

Ancient mitochondrial DNA and morphology elucidate an extinct island radiation of Indian Ocean giant tortoises (*Cylindraspis*)

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Ancient mitochondrial DNA sequences were used for investigating the evolution of an entire clade of extinct vertebrates, the endemic tortoises (*Cylindraspis*) of the Mascarene Islands in the Indian Ocean. Mitochondrial DNA corroborates morphological evidence that there were five species of tortoise with the following relationships: *Cylindraspis triserrata* ((*Cylindraspis vosmaeri* and *Cylindraspis peltastes*) (*Cylindraspis inepta* and *Cylindraspis indica*)). Phylogeny indicates that the ancestor of the group first colonized Mauritius where speciation produced *C. triserrata* and the ancestor of the other species including a second sympatric Mauritian form, *C. inepta*. A propagule derived from this lineage colonized Rodrigues 590 km to the east, where a second within-island speciation took place producing the sympatric *C. vosmaeri* and *C. peltastes*. A recent colonization of Réunion 150 km to the southwest produced *C. indica*. In the virtual absence of predators, the defensive features of the shells of Mascarene tortoises were largely dismantled, apparently in two stages. ‘Saddlebacked’ shells with high fronts evolved independently on all three islands. This and other features, such as a derived jaw structure and small body size, may be associated with niche differentiation in sympatric species and may represent a striking example of parallel differentiation in a large terrestrial vertebrate. The history of Mascarene tortoises contrasts with that of the Galápagos, where only a single species is present and surviving populations are genetically much more similar. However, they too show some reduction in anti-predator mechanisms and multiple development of populations with saddlebacked shells.

Keywords: ancient DNA; island radiation; giant tortoise; *Cylindraspis*; colonization; Mascarene islands

1. INTRODUCTION

In historical times, giant tortoises (Testudinidae) existed on three widely separated groups of islands: (i) the Galápagos (a *Chelonoidis*), (ii) the Seychelles and other islands north of Madagascar (at least one species of *Aldabrachelys*) and (iii) the Mascarene Islands, which are a group of three volcanic islands lying 700–1400 km east of Madagascar in the southwest Indian Ocean (up to five species of *Cylindraspis*). When first discovered around 1500, the Mascarenes had very large tortoise populations (Leguat 1708; Bour 1980; North-Coombes 1986; Cheke 1987). These were heavily exploited after human settlement and eventually exterminated: by 1725 on Mauritius and its satellite islets, by 1795 on Rodrigues and by 1830 on Réunion. In spite of their original abundance very few specimens from living populations found their way into natural history collections. The majority of these are isolated shells that lack firm locality data. Most certain anatomical knowledge of Mascarene tortoises has consequently come from subfossil remains, which are abundant in swamps and caves on the islands (Günther 1877; Gadow 1894; Arnold 1979; Mourer-Chauviré *et al.* 1999). These subfossils suggest that there were two very distinct tortoise species on Mauritius, namely *Cylindraspis inepta* and *Cylindraspis triserrata*. Rodrigues probably also had two, namely *Cylindraspis vosmaeri* and *Cylindraspis peltastes*, although they were much more similar to each other than

the Mauritian pair and many of their skeletal elements cannot be easily distinguished. Réunion appears to have had a single variable species, namely *Cylindraspis indica*. Morphology indicates that *Cylindraspis*, which is confined to the Mascarenes, is a clade (Bour 1984) and supports the pattern of relationships among the different species shown in figure 1.

Evidence for the affinities of *Cylindraspis* to other tortoises is not strong. A relationship to the southern African *Geochelone pardalis* has been suggested on the basis of morphology (Bour 1984, 1992), but, because tortoises are often individually very variable, it is difficult to assess the robustness of many of the supposed synapomorphies involved. Short mitochondrial DNA (mtDNA) sequences from a wide range of tortoises weakly associate *C. vosmaeri* with the Madagascan *Astrochelys radiata*, which in turn groups with the African *Geochelone pardalis* and Aldabran *Aldabrachelys gigantea* (Momont 1998). Available evidence consequently suggests that the nearest relatives of *Cylindraspis* occur to the west of the Mascarene Islands.

Ancient mtDNA sequences are recoverable from a proportion of subfossil bones and archaeological and museum specimens (e.g. Poinar *et al.* 1996; Glenn *et al.* 1999), but have rarely been obtained from more than one or two extinct species within a particular group. MtDNA sequences extracted from subfossil bones and old museum specimens of *Cylindraspis* have already been used for relating enigmatic type specimens to their islands of origin (Austin *et al.* 2002). Here we use ancient DNA for the first time for investigating an entire group of extinct animals, assessing the validity of the various species of *Cylindraspis* and testing the hypothesis of their

