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Morphology of hatchling *Dipsochelys* giant tortoises

Abstract

Three living taxa of Aldabra and Seychelles giant tortoises (*Dipsochelys dussumieri, D. hololissa* and *D. arnoldi*) have been recognised recently. Some of the characters on which the adults are recognised have previously been suggested to be due to dietary distortion. The study of hatchlings of all three morphotypes confirms that these taxa are morphologically and taxonomically distinct. These taxa have not been distinguished by recent molecular genetic studies, highlighting that recently evolved species may not be accurately identified by molecular methods. This represent a limit to these now widely applied techniques which is relevant to the current debate on the value of molecular taxonomy.

Introduction

In recent years there has been an increase in the application of molecular methods to phylogenetic questions, with some proposals that taxonomy should rely largely on molecular techniques (TAUTZ et al. 2002, 2003; HEBERT et al. 2003) although some authors have disputed this suggestion (SEBERG et al. 2003; LIPSCOMB et al. 2003). In highly disputed taxa such
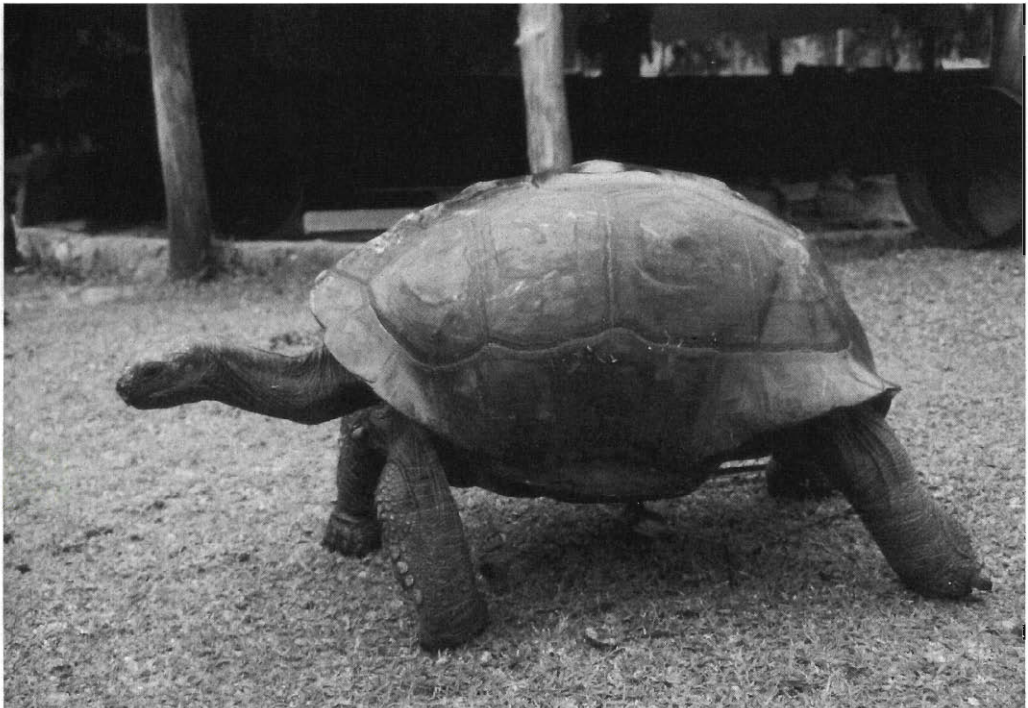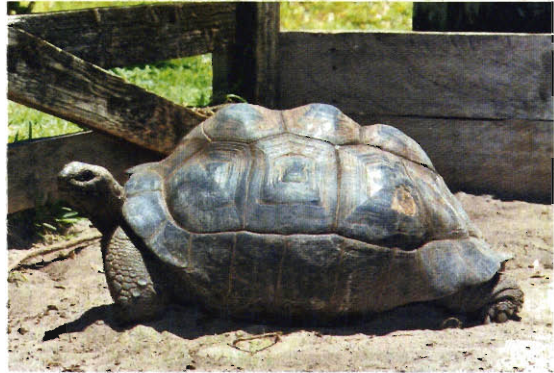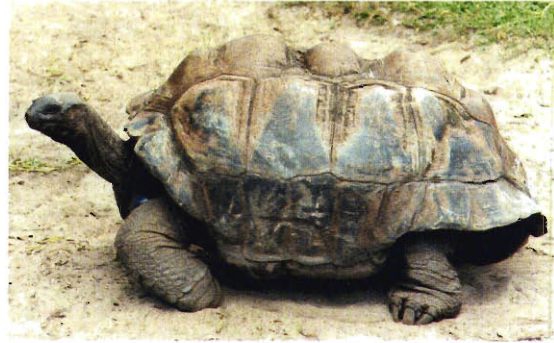

