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Relationships and evolution of the North African geckos, *Geckonia* and *Tarentola* (Reptilia: Gekkonidae), based on mitochondrial and nuclear DNA sequences

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Abstract

Mitochondrial (cytochrome *b* and 12S rRNA) and nuclear (c-*mos*) genes, analyzed by a variety of methods, indicate that the distinctive northwest African gecko *Geckonia chazaliae* is a member of the *Tarentola* clade, being most closely related to the species of the western Canary and Cape Verde islands. Relationships in *Tarentola* as a whole are as follows: (*T. americana* ((*T. mauritanica*, *T. angustimentalis*) ((*T. deserti*, *T. boehmei*) ((*T. b. boettgeri*—South (*T. b. boettgeri*—North (*T. b. bischoffi*, *T. b. hierrensis*))) ((*T. annularis*, *T. ephippiata*) (*Geckonia*, *T. delalandii*, *T. gomerensis*, Cape Verde species)))))); nearly all nodes have high bootstrap support. Results confirm that *T. americana* of Cuba and the Bahamas separated at the most basal dichotomy of the phylogeny and give no positive support for the monophyly of the subgenera *Tarentola* s. str. and *Makariogecko*. The latter includes *Geckonia* and the subgenus *Sahelogecko*. Continental *Tarentola* appear to have invaded the Sahara desert from its northern edge. They have also colonized groups of Atlantic islands five times: a single invasion of the West Indies and three of the Canary islands, one of which then went on to invade the Cape Verde archipelago. The phylogeny corroborates anatomical evidence that the ground-dwelling *Geckonia* had a climbing ancestry, something that is paralleled in some southern African terrestrial gekkonids related to *Pachydactylus*. Distinctive derived features of *Geckonia* occur in other gekkonids that are ground dwelling in arid habitats and may be functionally related to this environment. The evolution of such features indicates that, although *Tarentola* is generally very uniform and may have been so for over 10 million years, this is not due to any overwhelming phylogenetic constraint. *G. chazaliae* should be included in *Tarentola*, as *Tarentola chazaliae*.

Keywords: Phylogeny; Biogeography; Colonization; Ecology; Adaptation

1. Introduction

The gekkonid lizard, *Geckonia chazaliae* Mocquard, 1895, sometimes called the casqued or helmeted gecko, is a distinctive northwest African species that is the only member of its genus. As its vernacular names suggest, *Geckonia* has a large head with a modified skull and a number of other derived features that occur only in a minority of gekkonids. These include a short plump body in which the usual number of presacral vertebrae is reduced from the primitive gekkonid number of 26 to 25, slender limbs, reduction of the usual number of pygal vertebrae from five to four, a short slender tail, and restriction of autotomy to the tail base. *Geckonia* occurs along the arid Atlantic coast of North Africa, from just south of Agadir in Morocco to the north of Senegal (Bons and Geniez, 1996). Over this distance of more than 2000 km, it is usually found within 25 km of the sea, living on the ground on sandy soils and dune formations in steppe with occasional stones and a few dispersed trees (especially *Euphorbia echinus*) (Bons and Geniez, 1996; Mellado and Mateo, 1992; Schleich et al., 1996). Like most other geckos, *Geckonia* is nocturnal but is in the minority in being ground dwelling and not very fast in its movements, often hunting by walking

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slowly on extended legs, and in its antipredator behavior, in which the animal attacks while squeaking and exposing the dark internal coloring of the mouth.

Geckonia is regarded the sister taxon of *Tarentola* (Joger, 1985; Kluge and Nussbaum, 1995; Russell, 1976). This is a group of about 22 quite similar species, which occur in North Africa, the coastal districts of the Mediterranean sea, Macaronesia (the Selvages, Canary, and Cape Verde islands), and Cuba and the Bahamas in the West Indies. The relationship is suspected on the basis of geographical proximity (Fig. 1) and overall morphological resemblance and receives some support from a putative synapomorphy: the presence of true osteoderms (bony elements within the dermis) in the skin of the supraorbital region (Bauer and Russell, 1989). Claw reduction also occurs in both *Geckonia* and *Tarentola*. Claws have been lost, or greatly reduced, on digits 1, 2, and 5 (not 1, 2, and 3 as indicated by Kluge and Nussbaum, 1995) but not on 3 and 4. Outgroup comparison indicates that the derived feature is loss or reduction of claws on digits 1, 2, and 3 and does not involve their presence (retention) of 3 and 4. A number of other geckos show claw loss on digits 1, 2, and 3, so this feature is not a putative synapomorphy that specifically supports the relationship of *Geckonia* and *Tarentola*. Although these two genera may form a clade, a sister relationship cannot be assumed, as *Tarentola* lacks obvious defining morphological synapomorphies, so the possibility cannot be excluded that *Geckonia* arose within it.