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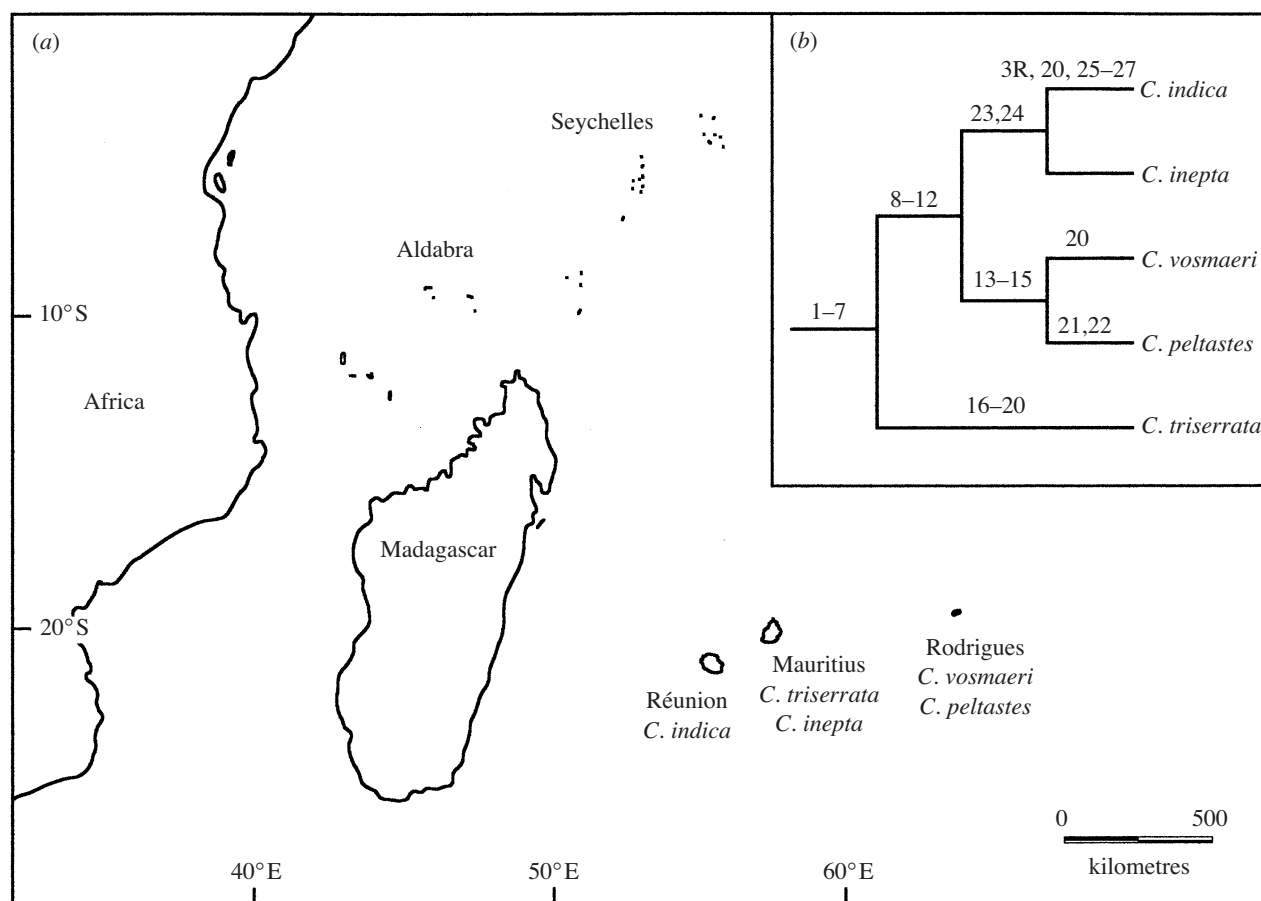


Figure 1. (a) Map of the southeast Indian Ocean showing the distribution of the five species of Mascarene tortoises (*Cylindraspis*).

(b) Relationships of the species of Mascarene tortoises (*Cylindraspis*) based on their morphology. The numbers indicate shared derived characters: 1, a sharp crest on the parietal bone running upwards and forwards between the exit of the main trunk of the trigeminal nerve and that of its maxillary branch; 2, the labial border of each maxilla often strongly denticulated, with a prominent downward point anteriorly so that the upper jaw has two tooth-like processes that are often better developed than in other tortoises; 3, odontoid tubercles present on the inner face of the maxilla or an extra row of tubercles present; 4, at least an indication of an extra triturating ridge on the external surface of the dentary; 5, bones of the shell thin; 6, fusion of the shell bones in adults with either partial fusion common or complete fusion common; 7, a caudal spur made up of fused vertebrae, present at least in males; 8, paired gular scutes fused into a single scale; 9, plastron small with length less than 80% that of the carapace; 10, shell openings markedly enlarged; 11, flattening of the crest of the cavum tympani anterior the incisure of the stapes; 12, one trochanter on the proximal femur markedly lower than the other; 13, stapediotemporal foramen small and impressions of blood vessels on the parietal and supraoccipital bones weak or absent; 14, foramen caroticum laterale large; 15, gular scute reduced in size; 16, three well-developed denticulated triturating ridges on the maxilla instead of two and the dentary modified correspondingly; 17, inguinal scute more or less absent (based on the type of *Testudo schweigeri*); 18, paired gular scutes pointed and diverging; 19, posterior plastron with large callose excrescences, at least in adult males; 20, carapace with raised anterior section ('saddleshaped'); 21, small body size; 22, short supraoccipital crest; 23, bones of the skull usually fused in adults; 24, vomer broad; 25, basioccipital tubercles reduced; 26, anal scutes elongate; 27, separation of the anterior surfaces of the maxillae. Character 20 has evolved three times and is variable in *C. indica*. The characters are taken mainly from Bour (1984) and have been checked against material of *Cylindraspis* in the zoology and palaeontology collections of the Natural History Museum, London. Phylogenetic analysis of informative characters using PAUP* (Swofford 2000) produces a tree of 20 steps with bootstrap (1000 pseudo-replicates) support of 93% for the *C. vosmaeri*–*C. peltastes*–*C. inepta*–*C. indica* clade and 70% for the *C. vosmaeri*–*C. peltastes* clade. R, reversal.

