

Radiation, multiple dispersal and parallelism in the skinks, *Chalcides* and *Sphenops* (Squamata: Scincidae), with comments on *Scincus* and *Scincopus* and the age of the Sahara Desert

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Abstract

Phylogenetic analysis using up to 1325 base pairs of mitochondrial DNA from 179 specimens and 30 species of *Chalcides*, *Sphenops*, *Eumeces*, *Scincopus* and *Scincus* indicates that *Sphenops* arose twice independently within *Chalcides*. It is consequently synonymized with that genus. *Chalcides* in this broader sense originated in Morocco, diversifying into four main clades about 10 Ma, after which some of its lineages dispersed widely to cover an area 40 times as large. Two separate lineages invaded the Canary Islands and at least five main lineages colonized southern Europe. At least five more spread across northern Africa, one extending into southwest Asia. Elongate bodies with reduced limbs have evolved at least four times in *Chalcides*, mesic ‘grass-swimmers’ being produced in one case and extensive adaptation to life in loose desert sand in two others.

In clade, *Chalcides striatus* colonized SW Europe from NW Africa 2.6 Ma and *C. chalcides* mainland Italy 1.4 Ma, both invasions being across water, while *C. c. vittatus* reached Sardinia more recently, perhaps anthropogenically, and *C. guentheri* spread 1200 km further east to Israel. *C. minutus* is a composite, with individuals from the type locality forming a long independent lineage and the remaining ones investigated being most closely related to *C. mertensi*. In the Northern clade, *C. boulengeri* and *C. sepsoides* spread east through sandy habitats north of the Sahara about 5 Ma, the latter reaching Egypt. *C. bedriagai* invaded Spain around the same time, perhaps during the Messinian period when the Mediterranean was dry, and shows considerable diversification. Although it is currently recognized as one species, the *C. ocellatus* clade exhibits as much phylogenetic depth as the other main clades of *Chalcides*, having at least six main lineages. These have independently invaded Malta and Sardinia from Tunisia and also southwest Arabia. *C. o. humilis* appears to have spread over 4000 km through the Sahel, south of the Sahara quite recently, perhaps in the Pleistocene. In the Western clade of *Chalcides*, *C. delislei* appears to have dispersed in a similar way. There were also two invasions of the Canary Islands: one around 5 Ma by *C. simonyi*, and the other about 7 Ma by the ancestor of *C. viridanus* + *C. sexlineatus*. *C. montanus* was believed to be related to *C. lanzai* of the Northern clade, but in the mtDNA tree it is placed within *C. polylepis* of the Western clade, although this may possibly be an artifact of introgression.

The *Eumeces schneideri* group, *Scincopus* and *Scincus* form a clade separate from *Chalcides*. Within this clade, the geographically disjunct *E. schneideri* group is paraphyletic. One of its members, *E. algeriensis* is the sister taxon to *Scincopus*, and *Scincus* may also be related to these taxa. The phylogeny suggests *Scincopus* entered desert conditions in Africa, up to 9.6 Ma and the same may have been true of *Scincus* up to 11.7 Ma. *Scincus* appears to have diversified and spread into Arabia around 6 Ma. Dates of origin and divergence of these skinks, desert *Chalcides* and other squamates agree with recent geological evidence that the Sahara is at least 5–7 My old.

The subspecies *Chalcides viridanus coeruleopunctatus* is upgraded to the species level as *C. coeruleopunctatus* stat nov., on the basis of its large genetic divergence from *C. v. viridanus*.

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1. Introduction

North Africa, including its large arid regions has a distinctive non-lygosomine skink fauna that includes members of *Chalcides* Laurenti, 1768, *Sphenops* Wagler, 1830, *Scincus* Laurenti, 1768, *Scincopus* Peters, 1864 and the *Eumeces schneideri* group (Daudin, 1802). As presently understood, *Chalcides* is a group of around 24 species, most of which occur in Morocco and surrounding areas, although the genus extends across northern Africa east as far as Somalia and Kenya, and through the Levant to Turkey, Iraq, Arabia, and coastal Iran and Pakistan; it also occurs in southern Europe. Four more species originally placed in *Chalcides* were separated and placed in *Sphenops* (Doumergue, 1901; Pasteur and Bons, 1960), but morphology (Caputo, 2004), chromosomes (Caputo and Odierna, 1992; Caputo et al., 1993a,b,c; Kalboussi et al., 2006) and mitochondrial DNA sequence (Brandley et al., 2005) indicate they are closely related to *Chalcides*. It and *Sphenops* are also part of a clade containing 15 or so other African, Madagascan, Mascarene and Seychelles genera (based on mitochondrial DNA—Brandley et al., 2005; and morphology—Greer and Shea, 2000). The *Chalcides*–*Sphenops* assemblage shows considerable morphological variation. In particular, some species are very elongate with varying degrees of limb reduction, a trend that reaches its extreme in *C. guentheri* Boulenger, 1887, which has minute external limb remnants without digits (Fig. 1B). The taxonomy of all or part of *Chalcides* has been revised successively by Boulenger (1887, 1890, 1896, 1898), Boulenger (1920), Lanza (1957), Pasteur (1981), Caputo (1993), Mateo et al. (1995), Greenbaum (2005), and Greenbaum et al. (2006). However, it has

not proved possible to construct a phylogeny from morphological features and there is still some uncertainty about some species boundaries. Morphology indicates that the *E. schneideri* group, *Scincopus* and *Scincus* form a clade (Arnold and Leviton, 1977; Griffiths et al., 2000). This is also supported by mitochondrial DNA sequences (Schmitz et al., 2004; Brandley et al., 2005) and chromosomes (Caputo et al., 1994), but detailed relationships remain unresolved.

As it is uncertain how the component species are related to each other in both main assemblages of skinks in North Africa, some 179 individuals including 80% of all recognized species and subspecies of *Chalcides* are investigated here, using up to 1325 base pairs (bp) of mitochondrial DNA (cytochrome *b*-396 bp; 12S rRNA-392 bp; 16S rRNA-537 bp). These sequences are employed to construct a phylogeny of the two assemblages, which is then used to explore their evolutionary and biogeographical history and aspects of their taxonomy.

2. Material and methods

2.1. Samples, DNA extraction and amplification

The 179 specimens used in this study comprise 146 *Chalcides*, 11 *Sphenops*, 10 *Eumeces*, 11 *Scincus* and 1 *Scincopus fasciatus* (Peters, 1864). Specimen data and GenBank accession numbers of the gene fragments sequenced for these are given in Table 1. All 179 specimens were sequenced for the cytochrome *b* (*cytb*) and 12S rRNA mitochondrial genes and a subset of 68, which included representatives from all independent lineages recovered

Fig. 1. (A) Maximum-likelihood tree for a combination of the *cytb* and 12S rRNA mtDNA sequences, showing broad relationships of *Eumeces*, *Scincus*, *Scincopus* and *Chalcides* (including the synonymized *Sphenops*). Numbers above branches indicate bootstrap support values for ML analyses (left) and posterior probability (pp) values for the Bayesian analyses (right). Numbers below the branches are the same parameters for analyses of a combination of the *cytb*, 12S and 16S mtDNA sequences of a subset of 68 specimens (see Section 2). A single number above or below a branch indicates both the bootstrap support and posterior probability values which are the same. The symbol “-” indicates that the particular method indicated did not support the topology presented in this figure. Numbers preceding taxa refer to Fig. 1B. Numbers by some selected internal nodes highlighted with a small filled circle indicate their ages in millions of years. (B) Distribution, morphology and habitat of skinks included in the phylogeny shown in (A). Photographs show general appearance, degree of body elongation and relative limb lengths. *Body length*: distance from snout tip to vent, in larger mature animals. *Taillbody ratio*: tail length divided by distance from snout tip to vent, in larger mature animals. *Fingers*: number of digits on anterior limbs (where known, number of phalanges on digits 1–5 is shown in brackets). *Toes*: number of digits on posterior limbs (where known, number of phalanges on digits 1–5 is shown in brackets). *Body vertebrae*: number of vertebrae from skull to sacrum. *Ear*: the ear drum may be entirely exposed (normal) or partly hidden by scales projecting across the ear opening, or almost or entirely hidden by them. *Snout*: snouts are often rounded in lateral view and roughly cylindrical in cross section, but may be wedge-shaped with the rostral scale often having a sharp horizontal edge, the upper labial scales angled and the anterior lower jaw countersunk into the upper one. *Habitat*: all taxa are ground dwelling, with *Eumeces* occurring in relatively open situations and most *Chalcides* in more enclosed ones where they often occur near vegetation or close to the ground plant interface. Members of the Grass-swimming clade often live among herbaceous vegetation, such as grass in which they “swim”. Other forms are associated with aeolian sand, but vary considerably in their tendency to burrow into it when taking shelter or foraging (data from Schleich et al., 1996; Bons and Geniez, 1996; Mateo et al., 1995; Caputo, 1993; Caputo and Lanza, 1992; Caputo et al., 1995, 2000; Greer et al., 1998 and personal observations).

by the analysis of these two genes, was also sequenced for the mitochondrial gene 16S rRNA.