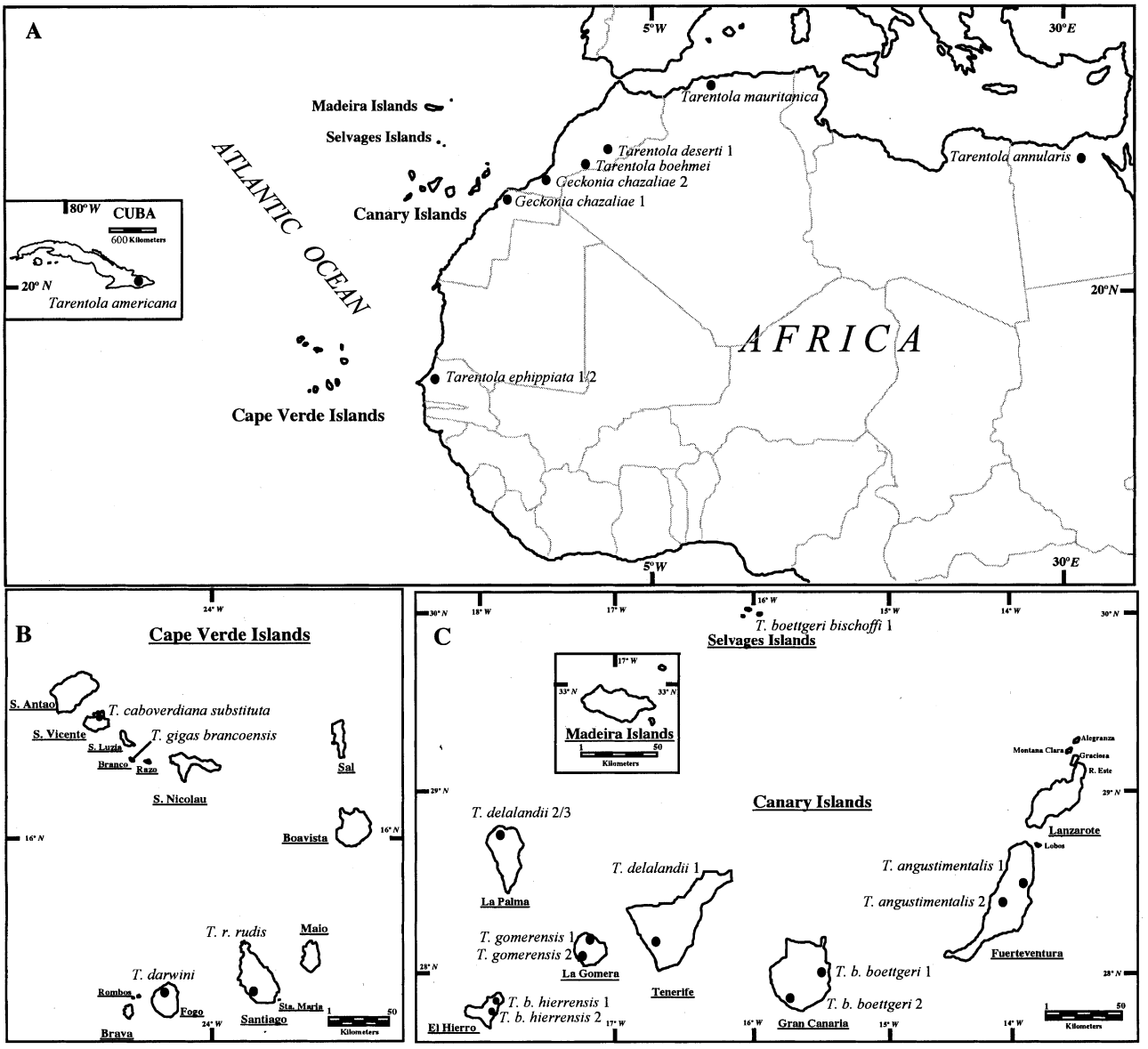

Fig. 1. Localities for *Tarentola* and *Geckonia* samples; see Table 1 for details. (A) North Africa and Cuba; (B) Cape Verde Islands; (C) Canary, Madeira, and Selvages Islands.

Results from albumin immunology suggest that *Tarentola* is related to other geckos found in the arid regions of North Africa, specifically *Stenodactylus* and *Ptyodactylus* (Joger, 1985), but mitochondrial and nuclear DNA sequence gives no support for this association (J.J. Austin, S. Carranza, and E.N. Arnold, unpublished). This is also true of morphology, which instead supports a relationship of *Geckonia* and *Tarentola* to the southern African *Pachydactylus* group of geckos, which includes *Pachydactylus*, *Chondrodactylus*, *Colopus*, *Palmatogecko*, and *Rhoptropus* (Kluge and Nussbaum, 1995). All these forms possess an extra phalanx on digit 1 of manus (hand) and pes (foot) and show great similarity in digital musculature (Russell, 1972, 1976) and reduction of claws on digits 1, 2, and 5 of manus and pes.

Relationships within *Tarentola* have also been investigated using albumin immunology (Joger, 1984a,b) and mtDNA sequences (Carranza et al., 2000; Nogales et al., 1998). However, the latter studies concentrated on the Macaronesian species and did not include *Geckonia* or enough North African species to be able to clarify the overall internal relationships of *Tarentola*. The present investigation incorporates these requisite taxa and investigates the phylogeny of *Geckonia* and *Tarentola* using partial sequences of two mitochondrial genes (cytochrome *b* and 12S rRNA) and one nuclear gene (c-*mos*). Aspects of the phylogeography and evolution of these lizards are also explored.

2. Material and methods

2.1. Samples and localities

A total of 28 individual geckos assignable to 18 species were included in the present study. They included 2 individuals of *G. chazaliae* and 23 assigned to 14 species of *Tarentola* (allocated to the subgenera *Tarentola* s. str., *Makariogecko*, *Neotarentola*, and *Sahelogecko* by Joger (1984c); see Table 1); 1 *Pachydactylus turneri* and individuals of two *Hemidactylus* species were used as outgroups. Specimen data are given in Table 1.

2.2. Extraction of DNA

Genomic DNA was extracted from 2–3 mm^3 of tail tissue following standard protocols described elsewhere (Carranza et al., 2000; Harris et al., 1998). Primers used in both the amplification and the sequencing were cytochrome b_1, cytochrome b_2 (Kocher et al., 1989), the forward primer of cytochrome b_2 and CB3-3R (Palumbi, 1996) for the cytochrome *b* gene, 12Sa and 12Sb for the 12S rRNA gene (Kocher et al., 1989), and G73 and G78 (Saint et al., 1998) for the nuclear c-*mos* gene. The three gene fragments were amplified by the polymerase chain reaction (PCR) and the resultant DNA was sequenced using the same standard protocols and conditions described by Carranza et al. (2000).

2.3. Alignment

DNA sequences were aligned by hand using an alignment editor (GDE; Smith et al., 1994). All the cytochrome *b* sequences had the same length and therefore no gaps were postulated. These sequences were translated into amino acids using the vertebrate mitochondrial code and no stop codons were observed. The 12S rRNA sequences were aligned with reference to the published secondary structure (Hickson et al., 1996). Gaps were inserted to resolve length differences between individual sequences and those positions that could not be unambiguously aligned were excluded. These were all in loop regions (between helices 36 and 38, 38 and 39, 40′ and 39′, 39′ and 42, 42 and 42′, 45 and 45′, 45′ and 47, and 48 and 48′; as given by Hickson et al., 1996) and involved length variation.

The c-*mos* nuclear gene sequences of all *Tarentola*, *Geckonia*, and *Pachydactylus* used in this study had the same length but were 12 nucleotides (4 amino acids) longer than the two *Hemidactylus* sequences employed as outgroups. A comparison with other reptile taxa (Saint et al., 1998) indicates that *H. turcicus* and *H. parkeri* have suffered a deletion in the c-*mos* gene. Sequences were first translated into amino acids, and a region that could not be unambiguously aligned (24 bp of the sequences without the deletion and 12 bp of the *Hemidactylus* c-*mos* sequences) was excluded from the analysis.