relationships based on morphology. The phylogeny is then used for reconstructing the colonization sequence and the development of ecomorphs within the Mascarene Islands.

In this paper the names *Astrochelys*, *Aldabrachelys*, *Chelonoidis* and *Geochelone* are all used as full genera in order to emphasize differences within the tortoise clade. Some workers treat some or all of these units as subgenera of a probably paraphyletic *Geochelone*, often referring all the tortoise species mentioned here to that genus.

2. MATERIAL AND METHODS

(a) Material

Material of all presently recognized Mascarene forms was investigated (table 1). This included a stuffed specimen and six shells in museums that were originally taken from living populations more than 170 years ago. Several of these are holotypes. Among these is the type of *Testudo schweigeri*, a shell that has been in London museums since at least the eighteenth century, the morphology of which indicates that it is conspecific

Table 1. Material from the extinct Mascarene *Cylindraspis* (Rodrigues, Mauritius and Réunion) and living and extinct non-Mascarene (Aldabra/Seychelles, Madagascar and Africa) tortoises investigated.

(BMNH, Natural History Museum, London; MNHN, Muséum National d'Histoire Naturelle, Paris; NNML, Nationaal Natuurhistorisch Museum, Leiden; NMV, Naturhistorisches Museum, Vienna. BMNH R3992 of *Cylindraspis triserrata* from Mauritius only had a partial sequence of 103 bp. *Cylindraspis indica* from Réunion was previously recognized as *Cylindraspis borbonica*. MNHN 7819 is the type of *Testudo indica* and MNHN 9374 is the type of *Testudo graii*. Molecular data has synonymized all three taxa under the name of *C. indica* (see Austin *et al.* 2002). The sequence of *Astrochelys yniphora* was taken from GenBank, accession number AF020896.)

	locality	element	tissue source	registration number	DNA
Rodrigues					
<i>C. vosmaeri</i>	?	carapace	dried tissue	NNML 6001, type	yes
	?	carapace	dried tissue	NMV 1461	yes
	?	stuffed specimen	dried skin	MNHN 1883.558	yes
	Caverne Patate	fragment of plastron	bone	BMNH 2000.50	yes
<i>C. peltastes</i>	?	carapace	dried tissue and bone	MNHN 7831, type	yes
	Caverne Patate	fragment of carapace	bone	BMNH 2000.51	yes
	Caverne Bambara	fragment of plastron	bone	BMNH 2000.52	yes
	Caverne Bambara	fragment of plastron	bone	BMNH 2000.53	yes
Mauritius					
<i>C. inepta</i>	Mare aux Songes	femur	bone	BMNH R4021	yes
	Mare aux Songes	femur	bone	BMNH R3991	no
	Mare aux Songes	femur	bone	BMNH 1977.663	no
	Mare aux Songes	skull	bone	BMNH 1977.549	no
	Mare aux Songes	skull	bone	BMNH 1977.572	no
	Mare aux Songes	femur	bone	BMNH 1977.558	no
	Mare aux Songes	shoulder girdle	bone	BMNH R4661	no
	Flic en Flaq	femur	bone	BMNH 76.11.4.17, type of <i>Testudo leptocnemis</i>	no
<i>C. triserrata</i>	Mare aux Songes	femur	bone	BMNH R3992	yes
	Mare aux Songes	skull	bone	BMNH 1977.569	no
	Mare aux Songes	femur	bone	BMNH 1977.661	no
	?	carapace	dried tissue	BMNH 1947.3.5.5, type of <i>Testudo schweigeri</i>	yes
<i>Cylindraspis</i> sp.	Ile aux Aigrettes	vertebra	bone	BMNH 2000.55	yes
	Ile aux Aigrettes	vertebra	bone	BMNH 2000.54	yes
	Mare aux Songes	femur	bone	BMNH R4687	no
	Mare aux Songes	pelvis	bone	BMNH 1977.641	no
	Mount Zaco	fragment of carapace	bone	—	no
	La Prairie	fragment of carapace	bone	—	no
Réunion					
<i>C. indica</i>	?	carapace	dried tissue and bone	MNHN 7819, type	yes
	?	carapace	dried tissue and bone	MNHN 9374	yes
	Marais de l'Ermitage	humerus	bone	BMNH 2000.47	yes
	Marais de l'Ermitage	humerus	bone	BMNH 2000.48	yes
	Marais de l'Ermitage	humerus	bone	BMNH 2000.49	yes
Aldabra/Seychelles					
<i>Aldabrachelys gigantea</i>	Aldabra	skull	bone	—	yes
	Seychelles?	—	blood	—	yes
Madagascar					
<i>Aldabrachelys grandidieri</i>	Ambatumifuku	humerus	bone	BMNH 92.6.5.1	yes
<i>Astrochelys radiata</i>	?	stuffed specimen	dried skin	—	yes
<i>Astrochelys yniphora</i>	?	—	blood	—	yes
Africa					
<i>Geochelone pardalis</i>	? (captive)	—	blood	—	yes

