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Study of the Carapace Shape and Growth in Two Galápagos Tortoise Lineages

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ABSTRACT Galápagos tortoises possess two main shell forms, domed and saddleback, that correlate with the biogeographic history of this species group. However, the lack of description of morphological shell variation within and among populations has prevented the understanding of the contribution of evolutionary forces and the potential role of ontogeny in shaping morphological shell differences. Here, we analyze two lineages of Galápagos tortoises inhabiting Santa Cruz Island by applying geometric morphometrics in combination with a photogrammetry 3D reconstruction method on a set of tortoises of different ages (from juvenile to adult). The aim of this study is to describe morphological features on the carapace that could be used for taxonomic recognition by taking into account confounding factors, such as the morphological changes occurring during growth. Our results indicate that despite the shared similarities of growth patterns and of morphological changes observed during growth, the two lineages and the different sexes can be distinguished on the basis of distinct carapace features. Lineages differ by the shape of the vertebral (especially concerning their width) and pleural scutes, with one lineage having a more compressed carapace shape, whereas the other possesses a carapace that is more elongated and expanded toward the sides as well as an higher positioning of the first vertebral scute. Furthermore, females have a more elongated and wider carapace shape than males. Finally, carapace shape changes with growth, with vertebral scutes becoming narrower and pleural scutes becoming larger during late ontogeny. J. Morphol. 272:379–386, 2011. © 2011 Wiley-Liss, Inc.

KEY WORDS: giant tortoises; *Chelonoidis*; photogrammetry; turtles

INTRODUCTION

Galápagos tortoises (*Chelonoidis* spp.) are one of the two (the other being their distinct relatives, the Aldabra tortoises) remaining groups of giant tortoises still existing in the world. Genetic data suggest that these animals diversified from their closest relative (*Chelonoidis chilensis*) about 6–12 mya (Caccone et al., 1999) and colonized the existing islands following the temporal formation of the archipelago, from the older islands on the East to the younger islands on the West (Caccone et al., 2002). Galápagos tortoises have evolved a highly variable shell morphology with animals being domed (rounded cupola-like carapace form), saddleback (high anterior opening of the shell, which is more compressed on the sides), or of intermediate morphology between the two. Van Denburgh (1914) and Fritts (1983, 1984) studied morphological differences in these animals using, among other characters, linear and curved measurements taken on the shell of more than 100 animals, highlighting the existence of distinct tortoise morphotypes within and among Galápagos islands. The islands of Floreana, Fernandina, Espanola, Pinta, Pinzon, Santa Fe, and San Cristobal harbor (or harbored in the case of Floreana, Fernandina, and Santa Fe) saddleback tortoises (San Cristobal also had domed tortoises, now extinct), Santiago has intermediate shell form tortoises, whereas Isabela and Santa Cruz have both saddleback and domed tortoises (the tortoises on Volcano Wolf on Isabela are highly heterogeneous in morphology, including intermediate shell forms; Fritts, 1983). Genetic analyses support the distinction of these morphotypes as separate lineages (Caccone et al., 2002; Beheregaray et al., 2004), with Santa Cruz and Isabela Islands being inhabited by multiple lineages, and 15 evolutionary units (indicated as separate species by Russello et al., 2010) are currently

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recognized. When mapping these distinct shell morphologies on a phylogenetic tree encompassing all extant and most of the extinct taxa, it seems that the saddleback and domed shell forms evolved independently multiple times in the Galápagos archipelago (Caccone et al., 2002; Poulakakis et al., 2008).

Although the Galápagos tortoise radiation is one of the most emblematic in evolutionary biology, Austin and Arnold (2001) noted that morphological diversity within islands is lower than in the extinct Mascarene giant tortoises, where three islands harbored both domed and saddleback shell forms within each island. Yet, there is now evidence that even if the same island rarely harbors saddleback and domed shells as in the Mascarene islands, intraisland genetic and morphological variation in Galápagos tortoises have been underestimated (Caccone et al., 2002; Ciofi et al., 2002; Beheregaray et al., 2003, 2004; Russello et al., 2005; Chiari et al., 2009). In fact, recent genetic analyses demonstrated the existence of two deeply genetically divergent lineages inhabiting the island of Santa Cruz with apparently similar domed shell morphologies (Beheregaray et al., 2003; Russello et al., 2005). This finding prompted the first direct comparative study of patterns and levels of shell morphological and genetic variation within and between Galápagos tortoise lineages inhabiting the same island (Chiari et al., 2009). This study, based on linear and curved shell measurements, showed a different overall shell shape between the two genetically distinct lineages. It also highlighted a correlation between levels of morphological (shell shape) and genetic variation that could not be explained only by a combination of historical or demographic factors (e.g., different colonization times or population sizes), suggesting that other factors (e.g., selection, plasticity) may also play an important role in shaping morphological shell differences.