2.4. Phylogenetic analyses

The three gene fragments comprising the data set (cytochrome *b*, 12S rRNA, and c-*mos*) were tested for incongruence using the incongruence length difference test (ILD) (Farris et al., 1994; Mickevich and Farris, 1981). Ten thousand heuristic searches were used, the invariable characters being removed before starting the analysis (Cunningham, 1997). The results of the ILD test (ILD, $P > 0.99$) clearly showed that the three gene fragments are congruent and can consequently be combined in a total-evidence analysis. A saturation analysis for each of the three gene fragments was carried out by plotting the observed proportions of transitions (ts) and transversions (tv) against the uncorrected genetic distances. The 1st and 2nd codon positions of the cytochrome *b* and c-*mos* coding genes were plotted independently from the highly variable 3rd codon positions. The results, presented in Fig. 2, indicate that the cytochrome *b* 3rd codon transitions showed some saturation when genetic distances were $\geqslant$15%. Therefore, subsequent phylogenetic analyses were performed both

Table 1
Details of material and sequences used in the present study

Species	Locality	GenBank Accession Nos. 12S rRNA/ cytochrome *b*/c-*mos*
Hemidactylus parkeri	Dhafra beach, near Ruwais, Abu Dabi (UAE) (BMNH 1996.169)	AF186117/AF184989/AF363541
Hemidactylus turcicus	Las Palmas, Gran Canaria, Canary Islands (Spain)	AF363568/AF364319/AF363540
Pachydactylus turneri (formerly *P. laevigatus*)	Richtersveldt, NW Cape Province, South Africa	AF186118/AF184990/AF363567
Geckonia chazaliae 1	Laâyoune, Morocco	AF363574/AF364325/AF363555
Geckonia chazaliae 2	Tan-Tan Plage, Morocco	AF363575/AF364326/AF363556
Genus *Tarentola*		
Subgenus *Neotarentola*		
T. americana	Guantánamo, Cuba	AF186119/AF184991/AF363542
Subgenus *Tarentola* s. str.		
Mainland		
T. boehmei	Akka Ighane, Morocco (BEV.2177)	AF363569/AF3644320/AF363543
T. deserti	Erfoud area, Morocco (BEV.2207)	AF363570/AF364321/AF363544
T. mauritanica	Abdelmaleh Rahmd, Algeria (BEV.2136)	AF363576/AF364327/AF363566
Canary Islands		
T. angustimentalis 1	Fuste, Fuerteventura	AF186120/AF184992/AF363545
T. angustimentalis 2	Pájara, Fuerteventura	AF186122/AF184994/AF363546
Subgenus *Sahelogecko*		
T. annularis	Egypt	AF363571/AF364322/AF363552
T. ephippiata 1	M'Bour, ORSTOM institute, Senegal (BEV.2226)	AF363572/AF364323/AF363553
T. ephippiata 2	M'Bour, ORSTOM institute, Senegal (BEV.2227)	AF363573/AF364324/AF363554
Subgenus *Makariogecko*		
Canary Islands		
T. delalandii 1	Adeje, Tenerife	AF186131/AF185003/AF363559
T. delalandii 2	Puntagorda, La Palma	AF186129/AF185001/AF363557
T. delalandii 3	Puntagorda, La Palma	AF186130/AF185002/AF363558
T. gomerensis 1	Playa Hermigua, La Gomera	AF186132/AF185004/AF363560
T. gomerensis 2	La Rajita, La Gomera	AF186134/AF185006/AF363561
T. boettgeri boettgeri 1	Arinaga, Gran Canaria	AF 186125/AF184997AF363548
T. boettgeri boettgeri 2	Tauro, Gran Canaria	AF186124/AF184996/AF363547
T. boettgeri hierrensis 1	Tamaduste, El Hierro	AF186126/AF184998/AF363549
T. boettgeri hierrensis 2	Los Llanillos, El Hierro	AF186127/AF184999/AF363550
Selvages Islands		
T. boettgeri bischoffi	Selvagem Grande (BEV.2239)	AF186128/AF185000/AF363551
Cape Verde Islands		
T. darwini	Ribeira ilheu, Fogo (BMNH1998.356)	AF186175/AF185047/AF363565
T. caboverdiana substituta	Bahia das Gatas, S. Vicente (BMNH1988.364)	AF186159/AF185031/AF363564
T. rudis rudis	Cidade Velha, Santiago (BMNH1988.365)	AF186140/AF185012/AF363562
T. gigas brancoensis	Branco	AF186145/AF185017/AF363563

Note. BMNH prefixes the accession numbers of voucher specimens deposited in the Natural History Museum, London. BEV prefixes accession numbers of voucher specimens deposited in the Laboratoire de Biogeographie et Ecologie des Vertébrés, Universite de Montpellier II, France.

with and without 3rd codon positions (or 3rd codon transitions for the maximum-parsimony (MP) analyses).

A variety of methods of phylogenetic reconstruction are available, often involving different models of evolutionary change. If different methods give similar or identical tree topologies it increases confidence that these are representative of the evolutionary history of the gene fragments that comprise the data set. If on the other hand results differ, this indicates that different interpretations are possible. Therefore, three different basic methods were used: maximum-parsimony (MP), maximum-likelihood (ML), and minimum-evolution (ME).

MP analyses involved heuristic searches with 100 random stepwise additions of taxa, tree bisection and reconnection (TBR), and branch swapping. The weight of transversions was varied relative to that given to transitions, being allocated the same, two times, and four times the weight of transitions in different analyses. The cytochrome *b* 3rd codon transitions were given a weight of 0 in some analyses. Gaps were treated as a fifth state. The weight of the gaps was always equal to the maximum weight given to either ts or tv.

For ML and ME analysis, there is an array of possible models. Models with few parameters are not likely to be very realistic and tend to give inaccurate estimates of evolution. Adding extra parameters produces more realistic models but increases sampling errors and the uncertainty of the resultant estimates; it also decreases statistical power. Because of this conflict, two contrasting models of sequence evolution were used: (1) the complex General Time Reversible (GTR) model which

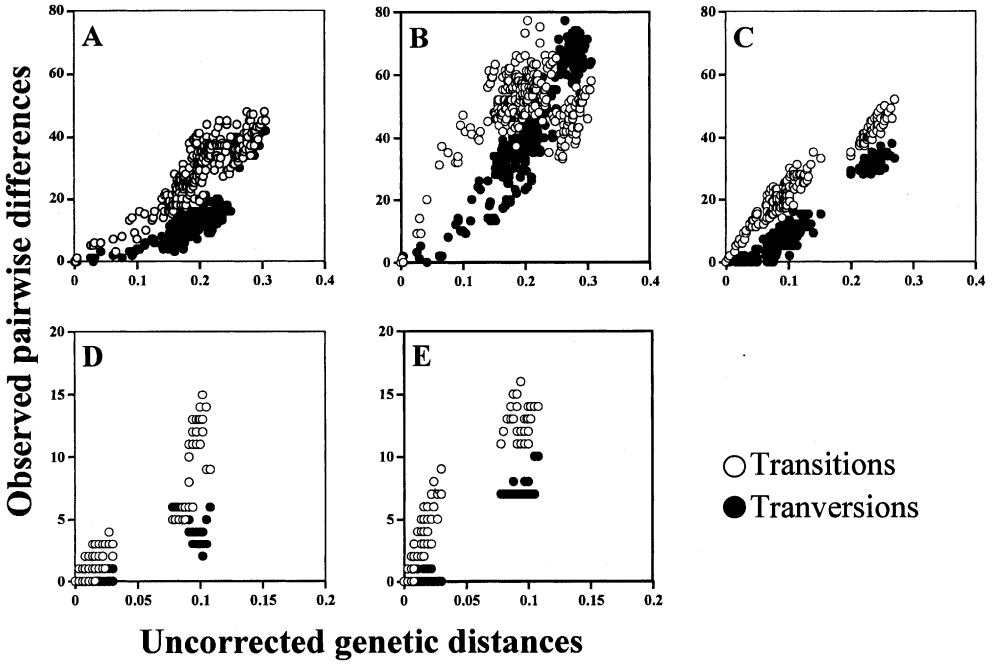

Fig. 2. Observed number of transitions (ts) and transversions (tv) plotted against incorrected genetic distances for the cytochrome *b*, 12S rRNA, and c-*mos* genes. (A) cytochrome *b* first and 2nd codon ts and tv; (B) cytochrome *b* 3rd codon ts and tv; (C) 12S rRNA ts and tv; (D) c-*mos* first and 2nd codon ts and tv (E) c-*mos* 3rd codon ts and tv.