Genomic DNA was extracted from tissue samples following standard protocols described elsewhere (Carranza

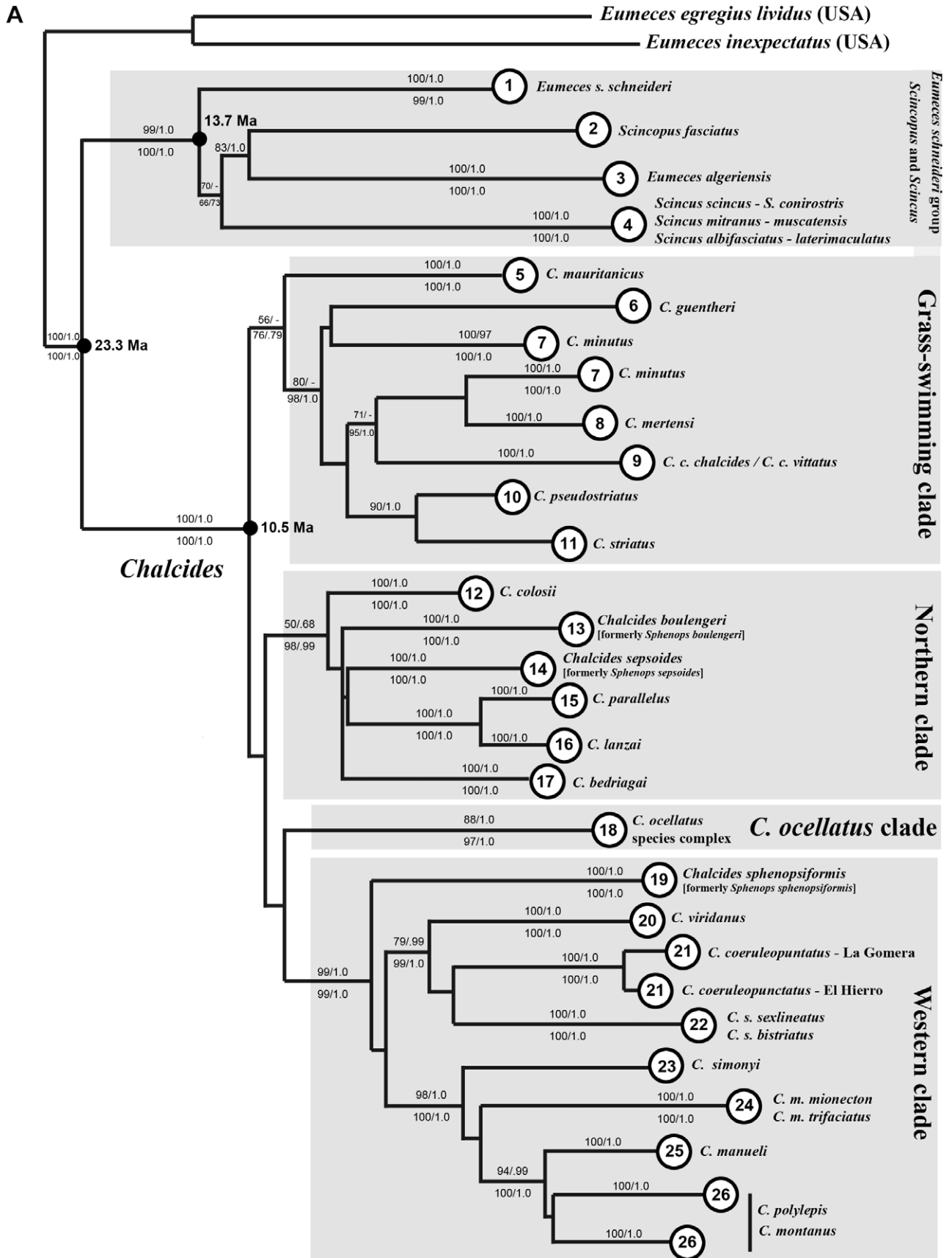




Fig. 1 (continued)

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

Taxa	Locality	GenBank Accession Nos. cytb/12S/16S	Reference (Museum number)
<i>Eumeces egregius</i>	North America (USA)	NC_000888 (mtDNA genome)	Kumazawa and Nishida (1995)
<i>Eumeces inexpectatus</i>	Florida, Duval Co. Little Talbot Island (USA)	AY217837/AY218040/AY217990	Whiting et al. (2003)
<i>Eumeces schneideri princeps</i>	10Km after Igdir, direction Dogubayazit (Turkey)	EU278238/EU278006/EU278073	E3091.12
<i>Eumeces schneideri pavimentatus-1</i>	Karaotlak in the Euphrates valley (Turkey)	EU278234/EU278002/EU278069	E8121.16 (BEV1594)
<i>Eumeces schneideri pavimentatus-2</i>	Coastal dunes after Karatas (Turkey)	EU278235/EU278003/EU278070	E8121.17 (BEV1566)
<i>Eumeces schneideri schneideri-1</i>	(Egypt)	EU278236/EU278004/EU278071	E1009.7
<i>Eumeces schneideri schneideri-2</i>	(Egypt)	EU278237/EU278005/EU278072	E1009.6
<i>Eumeces algeriensis algeriensis-1</i>	Massa (Morocco)	EU278253/EU278021/EU278086	E3091.11
<i>Eumeces algeriensis algeriensis-2</i>	(Morocco)	EU278249/EU278017/EU278082	E1009.4
<i>Eumeces algeriensis algeriensis-3</i>	(Morocco)	EU278250/EU278018/EU278083	E1009.5
<i>Eumeces algeriensis algeriensis-4</i>	Ras el Ma, W. of Saidia (Morocco)	EU278251/EU278019/EU278084	E3091.9
<i>Eumeces algeriensis algeriensis-5</i>	Essauira (Morocco)	EU278252/EU278020/EU278085	E3091.10
<i>Scincopus fasciatus</i>	(Tunisia)	EU278254/EU278022	E04045.6
<i>Scincus albifasciatus albifasciatus-1</i>	Boudarga (Mauritania)	EU278243/EU278011/EU278078	E0602.19
<i>Scincus albifasciatus albifasciatus-2</i>	Oued Choum, Adrar (Mauritania)	EU278241/EU278009/EU278076	E0602.20
<i>Scincus albifasciatus albifasciatus-3</i>	Around Ayoun el Atrous (Mauritania)	EU278239/EU278007/EU278074	E0602.16
<i>Scincus albifasciatus albifasciatus-4</i>	Around Ayoun el Atrous (Mauritania)	EU278240/EU278008/EU278075	E0602.17
<i>Scincus albifasciatus laterimaculatus</i>	Mahmid, South of Zagora (Morocco)	EU278242/EU278010/EU278077	E3007.8
<i>Scincus conirostris</i>	Jabal Dannah (UAE)	EU278248/EU278016/EU278081	E3007.14
<i>Scincus scincus-1</i>	(Egypt)	EU278244/EU278012/EU278079	E8121.21
<i>Scincus scincus-2</i>	Unknown	AY217822/AY218025/AY217976	Whiting et al. (2003)
<i>Scincus mitranus mitranus</i>	Al Ain (UAE)	EU278247/EU278015/EU278080	E3007.13
<i>Scincus mitranus muscatensis-1</i>	Muscat (Oman)	EU278245/EU278013	E1026.7
<i>Scincus mitranus muscatensis-2</i>	Oasis Behind Hotel Al Diyah; close to Nizwa (Oman)	EU278246/EU278014	E1026.8
<i>C. boulengeri-1</i> [formerly <i>Sphenops</i>]	Oued Shili (Tunisia)	EU278156/EU277924/EU278045	E3107.4
<i>C. boulengeri-2</i> [formerly <i>Sphenops</i>]	Oued Shili (Tunisia)	EU278154/EU277922	E3107.2
<i>C. boulengeri-3</i> [formerly <i>Sphenops</i>]	Gafsa (Tunisia)	EU278155/EU277923/EU278044	E3107.3
<i>C. sepsoides-1</i> [formerly <i>Sphenops</i>]	(Egypt)	EU278157/EU277925/EU278046	E8121.24
<i>C. sepsoides-2</i> [formerly <i>Sphenops</i>]	Zaranik (Egypt)	EU278158/EU277926/EU278047	E2002.5
<i>C. sphenopsiformis-1</i> [f. <i>Sphenops</i>]	Laayoune (Western Sahara)	EU278105/EU277873	E4113.37
<i>C. sphenopsiformis-2</i> [f. <i>Sphenops</i>]	1 km after Massa, direction Sidi Rbat (Morocco)	EU278107/EU277875/EU278031	E8121.26
<i>C. sphenopsiformis-3</i> [f. <i>Sphenops</i>]	1 km after Massa, direction Sidi Rbat (Morocco)	EU278108/EU277876/EU278032	E8121.27 (BEV6403)
<i>C. sphenopsiformis-4</i> [f. <i>Sphenops</i>]	Tan-Tan (Morocco)	EU278106/EU277874	E4113.33
<i>C. sphenopsiformis-5</i> [f. <i>Sphenops</i>]	Laayoune (Western Sahara)	EU278109/EU277877	E4113.35
<i>C. sphenopsiformis-6</i> [f. <i>Sphenops</i>]	Laayoune (Western Sahara)	EU278110/EU277878	E4113.36
<i>C. mauritanicus-1</i>	Coast, 8 km W of Ras el Ma (Morocco)	EU278203/EU277971/EU278060	E2506.3
<i>C. mauritanicus-2</i>	Coast, 2 km W of Ras el Ma (Morocco)	EU278201/EU277969	E2506.6
<i>C. mauritanicus-3</i>	Coast, Ras el Ma (Morocco)	EU278200/EU277968	E2506.4
<i>C. mauritanicus-4</i>	Coast, 8 km W of Ras el Ma (Morocco)	EU278202/EU277970	E2506.5
<i>C. guentheri</i>	Judean Mountains, near Nahal Qetalav (Israel)	EU278233/EU278001	E04045.1 (HUIR-23322)
<i>C. minutus-1</i>	5 km after Debdou, Direction Mountain (Morocco)	EU278204/EU277972/EU278061	E2506.7
<i>C. minutus-2</i>	Taffert, Jebel Bou Iblane (Morocco)	EU278205/EU277973/EU278062	E2506.9
<i>C. minutus-3</i>	(Azrou, Morocco)	EU278206/EU277974/EU278063	E5055.1
<i>C. mertensi-1</i>	Ain Soltane (Tunisia)	EU278209/EU277977	E08045.3
<i>C. mertensi-2</i>	Ain Soltane (Tunisia)	EU278207/EU277975/EU278064	E08045.1
<i>C. mertensi-3</i>	Ain Soltane (Tunisia)	EU278208/EU277976	E08045.2
<i>C. chalcides chalcides-1</i>	Piombino (Italy)	EU278212/EU277980	E8121.2 (BEV5996)