Although the work of Chiari et al. (2009) provided valuable insights in demonstrating the existence of previously undetected shell shape variation within and among lineages, it did not recognize lineage-specific morphological characters on the shell between the two studied taxa and did not describe shape changes during late ontogeny, which could represent a confounding factor for lineage identification. The detection of distinct morphological features on the shell would be extremely helpful to clarify the taxonomic status of these animals [by applying an integrative taxonomic approach, as suggested by Padial et al. (2010)].

In this study, we use geometric morphometrics in combination with a photogrammetry 3D reconstruction method (Chiari et al., 2008) to find detailed carapace differences in the same two tortoise lineages from Santa Cruz studied by Chiari et al. (2009) using traditional morphometrics. Various studies (e.g., Adams and Rohlf, 2000; Parson et al., 2003; Trapani, 2003; Bernal, 2007; Kaliontzopoulou et al., 2007; de Oliveira et al., 2008) highlighted the superiority of geometric morphometrics in better describing shape differences than traditional morphometrics. We here report on the detailed morphological differences in the carapace shape that could be used to distinguish between the two lineages and between the sexes and on the changes occurring during growth in females and males of each of the two lineages.

MATERIALS AND METHODS

Sampling

Animal procedures were carried out in this study following the ethics guidelines on animal handling as required by Yale University (IACUC permit 10825).

Tortoises were sampled in August 2006 on Santa Cruz Island in the Galápagos archipelago in the known distribution area of the two studied lineages, Cerro Fatal and La Reserva (additional sampling information in Chiari et al., 2009). Digital images to be used for 3D carapace reconstruction were obtained following Chiari et al. (2008, photogrammetry method) for all the encountered individuals, excluding the ones with major shell injuries or deformities. Three-dimensional reconstruction of the carapace was successful for 60 individuals (33 and 27 from Cerro Fatal and La Reserva lineages, respectively). We used for this work animals of different ages (from juveniles to adults) for which sex could be determined without any doubt (see Chiari et al., 2009, for sex discrimination) for a total of 14 males and 19 females and 10 males and 17 females belonging to the lineages of Cerro Fatal and La Reserva, respectively. Some of these animals were the same as in Chiari et al. (2009; 58% and 89% of Cerro Fatal and La Reserva samples, respectively), but here we also included turtles with a curved carapace length below 580 mm (here defined as juveniles; measurement obtained as described in Chiari et al., 2009). The juveniles included in our dataset were six from Cerro Fatal (three females and three males) and one from La Reserva (a female).

Data Acquisition

Digital images of the carapace of the animals and of a predefined calibration target (see Chiari et al., 2008 for additional information) were obtained with a Pentax Optio W10 (pixel resolution of 1600 × 1200), following Chiari et al. (2008, photogrammetry method). We used eight digital images for the camera calibration, whereas between 9 and 20 digital images were used for each 3D carapace reconstruction done with PhotoModeler® Pro 5.2.3 (Eos System) following Chiari et al. (2008, photogrammetry method). Each 3D carapace reconstruction was scaled to the "real" animal size using as Euclidean distance the straight width of the second vertebral scute of each individual (Fig. 1, distance between the landmarks 4 and 17 obtained with a caliper with resolution of 0.1 mm).

Morphometrics

The 3D coordinates of 25 landmarks (Fig. 1), giving 75 variables (25 landmarks with three coordinates for each landmark), were exported from PhotoModeler® and analyzed in the R environment (version 2.7.2, 2010) to perform all the analyses described below. Twenty-three missing landmarks occurred in 18 of the 60 individuals analyzed for this work. The coordinates of the missing landmarks were estimated by using the symmetrical counterpart, assuming for this estimation that the