was selected from several available models by Modeltest v. 3.0 (Posada and Crandall, 1998) as one fitting the data set best and (2) the simpler Kimura two-parameter (K2P) model, which takes into account the differences between the number of transitions and that of transversions (ts/tv ratio), but assumes equal frequencies for the four types of nucleotides. Both ME and ML analysis involved heuristic searches with TBR branch swapping. A hundred random stepwise additions of taxa were used in the ME analyses and 10 in ML ones. The cytochrome *b* 3rd codon positions were excluded in some analyses.

In total, 14 different analyses were used, varying in basic method and the parameters involved. These were (1) MP (ts = tv), (2) MP (ts = 1, tv = 2), (3) MP (ts = 1, tv = 4), (4) MP (ts = tv, cytocrome *b* 3rd codon ts = 0), (5) MP (ts = 1, tv = 2, cytochrome *b* 3rd codon ts = 0), (6) MP (ts = l, tv = 4, cytochrome *b* 3rd codon ts = 0), (7) ML (GTR) including cytochrome *b* 3rd codon positions, (8) ML (GTR) excluding cytochrome *b* 3rd codon positions, (9) ML (K2P) including cytochrome *b* 3rd codon positions, (10) ML (K2P) excluding cytochrome *b* 3rd codon positions, (11) ME (GTR) including cytochrome *b* 3rd codon positions, (12) ME (GTR) excluding cytochrome *b* 3rd codon positions, (13) ME (K2P) including 3rd codon positions, and (14) ME (K2P) excluding cytochrome *b* 3rd codon positions. All analyses were performed using PAUP* (Swofford, 1998) except where stated. Robustness of trees was assessed by bootstrap analysis (Felsenstein, 1985) which involved 1000 heuristic pseudo-replications for the MP and ME trees and 250 for the ML trees.

2.5. Molecular clock considerations

Divergence times on trees were estimated using the nonparametric rate smoothing (NPRS) method implemented in the program r8s (Sanderson, 1997). Given that the NPRS method for estimating divergence times is dependent on both topology and branch lengths, age ranges were calculated for each node based on all different topologies obtained and three different branch length optimization methods (MP (ACCTRAN and DELTRAN) and ML (GTR)). The age of El Hierro island in the Canary archipelago, estimated by Guillou et al. (1996) as 1.1 My, was used for calibration, on the assumption that the ancestor of the resident *T. boettgeri hierrensis* colonized soon after island formation. The problems of sampling artifacts, existing genetic diversity in the ancestral population and extinct lineages, when calibrating clocks, has been discussed by Emerson et al. (2000a) using *Brachyderes* beetles as examples. To limit unappreciated effects of these factors, these authors suggest that two calibrations should be made using two different events on the phylogeny. These are the separation of lineages within the colonized island, and the separation of the ancestral lineage of these from that of the supposed source population. In *Tarentola* the first calibration, based on the two samples available from El Hierro, gives a rate of sequence evolution of only 0.218% per My. This would entail a divergence rate 10 times slower than that calculated for other lizards (Carranza et al., 2000) and arthropods (Brower, 1994; DeSalle et al., 1987) and would indicate that the Canary

Islands were colonized many millions of years before their formation. The second calibration was consequently used very low genetic variability seems to be common in lizard species and subspecies within small islands of the Canaries such as El Hierro (Brown and Pestano, 1998; S. Carranza and E.N. Arnold, pers. obs.; Carranza et al., 2000, 2001; González et al., 1996; Rando et al., 1997), thus reducing the problems of sampling artifacts when calibrating divergence events. This contrasts with the usually higher intraisland variability found within species and subspecies of coleopterans in the Canary Islands (Emerson et al., 2000a,b; Juan et al., 1998) and the associated dangers of missing unsampled and extinct mitotypes (Emerson et al., 2000a). The lower genetic variability of lizards on small islands of the Canaries is probably related to their higher mobility and broader ecological niche, both promoting genetic homogenization and precluding further differentiation into distinct populations.

3. Results

A total of 1355 bp of sequence were used to infer the phylogenetic position of the enigmatic *G. chazaliae* and to analyze the radiation of *Tarentola*. Of the 1355 bp (684 bp from cytochrome *b*, 320 bp from 12S rRNA, and 351 bp from c-*mos*), 592 bp were variable and 486 bp parsimony informative. The strict consensus of the 20 trees obtained from the 14 different analyses used is shown in Fig. 3. Ranges of bootstrap support for specific nodes are indicated in this figure and levels of support for particular analyses given in Table 2. The strict consensus clearly places *Geckonia* within *Tarentola* in a clade with *T. delalandii*, *T. gomerensis*, and the species from the Cape Verde archipelago.

In fact, it was not possible to amplify the first 303 bp of the cytochrome *b* gene (from primers cytochrome b_1 to cytocrome b_2) of one of the *Geckonia* specimens, *G. chazaliae*. 2. A second analysis including only *G. chazaliae* 1 was consequently performed to check whether the absence of this sequence fragment was affecting the topology of the trees. In this analysis the number of variable positions decreased to 582 and parsimony-informative ones to 480. However, the strict consensus topology derived from the 23 trees obtained using the same 14 kinds of analyses was identical to the initial one, all the nodes showing very similar levels of bootstrap support (data not shown).

In separate analyses of the different gene fragments, the combination of the two mitochondrial genes, cytochrome *b* and 12S rRNA (663 of 1004 bp variable and 454 bp parsimony informative), produced a well-supported consensus topology similar to that of the three gene fragments together (Fig. 4a). Independent analysis of the cytochrome *b* (518 of 684 bp variable and 344 bp parsimony informative) and the 12S rRNA (145 of 320 bp variable and 110 bp parsimony informative) gave near-identical topologies (data not shown). The consensus for the c-*mos* sequence (55 of the 351 bp variable and 32 bp parsimony informative) was largely uninformative but congruent with other treatments and included an unresolved but highly supported polytomy made up of *Geckonia* and all the *Tarentola* species included in the study, confirming that members of these two genera are more closely related to each other than to *Pachydactylus* (Fig. 4b).

In all these analyses, the bootstrap support for an orgin of *Geckonia* within *Tarentola* is very high, approaching 100% in all cases. The fact that many different analyses were used, and that gene fragments were analyzed together and separately with congruent results, increases our confidence that *Geckonia* is a member of *Tarentola* and that the strict consensus tree shown in Fig. 3 reflects the true phylogeny of the three combined genes.