(continued on next page)

Table 1 (continued)

Taxa	Locality	GenBank Accession Nos. cytb/12S/16S	Reference (Museum number)
<i>C. chalcides chalcides-2</i>	(Italy)	EU278210/EU277978	E8121.1 (BEV5997)
<i>C. chalcides chalcides-3</i>	Giglio Island (Italy)	EU278211/EU277979	E2411.2
<i>C. chalcides vittatus-1</i>	Tunis (Tunisia)	EU278216/EU277984/EU278065	E8121.5 (BEV6013)
<i>C. chalcides vittatus-2</i>	Between Nuoro and Pto. Marieri, Sardinia (Italy)	EU278215/EU277983	E23056.2
<i>C. chalcides vittatus-3</i>	Sardinia (Italy)	EU278213/EU277981	E8121.4 (BEV5999)
<i>C. chalcides vittatus-4</i>	Between Nuoro and Pto. Marieri, Sardinia (Italy)	EU278214/EU277982	E23056.1
<i>C. pseudostriatius-1</i>	3 km after Skhirat, direction Sidi Bettache (Morocco)	EU278217/EU277985/EU278066	E2806.4
<i>C. pseudostriatius-2</i>	Tarmilete, after Quimes (Morocco)	EU278218/EU277986	E8121.7 (BEV6015)
<i>C. striatus-1</i>	Tarifa (Spain)	EU278232/EU278000/EU278068	E3007.4
<i>C. striatus-2</i>	Puerto de Santa María, Cádiz (Spain)	EU278231/EU277999	E3007.5
<i>C. striatus-3</i>	Páramo de Masa, Burgos (Spain)	EU278230/EU277998	E1026.4
<i>C. striatus-4</i>	South of Pamplona, Navarra (Spain)	EU278223/EU277991	E1026.6
<i>C. striatus-5</i>	Dam of Gamboa, Vitoria (Spain)	EU278222/EU277990	E1026.5
<i>C. striatus-6</i>	Cazeville, Hérault (France)	EU278221/EU277989	E3007.3
<i>C. striatus-7</i>	Montesalgueiro, Galicia (Spain)	EU278220/EU277988	E8121.12
<i>C. striatus-8</i>	South of Cabanes de Cambon, Hérault (France)	EU278219/EU277987	E8121.9 (BEV6012)
<i>C. striatus-9</i>	Gibraleón, Huelva (Spain)	EU278228/EU277996	E8121.11
<i>C. striatus-10</i>	San Juan del Puerto, Huelva (Spain)	EU278229/EU277997	E8121.13
<i>C. striatus-11</i>	Candelario, Salamanca (Spain)	EU278227/EU277995/EU278067	E3007.6
<i>C. striatus-12</i>	Bienservida, Albacete (Spain)	EU278226/EU277994	E8121.15
<i>C. striatus-13</i>	Torcal de Antequera, Málaga (Spain)	EU278224/EU277992	E8121.10
<i>C. striatus-14</i>	Puerto de Tiscar, Jaén (Spain)	EU278225/EU277993	E8121.14
<i>C. colosii-1</i>	Mokrisset (Morocco)	EU278162/EU277930/EU278049	E3091.4
<i>C. colosii-2</i>	12 km SE. of Tetouan (Morocco)	EU278161/EU277929/EU278048	E3091.3
<i>C. colosii-3</i>	12 km SE. of Tetouan (Morocco)	EU278159/EU277927	E3091.1
<i>C. colosii-4</i>	12 km SE. of Tetouan (Morocco)	EU278160/EU277928	E3091.2
<i>C. parallelus</i>	2 km E. Ras el Ma (Morocco)	EU278153/EU277921/EU278043	E2806.1
<i>C. lanzai-1</i>	Azrou (Morocco)	EU278152/EU277920/EU278042	E2506.25
<i>C. lanzai-2</i>	Azrou (Morocco)	EU278151/EU277919	E2506.24
<i>C. lanzai-3</i>	Azrou (Morocco)	EU278149/EU277917	E2506.22
<i>C. lanzai-4</i>	Azrou (Morocco)	EU278150/EU277918	E2506.23
<i>C. bedriagai bedriagai-1</i>	Cardena, Córdoba (Spain)	EU278147/EU277915	E7061.13
<i>C. bedriagai bedriagai-2</i>	La Aliseda, Jaén (Spain)	EU278148/EU277916/EU278041	E1106.9
<i>C. bedriagai bedriagai-3</i>	Rodalquilar, Almería (Spain)	EU278145/EU277913	E7061.5
<i>C. bedriagai bedriagai-4</i>	Castro de Filabres, Almería (Spain)	EU278146/EU277914	E7061.6
<i>C. bedriagai bedriagai-5</i>	Calar del Mundo, Albacete (Spain)	EU278143/EU277911	E7061.3
<i>C. bedriagai bedriagai-6</i>	El Alquian, Almería (Spain)	EU278144/EU277912	E7061.4
<i>C. bedriagai bedriagai-7</i>	Caravaca de la Cruz, Murcia (Spain)	EU278141/EU277909	E1106.13
<i>C. bedriagai bedriagai-8</i>	Sierra de Espuña, Murcia (Spain)	EU278142/EU277910	E21095.1
<i>C. bedriagai cobosi-9</i>	El Rincón, Huelva (Spain)	EU278140/EU277908	E4113.8
<i>C. bedriagai cobosi-10</i>	La Rábida, Huelva (Spain)	EU278139/EU277907/EU278040	E1106.2
<i>C. bedriagai cobosi-11</i>	Vilareal de Santo Antonio, Algarve (Portugal)	EU278137/EU277905	E7061.1
<i>C. bedriagai cobosi-12</i>	Pavia (Portugal)	EU278138/EU277906	E7061.2
<i>C. bedriagai cobosi-13</i>	Marbella, Málaga (Spain)	EU278135/EU277903	E1106.10
<i>C. bedriagai cobosi-14</i>	Sayalonga, Málaga (Spain)	EU278136/EU277904	E1106.11
<i>C. bedriagai cobosi-15</i>	Venta del Pobre, Granada (Spain)	EU278134/EU277902	E7061.15
<i>C. bedriagai cobosi-16</i>	Barbate, Cádiz (Spain)	EU278133/EU277901	E7061.7
<i>C. bedriagai cobosi-17</i>	Punta Camarinal, Cádiz (Spain)	EU278131/EU277899	E7061.9