with *C. triserrata* (Arnold & Austin 2002). Recent subfossil bones from both caves and swamps and older mineralized bones from sand dunes were also examined. Sources included the limestone caves of Patate and Bambara on the Plaine Corail (southeast Rodrigues), a small limestone cave on Ile aux Aigrettes (southeast Mauritius), volcanic caves at Zaco, Black River Gorges (southwest Mauritius) and at La Prairie (west Mauritius), the

swamps of Mare aux Songes (southeast Mauritius) and Marais de l'Hermitage (west Réunion) and the calcareous sand dunes at Flic en Flaq (west Mauritius), a deposit which no longer exists (C. G. Jones, personal communication). Mascarene specimens were allocated to species on the basis of their morphology. Material from a number of living and extinct African, Madagascan and Aldabran testudinid species were used as outgroups (table 1).

(b) Laboratory procedures

Techniques appropriate for ancient DNA (Austin *et al.* 1997*a,b*, 2002) were used for obtaining sequences spanning a 428 bp (base pair) fragment of the mitochondrial tRNA-Glu and cytochrome *b* genes. The methods of sample preparation, DNA extraction, polymerase chain reaction (PCR) amplification and DNA sequencing are described elsewhere (Austin *et al.* 2002). The molecular work on all the museum and subfossil material was carried out in a laboratory dedicated to the study of ancient DNA, which was physically separated from all other molecular biology facilities. Work followed strict procedures in order to minimize contamination (Austin *et al.* 1997*a,b*). Replicate and independent DNA extractions and PCR amplifications were conducted on all museum specimens and subfossil bones in order to authenticate sequences obtained from initial extractions. DNA extractions and PCR amplifications from modern samples were carried out in a separate laboratory using similar procedures, but without the need for secondary PCR amplification and short PCR amplicons. All sequences were deposited in GenBank (accession numbers AF371238–AF371260).

(c) Phylogenetic analyses

Sequences were aligned by eye, with a single short (2–3 bp) indel at the tRNA-Glu/cytochrome *b* junction being required in some species. Only one sequence was included in the phylogenetic analyses when sequences of individual tortoises were identical, resulting in 15 unique sequences with each representing one to four individuals. Maximum-parsimony (MP), maximum-likelihood (ML) and neighbour-joining (NJ) analyses were performed using PAUP* 4.0b8 (Swofford 2000). The MP analyses employed branch and bound searches with the MULTREES option in effect. Transversions were given one, two, five and 10 times the weight of transitions in different analyses via the STEPMATRIX option in order to explore the effect of such weighting. Gaps were treated as a fifth state in unweighted MP searches. The ML analyses used a Tamura–Nei (Tamura & Nei 1993) model with empirically determined base frequencies, rates for each class of transition and gamma-distributed rate heterogeneity between sites. This model was selected on the basis of a hierarchical likelihood ratio test of alternative models that was implemented in the program MODELTEST 3.0 (Posada & Crandall 1998). Heuristic searches were conducted using 10 random addition sequences with branch swapping using tree bisection–reconnection and the MULTREES option. The NJ analyses were performed using ML-corrected distance matrices that incorporated the same model of sequence evolution as in the ML searches. Support for the tree topology was inferred using bootstrapping (Felsenstein 1985) with 1000 pseudo-replicates for each of the MP, ML and NJ analyses.

3. RESULTS**(a) Ancient mtDNA sequences recoverable from old remains**

DNA sequences were successfully obtained from 18 out of 31 specimens of *Cylindraspis*. These included the seven museum specimens originally taken from living populations, all six subfossil bones collected in the limestone caves on Rodrigues and Mauritius and all three subfossil bones from the marsh site on Réunion (table 1). By contrast, DNA could only be extracted from 2 out of the 12 samples of subfossil bone material from the marsh of Mare aux Songes on Mauritius and none at all from the

material from the volcanic caves and sand dunes on that island. The radiocarbon ages for the tortoise bones from the Mauritius and Réunion marshes are similar (Mauritius 1490 ± 230 years and 1580 ± 250 years (Burleigh & Arnold 1986) and Réunion 915 ± 120 years and 1755 ± 40 years (Mourer-Chauviré *et al.* 1999)), so the variation in the success rates of extracting DNA from bones from these sites (3 out of 3 for Réunion against 2 out of 12 for Mauritius) (exact χ^2 -test, $p \leq 0.01$) is likely to result from differences in the conditions of preservation. All DNA sequences were judged to be authentic on the basis of their absolute similarity and reproducibility between different specimens of the same species, different samples from the same specimen and overlapping regions of different PCR amplicons.