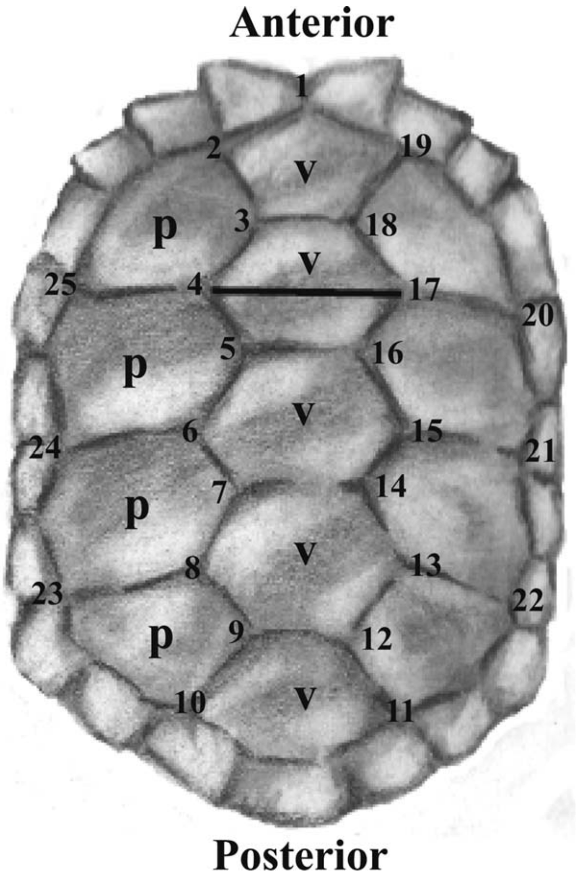

Fig. 1. Carapace of a Galápagos tortoise with the landmarks used for this study indicated by numbers. Horizontal black line between landmark 4 and 17 indicates the linear measurement used to scale to "real" size the 3D reconstructions. "v" and "p" indicate the vertebral and pleural scutes, respectively. Pleural scutes are indicated only for one side of the carapace.

two halves of the carapace, from head (landmark 1 in Fig. 1) to tail, were symmetrical. To get the coordinates of these missing landmarks, symmetric-paired landmarks (every landmark except the number 1, Fig. 1 and the ones for which the symmetric landmark was missing) were used to compute their mean and then combined with the coordinates of landmark 1 to find the coordinates of points expected to lie on the symmetric plan (Fig. 1). Because there is always some degree of variation in the position of the landmarks relative to the symmetric plane, a principal component analysis (PCA) decomposition was then run on the coordinates of the symmetric plan, giving a double weight to the average of the symmetric-paired landmarks (as there is one symmetric landmark for each half side of the carapace relative to the symmetric plan), to find the first three principal components (PCs) of which the first two should correspond to the two axes defining the symmetry plan and the third one reflects local asymmetries. Coordinates of the missing landmarks were then estimated as being equal to the coordinate of their symmetrical counterpart by multiplying the third coordinate by -1 (see also Claude, 2008 for more general information on the missing landmarks calculation).

Once the coordinates of the missing landmarks were estimated for each carapace, all configurations of landmarks were superimposed using a partial-generalized Procrustes superimposition (Dryden and Mardia, 1998; Claude, 2008), which has the advantage of decomposing the studied form into size and several shape components. This procedure allows scaling, rotation, and translational effects to be removed. Size was then estimated as the centroid size, whereas the shape was given by the coordinates of the superimposed landmarks. Due to the fact that our dataset contained a much larger number of variables (75) versus individuals (60), a PCA on landmark coordinates was run to reduce the number of variables to the first 16 PCs, which accounted for more than 90% of the variation in our dataset. Thus, shape coordinates were summarized by these first 16 PCs. The reduction of shape components also allowed removing part of the variation that could be considered as a noise and the redundancy between variables (e.g., due to the superimposition process and to the fact that left and right sides of the carapace can partly be considered redundant).

Statistical Shape Analyses

To test whether lineages and sexes (considered as separate factors) differed in shape, we performed a multivariate analysis of variance (MANOVA) using the Pillai statistics and the type II sums of squares and cross products on the first 16 PCs. A Bonferroni correction was applied for multiple comparisons. Centroid size was introduced as a covariate to estimate whether allometry was different between sexes or lineages. Interactions among the above mentioned factors (sex, lineage, and size) allowed then to consider differences in sexual dimorphism or in allometry between groups (see below for analyses done after removing allometric effects). A linear discriminant analysis was applied using the four groups (the two lineages subdivided by the two sexes).

