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Ibanez, C. M., Sepúlveda, R. D., & Sigwart, J. D. (2017). Comparative allometric variation in intertidal chitons (Polyplacophora: Chitonidae). *Zoomorphology*. Advance online publication. https://doi.org/10.1007/s00435-017-0387-2

Published in:
Zoomorphology

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

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Download date:11. Jul. 2024

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No H2020-MSCA-IF-2014-655661.

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Comparative allometric variation in intertidal chitons (Polyplacophora: Chitonidae)

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Abstract Allometry involves the study of the relationship between size and shape of an individual, and in particular, the manner in which shape depends on size. Animals with multi-element skeletons may have differing growth allometries in different parts of the body. Chitons, for example, have eight overlapping shell plates or valves of three distinct types: head (one plate), intermediate (six plates), and tail (one plate). The overall chiton body is ellipsoidal and different species differ in their eccentricity. The aim of this study was to examine overall allometry in size and shape over adult ontogeny, and how these patterns vary among four closely-related species of intertidal chitons from Southeastern

Pacific Ocean. For each specimen (n=407), measurements were taken of total body length and the exposed anterio-posterior lengths of the eight shell plates. Multivariate allometry was evaluated by means of a Principal Component Analysis for each species separately, and for the total. The results showed differential allometric growth of specific skeletal elements, which varied among species; however, there was no clear evidence for specific differentiable growth stages. The overall trend among the combined species was for weakly positive allometry of shell plate widths, but isometric growth of total length and width; thus, the lateral proportion of the animal occupied by shell increases over growth and conversely "thinner looking" girdles may be generally indicative of older animals.

Keywords: Allometry, shell shape, growth, polyplacophorans, morphometry, Southeastern Pacific.

Introduction

The form of an organism corresponds to the integration of size and shape. By definition, shape consists of those aspects of form that remain when size is removed (Mosimann 1970; Bookstein 1991). Intra-specific morphological variation among natural populations has been frequently observed and well documented, particularly, in shelled gastropods (e.g. Rolán et al. 2004; Conde-Padín et al. 2007; Sepúlveda and Ibáñez 2012; Avaca et al. 2013). Morphological variation within species is determined, in first instance, by the variation in body size of the individuals that compose a population (Huxley 1932; Kemp and Bertness 1984). This association between shape and size implies quantitative scaling relationships that can explain or even determine some processes within a population (Gayon 2000; Economo et al. 2005).

Allometry deals with variation of traits associated with variation of the overall size of the organisms. The traits can be the size of parts, their shape, or physiological, ecological, and behavioural characteristics, but the range of traits considered differs among the various concepts of allometry (Klingenberg 1998). Growth is often accompanied by changes in proportion as well as in size, which is known as the phenomenon of relative or allometric growth (i.e. shape changes during growth). Isometric growth refers to structures that vary proportionally with overall body size (Klingenberg 1996; 1998). Allometric growth occurs when a structure does not co-vary in linear proportion with total body size (Huxley 1932; Gayon 2000). Basic descriptions of allometry can provide a foundation for understanding the potential predictive power of specific shape variables (Dryden and Mardia 1998; Klingenberg 1998).

Chitons are a marine molluscan group belonging to class Polyplacophora, which are relatively morphologically constrained among extant taxa (Sirenko 2006; Sigwart 2009). These animals have a biphasic life cycle, with a dispersing trochophore larva that settles to the benthos where grows the ventral foot and mineralises eight shell plates (Eernisse 2007). Chitons usually attach to hard substrates with their muscular foot, which is protected by their characteristic articulating eight-part shell armour. The first (anterior: head) and the last (posterior: tail) plates are approximately semi-circular, their breadths are usually smaller than the intermediate plates in keeping with the overall oval body form (Schwabe 2010). The six intermediate plates are similar in shape, though shell plate II (immediately behind the head) is anterio-posteriorly elongated compared to the others, and in many species (e.g., *Lepidochitona cinereus*, *Tonicella marmorea*), there is a clear difference in widths among plates in a single animal (Baxter 1982; Baxter and Jones 1986; Connors et al. 2012). These plates provide protection while still allowing some degree of flexibility during locomotion over uneven and rough surfaces, as well as when rolling defensively into a ball-like conformation when dislodged from a surface (Connors et al. 2012; Sigwart et al. 2015). This complex multi-element armature is a combination of hard and soft aspects, with shell plates surrounded by a flexible girdle, and there is potential inter-specific variability in growth of all the various components (Baxter and Jones 1986; Avila-Poveda and Abadia-Chanona 2013).