<i>C. bedriagai cobosi-18</i>	Vejer el Palmar, Cádiz (Spain)	EU278132/EU277900	E2411.1
<i>C. bedriagai pistaciae-19</i>	Mina de Valdeinfierno, Córdoba (Spain)	EU278130/EU277898/EU278039	E7061.12
<i>C. bedriagai pistaciae-20</i>	Lucena del Puerto, Huelva (Spain)	EU278126/EU277894	E1106.3
<i>C. bedriagai pistaciae-21</i>	Santa Olalla del Cala, Huelva (Spain)	EU278127/EU277895	E1106.7
<i>C. bedriagai pistaciae-22</i>	Las Chinas, Huelva (Spain)	EU278128/EU277896	E1106.5
<i>C. bedriagai pistaciae-23</i>	Isla de Faro, Pontevedra (Spain)	EU278129/EU277897	E1106.16
<i>C. ocellatus ocellatus-1</i>	Tata (Morocco)	EU278170/EU277938/EU278052	E1906.1
<i>C. ocellatus ocellatus-2</i>	Icht (Morocco)	EU278171/EU277939	E1906.2
<i>C. ocellatus tiligugu-3</i>	Algiers (Algeria)	EU278169/EU277937/EU278051	E0602.2
<i>C. ocellatus tiligugu-4</i>	Ras el Ma, W. of Saidia (Morocco)	EU278166/EU277934	E2006.10
<i>C. ocellatus subtypicus-5</i>	8 km N. of Taforalt (Morocco)	EU278168/EU277936/EU278050	E2006.2
<i>C. ocellatus tiligugu-6</i>	Ras el Ma, W. of Saidia (Morocco)	EU278167/EU277935	E2006.13
<i>C. ocellatus subtypicus-7</i>	(Morocco)	EU278165/EU277933	E2006.1
<i>C. ocellatus tiligugu-8</i>	Molouya bridge, close to Ras el Ma (Morocco)	EU278163/EU277931	E2006.12
<i>C. ocellatus subtypicus-9</i>	19 km W. of El Aioum (Morocco)	EU278164/EU277932	E0602.9
<i>C. ocellatus ocellatus-10</i>	Southern Egypt (Egypt)	EU278172/EU277940	E2411.19
<i>C. ocellatus ocellatus-11</i>	Around Ayoun, El Atrous (Mauritania)	EU278173/EU277941	E2002.1
<i>C. ocellatus ocellatus-12</i>	Around Ayoun, El Atrous (Mauritania)	EU278174/EU277942	E2002.2
<i>C. ocellatus ocellatus-13</i>	Negev Desert, Nahal Paran (Israel)	EU278183/EU277951/EU278055	E04045.4 (HUIR-19586)
<i>C. ocellatus ocellatus-14</i>	(Egypt)	EU278181/EU277949/EU278054	E1009.1
<i>C. ocellatus ocellatus-15</i>	Coastal Plain, Ziqqim sands (Israel)	EU278182/EU277950	E04045.2 (HUIR-19923)
<i>C. ocellatus ocellatus-16</i>	Negev Desert, Nizzana sands (Israel)	EU278184/EU277952	E04045.5 (HUIR-19328)
<i>C. ocellatus ocellatus-17</i>	Karaotlak in the Euphrates valley (Turkey)	EU278180/EU277948	E2411.18 (BEV1595)
<i>C. ocellatus ocellatus-18</i>	(Cyprus)	EU278175/EU277943/EU278053	E2006.3
<i>C. ocellatus ocellatus-19</i>	(Cyprus)	EU278176/EU277944	E2006.4
<i>C. ocellatus ocellatus-20</i>	Gizeh (Egypt)	EU278178/EU277946	E1906.9
<i>C. ocellatus ocellatus-21</i>	(Egypt)	EU278177/EU277945	E1009.3
<i>C. ocellatus ocellatus-22</i>	(Egypt)	EU278179/EU277947	E1009.2
<i>C. ocellatus ocellatus-23</i>	Tozeur (Tunisia)	EU278198/EU277966/EU278059	E1906.7
<i>C. ocellatus ocellatus-24</i>	Tozeur (Tunisia)	EU278199/EU277967	E1906.8
<i>C. ocellatus ocellatus-25</i>	140 km from Sfax, direction to Gafsa (Tunisia)	EU278193/EU277961/EU278058	E1906.6
<i>C. ocellatus tiligugu-26</i>	(Malta)	EU278192/EU277960	E2006.9
<i>C. ocellatus tiligugu-27</i>	Sousse (Tunisia)	EU278197/EU277965	E2006.19
<i>C. ocellatus ocellatus-28</i>	Kerkenah Island (Tunisia)	EU278196/EU277964	E1906.5
<i>C. ocellatus ocellatus-29</i>	Kerkenah Island (Tunisia)	EU278194/EU277962	E1906.3
<i>C. ocellatus ocellatus-30</i>	Kerkenah Island (Tunisia)	EU278195/EU277963	E1906.4
<i>C. ocellatus tiligugu-31</i>	Gerouga (Tunisia)	EU278188/EU277956	E2006.14
<i>C. ocellatus tiligugu-32</i>	Nebur (Tunisia)	EU278189/EU277957	E2006.17
<i>C. ocellatus tiligugu-33</i>	Ain Draham (Tunisia)	EU278190/EU277958/EU278057	E2006.15
<i>C. ocellatus tiligugu-34</i>	Tabarka (Tunisia)	EU278191/EU277959	E2006.18
<i>C. ocellatus tiligugu-35</i>	Sardinia (Italy)	EU278187/EU277955	E2006.7
<i>C. ocellatus tiligugu-36</i>	Sta. Teresa, North of Sardinia (Italy)	EU278185/EU277953	E2006.5
<i>C. ocellatus tiligugu-37</i>	Sardinia (Italy)	EU278186/EU277954/EU278056	E2006.8
<i>C. viridianus-1</i>	Anaga, Tenerife, Canary Islands (Spain)	EU278117/EU277885/EU278036	E2806.10
<i>C. viridianus-2</i>	La Laguna, Tenerife, Canary Islands (Spain)	EU278116/EU277884	E2806.12
<i>C. viridianus-3</i>	Teno, Tenerife, Canary Islands (Spain)	EU278114/EU277882	E2806.11
<i>C. viridianus-4</i>	La Laguna, Tenerife, Canary Islands (Spain)	EU278115/EU277883/EU278035	E2806.14
<i>C. sexlineatus sexlineatus-1</i>	Gran Canaria, Canary Islands (Spain)	Z98038/Y14453/	Cabrera et al. (unpublished)
<i>C. sexlineatus sexlineatus-2</i>	Tauro, Gran Canaria, Canary Islands (Spain)	EU278112/EU277880/EU278034	E2806.8
<i>C. sexlineatus sexlineatus-3</i>	Tauro, Gran Canaria, Canary Islands (Spain)	EU278113/EU277881	E2806.9
<i>C. sexlineatus bistriatus-4</i>	San Nicolas, Gran Canaria, Canary Islands (Spain)	AF054558/AF054530/AF054544	Brown and Pestano (1998)
<i>C. sexlineatus bistriatus-5</i>	Santa Lucía, Gran Canaria, Canary Islands (Spain)	AF054559/AF054531/AF054545	Brown and Pestano (1998)

(continued on next page)

Table 1 (continued)