To avoid branch-length artifacts when estimating divergence times, a data set that excluded *G. chazaliae* 2, for which cytochrome *b* sequence was incomplete was used. When the procedure described under Section 2.5 was applied to the 23 different topologies resulting from the 14 different analyses used, a range of possible dates for each node was obtained. These are shown in Fig. 3 and Table 3.

4. Discussion

4.1. Groupings within Tarentola

In all the individual analyses, *T. americana*, the sole member of the subgenus *Neotarentola*, separates first, corroborating a recent investigation involving fewer species (Carranza et al., 2000). This is followed by four members of the subgenus *Tarentola* s. str., which form two clades: *T. mauritanica* + *T. angustimentalis* and *T. deserti* + *T. boehmei*. In the 20 trees produced by the 14 separate analyses of the total data set, only the four ME trees support monophyly of these members of the subgenus *Tarentola* s. str. Among the remaining, MP and ML, analyses there are none where *T. deserti* + *T. boehmei* is basal and all put the *T. mauritanica* + *T. angustimentalis* clade in this position (see Fig. 5). Nonmonophyly of *Tarentola* s. str. would be unsurprising, as the morphological evidence for this supposed clade is slight. Its members do have a distinctive horseshoe of slightly enlarged scales around each big dorsal tubercle (Joger, 1984c) but this condition occurs sporadically within other sections of *Tarentola* sens. lat (pers. obs.).

In the strict consensus tree of all analyses (Fig. 3), a trichotomy is formed by *T. annularis* + *T. ephippiata*

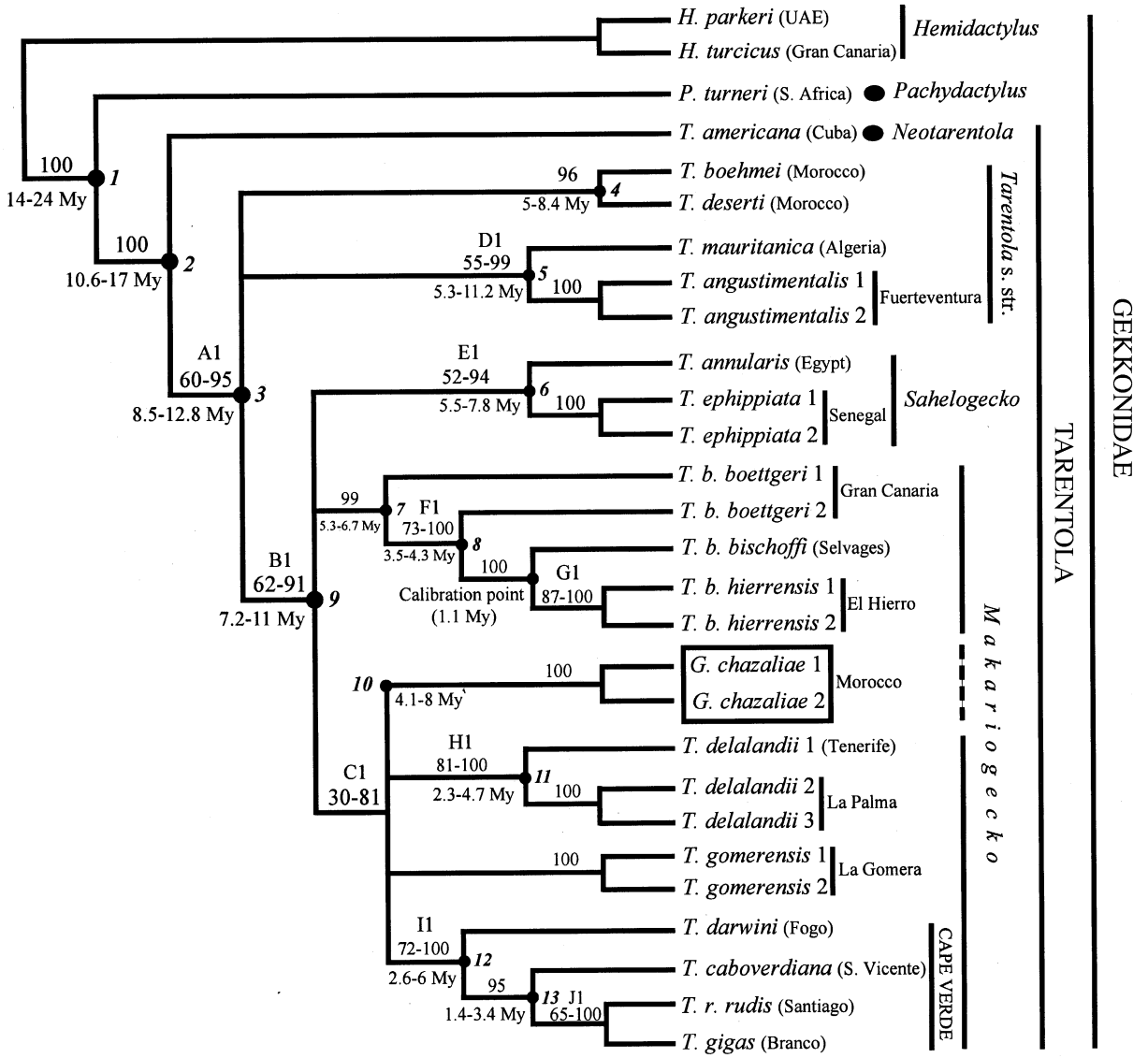

Fig. 3. Strict consensus of the 20 trees obtained as a result of the combined analysis of all 28 taxa including 1355 bp of sequence (684 bp from cytochrome *b*, 320 bp from 12S rRNA, and 351 bp from the nuclear gene c-*mos*) using various methods and different parameters (see Section 2.4 for more details). Numbers above the nodes indicate the minimum and maximum bootstrap support for all methods and parameters used. When the differences between all the bootstrap support values was <10% only the average value is shown. Details of the specific bootstrap supports for the nodes A1 to J1 are shown in Table 2. Numbers below the nodes are the estimated range of the ages (in millions of years) of the speciation events concerned. Numbers in italics refer to Table 3.

(members of the subgenus *Sahelogecko*), *T. boettgeri*, and a clade comprising *Geckonia*, *T. delalandii*, *T. gomerensis*, and Cape Verde species. However, in none of the component trees is *T. annularis* + *T. ephippiata* basal and it is sister to *T. boettgeri* in only 4 of them (2 ME and 2 ML). In the remaining 16 trees *T. boettgeri* is basal and *T. annularis* + *T. ephippiata* is sister to the remaining clade (Fig. 5). This arrangement places the subgenus *Sahelogecko* and the genus *Geckonia* within the subgenus *Makariogecko*, indicating that the latter is not a clade. Again, morphology does not strongly contradict this conclusion. *Makariogecko* is said to be defined by enlarged scales along the edge of the supraocular region (Joger, 1984c), but this feature shows significant variation both within the species assigned to *Makariogecko* and elsewhere (pers. obs.).