Ill. 1.   Aldabra giant tortoise, *Dipsochelys dussumieri.*

as Chelonia molecular phylogenies are now being adopted as systematic schemes.

The external appearance of the carapace has been the primary character used to distinguish different chelonian taxa since the first taxonomic description (Linnaeus 1758). Over nearly 250 years a large number of tortoise species has been diagnosed on the basis of overall shape, scute arrangements and colouration. Whilst some are highly distinctive monotypic genera, others such as *Testudo* include a wide range of similar species (Gmira 1993; Perälä 2002a,b,c). For many taxa taxonomically useful characters may be obscured by individual morphological variation and abnormal morphotypes (Lambert 1995). In species with large numbers of captive individuals a further complication is added by the possibility of distortion induced by captive conditions. It has been reported that unnatural captive diets can have major impacts on the appearance of tortoise carapaces (Arnold 1979) although there are few detailed studies of the precise nature of this distortion and its impact on taxonomic characters (Gerlach 2003b).

Captive conditions may induce abnormal morphology through ontogenetic processes due to nutritional imbalances during carapace growth, most obviously when calcium levels are low and phosphorus levels unnaturally high (Gerlach 2003b; Lambert et al. 1988; Barros 2001). Captive tortoises may play a role in clarifying the differences between similar species when captive-bred juveniles that have been bred and reared under identical conditions are available, thus eliminating the possibility of dietary distortion.

Although hatchlings morphology may be expected to be too conservative to allow closely related species to be distinguished the three extant *Dipsochelys* species have distinctive hatchling morphologies. All liv-

Ill. 2. Seychelles giant tortoise, *Dipsochelys hololissa*. a) male (Adam); b) female (Josephine). Photos: Fritz Wüthrich.

ing Indian Ocean giant tortoises were referred to the Aldabran giant tortoise species *D. dussumieri* (also known as *Geochelone gigantea* or *D. elephantina*) (Ill. 1) until 1998 when the genus was revised and living tortoises assigned to *D. dussumieri* and two supposedly extinct species *D. hololissa* (Ill. 2) and *D. arnoldi* (Gerlach & Canning 1998) (Ill. 3). As these three taxa are morphologically distinct as adults but currently genetically indistinguishable (Palcovaks et al. 2002, 2003; Austen et al. 2003) the morphology of captive bred hatchlings is of considerable interest in terms of their taxonomy, evolution and conservation.

Ill. 3. Arnold's giant tortoise, *Dipsochelys arnoldi*. a) male (Hector); b) female (Betty). Photos: FRITZ WÜTHRICH.

Methods

A range of juvenile *Dipsochelys* tortoises hatched in Seychelles were examined. *D. hololissa* and *D. arnoldi* were captive bred by the *Nature Protection Trust of Seychelles* from adults assigned to these species (Ill.2 & 3) (GERLACH & CANNING 1998). *D. dussumieri* were captive bred by private breeders in Seychelles and hatched from wild nests on Curieuse island.

Measurements were taken after the hatchling had reached 10 days of age (incubator hatched) or on emergence from a natural nest (at which time they would be expected to be approximately 10 days old; SWINGLAND & COE 1978). For each individual all scutes were measured, as well as the straight carapace lengths, height, costal height (from the plastron to the top of the costal scutes at the carapace midpoint – see Ill. 4), width at the midpoint and at the anterior of the shell (at the suture between the 2nd and 3rd marginals), plastron length and depth of the anal notch. The colour of the carapace and plastron were noted. The number and origins of the juveniles are given in Table 1. The morphometrics are summarised in Table 2 and were analysed statistically using principal component analysis.