Particularly, allometry of body shape and size in chitons has been described as a tool to examine plasticity, and as a potential source of characters to differentiate between similar species during their adult ontogeny (Baxter 1982; Baxter and Jones 1986), and to determine relationships of size allometry in the length-weight relationship (e.g., *Chiton albolineatus*) to relate differential growth rates of the different components of the chiton body (Baxter & Jones 1986; Flores-Campaña et al. 2012).

Herein, we explored the allometric and morphological variation of shell plates of four common intertidal polyplacophoran species from the same family Chitonidae, but covering multiple genera. These species have differing but largely overlapping ranges in the shallow southeast Pacific (Araya and Araya 2015). The four chiton species selected belong to the same taxonomic family (Chitonidae), yet the conformation and size of shell plates are very different: *Acanthopleura echinata* (Barnes, 1824) and *Enoplochiton niger* (Barnes, 1824) have larger size and narrower plates, while *Chiton granosus* Frembly, 1827 and *Tonicia elegans* Frembly, 1827 have smaller size and wider plates. Following these observations of shape, we used whole animals in dorsal view (flat, intact animals with shell plates in place) to test whether the relative proportions of shell plates shifts as animals get larger, and how these patterns vary among species. The comparative allometry of overall body shape in these four species during ontogeny provides a strong basis to establish a potential generalised allometric relationship between shape variables and body size in chitons and contributes to understanding of growth laws in marine invertebrates.

Materials and methods

Study areas and sample collection

A total of 407 adult specimens (≥10 mm) belonging to four species of intertidal chitons were obtained through original fieldwork between 2011 to 2016 and identified as *Acanthopleura echinata, Chiton granosus, Enoplochiton niger*, and *Tonicia elegans* (Figure 1). The inclusion of "adult specimens" is referred to the exclusion of larvae and extremely small specimens (<10 mm), and is not related to their sexual maturity. Size at maturity in chitons has been reported for species of the genus *Chiton* from Mexico, Peru and Chile, and these studies suggested that chitons mature at small body size (<30 mm: Sotil 2004; Avila-Poveda and Abadia-Chanona 2013; Vélez-Arellano et al. 2014; Brito 2017). Animals were collected on intertidal rocky shores and subtidal shallow waters until five meters depth at 14 locations along the Southeastern Pacific Ocean within their overlapping geographical distribution, which ranges between 4°S and 42°S latitude (Araya and Araya 2015) over more than 4,500 km of coastline (Table 1, Figure 2A). Conspecific individuals from all localities were combined for morphometric analyses, aimed to include all shape and size variation along the gradient among all localities. All specimens measured in this study were deposited at the Museo Nacional de Historia Natural, Chile (MNHNCL).

Allometric analysis

To analyse the morphological variation of each species of chiton, the following 12 distance variables were measured on ethanol preserved specimens through a digital calliper (precision: ±1mm): total length (TL), total width (TW), length of plate I and plate VIII (the terminal shell plates), and widths of each shell plate (I to VIII) (Figure 2B). We made a correction of body length by standardized width of each plate dividing each plate width by total length to compare shell plates standardized width across species. To avoid morphometric bias or skew, we used only flattened specimens and did not take any measurements from curled specimens. All statistical analyses were performed in R (ver. 3.1.2, R Core Team 2014), and specific commands are noted below to avoid possible ambiguity about interpretation of results.

First, a bivariate approach was used to determine the standard allometric coefficient for each variable with respect to total length in each species. The slope coefficient and 90% and 95% confidence intervals of the standard allometric equation $\log(x) = \log(a) + b\log(\mathrm{TL})$ (Huxley 1932), were calculated via ordinary least squares regression. These calculations determine whether the ontogeny of individual measurements is isometric ($b = 1$) with respect to body length. When a 95% confidence interval for the allometric coefficient does not overlap over the null hypothesis ($b = 1$, isometric growth), then the slope of the variable indicates allometric growth ($b \neq 1$). Moreover, when the allometric growth is defined, then we may infer hypo-allometric growth or negative allometry ($b < 1$) or hyper-allometric growth or positive allometry ($b > 1$) over ontogeny for that variable.

Second, a multivariate approach was employed to explore potential shifts in shape-space over ontogeny within each species, using Principal Component Analysis (PCA). In a dataset comprising multiple ontogenetic sets (species, or variables), the first component (PC1) summarises changes in size, while second (PC2) and later components reflect variation in shape trajectories (Shea 1985). Therefore, in a PCA combining data from multiple ontogenetic stages, any shifts in growth patterns would be indicated by changes in the relationship of PC2 to PC1 or to total length (i.e. asymmetric distribution, or clear breaks in the distribution of plotted data; Nikolioudakis et al. 2010).

The original measurement data for 12 variables were log-transformed and subjected to a PCA for each species separately, specifying a variance-covariance matrix (R command prcomp). The distribution of PC2 values calculated for each single-species' dataset was visually inspected in relation to individual values for PC1 and specimen size (TL) to identify potential breaks or shifts in allometry that would indicate differential growth stages.