Sequences were obtained for four specimens of each of the two supposed Rodrigues species *C. vosmaeri* (three identical) and *C. peltastes* (all four identical) and for five specimens of the Réunion species *C. indica* (two identical). Only one bone out of seven investigated and certainly identified as the Mauritian *C. inepta* produced DNA. However, sequences were also obtained from two unidentified *Cylindraspis* vertebrae from Ile aux Aigrettes. These are identical to each other and differ from the *C. inepta* sequence by only two transition substitutions, so are likely to represent a second independent sample for this species. The only complete apparent *C. triserrata* sequence came from the type of *T. schweigeri* that was originally taken from a living population. Just one of the three subfossil specimens of *C. triserrata* yielded a sequence, which was a 103 bp fragment that was identical to the homologous region of the *T. schweigeri* type. The percentage differences between the DNA sequences of the species of *Cylindraspis* are shown in table 2.

(b) Relationships among *Cylindraspis* tortoises

The alignment consisted of 428 characters comprising 17 bp of the tRNA-Glu gene, 6 bp of intergenic sequence and 405 bp of the cytochrome *b* gene. One hundred and one sites were variable and 57 were parsimony informative (including two gap sites). The majority of substitutions were transitions: 82 variable sites involved transitions only, eight sites involved transversions only and overall there was a sixfold excess of transitions over transversions.

The MP analysis with equal weighting of transversions and transitions found 24 trees of 163 steps. Different weighting of transversions relative to transitions found a subset of 12 of these trees, regardless of the transversion weight applied. The NJ and ML trees and two of the MP trees were identical in topology. The ML tree is shown in figure 2. All of the Mascarene sequences form a well-supported clade, excluding the outgroups. *Cylindraspis vosmaeri*, *C. peltastes* and *C. inepta* each constitute well-supported monophyletic units and these are genetically differentiated from their respective sympatric species (figure 2 and table 2). *Cylindraspis vosmaeri* and *C. peltastes* form a clade that is sister to *C. indica* and *C. inepta* and *C. triserrata* is basal to these. The MP trees differ from the ML and NJ trees only in their relationships between *C. indica* sequences and their relationships among the outgroup taxa. The sequences of *C. indica* form a clade in half of the shortest MP trees, but are paraphyletic relative

Table 2. Mean (\pm s.d.) pairwise intraspecific (along diagonal) and interspecific (below diagonal) percentage divergences for 428 bp of mtDNA sequence from five extinct Mascarene tortoise species (*Cylindraspis*).

(The pairwise differences between *Cylindraspis* species and five outgroup taxa are included for comparison. Genetic distances were calculated using the Tamura–Nei model with gamma-corrected rate heterogeneity between sites.)

	number of sequences	1	2	3	4	5
1 <i>C. indica</i>	5	0.55 (0.43)	—	—	—	—
2 <i>C. triserrata</i>	2	11.92 (1.06)	0.00 (0)	—	—	—
3 <i>C. inepta</i>	3	1.98 (0.32)	12.31 (0.84)	0.49 (0.00)	—	—
4 <i>C. peltastes</i>	4	7.52 (0.28)	16.95 (0.00)	8.79 (0.04)	0.00 (0)	—
5 <i>C. vosmaeri</i>	4	7.78 (0.34)	15.50 (0.34)	9.06 (0.23)	2.51 (0.13)	0.37 (0.41)
6 outgroups	5	16.38 (3.08)	17.42 (2.25)	16.20 (2.84)	17.08 (2.38)	17.60 (2.45)

to the *C. inepta* clade in all other trees (MP, ML and NJ). Thus, they share a common ancestor with *C. inepta*, but lack any consistent and well-supported topology. However, monophyly of the *C. indica* sequences cannot be rejected by the present dataset. Trees in which *C. indica* sequences are monophyletic or paraphyletic are equally parsimonious and a constraint tree in which *C. indica* sequences are monophyletic is not significantly different from the ML tree (Kishino–Hasegawa test, $\Delta\ln L=1.66$ and s.d. = 4.67 and one-tailed *t*-test, $t=0.37$ and $p=0.36$). The relationships of outgroup taxa to the Mascarene clade and, in most cases, among themselves are poorly unresolved.

4. DISCUSSION

(a) Identification of *T. schweigeri*

The identity of the 103 bp of the cytochrome *b* sequence extracted from a subfossil *C. triserrata* bone from Mare aux Songes along with the homologous region obtained from the type of *T. schweigeri* corroborates the morphological evidence (Arnold & Austin 2002) that these forms are conspecific. Given our strict adherence to contamination controls, this is unlikely to be an artefact caused by contamination of the *C. triserrata* sample by DNA from the *T. schweigeri* type. No DNA contamination was detected in any other extractions or PCR amplifications and no DNA from *T. schweigeri* was ever handled concurrently with the *C. triserrata* specimens. The sequence from the *T. schweigeri* type differs by 6–16% (uncorrected sequence difference) from homologous sequences of 26 other tortoise species, which comprise the four other *Cylindraspis* and 22 non-Mascarene tortoises (data not shown). Among these is *Gopherus polyphemus* with which the *T. schweigeri* type has been tentatively identified (Pritchard 1996).