Results

The only discrete character investigated was colouration. All hatchlings appeared brown in comparison with the generally dark grey or black adults. In all *D. dussumieri* the hatchlings were dark brown and skin, carapace and plastron darkened to black within one week. *D. hololissa* were a lighter brown and retained that colour for 3-4 weeks before darkening to blackish brown (Ill. 5 & 6). *D. arnoldi* were light brown on hatching, with darker edges to the carapace scutes and some grey spots (Ill. 7 & 8). Skin colour was light brown on the head and limbs, the neck and tail were grey. The plastron was pale brown. This colour was retained for three weeks, after which time progressive dark-

Table 1. Origins of tortoises examined.

Species	Location	Number
D. hololissa	private breeders	2
D. arnoldii	private breeders	3
D. dussumieri	private breeders	15
"	Curieuse	10

	PC1	PC2
Eigenvalue	7.620	2.809
Cummulative proportion of variance	0.476	0.652
Straight length (SL)	-0.010	-0.492
Curved length (CL)	0.161	-0.417
Height (H)	0.293	0.069
Costal height (CH)	0.327	0.066
Width (W)	0.191	-0.294
Anterior width (AW)	0.267	-0.175
Vertebral 1 (V1)	0.342	-0.038
Vertebral 2 (V2)	0.306	-0.167
Vertebral 3 (V3)	-0.205	-0.123
Vertebral 4 (V4)	0.180	-0.363
Vertebral 5 (V5)	-0.270	-0.276
Costals 1 (C1)	0.019	0.161
Costals 2 (C2)	0.216	0.012
Plastron (P1)	-0.233	-0.386
Anals (A)	-0.308	-0.177
Notch (N)	-0.347	-0.008

Table 2. Factor loadings of principal components of variation in *Dipsochelys* hatchlings

ening was observed. The colour patterns at one week old are shown in Ill. 9.

Principal component analysis (Table 2; Ill. 10) identifies two morphological groupings: *D. dussumieri* (PC2) and a combined grouping of *D. arnoldi* and *D. hololissa* (PC1). The summarised morphometrics (Table 3; Ill. 11) show that hatchling *D. dussumieri* are relatively high, with a low costal height, a carapace that is narrowed at the front, second costal longer than the first, long plastron and deep anal notch. Hatchling *D. hololissa* have a low carapace height and high costal, a narrow front to the carapace, long second costal, slightly shortened plastron and a very shallow anal notch. *D. arnoldi* have a high carapace with a low costal height, the carapace is widest at the front, the second costal shorter than the first, the plastron slightly reduced and the anal notch indistinct.

Discussion

It has been suggested that many of the different morphotypes of genera such as *Dipsochelys* and *Testudo* are the result of captive conditions (ARNOLD 1979; LAMBERT 1995). In the case of *Dipsochelys* recent molecular genetic analyses have failed to identify any consistent groupings in living individuals or recent museum specimens (AUSTEN & ARNOLD 2001;

Species	Length (mm)	Proportion						
		H/L	CH/H	AW/W	C2/C1	PI/L	A/PI	N/A
D. h.	62.2-64.0	0.45-0.47	0.83-0.89	0.78-0.82	0.98-1.00	0.85-0.87	0.11-0.13	0.08-0.11
D. a.	66.9-69.2	0.51-0.53	0.81-0.84	1.04-1.05	0.93-0.94	0.85-0.86	0.12-0.13	0.02-0.12
D. d.	61.0-70.5	0.50-0.58	0.41-0.63	0.74-0.85	1.00-1.13	0.88-0.98	0.17-0.18	0.50-0.83

Table 3. Summary of morphometrics distinguishing hatchling *Dipsochelys* (abbreviations from Table 2)

PALKOVACS et al. 2002, 2003; AUSTEN et al., 2003). It has been suggested that this may indicate that the individuals examined belong to a single species or that any divergence has been very recent (GERLACH 2003a). The comparison of hatchling *Dipsochelys* tortoises (Ill. 11) provides an unequivocal demonstration that the morphological variations found in the three recently defined living species (GERLACH & CANNING 1998) are not due to external factors such as diet but are genetically controlled and that they represent distinct taxa despite their lack of genetic divergence in the neutral genetic regions studied. It is notable that the differences observed include hatchling specific features not seen in the adult tortoises (colouration)

as species. A more equivocal case is seen in Galapagos tortoise populations from the south of Isabela island (*Chelonoidis nigra microphyes, Ch. n. vandenburghi, Ch. n. guentheri* and *Ch. n. vicina*). Despite distinctive morphology in the wild populations (GUYOT & PRITCHARD 1999) molecular data could not distinguish these taxa (LOUIS 1997; CACCONE et al. 1999). Although at least some are generally considered distinct there are some analyses that have considered them to be of doubtful validity (PRITCHARD 1996).