The relationships just described, which are supported by the great majority of component trees, are apparent in the 50% majority rule consensus tree calculated by taking the 14 strict consensus trees of each individual analysis (see Fig. 5). Inter-relationships of *Geckonia*, *T. delalandii*, *T. gomerensis*, and the Cape Verde species in the component trees are very variable so no additional resolution is possible. Alternative relationships of the *T. annularis* + *T. ephippiata* and the *T. boehmei* + *T. deserti* clades have been highlighted.

Table 2
Bootstrap support for specific nodes

	A1	B1	C1	D1	E1	F1	G1	H1	I1	J1	A2	B2	C2	D2	E2	F2	G2	H2	I2
MP(ts = tv)	77	62	52	55	70	98	99	100	85	100	75	62	51	95	54	68	98	100	84
MP(ts = 1, tv = 2)	80	73	72	59	87	99	99	100	80	100	79	75	71	95	57	87	100	100	82
MP(ts = 1, tv = 4)	73	76	81	65	89	98	99	100	72	100	74	77	80	96	56	85	98	100	72
MP(ts = tv, cytb 3rd codon ts = 0)	90	91	73	83	94	96	97	94	75	85	89	88	78	96	86	93	96	93	79
MP(ts = 1, tv = 2, cytb 3rd codon ts = 0)	87	87	74	89	91	98	96	96	78	90	82	87	75	89	89	91	98	96	81
MP(ts = 1, tv = 4, a cytb 3rd codon ts = 0)	73	69	64	89	80	98	97	96	79	89	72	75	66	74	90	82	98	94	80
ME(K2P) including cytb 3rd codon positions	95	63	71	95	52	95	100	100	100	100	92	63	74	100	94	54	96	100	100
ME(K2P) excluding cytb 3rd codon positions	90	75	79	99	60	73	98	99	100	65	78	84	85	95	99	58	85	96	98
ME(GTR) including cytb 3rd codon positions	96	78	70	97	67	97	100	100	100	100	92	79	78	100	96	68	96	100	100
ME(GTR) excluding cytb 3rd codon positions	92	78	77	100	64	72	100	99	100	65	86	72	81	98	100	62	72	97	100
ML(GTR) including cytb 3rd codon positions	76	82	62	73	89	100	97	100	77	100	69	75	61	100	78	87	100	100	84
ML(GTR) excluding cytb 3rd codon positions	60	67	30	96	67	89	87	83	76	76	65	72	28	94	92	69	88	79	68
ML(K2P) including cytb 3rd codon positions	74	84	62	71	89	99	100	100	79	100	72	80	58	95	73	88	100	100	73
ML(K2P) excluding cytb 3rd codon positions	63	66	31	96	65	87	86	82	72	75	53	57	30	82	96	62	91	83	76

Note. A1–J1 nodes in Fig. 3; A2–I2, nodes in Fig. 4a; cytb, cytochrome *b*.

4.2. Phylogeography

The estimated age of the separation of the West Indian *Tarentola americana*, 10.6–17 My, makes it certain that the ancestor of this gecko reached the Neotropics by natural transmarine colonization. Separation of this lineage by plate tectonics can be excluded as Africa and southern America broke apart around 100 My ago (Smith and Briden, 1977). Similarly, human transportation is ruled out by the estimated age of separation and the morphological distinctiveness of *T. americana*.

The North African continental species form a sequence of branches (Fig. 5): *T. mauritanica*, *T. boehmei* + *T. deserti*, *T. annularis* + *T. ephippiata*, and *Geckonia*. The geographical distributions of successive branches are placed increasingly southwards from the Mediterranean coast, shifting from relatively mesic situations typical of most gekkonid lizards to increasingly hot and arid habitats. Such a phylogenetic pattern may reflect the way that severe habitats of relatively recent origin such as deserts can be colonized from their edges by an iterative process of speciation, displacement, and adaptation (Arnold, 1981). The estimated ages of the speciation events concerned (see Fig. 5) may possibly reflect the timing of stages of aridification of the Sahara desert. The area phylogeny of continental *Tarentola* does not fit any general pattern among sympatric taxa, so is unlikely to be a result of vicariance.

Tarentola colonized the Canary islands more than once (Carranza et al., 2000; Joger, 1984a; Nogales et al., 1998). *T. angustimentalis*, the sister of the continental *T. mauritanica*, reached the eastern islands of Fuerteventura and Lanzarote while the other islands are occupied by species assigned to the subgenus *Makariogecko*. These later forms were regarded the descendants of a single colonizing species, but the phylogeny in Fig. 5 indicates that there were two separate invasions: one by *T. boettgeri* to the Selvages, Gran Canaria, and El Hierro and another by the ancestor of *T. delalandii*, *T. gomerensis*, and the Cape Verde species to the western islands of Tenerife, La Gomera, La Palma, and El Hierro. Other possible scenarios involving fewer invasions of the Canary islands are less parsimonious overall and would involve reinvasion of the North African mainland. This is unlikely on other grounds. Currents and winds liable to have carried propagules run from the vicinity of North Africa southwestward toward the Canaries, rather than in the direction of the mainland (Juan et al., 2000). Also, while mainland reptile taxa frequently invade islands, those that have been subjected to insular conditions for significant periods rarely if ever invade continental areas (Arnold, 2000). Although the lacertid clade *Gallotia* appears to have colonized the Canary archipelago in a single sequence from east to west (S. Carranza and E.N. Arnold, pers. obs.; González et al., 1996; Rando et al., 1997), it seems probable that the three independent invasions of *Tarentola* arrived via