(b) Validity of the species of *Cylindraspis*

DNA evidence clearly corroborates the validity of four of the Mascarene tortoise species already recognized on morphological grounds. They each form reciprocally monophyletic clades and there are substantial genetic differences between species (table 2). The lack of unequivocal support for monophyly of *C. indica* does not invalidate this species given its apparent morphological synapomorphies (figure 1). Rather it is consistent with a recent colonization of Réunion in which mtDNA lineage sorting in the founder population had not yet reached

monophyly (Patton & Smith 1994; Avise 2000). Mourer-Chauviré *et al.* (1999) independently suggested a recent origin for *C. indica* on the basis of morphological similarities between *C. indica* and *C. inepta* and the restricted distribution of tortoises on Réunion. Several other groups, including bats, birds and geckos, also appear to have colonized Réunion in relatively recent times (Cheke 1987; Mourer-Chauviré *et al.* 1999; J. J. Austin and E. N. Arnold, unpublished data).

(c) Origin of the Mascarene tortoises

As noted, previous molecular data (Momont 1998) and morphology (Bour 1984, 1992) provide weak evidence that *Cylindraspis* is related to tortoises living to the west of the Mascarenes in Madagascar, the Seychelles and Africa. Other heterotherm taxa (gekkonid and scincid lizards) have reached the Mascarenes from both Madagascar and nearby islands in the west and Australasia and adjoining southeast Asia in the east (J. J. Austin and E. N. Arnold, unpublished data). However, the distance from the latter region is far in excess of the transmarine journeys that are known to have been made by floating tortoises. The voyage from Java, where Pleistocene tortoises are known (Hooijer 1971), to the Mascarenes would be at least 5500 km. The longest known dispersals made to oceanic islands by tortoises are up to 2000 km from North America to Bermuda (Meylan & Sterrer 2000) and *ca.* 1000 km from South America to the Galápagos Islands. This makes the western origin of Mascarene tortoises suggested by other evidence more likely.

(d) Inter-relationships among the Mascarene species and patterns of colonization

Morphology (figure 1) and mtDNA (figure 2) have produced estimates of *Cylindraspis* relationships that are fully congruent. As these two character sources are independent, it is likely that the common topology represents the actual phylogeny. The most parsimonious interpretation of this pattern of relationships in terms of colonization pattern is that the common ancestor of *Cylindraspis* arrived first on Mauritius, where speciation produced *C. triserrata* and the ancestor of the remaining Mascarene tortoise species (figure 3). This lineage led to *C. inepta* on Mauritius and was also the source of a propagule that colonized Rodrigues, where a second within-island speciation event occurred resulting in *C. vosmaeri*