These conflicts between morphological taxon assignments and molecular phylogenies are all cases where recent divergence may have occurred (living *Dipsochelys* within the last 18,000 years [GER-
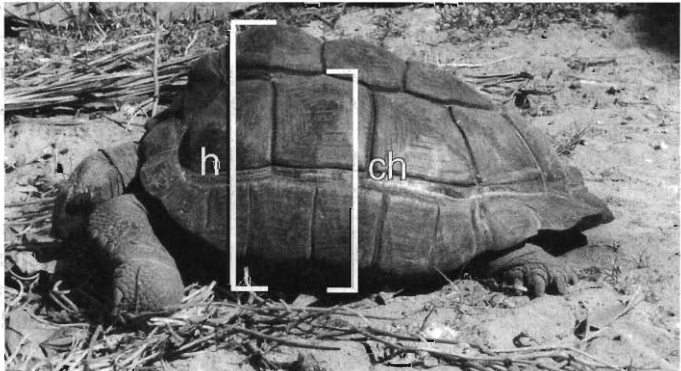
Ill. 4. Lateral view of subadult male Seychelles giant tortoise, *Dipsochelys hololissa*, showing the difference between height (H) and costal height (CH) measurements.

and features also found in adults: relative costal heights, costal scute proportions and depth of the anal notch. It is inevitable that hatchling morphology must be a precursor to adult morphology but the cause or function of the colouration differences are not known at present.

A similar situation is found in the available molecular genetic data on *Testudo* (KUYL et al. 2001; ÁLVAREZ et al. 2000) which do not provide support for some identifiable populations recently described

LACH 2003a]; *Testudo weissingeri* within 1 million years [KUYL et al. 2000]; Isabela *C. nigra* less than 500,000 years [CACCONE et al. 1999]). The use of neutral regions of the genome in molecular systematics allows relatively objective phylogenies to be constructed but can only provide a measure of degree and timing of divergence and not speciation itself. Neutral regions are unlikely to reflect small, recent genetic changes in functional regions. Although small and recent in origin such changes

Ill. 5. Week-old *Dipsochelys hololissa* hatchling with adult male.