To remove the contribution on possible shape differences due to allometric growth, we first regressed the Procrustes-superimposed shape variables on the size (see above) for each of the four groups (two sexes per two lineages) and then applied a Burnaby correction (Burnaby, 1966), taking into account the four different growth curves. This approach allows consideration of the variation that is independent of growth only. Also in this case, the first 16 principal components of shape variation (free from allometric effects) were selected to account for 90% of the remaining variation. A MANOVA was thus applied on shape variation summarized by these 16 PCs as described above. A Bonferroni correction was applied for multiple comparisons. A linear discriminant analysis was performed on the 16 first PCs using the four groups (two sexes per two lineages). To depict the differences in carapace morphology among the four groups, variation along discriminant axes was estimated from extreme values found on each discriminant axis. The carapace morphologies corresponding to these extreme values were obtained by recovering the scores of the 16 corresponding PCs, following the procedure for reconstructing shape from linear discriminant analysis described in Claude (2008). Finally, shape differences associated with growth for each of the four groups (male and females for each lineage) were described by regressing for each group the shape coordinates on the size. Fitted values for the smallest and largest individuals in each sex–lineage subgroup allowed visualizing shape changes occurring during growth.

RESULTS

Table 1 shows that carapace shape is strongly correlated to size (size as a factor in the table), suggesting allometric growth for the carapace. Furthermore, Cerro Fatal and La Reserva differ in their mean carapace shape (lineage as a factor, Table 1), whereas sexual dimorphism plays a marginal role in explaining mean shape differences ($0.05 < P$-value < 0.1, sex as a factor, Table 1). Allometric growth effect is different between females and males (interaction size × sex, Table 1)

TABLE 1. Mean carapace shape distinction between La Reserva and Cerro Fatal lineages on Santa Cruz Island

Effect	*df*	Pillai	Approx *F*	*df* num	*df* den	*P*-value
Size	1	0.8650	14.822	16	37	1.753×10^{-11}**
Sex	1	0.4358	1.786	16	37	0.072
Lineage	1	0.7605	7.345	16	37	3.056×10^{-7}**
Size × Sex	1	0.5764	3.147	16	37	0.002**
Size × Lineage	1	0.7117	5.708	16	37	6.170×10^{-06}**
Sex × Lineage	1	0.3098	1.038	16	37	0.443
Size × Sex × Lineage	1	0.5476	2.799	16	37	0.005**
Error term	52					

Two-way MANOVA on shape variables with size (Size), lineage (Lineage), and sex (Sex) as factors.
*indicates significant *P*-value ($P < 0.05$).
**indicates significant *P*-value after Bonferroni correction with $n = 6$ ($P < 0.008$).
"x" indicates the interaction between factors. *df*, degree of freedom; *df* num, degree of freedom numerator; *df* den, degree of freedom denominator.

and between the two lineages (interaction size × lineage, Table 1). Sexual dimorphism (difference in the carapace mean shape between the two sexes) is similar between lineages (interaction sex × lineage, Table 1). Finally, allometric growth differences between sexes are not the same between the two lineages (interaction size × sex × lineage, Table 1).

Table 2 shows that the two lineages, Cerro Fatal and La Reserva, still differ in their mean shape when shape differences due to allometric growth effects are removed (lineage as a factor, Table 2). Furthermore, carapace mean shape is different between the sexes (sex as a factor, Table 2), indicating a sexual dimorphism for the carapace, which is expressed in the same way between the two lineages (interaction sex × lineage, Table 2). The discrepancy of the result concerning the sexual dimorphism (significant only when allometric growth is removed) could be the result for example of small individuals (e.g., juveniles) of one sex being similar in carapace shape to larger individuals (e.g., adults) of the other sex.

A linear discriminant analysis clearly separates the two lineages and sexes along the first and second axes, respectively (discriminate power associated with the first axis was 72%, whereas the one associated with the second axis was 19%, data not shown). Eleven individuals were misclassified by this analysis. Of these, five individuals (one juvenile) were misclassified only in terms of lineage and six (two juveniles) in terms of sex. Misclassification of individuals for either lineage or sex was around 18%, while it was around 10% for only one of the two factors (lineage or sex). Figure 2 shows the results of the linear discriminant analysis after removing allometric growth effects. Twelve individuals were misclassified by this analysis, five in terms of lineage (and two of them also in terms of sex) and the rest (of which two were juveniles) in terms of sex. Figure 3 shows the superimposition of the reconstructed extreme (most different) carapace shapes (see Materials and Methods for additional explanation) in three dimensions along the first (which reflects differences between lineages, Fig. 3Aa and b) and the second (which reflects differences between sexes, Fig. 3B a and b) discriminant axes, after allometric growth effects are removed. Tortoises from Cerro Fatal show a higher and more compressed carapace than tortoises from La Reserva (Figs. 3A and 4), which instead appear to have a carapace that is more elongated and expanded on the sides (Fig. 3A). Tortoises from La Reserva also show a higher position of the first vertebral scute compared with the ones from Cerro Fatal (Fig. 3Ab). Furthermore, tortoises from Cerro Fatal have generally larger (wider) vertebral scutes, especially for the fourth one (Fig. 3Aa), which tends to be hexagonal in Cerro Fatal and more pentagonal in La Reserva, and smaller pleural scutes. The sulcus of the second and third pleural scutes to the third vertebral scute is higher in