Third, the multivariate analysis was extended to a simultaneous PCA for the four species, to test whether shape could be used to differentiate species. As before, log-transformed data were subjected to PCA. Loading (rotation) values for PC1 for each variable were compared to the expected value, by calculating a 95% confidence interval on 10,000 bootstrap replicates (boot.ci, using type "basic"). When the confidence interval includes the expected variable factor loading value $(1/12)^{0.5}$ for an element in an analysis of 12 component variables, this would indicate isometry of that variable with respect to overall shape (Shea 1985).

Results

The size range (TL) of chitons measured and used in this study varied between 24 and 141 mm (mean 76.9 ± 32.9 S.D.) for *Acanthopleura echinata*, between 14 and 79 mm (mean 44.0 ± 5.0 S.D.) for *Chiton granosus*, between 44 and 110 mm (mean 75.9 ± 18.8 S.D.) for *Enoplochiton niger*, and between 10 and 58 mm (mean 31.2 ± 11.1 S.D.) for *Tonicia elegans* (Figure 1 and 2B). In all four species, as in all typical chitons, the terminal shell plates were the narrowest, and the central shell plates (IV-VI) were wider, though the widths of various features generally differed among species (Figure 3).

Bivariate comparisons of each individual component to overall body length indicated varying patterns of growth, which were not consistent among taxa but corresponded to observed patterns in morphology (Table 2). In particular, the posterior parts of the armature of *E. niger* and *A. echinata* had significantly positive allometry, thus the widths of posterior shell plates get wider more rapidly as overall body length increases; while the most shell plates in *T. elegans* showed significantly negative allometry relative to body length, indicating that the overall body size increases more rapidly than the widths of the shell plates. *A. echinata* and *C. granosus* showed isometric growth in the anterior and posterior shell plates, respectively (Table 2).

In multivariate analyses, first principal component (PC1) in species-specific analyses accounted for more than 92% of variation. The signs of the PC1 loadings were consistent within each species (either all positive, or all negative), indicating that PC1 distributes specimens according to length. The second principal component (PC2) reflects changes in shape; this accounted for between 0.9% (*A. echinata*) to a maximum of 4% (*E. niger*) of the variation. Comparison of PC2 values with body length (TL) and PC1 values showed a symmetrical uncorrelated distribution with no evidence of any ontogenetic shifts. The other components (PC3-PC12) have little variation (<1%).

Multi-species PCA also recovered a first principal component accounting for 95.3% of variability. The PC1 loadings for all 12 variables were positive and of similar values, indicating this component is a length axis reflecting scaling of features with body length more than shape change; several features were positively allometric, although others showed isometry (Table 2). The second component PC2 contributes to the separation of species according to shape, especially *T. elegans* and *A. echinata* (Figure 4). The factors with relatively larger loadings for PC2 indicate which are potentially more relevant to shape variation: these features are total length (0.42), total width (0.24), and the length of shell plate VIII (-0.86). For PC3 larger loadings are total width (-0.43), and the length of shell plate I (0.84).

Discussion

This study gives us strong evidence that shape differences and allometry even among closely related, ecologically similar taxa have species-specific patterns that were previously unappreciated. These results are concordant to Klingenberg (1996; 2010), who indicated a multidimensional inherent growth even when simple shapes vary in many different ways. Quantitatively, the features that contribute most to shape variation (i.e., total length, total width, and the length of shell plates I and VIII) all increase isometrically on average (Table 2).

These intertidal chitons showed different types of allometry among their shell plates, a pattern previously reported in other species (Saad 1997). There is weak evidence that the terminal plates have a less positively allometric growth than intermediate plates: the widths of terminal plates have lower values for the allometry coefficients, compared to intermediate plates in the multi-species PCA (Table 2), and the lengths of the terminal plates grow isometrically with respect to total size. These differences may be a consequence of their terminal location; in the tail plate, growth is holoperipheral and both terminal plates superficially are based on a more elliptic shape.

The shape of chitons is more or less oval in outline, but among the 1000 living species this presents a wide variation from broad oval to worm like (vermiform) body shape

(Schwabe 2010). Shape allometry, changes in the outline shape during adult growth, varies among species and can potentially vary in separate populations (Emam and Ismail 1993). This variation is in part related to niche specification, both in specific adaptations of overall body size and in terms of shell construction and material strength (Sigwart et al. 2015).

Acanthopleura echinata has a size-segregating vertical distribution, in that the largest individuals of the species are found relatively lower (Otaíza and Santelices 1985). Our sampling nonetheless covered the whole vertical range of that species. Among the species studied, three are in the exposed intertidal but one species is found in lower intertidal to shallow subtidal waters (*T. elegans*). *Tonicia* spp. generally lack complex shell sculpture and the features of the girdle perinotum are often so diminutive that the dorsal girdle surface seems to be nude. This suggests both morphological separation and ecological separation correlated to a distinct allometric pattern in this species that differs from the other three.