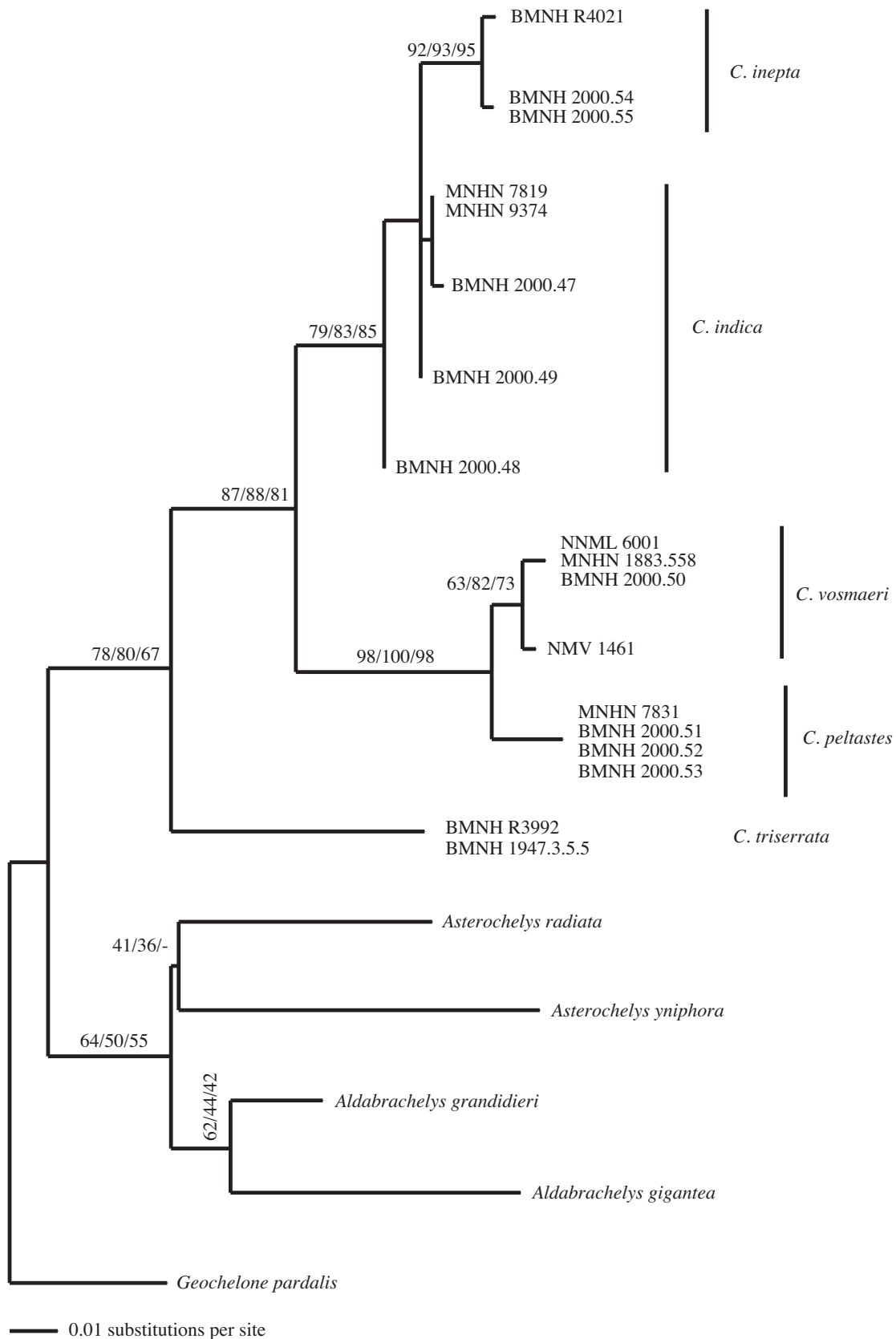


Figure 2. Molecular phylogeny of Mascarene tortoises based on 428 bp of the mitochondrial tRNA-Glu and cytochrome *b* genes. The ML tree ($-\ln$ likelihood = 1326.25) is shown. The numbers above the branches are the ML, NJ and MP bootstrap values, respectively. Multiple samples listed together shared identical sequences.

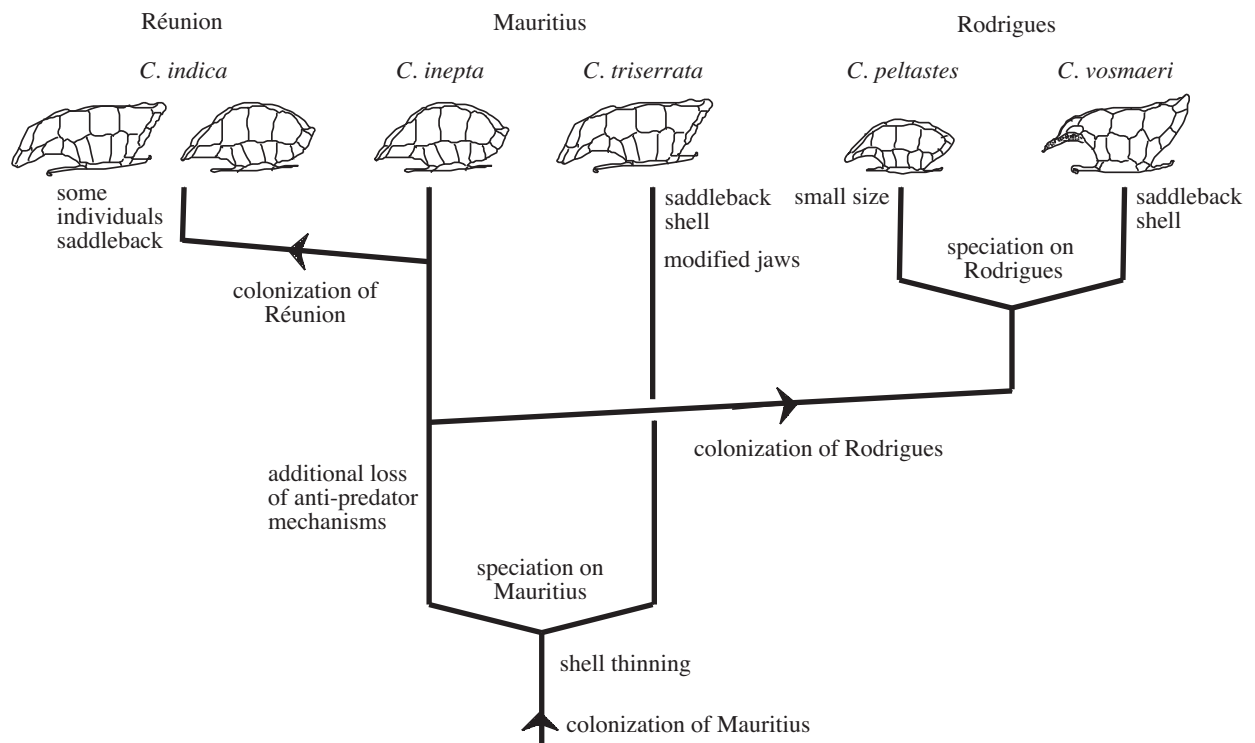


Figure 3. Principal events in the history of Mascarene tortoises. The arrowed lines show colonization events.

and *C. peltastes*. Finally, a recent colonization of Réunion from the *C. inepta* lineage produced *C. indica*.