TABLE 2. Carapace mean shape distinction between La Reserva and Cerro Fatal lineages on Santa Cruz Island after removal of allometric growth effects

Effect	*df*	Pillai	Approx *F*	*df* num	*df* den	*P*-value
Sex	1	0.4711	2.283	16	41	0.017**
Lineage	1	0.7057	6.144	16	41	1.33×10^{-6}**
Sex × Lineage	1	0.2693	0.944	16	41	0.530
Error term	56					

Two-way MANOVA on shape variables lineage (Lineage) and sex (Sex) as factors.
*indicates significant *P*-value ($P < 0.05$).
**indicates significant *P*-value after Bonferroni correction with $n = 2$ ($P < 0.025$).
"x" indicates the interaction between factors. *df*, degree of freedom; *df* num, degree of freedom numerator; *df* den, degree of freedom denominator.

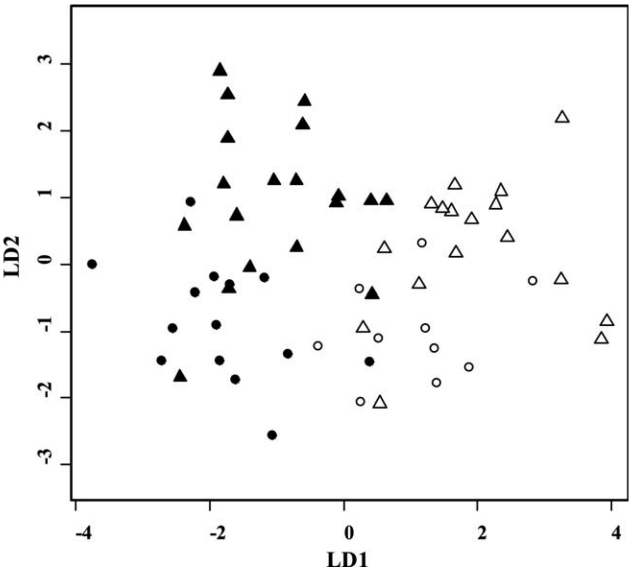

Fig. 2. Linear discriminant analysis along the first and second discriminant axes (LD1 and LD2, respectively) on the first 16th principal components shape coordinates (see text for further information) after removing allometric growth effects. Discriminate power associated with the first axis was 70%, whereas the one associated with the second axis was 20%. Black circles= Cerro Fatal males. White circles= La Reserva males. Black triangles= Cerro Fatal females. White triangles= La Reserva females.

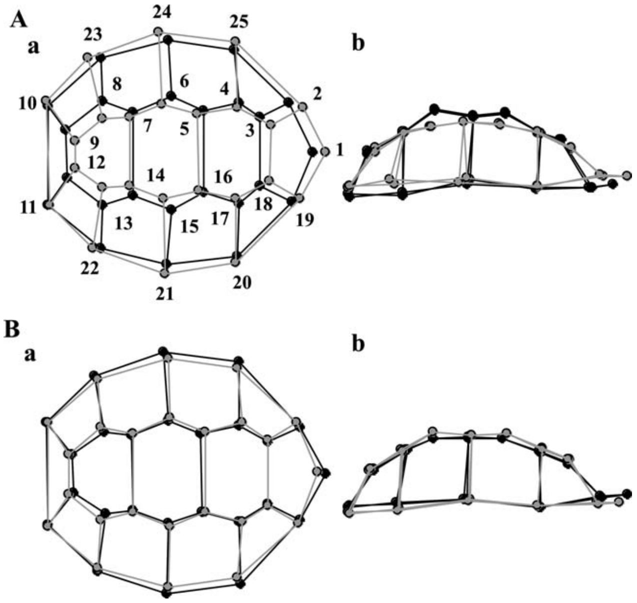

Fig. 3. 3D carapace shape differences for extreme values (most different shapes, see text for further explanations) between La Reserva and Cerro Fatal lineages on Santa Cruz island (**A**) and between sexes (**B**) after allometric growth effects were removed. Dots and numbers represent landmarks as in Figure 1. In A, black dots represent Cerro Fatal, whereas grey dots represent La Reserva lineages, respectively. In B, black dots represent females, whereas grey dots represent males, respectively. "a" represents the dorsal and "b" the lateral views.