Ill. 6 (left). Week-old *Dipsochelys hololissa* hatchling.
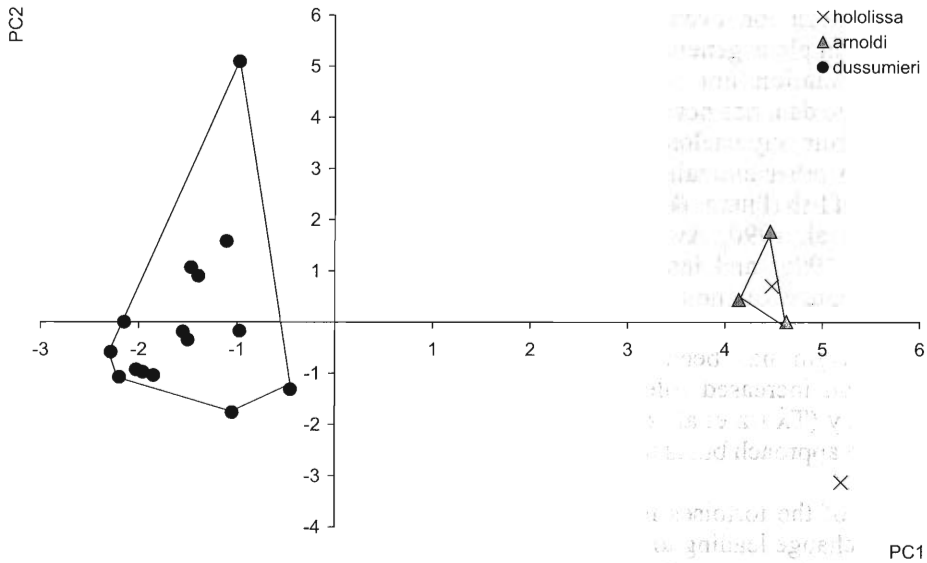Ill. 7 (left below). Week-old *Dipsochelys arnoldi* hatchling.
Ill. 8 (below). Hatchling *Dipsochelys arnoldi*.
Ill. 9 (next page). Week-old hatchlings of *Dipsochelys* species. a, d, g) *D. arnoldi* (carapace length 69.4 mm); b, e, h) *D. hololissa* (64.0 mm); c, f, i) *D. dussumieri* (66.5 mm).
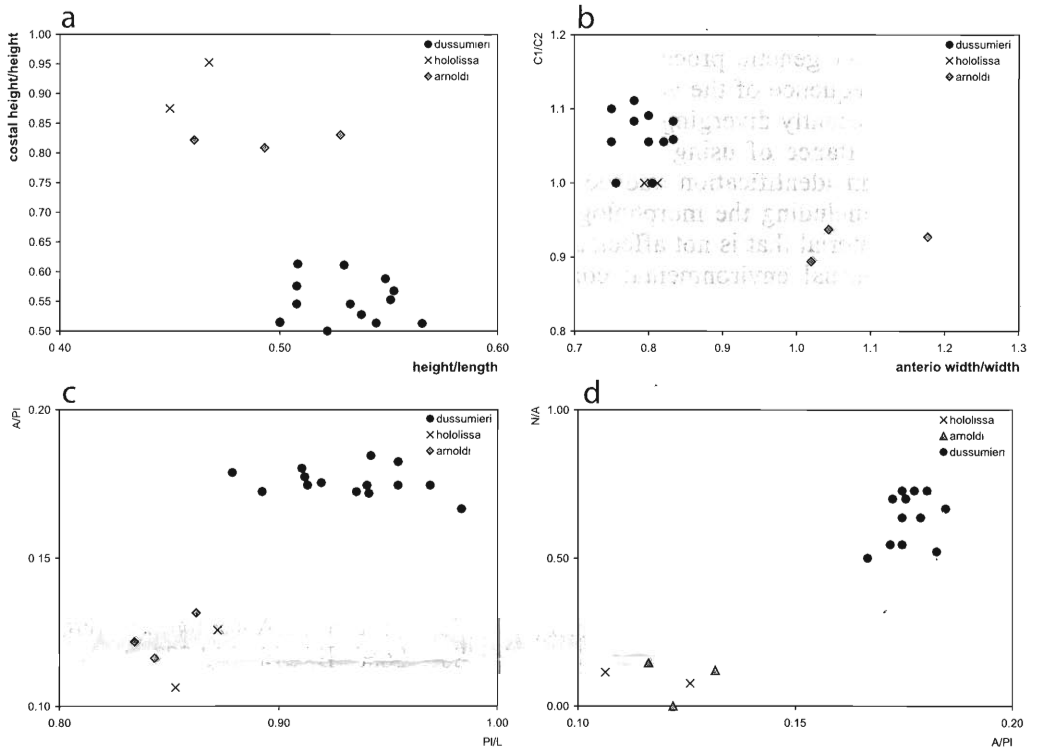
Ill. 10. Principal components plot.

Ill. 11. Comparison of carapace, plastron, and scute proportions.

can represent speciation events, even to the extent of complete genetic isolation. Such rapid speciation not reflected in molecular genetic data has not been previously reported from any chelonian species but is known in other animals, including several species of fish (PHELPS & ALLENDORF 1983; MEYER et al. 1990; AVISE 1990 & 1994; ROSSITER 1995) and insects (SHAW 2002). This problem of molecular methods failing to distinguish species of recent evolutionary origin has been noted by proponents of an increased role for molecular taxonomy (TAUTZ et al. 2003) as a limitation of the approach but is not widely reported.

In the cases of the tortoises mentioned above, a small change leading to dwarfism may account for the morphological distinctiveness of *Testudo weissingeri* (KUYL et al. 2000) and a series of functional changes lead to speciation in *Dipsochelys*. The morphology described above confirms this is a distinct genetic process and not merely a consequence of the environment alone. These recently diverging taxa highlight the importance of using a range of data sources in identification and definition of taxa, including the morphological analysis of material that is not affected by captive or unusual environmental conditions.

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