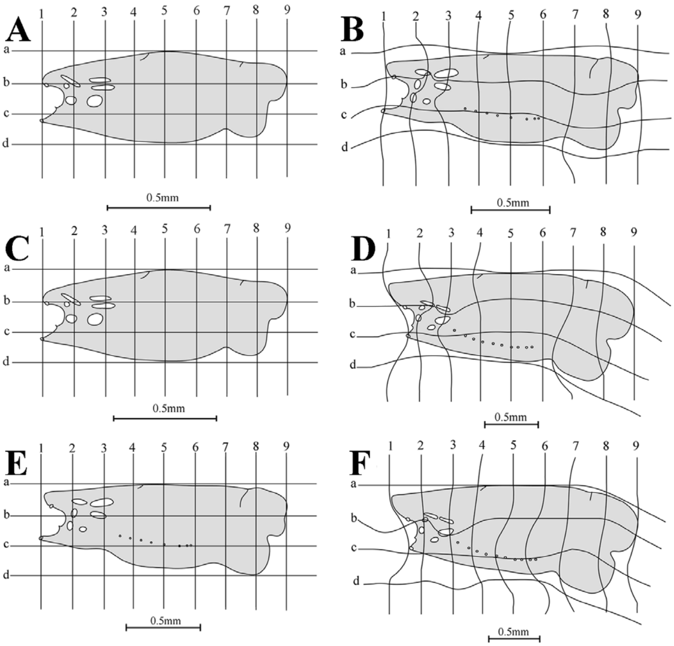

Figure 3. Distortion grids comparing lateral silhouettes of *Hydaticus bimarginatus* (Say) first (A) and second (B) instars; first (C) and third instars (D); and second (E) and third instars (F).

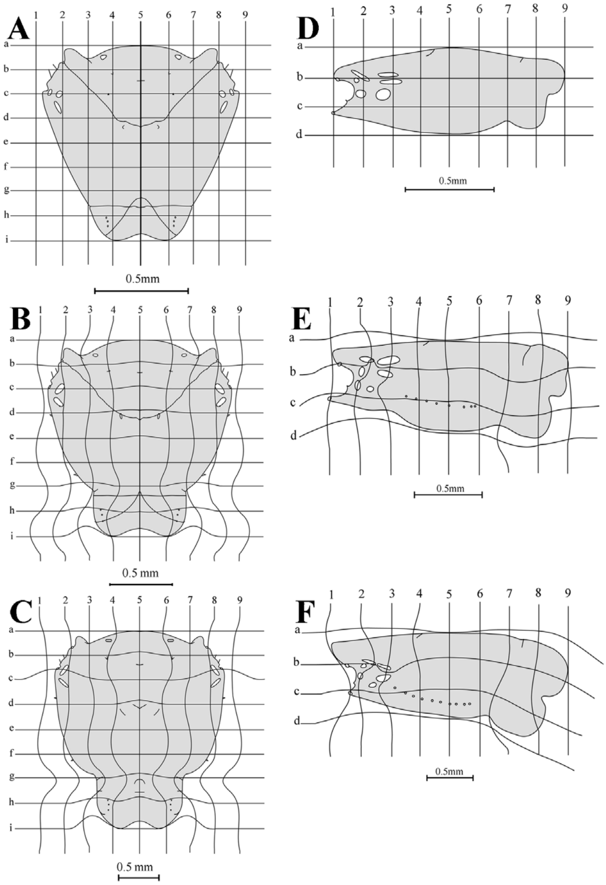

Figure 4. Distortion grids comparing dorsoventral (A-C) and lateral (D-F) silhouettes of *Hydaticus bimarginatus* (Say). Comparisons are first to second (AB; DE) and first to third (AC; DF).

DISSCUSSION

The analysis demonstrates that significant changes in cranial architecture are occurring during larval development of *H. bimarginatus* (Fig. 4). The successive dorsoventral transformation of *H. bimarginatus* is similar to that of *A. disintegratus* (6). Changes in the posterior and temporal cranial regions appear to be responses to accommodate subsequent and disproportional increases in the mass and volume of the mandibular adductor muscles, which serve to close the mandibles. The adductor muscles originate on large areas of the posterodorsal, posteroventral, and lateral interior walls of the head (20, 21, 22). The adductor muscles occupy a considerable portion of the cranial cavity, where the most significant dorsoventral ontogenetic changes occur (6). An increase in the adductor muscle size would serve to increase the out force applied at the mandible tip. Conversely, smaller adductor muscles would signify that the larvae would have a lesser out force at the mandible tip. The trapezoid shape of the head of the first instar is due to the smaller size of the adductor muscle mass indicating this larval stage is adapted for feeding on delicate prey. The expansion of the posterior lateral margin of the second and third instar is most likely due to an increase in the adductor muscles, allowing the larvae to consume more robust prey. Thus, the prey regime composition of the first stadium may be significantly different from that of the second and third stadia. These results also suggest that while size may be an important factor in prey selection by dytiscid larvae, prey resistance to capture may be a significant factor as well.

Changes in the lateral morphology of *H. bimarginatus* also suggest a shift in the prey regime during larval development. The change from the almost classical prognathous cranium (20) of the first and second instars to a cranial orientation that is almost intermediate between prognathic and hypognathic conditions (subprognathic, 5) in the mature larva is notable. The pronounced subprognathic orientation of the third instar cranium may be an adaptation to feeding on organisms most often found on a habitat substrate (e.g., leaves or sediments) below the long axis of the *H. bimarginatus* body. An apparent shift in the position of the fourth corneal lens to a more ventral position also supports this hypothesis. A subprognathic declination angle was described for the mature larvae of *Coptotomus lenticus*, which were collected at or near the bottom of its habitat (23). This subprognathy would potentially allow mature larvae to locate and exploit substrate surface dwelling prey more effectively. In contrast to this subprognathic species, *Thermonectus basillaris* (Harris) is somewhat hyperprognathous (5) with the first and third corneal lenses greatly enlarged and dorsally orientated, suggesting that this taxon is more suited for feeding in the open water column or at or near the water's surface (24).

Dorsoventral ontogenetic development in the anterior regions of crania of *H. bimarginatus* is nearly isometric, including intermandibular distances (Table I). Intermandibular distance (ID) is interpreted as an approximation of gape, the maximum distance between the mandibular apices when larvae are poised to strike. This is an important factor in dytiscid larval feeding because an increase in gape would allow the larvae to consume larger prey. The ontogenetic increase in gape suggests that the prey consumed is also increasing in size. However, noticeable variation in the cranial architecture of *H. bimarginatus*, specifically positive allometric growth in the posterior and temporal regions, is present. These accommodations are thought to be a response to an increase in the adductor muscles, which close the mandibles.

In summary, if previous studies are correct in assuming that dytiscid larvae are generalist and opportunistic predators and that competition is minimal, then there should be little variation in profiles of dytiscid cranium. However, this study shows that significant ontogenetic change in cranial architecture occurs during larval development of *H. bimarginatus* in both dorsoventral and lateral profiles. The biomechanical interpretation of these changes suggests that the degree of resistance offered by prey to capture and feeding by larvae of *H. bimarginatus* may be an important selective force in determining cranial architecture. Changes observed also indicate that second and third instars may occupy different microhabitats than do first instars. Thus, the prey regime encountered by later instars would be significantly different than those exploited by first instars. Although this morphological variation does not permit identification of specific prey, it does suggest that larval prey regimes are far more complex than previously thought. Habitat preference studies and analysis of ontogenetic mandibular variation among instars are needed in order to fully understand the degree to which these instars partition their food resources.

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