tortoises from Cerro Fatal than tortoises from La Reserva (Fig. 3Ab), producing a more pentagonal shape of the second and third pleural scutes in Cerro Fatal, compared with the more rectangular ones of individuals from La Reserva. In La Reserva tortoises, the sulcus point between the marginal and the pleural scutes lie on the same line in the lateral view (Fig. 3Ab), in comparison to tortoises from Cerro Fatal, where the sulcus of the sixth and seventh marginal scutes to the second and third pleural scutes is in a slightly higher position compared with the other connections between marginal and pleural scutes. Furthermore, in Cerro Fatal individuals, the second and third pleural scutes extend more ventrally and internally than in La Reserva tortoises, with the sulcus between these scutes being perpendicular to the symmetry axis for Cerro Fatal, and more

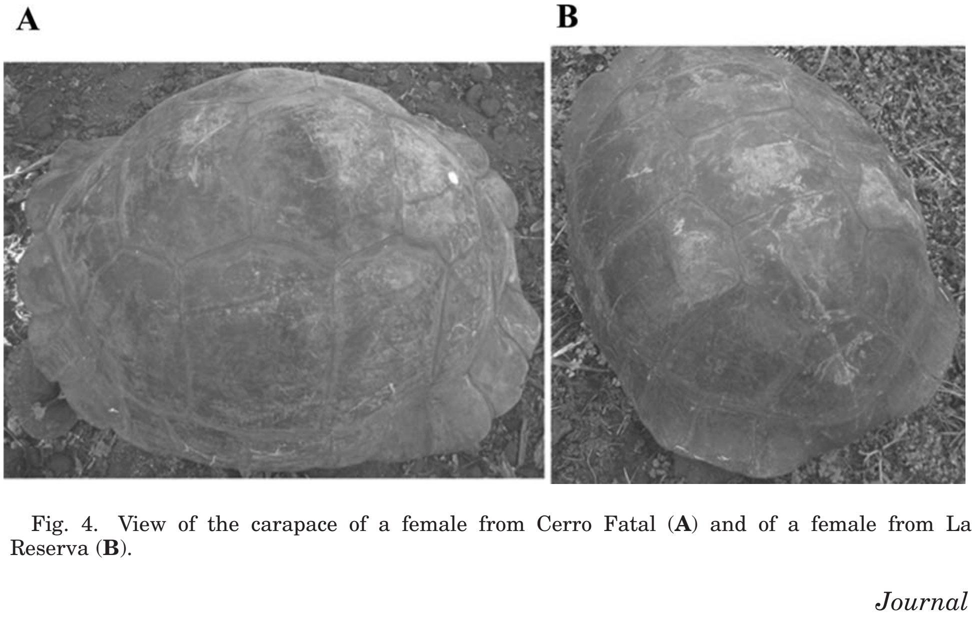

Fig. 4. View of the carapace of a female from Cerro Fatal (**A**) and of a female from La Reserva (**B**).