Taxa	Locality	GenBank Accession Nos. cytb/12S/16S	Reference (Museum number)
<i>C. sexlineatus bistriatus-6</i>	Valleseco, Gran Canaria, Canary Islands (Spain)	EU278111/EU277879/EU278033	E2806.6
<i>C. sexlineatus bistriatus-7</i>	San Andrés, Gran Canaria, Canary Islands (Spain)	AF054561/AF054533/AF054547	Brown and Pestano (1998)
<i>C. coeruleopunctatus-1</i>	Hermigua beach, La Gomera, Canary Islands (Spain)	EU278124/EU277892/EU278038	E2806.22
<i>C. coeruleopunctatus-2</i>	Hermigua beach, La Gomera, Canary Islands (Spain)	EU278125/EU277893	E2806.23
<i>C. coeruleopunctatus-3</i>	El Matorral, El Hierro, Canary Islands (Spain)	EU278122/EU277890	E2806.19
<i>C. coeruleopunctatus-4</i>	San Salvador, El Hierro, Canary Islands (Spain)	EU278123/EU277891/EU278037	E2806.20
<i>C. coeruleopunctatus-5</i>	Los Llanillos, El Hierro, Canary Islands (Spain)	EU278118/EU277886	E2806.13
<i>C. coeruleopunctatus-6</i>	San Andres, El Hierro, Canary Islands (Spain)	EU278119/EU277887	E2806.16
<i>C. coeruleopunctatus-7</i>	San Andres, El Hierro, Canary Islands (Spain)	EU278120/EU277888	E2806.17
<i>C. coeruleopunctatus-8</i>	San Andres, El Hierro, Canary Islands (Spain)	EU278121/EU277889	E2806.18
<i>C. simonyi</i>	Fuerteventura, Canary Islands (Spain)	EU278104/EU277872/EU278030	E3007.2
<i>C. mionecton trifasciatus-1</i>	Sidi Ifni (Morocco)	EU278102/EU277870/EU278029	E2506.18
<i>C. mionecton trifasciatus-2</i>	21 km North of Tiznit (Morocco)	EU278101/EU277869	E2506.17
<i>C. mionecton trifasciatus-3</i>	Tamrhakht, Close to Agadir (Morocco)	EU278103/EU277871	E2506.16
<i>C. mionecton mionecton-1</i>	Cap Rhir (Morocco)	EU278099/EU277867	E2506.11
<i>C. mionecton mionecton-2</i>	16 km S. of Essaouira (Morocco)	EU278100/EU277868/EU278028	E2506.12
<i>C. mionecton mionecton-3</i>	Marrakech (Morocco)	AF054555/AF054527/AF054541	Brown and Pestano (1998)
<i>C. mionecton mionecton-4</i>	Cap Rhir (Morocco)	EU278098/EU277866/EU278027	E2506.10
<i>C. mionecton mionecton-5</i>	16 km S. of Essaouira (Morocco)	EU278096/EU277864	E2506.13
<i>C. mionecton mionecton-6</i>	South of Safi (Morocco)	EU278097/EU277865	E2411.4
<i>C. manueli-1</i>	Sidi Ifni (Morocco)	EU278089/EU277857/EU278023	E2506.1
<i>C. manueli-2</i>	Sidi Ifni (Morocco)	EU278087/EU277855	E2506.2
<i>C. manueli-3</i>	Sidi Ifni (Morocco)	EU278088/EU277856	E4113.23
<i>C. polylepis-1</i>	Azemmour (Morocco)	EU278092/EU277860	E2806.2
<i>C. polylepis-2</i>	Marrakech (Morocco)	AF054556/AF054528/	Brown and Pestano (1998)
<i>C. montanus</i>	5 km N. of Oukaimeden (Morocco)	EU278090/EU277858	E2506.29
<i>C. polylepis-3</i>	25 km N. of Marrakech (Morocco)	EU278091/EU277859/EU278024	E14124.1
<i>C. polylepis-4</i>	Sidi Azigza (Morocco)	EU278093/EU277861/EU278025	E2506.21
<i>C. polylepis-5</i>	Oulmes (Morocco)	EU278094/EU277862	E2806.3
<i>C. polylepis-6</i>	(Morocco)	EU278095/EU277863/EU278026	E14124.2
<i>C. polylepis-7</i>	(Morocco)	Z98039/Y14448/	Cabrera et al. (unpublished)

Acronyms:BEV:Herptological Collection of the Ecologie et Biogéographie des Vertébrés team of EPHE-UMR 5175, Montpellier, France; HUIR: The Scientific Collection of Natural History of the Hebrew University of Jerusalem.

et al., 1999, 2000). Primers used in both amplification and sequencing were S1F (5'-TTC AAC TAC AAA AAC CTA ATG ACC C-3') and cytochrome *b2* (Kocher et al., 1989) for cytochrome *b*; 12Sa and 12Sb (Kocher et al., 1989) for the 12S rRNA gene and 16Sar-5' and 16Sbr-3' (Palumbi, 1996) for the 16S rRNA gene.

2.2. Phylogenetic analyses

DNA sequences were aligned using ClustalX (Thompson et al., 1997) with default parameters (gap opening = 10; gap extension = 0.2). All the *cytb* 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, suggesting that they were probably all functional. Although some gaps were postulated in order to resolve length differences in the 12S and 16S rRNA gene fragments, all positions could be unambiguously aligned and were therefore included in the analyses.

Phylogenetic analyses were performed using maximum likelihood (ML) and Bayesian analyses. MODELTEST (Posada and Crandall, 1998) was used to select the most appropriate model of sequence evolution using the Akaike information criterion. This was the Tamura and Nei (TrN) model, taking into account the shape of the gamma distribution (G) and the number of invariable sites (I) for the data set containing the *cytb* + 12S genes and the GTR + I + G model for the data set containing *cytb* + 12S + 16S, and for all the independent partitions (*cytb*, 12S, 16S). ML analyses were performed with PHYML v. 2.4.4 (Guindon and Gascuel, 2003) with model parameters fitted to the data by likelihood maximization. Reliability of the ML trees was assessed by bootstrap analysis (Felsenstein, 1985), involving 1000 replications.

Bayesian analyses were performed on MRBAYES v. 3.1.2 (Huelsenbeck and Ronquist, 2001). For the combined analysis each partition had its own model of sequence evolution and model parameters (see above). Four incrementally heated Markov chains with the default heating values were used. All analyses started with randomly generated trees and ran for 2×10^6 generations in two independent runs with samplings at intervals of 100 generations that produced 20,000 trees. After verifying that stationarity had been reached, both in terms of likelihood scores and parameter estimation, the first 3500 trees were discarded in both independent runs and the combined analyses, and a majority rule consensus tree was generated from the remaining 16,500 (post-burnin) trees. The frequency of any particular clade among the individual trees contributing to the consensus tree represents the posterior probability (pp) of that clade (Huelsenbeck and Ronquist, 2001); only values equal to or above 95% were considered to indicate sufficient support (Wilcox et al., 2002).

Topological incongruence among partitions was tested using a reciprocal 70% bootstrap proportion or a 95% pp

threshold (Mason-Gamer and Kellogg, 1996). Topological conflicts were considered significant if two different relationships for the same set of taxa were both supported with bootstrap values $\geq 70\%$ or pp values $\geq 95\%$.

Topological constraints to test alternative topologies were constructed using MacClade v.4.0 (Maddison and Maddison, 2000) and compared with optimal topologies using the Shimodaira–Hasegawa (SH) (Shimodaira and Hasegawa, 1999) test implemented in PAUP*4.0b10 (Swofford, 1998).

Parsimony reconstructions of ancestral states of morphological and ecological traits were performed in MacClade 4.0 (Maddison and Maddison, 2000).

2.3. Estimation of divergence times

Divergence times were estimated for the different lineages recovered by the analysis of the *cytb* + 12S data set using the computer program r8sb v1.6.4 (Sanderson, 1997, 2002). This program implements several methods for estimating absolute rates of molecular evolution, ranging from standard maximum likelihood ones to more experimental semiparametric and nonparametric methods, which relax the stringency of the clock assumptions using smoothing methods. One of the advantages of this program is that, through a cross-validation test, it allows the user to explore the fidelity with which any of these methods explain the branch length variation (Sanderson, 2002). This procedure removes each terminal branch in turn, estimates the remaining parameters of the model without that branch, predicts the anticipated number of substitutions on the pruned branch and reports the performance of these predictions as a cross-validation score, which allows the user to select the method that best explains the branch length variation (Sanderson, 2002).

To estimate absolute rates, we used a single calibration point based on the assumption that divergence between the *Chalcides* population endemic to the island of La Gomera (Canary Islands) and that on the island of El Hierro (Canary Islands) began approximately 1 Ma, soon after El Hierro was formed (Guillou et al., 1996). These populations are suitable for use in calibration as they form a clade with low intrapopulational variability (Brown and Pestano, 1998). Apart from the assumption that El Hierro was colonized soon after its origin, factors that could affect clock calibrations include stochastic variation at low levels of sequence divergence and the possibility of extinct or unsampled lineages (Emerson et al., 2000; Emerson, 2002), although there is no evidence of any of these factors acting in *Chalcides* (Brown and Pestano, 1998; this study). It is important to bear in mind that dates in general are maximal, as calibration of the molecular clock is based on the assumption that colonization of El Hierro Island in the Canaries by *Chalcides* occurred soon after the island rose from the sea about 1 Ma. If colonization actually occurred more recently than this, actual dates would be more recent. Within this constraint, a maximum date for

colonizations elsewhere is the time when an island endemic separated from its nearest relative. A minimum is provided by the date that any intra-island cladogenesis began, but this is likely to be an under-estimate, since not all living daughter lineages may have been sampled and many earlier ones may be extinct. The possibility of human introduction is based on very low divergence from the source area.

3. Results and discussion

Independent analyses of the three-gene fragments (*cytb*, 12S and 16S) showed that there is no topological conflict among partitions (Mason-Gamer and Kellogg, 1996, see above) and therefore the genes could be analyzed in combination. The data set consisting of *cytb* + 12S gene fragments for all 179 individual skinks comprised 788 bp of sequence (396 bp of *cytb* and 392 of 12S), of which 364 positions were variable (193 of *cytb* and 171 of 12S) and 333 parsimony-informative (176 of *cytb* and 157 of 12S). The results of the ML and Bayesian analysis of these data are shown in Figs. 1–6. The second data set comprised all three-gene fragments (*cytb* + 12S + 16S) from a subset of 68 individuals selected to represent the diversity shown in the two-gene tree. It consisted of 1325 bp of sequence

(396 *cytb*, 392 12S and 537 of 16S), of which 515 were variable (179 of *cytb*, 160 of 12S and 176 of 16S) and 464 parsimony-informative (166 *cytb*, 144 12S and 154 16S). ML and Bayesian analyses of this three-gene data set produced very similar topologies to those from the two-gene set, but bootstrap and Bayesian posterior probability values are often higher (see Figs. 1–6).