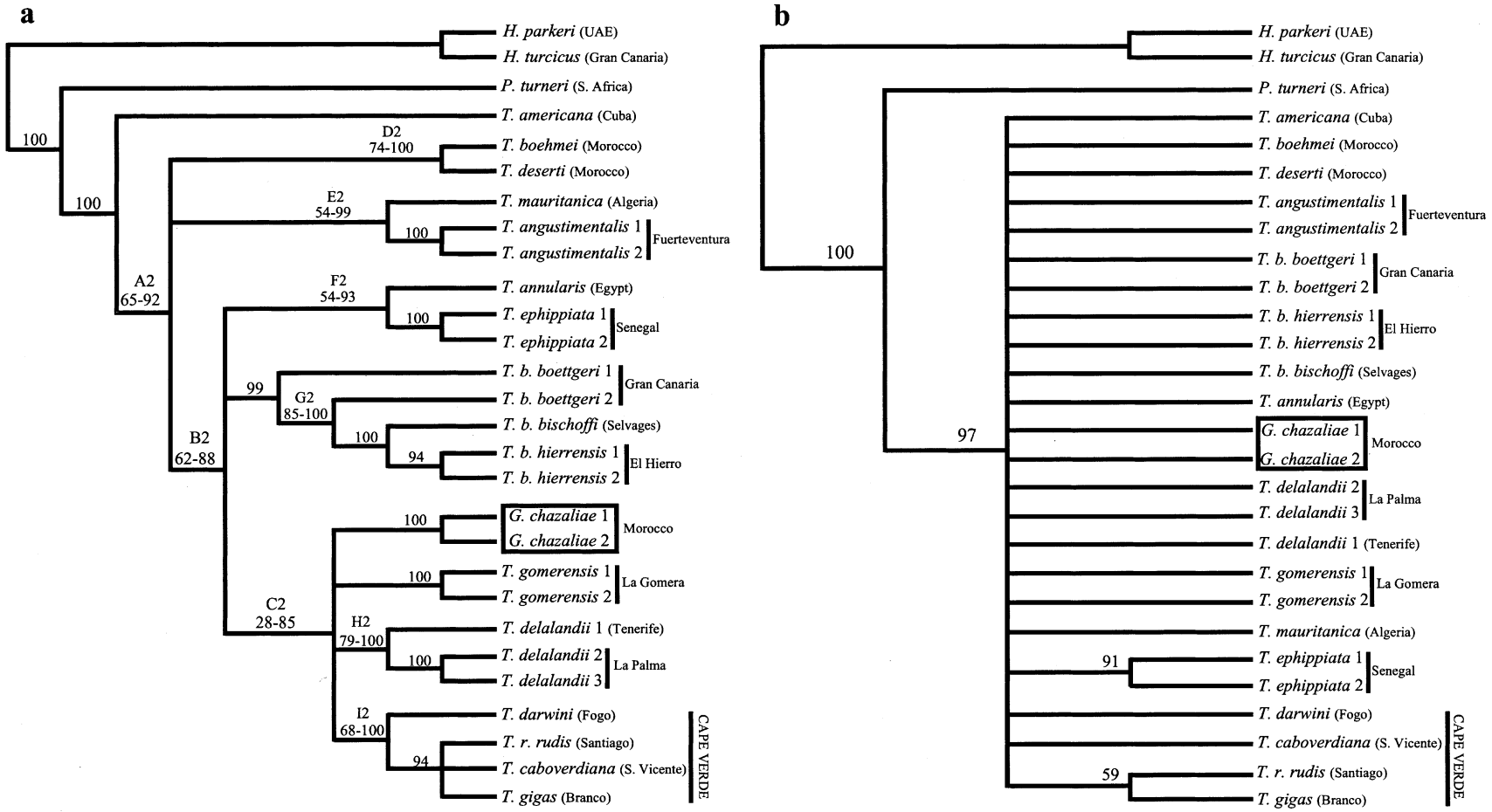

Fig. 4. (a) Strict consensus of the 18 trees obtained from the analysis of the two mitochondrial genes alone (684 bp of the cytochrome *b* and 320 bp of the 12S rRNA) using the same methods and parameters as those in Fig. 3; (b) strict consensus of the 108 trees obtained from the analysis of the c-*mos* nuclear gene alone (351 bp) using the following methods and parameters: MP (ts = tv), MP (ts = 1, tv = 2), MP (ts = 1, tv = 4), MP (GTR), ML (K2P), ME (GTR), and ME (K2P). Numbers above the nodes indicate the minimum and maximum bootstrap support for all the methods and parameters used. When the differences between the bootstrap support values was <10% only the average value is shown. Details of the specific bootstrap supports for the nodes A2 to I2 are shown in Table 2.

Table 3
Ages (in My) for some selected nodes on Figs. 3 and 5 obtained using three different branch-length calculation methods MP (ACCTRAN, DELTRAN) and ML (GTR)

Node	ACCTRAN	DELTRAN	ML (GTR)	Mean
1	15–22	14.1–15.6	19.7–24	18
2	11.3–16.7	10.67–11.8	13.6–17	13.3
3	9.5–12.5	8.5–9.3	11.1–12.8	10.3
4	5.4–8.4	5–6.1	6.1–8	6.6
5	5.3–10.8	6.1–8.6	7–11.2	8.8
6	5.9–7.7	5.5–6.2	6.7–7.8	6.3
7	6–6.3	5.3–5.8	6.5–6.7	6
8	3.6–3.8	3.5–3.8	4.2–4.3	3.8
9	7.2–10	6.8–8.2	8.8–11	8
10	4.1–8	4.7–6.5	6–8.5	5.8
11	2.3–4.7	3.3–4.1	3.5–4.7	3.6
12	2.6–4.8	3.3–4.1	4.5–6	4.1
13	1.4–2.7	1.9–2.9	2.6–3.4	2.4
14	10.9–13.7	9.5–10.3	12.9–14.3	11.54
15	9.8–12.5	8.5–9.3	11.6–12.8	10.3
16	7.2–7.7	6.8–7.6	8.7–8.9	7.8

Note. Ranges represent the maximum and minimum values for all 23 different topologies (see Section 3). Mean represents the average of all 69 (23×3) values obtained.

the southwest-running Canary current, which subsequently carried a single colonizing species from the western Canary islands a further 1400 km to the Cape Verde archipelago (Carranza et al., 2000). Members of *Tarentola* have thus been involved in as many as five long-distance transmarine colonizations of islands: the West Indies, the Canaries three times, and the Cape Verde archipelago. There is no evidence as to whether the invasions of the Canaries by the immediate ancestors of current taxa were of "empty" areas or whether replacement of one or more previous colonizations of *Tarentola* was involved. The relative ages of the *T. angustimentalis* lineage and of the taxa which are now present on Gran Canaria are similar, so there is no other indication of possible replacement.

Geckonia occupies a very narrow coastal strip on the edge of the huge Saharan arid region. As this latter area appears to include suitable habitat for *Geckonia*, its absence there requires explanation. One possible factor is the presence of similarly sized, ecologically analogous ground geckos of the genus *Stenodactylus* that are widespread in North Africa. Possibly *Geckonia* arose in the narrow coastal strip in more mesic times, when this dry area was cut off from others further east. Later aridification may have led to the spread of *Stenodactylus* so that *Geckonia* is now bordered by a competitor that prevents its eastward spread. Alternatively, *Geckonia* may have once been more widespread and has been restricted by the invasion of North Africa by *Stenodactylus*, the phylogeny of which suggests that it colonized the Saharan area from Arabia (D.J. Harris