The presence of sympatric species pairs on Mauritius and Rodrigues represents a striking example of parallel differentiation in a large terrestrial vertebrate. Speciation occurred independently on each island and resulted in very similar and apparently adaptive (see §4e) morphological divergence between species. This situation is paralleled by the adaptive radiations of some geckos in the Mascarenes (J. J. Austin and E. N. Arnold, unpublished data) and the *Anolis* lizards of the Greater Antilles (Losos *et al.* 1998). The evolution of the Mascarene tortoises contrasts with that of the tortoises of the Seychelles and Galápagos in which there are no intra-island speciations and morphological divergence is much more restricted (Arnold 1979; Pritchard 1996; Caccone *et al.* 1999; J. J. Austin and E. N. Arnold, unpublished data).

The similarity in evolutionary outcomes on two separate islands of the Mascarenes suggests that ecological factors played a substantial role in directing divergence and perhaps speciation as well. This may possibly have occurred parapatrically via divergent natural selection acting along ecological gradients or sympatrically via ecological displacement (Endler 1977). Mauritius originally had diverse habitats ranging from evergreen rainforest to open, dry palm savannah and also large topographic and seasonal variation in rainfall and temperature (Vaughan & Wiehe 1937; Vinson 1976). Rodrigues probably had a similar environmental diversity. These conditions could have produced sufficiently strong selective gradients for driving speciation. Alternatively, volcanic activity may have caused isolation and/or bottlenecks of ancestral populations (Carson *et al.* 1990; DeSalle & Templeton 1992; Pestano & Brown 1999),

which then diverged in allopatry before becoming sympatric. Intermittent volcanism occurred on Mauritius up until 0.2 million years ago (McDougall & Chamalaun 1969).

(e) *The evolution of ecomorphs*

Oceanic island tortoises have often evolved a distinctive shell facies, which can be seen to varying extents in the Galápagos, the Seychelles and the Mascarenes (Arnold 1979). Shells have become thinner and lighter in weight in all these archipelagos, their anterior and posterior openings enlarged and the lower part of the shell, the plastron, smaller. These shifts involve the dismantling of the anti-predator mechanisms that are typical of tortoises from continental areas where substantial predator pressure exists, something that is permitted by the absence or great reduction of such pressure on oceanic islands. The shifts are probably driven by natural selection resulting from increased intraspecific competition on islands as they reduce the costs of maintaining and transporting the shell and increase agility in reaching food (Arnold 1979).

The present phylogeny suggests that this reduction in defensive features evolved in two stages in Mascarene tortoises. A degree of shell thinning is found in all *Cylindraspis*, so it probably occurred in the common ancestor of the present species. The remaining modifications, which are more extensive than in any other island tortoises (Arnold 1979), took place on Mauritius after the origin of *C. triserrata*, but before colonization of Rodrigues and Réunion. These changes may have made such transmarine colonizations easier, as they increased buoyancy by reducing the weight of the shell.

Tortoises from islands in the Galápagos archipelago with rich plant growth tend to have shells that are simply domed, which enables the animals to move relatively

easily through dense vegetation. In contrast, shells are 'saddlebacked', that is with a raised front, on dry islands with sparse vegetation. This shell form is associated with the development of a long neck that permits extensive upward reach, something that is perhaps beneficial in stretching high for food and in agonistic display. Phylogeny indicates that saddlebacked shells, sometimes strikingly similar to those found in the Galápagos, evolved independently and in parallel on each of the three main islands of the Mascarenes (figure 3) in *C. vosmaeri*, *C. triserrata* and in some *C. indica*. In contrast to the situation in the Galápagos, Mascarene saddlebacked taxa occurred alongside ones with domed shells, which on Mauritius and Rodrigues belonged to separate species. In these cases, the differences in shell shape and other features may have been associated with interspecific niche differentiation. Other derived features that are also probably related to this include unique multicusped horny jaws in *C. triserrata* and a small body size in *C. peltastes*.

5. CONCLUDING REMARKS

Ancient mtDNA confirms that the Mascarene Islands were inhabited by a group of five tortoise species that first reached Mauritius and then invaded Rodrigues to the east and Réunion to the west. Intra-island speciation occurred on both Mauritius and Rodrigues and produced pairs of sympatric species that differed in morphological features that are probably associated with niche exploitation. The anti-predator features of the shell were dismantled to a greater extent than in any other island tortoises.

This history contrasts with that of Galápagos tortoises, which constitute only a single species, different island populations of which have diverged in only the last 2 million years (Caccone *et al.* 1999). The mtDNA genetic divergence in the Galápagos is slight compared with the species differences in the Mascarenes. However, both island groups show at least some reduction in anti-predator mechanisms and the multiple development of forms with saddlebacked shells that may confer advantage in dry open habitats.

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