The tree was rooted using two American skink species long assigned to the genus *Eumeces* and now sometimes placed in *Pleistodon* (see Brandley et al., 2005). It comprised two well-supported assemblages (Fig. 1A): one including members of the *E. schneideri* group, *Scincus* and *Scincopus*, and the other comprised representatives of *Chalcides* and *Sphenops*. The phylogenies of these units are discussed in the next section.

In order to clarify how well the data support the temporal inferences given in Section 3.1, we have compared the rate of molecular evolution of the mitochondrial region used for this study with the rate of molecular evolution in other studies. The results show that the substitution rate inferred from r8s for the concatenated *cytb* + 12S fragment of the present study is 1.35% per million years. This rate is comparable to the rates calculated for exactly the same mtDNA regions for amphibians of the genus *Pleurodeles* (1.46% per million

Grass-swimming clade of *Chalcides*

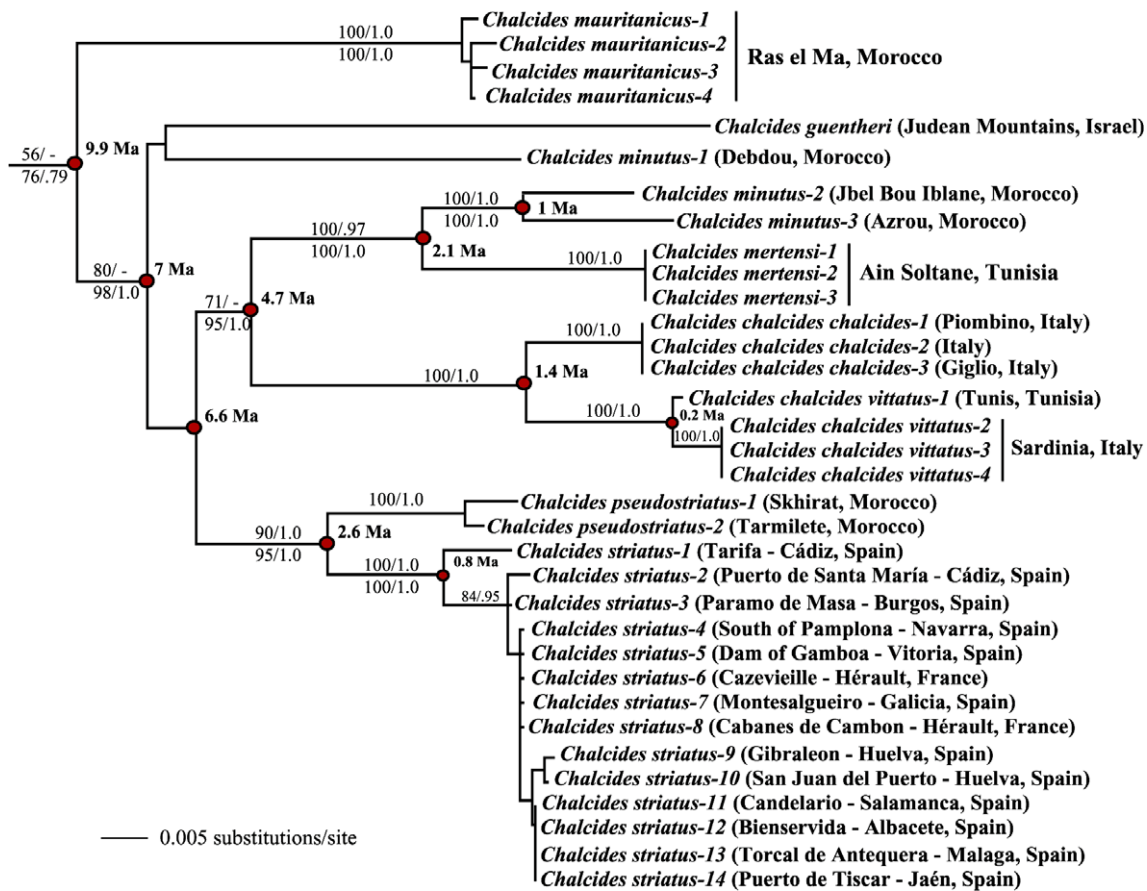


Fig. 2. Enlarged subsection of Fig. 1A showing the detailed phylogenetic relationships in the Grass-swimming clade. Figures above and below some branches and at some nodes are explained in the caption of Fig. 1A.

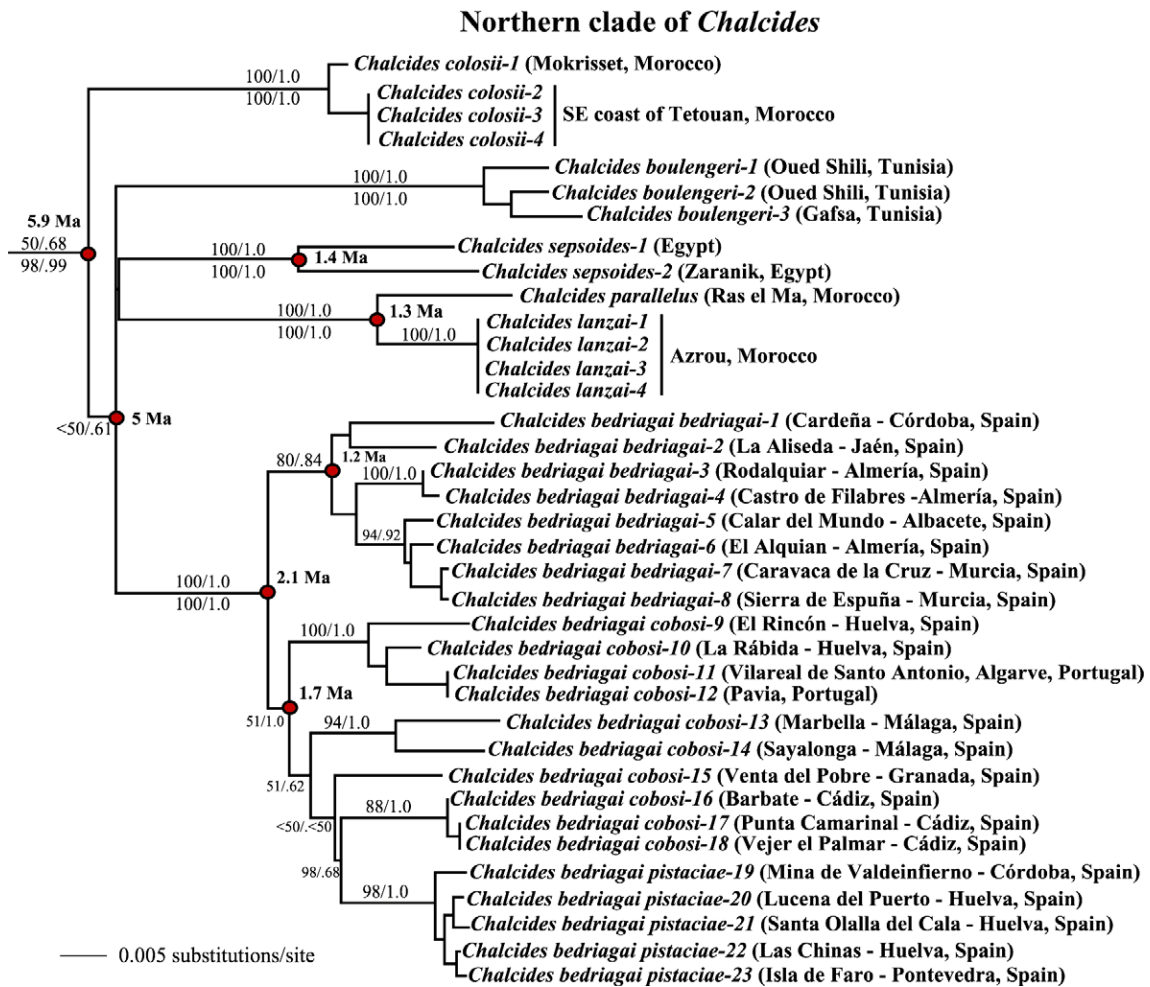


Fig. 3. Enlarged subsection of Fig. 1A showing the detailed phylogenetic relationships in the Northern clade. Figures above and below some branches and at some nodes are explained in the caption of Fig. 1A.

years; Carranza and Arnold, 2004) and *Hydromantes* (0.99% per million years; Carranza et al., in press) and the lacertid lizards of the tribe Lacertini (1.35% per million years; Carranza et al., 2004). In Table 2, we also present the corresponding percent sequence divergence of haplotypes between groups discussed in the section below.

3.1. Phylogeny and historical biogeography

3.1.1. The status of *Sphenops*

The species of *Chalcides* and *Sphenops* form a well-supported holophyletic group, in which *Sphenops* is polyphyletic. Two pairs of species within it, namely *S. boulengeri* plus *S. sepsoides*, and *S. sphenopsiformis* plus *S. delislei*, are more closely related to different groups of *Chalcides* than to each other (Fig. 1A). This makes *Chalcides* as currently recognized paraphyletic, and *Sphenops* is consequently synonymized with it, so *Chalcides* becomes a clade.