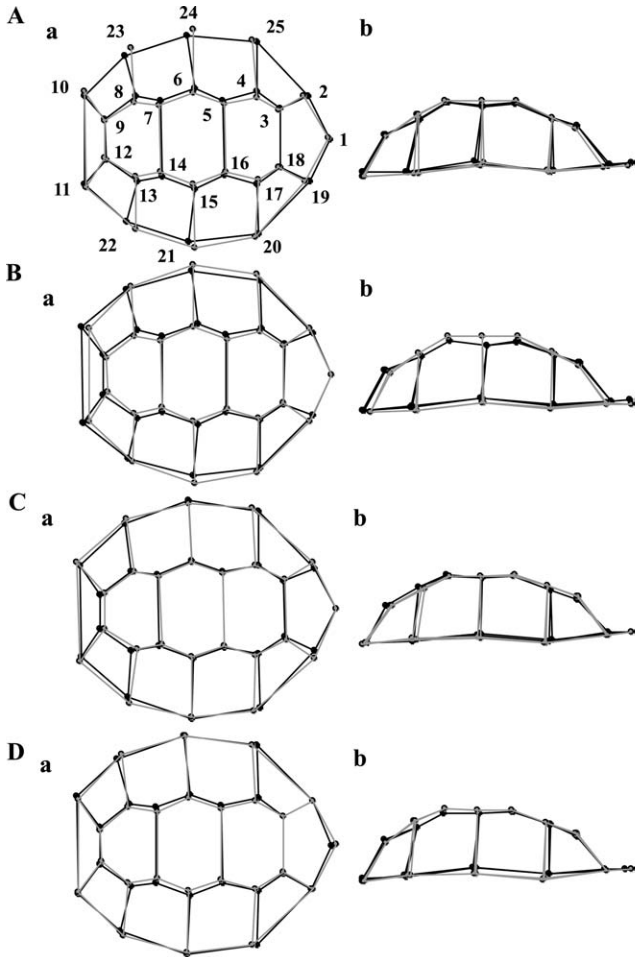

Fig. 5. 3D carapace shape differences at the extreme growth stages (smaller vs. larger) for each of the four groups (males and females of each lineage): (**A**) Males of Cerro Fatal; (**B**) Females of Cerro Fatal; (**C**) Males of La Reserva; (**D**) Females of La Reserva. Dots and numbers represent landmarks as in Figure 1. "a" represents the dorsal and "b" the lateral views. In grey, always the shape of the larger growth stage and in black always the one of the smaller.

oblique posterolaterally for La Reserva. Finally, the first pleural scutes are more elongated in tortoises from La Reserva than in tortoises from Cerro Fatal. Sexual dimorphism is less important, with females presenting a more elongated carapace, a slightly more expanded carapace on the sides, and smaller fourth and longer first vertebral scutes than males (Fig. 3B). Furthermore, carapace is slightly higher in females than males (Fig. 3Bb).

Carapace shape changes with growth in each of the four studied groups (Fig. 5). For all the groups, the second to the fourth vertebral scutes become relatively narrower (with the exception of the second and third vertebral scutes in males from La Reserva, Fig. 5C), whereas pleural scutes become relatively larger during late ontogeny (Fig. 5). For both sexes of Cerro Fatal tortoises, the carapaces becomes relatively wider with growth, especially at the level of the second and third pleural scutes (Fig. 5A,B). In Cerro Fatal females, the fifth vertebral scute is more bent ventrally with growth than in males (Fig. 5A,B). In La Reserva, carapace shape changes with growth are less expressed (may be because only one juvenile, a female, from La Reserva is included in our dataset) and located more in the posterior part of the carapace, which tends to be slightly wider at the level of the third and fourth pleural scutes (Fig. 5C,D).

DISCUSSION

A previous study based on traditional morphometrics recognized the two lineages of Galápagos tortoises from Santa Cruz, Cerro Fatal and La Reserva, as morphologically distinct (Chiari et al., 2009). The results obtained by using 3D geometric morphometrics only on carapace variables confirm the ones obtained by traditional morphometrics on the entire shell (carapace + plastron; Chiari et al., 2009). The two lineages differ in mean size and carapace shape (Table 1), and these shape differences remain even after allometric growth effects are removed (Table 2). However, although detailed and graphic visualization of specific morphological differences was difficult using traditional morphometrics, the use of 3D geometric morphometrics allowed us to better describe the morphological carapace differences between the two lineages (Fig. 3). Tortoises from Cerro Fatal appeared to have a higher and more compressed carapace shape and wider vertebral scutes compared with tortoises from La Reserva, which instead have a longer carapace, more expanded on the sides, with higher sulcus of the marginal the pleural scutes, and a relatively higher first vertebral scute. Our results support an early observation (Rodhouse et al., 1975) done on tortoises from La Reserva, for which some animals although having a characteristic general domed shell form, also showed a slight upward opening on the front of the carapace.