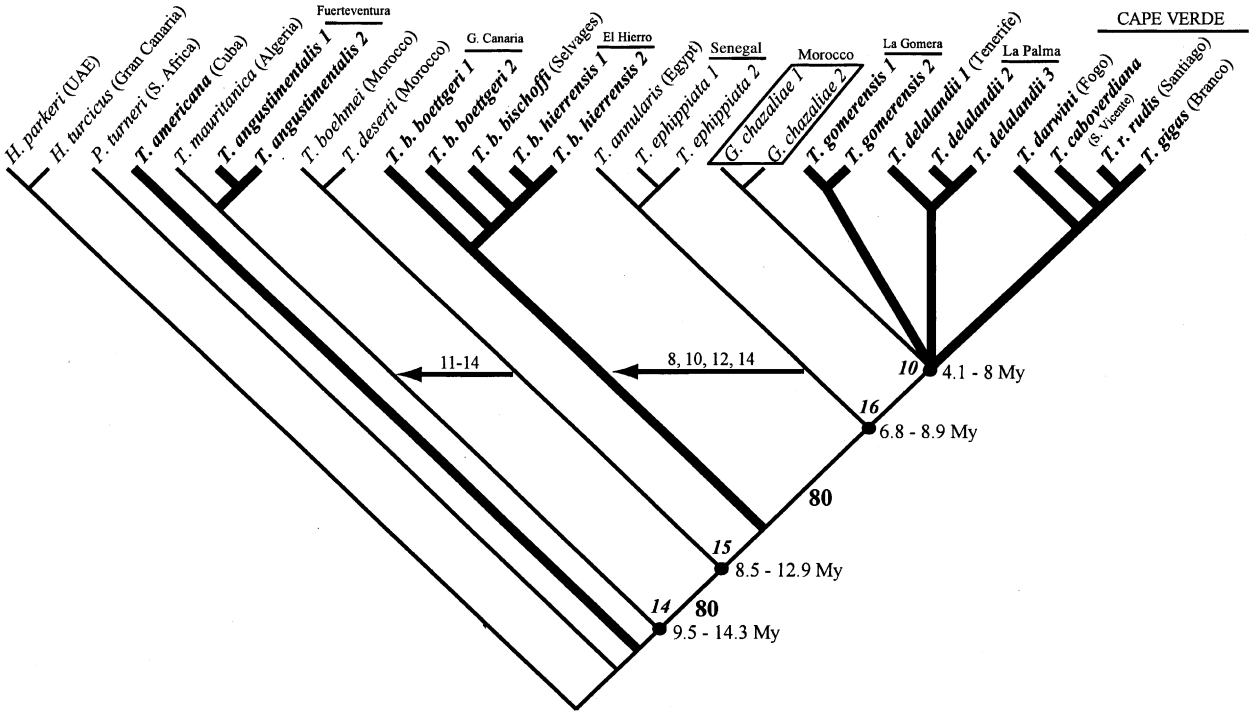

Fig. 5. Preferred phylogeny for *Tarentola*. Fifty percent majority rule consensus tree calculated from the 14 strict consensus trees of each individual analysis. All resolved nodes have a 100% support except where indicated. Alternative relationships at the two nodes with 80% support are marked by arrows. Numbers above them indicate which of the 14 types of analysis used (listed in Section 2.4) support these relationships. Exclusive lineages of insular taxa are marked in boldface; continental lineages are in lightface. Numbers by the nodes indicated by dots are the estimated range of the ages (in millions of years) of the speciation events concerned. Numbers in italics refer to Table 3.

and E.N. Arnold, unpublished). This latter hypothesis would be corroborated by the discovery of fossils or relicts of *Geckonia* east of its present range, and if divergence times suggest that *Stenodactylus* has entered North Africa quite recently, at least since the origin of the exclusive lineage of *Geckonia*.

4.3. Evolution of Geckonia

Geckonia has expanded subdigital lamellae with the hair-like setae usual in climbing geckos (Russell, 1972, 1976) and complex digital musculature that many such animals use to control these structures. As in *Tarentola* and *Pachydactylus*, the musculature is capable of producing hyperextension of the digits, the ability to turn their distal sections upward, which helps keep the expanded subdigital lamellae and their setae clean by holding them clear of dusty surfaces. In scansorial geckos, hyperextension also helps disengage the setae when the foot is lifted from the surface during climbing. It is difficult to explain the possession of these complex derived features if the *Geckonia* lineage was always entirely ground dwelling. So, on the basis of functional redundancy (Arnold, 2001), these structures suggest that *Geckonia* had ancestors with substantial climbing ability and that its ground-dwelling habits are secondary. The phylogeny provides strong corroboration of this interpretation, as there is a series of branches arising on the *Geckonia* lineage entirely composed of species of *Tarentola* that regularly climb.

The derived features that distinguish *Geckonia* from *Tarentola* may be related to the shift from a climbing to a ground-dwelling life mode in open areas. Similar shifts can be seen in ecologically analogous members of the gekkonid genus *Stenodactylus* of Arabia and North Africa. They include increase in head size, reduction in presacral vertebra number (Arnold, 1980), development of longer limbs, and evolution of a relatively short sometimes slender tail that autotomizes at the base (Arnold, 1980, 1984a). These morphological features are accompanied by a lack of speed, slow visual scanning (a hunting technique in which the lizards walk slowly with the body held high on extended legs; Arnold, 1984b), and distinctive antipredator behaviors that do not simply involve retreat (for instance *Stenodactylus leptocosymbotes* may slowly wave its distinctively colored tail, and *S. doriae* stands high on its legs with its tail raised and may attempt to bite if approached; Arnold, 1984b; pers. obs.). Reduced presacral vertebral number may be associated with less need to flex the body during locomotion in forms with long slender limbs related to hunting technique operating in open terrain. Aggressive or static antipredator behavior might be advantageous in relatively slow animals living in such places, where refuges cannot always easily be reached. These factors may also make shedding all of the tail from the base advantageous, since predators are "offered" a substantial distraction which may enable the owner of the tail to escape, even though it moves relatively slowly (Arnold, 1984a, 1988).

The ancestors of *Stenodactylus* may have climbed to some extent (D.J. Harris and E.N. Arnold, unpublished), but this lineage apparently never had the sophisticated toe structure found in *Tarentola* and *Geckonia*. However, the southern African *Pachydactylus* group parallels these latter North African geckos closely. One or more lineages have become ground dwelling, producing *Chondrodactylus*, *Colopus*, and *Palmatogecko* in coastal areas, all of which retain complex digital muscles (Russell, 1976). They also show a number of the other distinctive derived features shared by *Geckonia* and *Stenodactylus*.

Despite a relatively long history that probably exceeds 10 My and involves numerous speciation events and wide geographical spread, *Tarentola* is morphologically quite uniform. Species differ considerably in size but most other variation involves relatively minor differences in body proportions, scale counts, the shape and arrangement of the scales, and color and pattern. It might be thought that this morphological similarity among all the representatives of *Tarentola* was the result of a lineage effect established in the ancestor of the group. However, the fact that *Geckonia* which arises within *Tarentola*, has developed numerous derived states shows that there is no overwhelming phylogenetic constraint on producing such features.

4.4. Nomenclature

The phylogenetic position of *G. chazaliae*, embedded within *Tarentola*, makes the latter genus paraphyletic, something that can be avoided by transferring *G. chazaliae* to *Tarentola*, which would also make its relationships clear. Such a course would unfortunately also change a binomial that has been in use for over a century. But *G. chazaliae* has not been employed much outside the taxonomic literature, so any confusion produced would be quite restricted. The change is consequently recommended.

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