In this study, we found differences in shape and size for the four species. These species of chitons live at the intertidal zone often exposed to heavy surf or under boulders in the shallow subtidal waters (Araya and Araya 2015). The largest species (*A. echinata* and *E. niger*) showed lower variability in the standardized widths of shell plates, while the relatively smaller species (*C. granosus* and *T. elegans*) showed higher variability in the standardized width shell plates. These differences could be consequence of a phylogenetic separation, or may be a by-product of shallower niche specialisation.

It is not presently clear whether there is any ontogenetic shift in mechanical conformation of the chiton armature due to different life stages and their corresponding inner organization related to processes such as gonadal ontogenesis, gonad development stages, sexual differentiation, and onset of the first sexual maturity (Avila-Poveda and

Abadia-Chanona 2013). While we did not find any evidence for specific allometric shifts in any of the specific variables, it is clear that there is a strong interaction of size and shape.

Ontogenetic variation on shell shape has been found in many other molluscs, including intertidal snails showing a strong allometry (e.g., Kemp and Bertness 1984; Hollander et al. 2006; Avaca et al. 2013). Allometry can differ among species and reflect evolutionary change in growth patterns related to ecological or physiological factors (Gould 2002; Klingenberg 2010). In chitons, allometric growth could be related to their extraordinary morphology; articulated shell plates allow the chitons to fit within crevices to avoid predators, or (for intertidal species) to use rocks or under-boulders as refuge to avoid the sunlight (Otaíza and Santelices 1985; Flores-Campaña et al. 2012). Apparently, chiton allometry, comparatively to other molluscs (i.e., gastropods and bivalves), could be the result of a combination of shell plate shape, differential growth rates, and environmental influences (Baxter 1982; Baxter and Jones 1986; Flores-Campaña et al. 2012).

While chitons appear superficially similar, straightforward morphometry can indicate clear differences among even closely related species. Different aspects of the multielement chiton armature experience differential growth allometries, which apparently experience a continuous shift over post-settlement life. These points provide specific data that may be relevant to field identification of growth stages; the negative allometry of plate widths in *T. elegans* means that older specimens would have apparently relatively wider girdles, while the opposite is true in *E. niger*. We examined allometry in four common species; chitons are morphologically constrained, yet these species are clearly different in shape and size. Not only to they have a different shell plate morphometric pattern, but their allometry, the acquisition of a distinctive shape over ontogeny, is also variable. Within the chiton scleritome, individual elements experience independent but coordinated growth trajectories. Expanding further on this approach promises new insights to the functioning of chiton armour during growth.

Acknowledgements

This study was financially supported by FONDECYT #1130266 grant “Evolutionary biogeography of the Southeastern Pacific polyplacophorans” to C.M. Ibáñez. We are grateful to F. Alfonso, A. Cifuentes, S. Curaz, A. Fabres, A. Navarrete, M.C. Pardo-Gandarillas, V. Sanhueza, J. Salazar, C. Tobar and G. Torretti for their assistance in field and laboratory work.

Ethical standards

This research was approved by the Universidad Andres Bello ethical committee and the Chilean government through FONDECYT. The manuscript has not been submitted to more than one journal for simultaneous consideration nor has it been published previously.

Conflict of interest

The authors declare that they have no conflict of interest with any other projects, researchers or organizations, commercial or otherwise.

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Fig. 1 Pictures of dorsal view (shell plates I to VIII oriented from left to right) of the whole animal for A) *Acanthopleura echinata,* scale bar = 20 mm, B) *Chiton granosus*, scale bar = 10 mm, C) *Enoplochiton niger*, scale bar = 20 mm and D) *Tonicia elegans* scale bar = 20 mm

Fig. 2 Map of sampling sites along the Southeastern Pacific coast (A), and dorsal view of a generalized chiton (B) showing the main morphological measurements (white lines) used in this study. I-VIII = shell plates from anterior to posterior

Fig. 3 Standardized width (mm) of shell plates I to VIII for the four species of chitons used in this study: A) *Acanthopleura echinata*, B) *Chiton granosus*, C) *Enoplochiton niger*, and D) *Tonicia elegans*. The box-plots indicate the median, 25^{th} and 75^{th} (boxes) percentiles, 10^{th} and 90^{th} (whiskers) percentiles, and outliers of the size distribution of chitons

Fig. 4 Shape differences in four species of chitons, resulting from combined principal components analysis. The second component (PC2, responsible for shape) is related to total body size represented by body length (mm). The four species studied are shown in different colours