3.1.2. The main divisions of *Chalcides*

Within *Chalcides* in this broader sense, there are four well-supported units, which are named here the Grass-

swimming clade, the Northern clade, the *C. ocellatus* clade and the Western clade (see Fig. 1A). The detailed interrelationships among these units are not apparent in the present study, although an investigation of skink phylogeny, using about 2200 bp of mitochondrial DNA sequence but including no more than six *Chalcides* species, placed *C. ocellatus* outside a clade comprising members of the grass-swimming and the Western clades (Brandley et al., 2005).

Taxa of *Chalcides* sampled for this study began to diversify around 10 Ma, probably in the Moroccan region. This is the only area where all four main clades occur and also includes 16 of the 30 accepted species in *Chalcides*, by far the greatest number in any area of its size. Species in this region span the basal phylogenetic splits of the main groups of *Chalcides*. The degree of diversification in Morocco is surprising and much greater than other reptile clades in this region. Thus, among endemic taxa, *Agama impalearis* Boettger, 1874 comprises only two possible species (Brown et al., 2002); the geckos *Quedenfeldtia* Boettger, 1883 and *Saurodactylus* Duméril and Bibron, 1836 have, respectively, two and three species, while *Tarentola* Gray, 1825 has six but not all may have originated in Mor-

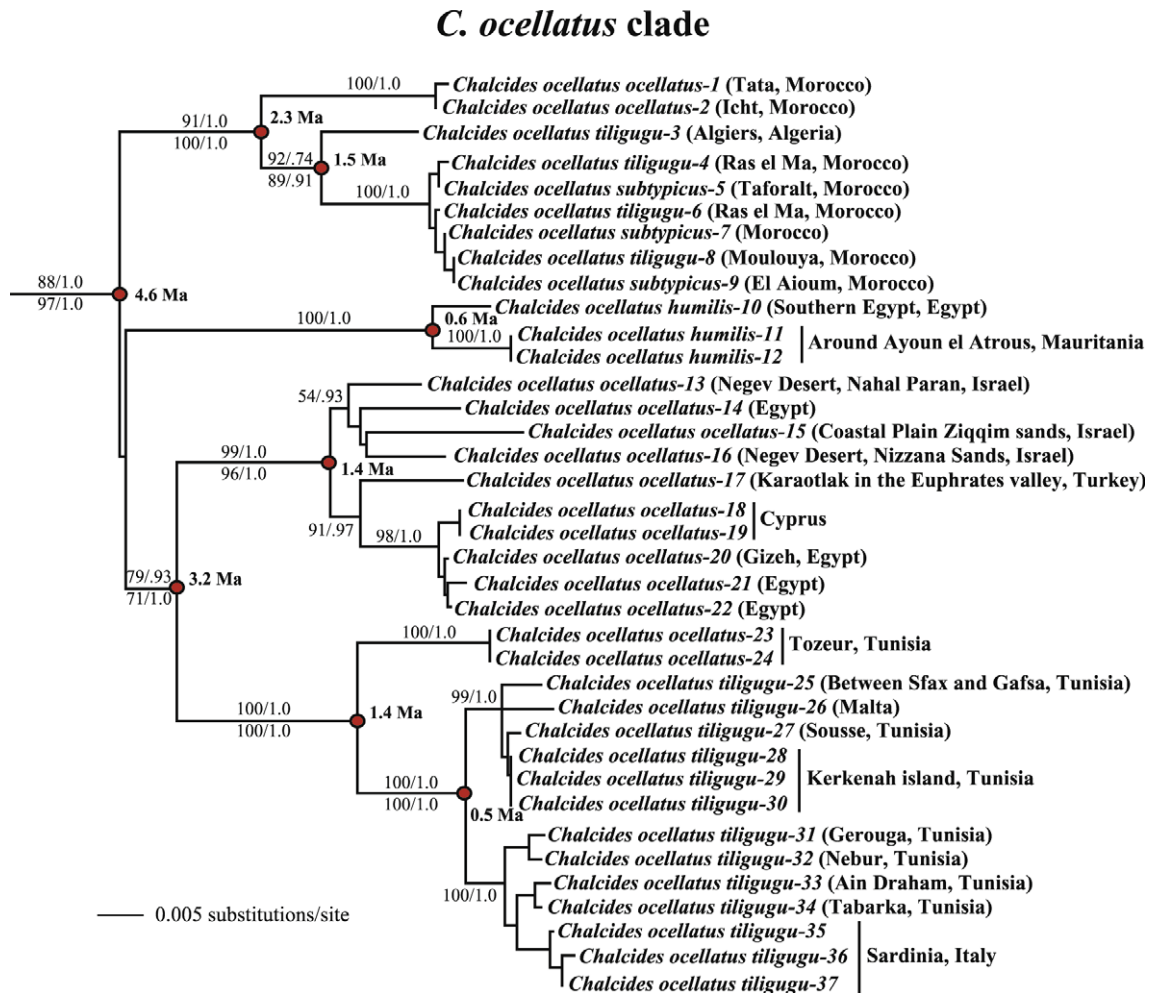


Fig. 4. Enlarged subsection of Fig. 1A showing the detailed phylogenetic relationships in the *C. ocellatus* clade. Figures above and below some branches and at some nodes are explained in the caption of Fig. 1A.

occo (see Bons and Geniez, 1996; Schleich et al., 1996; Carranza et al., 2000, 2002).

3.1.3. The Grass-swimming clade of chalcides (Fig. 2)

Species recognized: *Chalcides mauritanicus* (Duméril and Bibron, 1839); *C. chalcides* (Linnaeus, 1758); *C. minutus* Caputo (1993); *C. mertensi* Klausewitz, (1954); *C. pseudostriatum* Caputo (1993); *C. striatum* (Cuvier, 1829); *C. guentheri* Boulenger (1887).

Many of the phylogenetic results in the present study involving members of the Grass-swimming clade of *Chalcides* are similar to those of Caputo (1993) based on electrophoretic analysis of allozymes. The clade is formed almost exclusively of elongate forms living in mesic habitats (with the exception of *C. mauritanicus*, which is included in the Grass-swimming clade with limited support). It began to diverge soon after the initial breakup of *Chalcides*, around 10 Ma. The early stages appear to have occurred in the northern Maghreb and started with the separation of *C. mauritanicus*, which occurs mainly along a short strip of the coastal area of western Algeria and adjoining Morocco (Fig. 1B). The sister group of *C. mauritanicus* then split into

three units in the Maghreb around 7 Ma; the descendant populations in this region are: (1) *C. pseudostriatum* Caputo, 1993, (2) *C. minutus* s. str. Caputo, 1993, and (3) *C. mertensi* Klausewitz, 1954 + specimens assigned to *C. minutus* from the Middle Atlas, and *C. chalcides vittatus* (Leuckart, 1828) of Tunisia and northwest Libya. At this time, *C. guentheri*, which now occurs in Israel and adjoining areas, 1200 km away from the Maghreb, also separated. A propagule from the *C. pseudostriatum* lineage in Northwest Morocco invaded the Iberian Peninsula about 2.6 Ma to produce *C. striatum* (Cuvier, 1829). At this time, North Africa was separated from southwest Europe by sea, so colonization would have been transmarine.

The samples of *C. striatum* investigated here diversified into two clades around 1 Ma in the south of the Iberian Peninsula. One of the clades remained there, while the other spread to the north of the peninsula and into southern France and northwest Italy. The uniformity of this clade indicates that movement was comparatively rapid and recent, perhaps even being postglacial. It is surprising that the ancestor of *C. pseudostriatum* + *C. striatum*, which our phylogeny suggests might have been in the northern Mag-