Based on the little current ecological and morphological data available for Galápagos tortoises, it is not possible to infer the factors explaining the morphological differences observed at the carapace between the two studied lineages. The observed morphological differences may or may not be all adaptive, and only the presence of a robust phylogeny with dating and shell shape data for all the extant and extinct Galápagos tortoises, including outgroups, would allow us to better address this question. Previous studies on the two major morphotypes of the shell in giant tortoises from Galapagos and Indian Ocean islands suggested that the parallel evolution may have been associated with reduced predation pressure and to morphological niche speciation (e.g., adaptation to a more xeric versus a more humid environment; Arnold, 1979;

Fritts, 1984; Bour, 1987; Austin and Arnold, 2001). Bour (1987) and Fritts (1984) in their studies on the evolution of distinct shell ecomorphs on the tortoises of the Indian Ocean and Galápagos, respectively, propose two opposite conclusions concerning the morphological niche speciation. On Galápagos, saddleback tortoises, smaller in size and volume than domed tortoises, would be favored in a more xeric environment with less ecological diversity, characteristic of low elevation habitats (Fritts 1983, 1984). Instead, Bour (1987) highlights how, in the Indian Ocean, generally smaller and lighter tortoises inhabit humid regions at higher elevations, whereas bigger tortoises occupy drier habitats at lower altitudes; a distinction which he attributes to better thermal regulation for larger shells. Cerro Fatal and La Reserva, where the two lineages occur, present very different environments (not due to anthropogenic influence), with the first being more xeric and with no trees that could offer shade to the animals, and the second being more moist (YC personal observation). A robust molecular phylogeny of these animals and additional comparative data (currently not available) on the ecology, behavior, and shell morphology of Galápagos tortoises (possibly including extinct tortoises and the reconstruction of past environments) inhabiting similar and different environments could allow to infer the relationship, if any, between habitat in which the tortoises occur and their shell shape. Furthermore, although our 3D reconstruction of the carapace does not provide information on the actual space available for the rear legs of the animal, the higher connection of the rear pleural on the marginal scutes of the tortoises from La Reserva (Fig. 3Bb) could provide more liberty of movement for the rear legs, facilitating the tortoises' migration. Tortoises belonging to the two lineages could present a distinct trend of migration and dispersion patterns (data on this subject are currently being collected), which may influence their shell shape, as already observed for the giant tortoises from Aldabra (Swingland and Lessells, 1979; Swingland et al., 1989). To test this hypothesis and the possible advantage of having a higher freedom of movement for the rear legs, precise and long-term movement data on tortoises from the two lineages as well as comparative measure of the leg lengths and their motility in correlation to different carapace and pleural scute shapes would need to be collected.

Based on the dataset currently used, sexual dimorphism was found to occur, even if shape differences were less important than the ones obtained by using linear and curve measurements obtained on the carapace and the plastron (Chiari et al., 2009). Our results not only support the existence of a sexual dimorphism for a given size, but also indicate the existence of a similar carapace shape between males and females at different ontogenetic stages (e.g., females at a given ontogenetic stage can have similar shape to males at a different ontogenetic stage). It is therefore important to take into account size and shape together to correctly estimate the sexual dimorphism on the basis of its carapace morphology alone. The more significant sexual dimorphism recovered by using traditional versus geometric morphometrics is probably the consequence of focusing in the current study only on carapace shape, without including data from the plastron and from the pygal region, which are known to be related to sex dimorphism in turtles. Furthermore, the current dataset also included juvenile individuals, which, although considered already as sexually mature based on visual examination of the plastron and tail, may not have fully developed sexual dimorphic carapace shape. Finally, the results of the MANOVA analyses suggest that growth needs to be taken into account for differentiating sexes (smaller *P*-values for sex in Table 2 than in Table 1), indicating also that each sex and each lineage display different allometric patterns.

The comparison of carapace shape at different growth stages (minimum and maximum) for each of the distinct four groups (males and females of each lineage) suggest a posterior compression of the carapace for the females of Cerro Fatal. This latter could be correlated with the sexual maturity state reached by the females of this lineage and the necessary changes occurring to allow a functional and effective copulation in relationship to the relative size of the sexually mature sexes, the concavity of the plastron of the males (the posterior part of the female carapace needs to "accommodate" the plastron of the male during copulation). This result may not be appreciable for females from La Reserva due to the limited number of juveniles included in our analysis for this lineage. Furthermore, in Cerro Fatal tortoises, carapace becomes wider with growth, while in tortoises from La Reserva, the carapace does not seem to further develop on the sides during growth. Finally, in Cerro Fatal tortoises, the relative position of the connection of the lateral pleural scutes to the vertebral scutes is higher at later growth stages, producing the more compressed carapace shape of the adults as described above. Our data on the carapace shape changes occurring with growth suggest that in tortoises from Santa Cruz Island selection maintains during ontogenesis a domed developmental trajectory for tortoises of both lineages, with the animals from Cerro Fatal becoming "more domed" than the ones from La Reserva, which do not seem to experience major carapace shape changes from the juvenile to the adult stage.

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