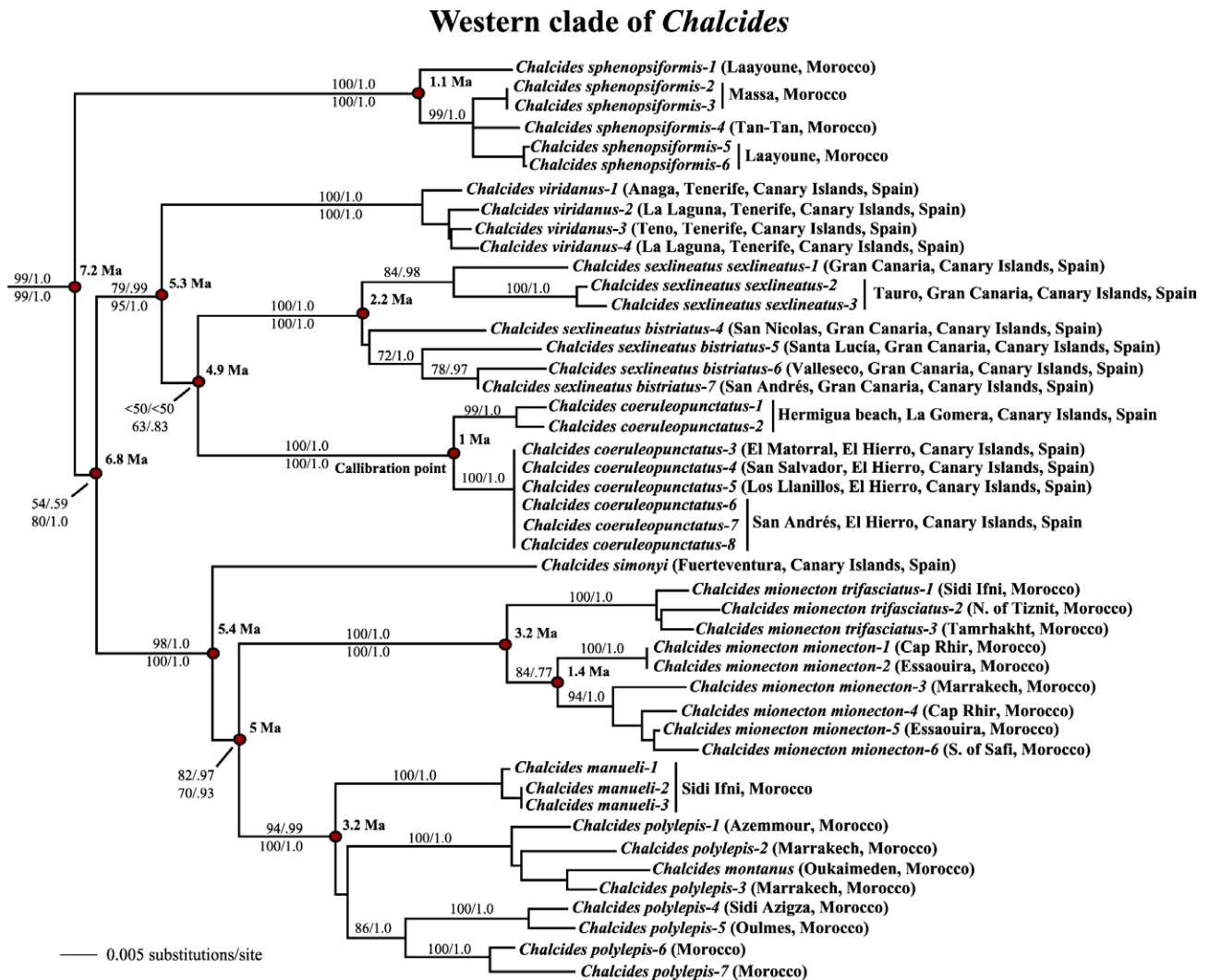


Fig. 5. Enlarged subsection of Fig. 1A showing the detailed phylogenetic relationships in the Western clade. Figures above and below some branches and at some nodes are explained in the caption of Fig. 1A.

hreb at the time, does not appear to have invaded the Iberian Peninsula during the Messinian period, at the end of the Miocene 5.3–5.9 Ma, when there was a land connection between the two areas (Hsü, 1972; Hsü et al., 1973; Krijgsman et al., 1999). It is of course possible that an early lineage of *C. striatus* did invade at this time, but that its descendants became extinct, to be replaced later by a second invasion leading to the present populations. This would parallel events in *Malpolon* snakes (Carranza et al., 2006). *Chalcides minutus* from Debdou, Morocco, the type locality of the species, occupies an isolated position in the Grass-swimming clade, while other specimens assigned to *C. minutus*, from Azrou and Jbel Bou Ibane in the Middle Atlas, are the sister taxon of the mainly Algerian *C. mertensi*. As they have diverged from typical *C. minutus* by more than 10% in *cytb* + 12S and by 5% from Tunisian samples of *C. mertensi*, it may be appropriate to name them as a separate species. But this should be delayed until investigation of the DNA of skinks in the 700 km wide area between these animals and the *C. mertensi* used here.

The unit formed by *C. mertensi* and ‘*C. minutus*’ from the Middle Atlas split from the more eastern *C. chalcides* 4.7 Ma. A propagule from the latter lineage invaded mainland Italy 1.4 Ma, giving rise to *C. c. chalcides*. As with *C. striatus* in Spain, this colonization would have been transmarine. Much more recently, *C. c. vittatus* reached Sardinia from the Tunisian region, possibly by human introduction (Giovannotti et al., 2007). This is also true of the Ocellated skink *Chalcides ocellatus* (Forskål, 1775) (see page 15) and may also be so for the Viperine snake *Natrix maura* (Linnaeus, 1758) in which the population on Sardinia also has its closest relatives in Tunisia (Guicking et al., 2003). The same may also apply to the Horseshoe whip snake *Hemorrhoids hippocrepis* (Linnaeus, 1758).

3.1.4. The Northern clade of *Chalcides* (Fig. 3)

Species recognized: *C. bedriagai* (Boscá, 1880); *C. bouleengeri* Anderson, 1896; *C. colosii* Lanza, 1957, *C. lanzai* Pasteur, 1967; *C. parallelus* (Doumergue, 1901); *C. sepsoides* (Audouin, 1829).

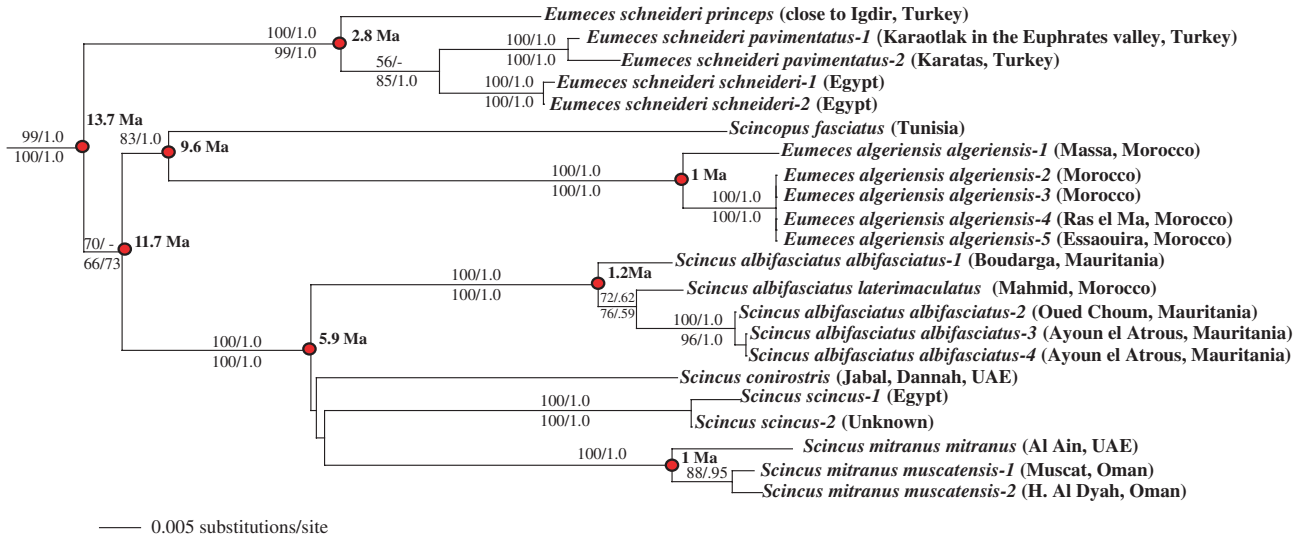
Eumeces schneideri group, *Scincopus* and *Scincus*

Fig. 6. Enlarged subsection of Fig. 1A showing the detailed phylogenetic relationships in the *Eumeces schneideri* group *Scincopus* and *Scincus*. Figures above and below some branches and at some nodes are explained in the caption of Fig. 1A.

Table 2

Percent sequence divergence for the different events discussed in Section 3.1

Event	Cytb + 12S	Cytb	12S
Divergence between the main units of <i>Chalcides</i>	11.1	14.6	7.7
Divergence between the main units of the Grass-swimming clade excluding <i>C. mauritanicus</i>	8.3	12.3	5.6
Divergence between N. African <i>C. pseudostriatum</i> and European <i>C. striatum</i>	3.9	5.8	2.1
Divergence between the two clades of European <i>C. striatum</i>	1.7	3.9	0.6
Divergence between <i>C. mertensilminutus</i> and <i>C. chalcides</i>	8.3	11.0	5.6
Divergence between the main units of the Northern clade of <i>Chalcides</i>	7.7	11.8	4.3
Divergence between <i>C. parallelus</i> and <i>C. lanzai</i>	2.5	4.5	0.6
Divergence of the main units of <i>C. bedriagai</i>	3.7	6.0	1.9
Divergence between xeric and mesic units of Moroccan <i>C. ocellatus</i>	3.9	5.3	2.8
Divergence between the lineages of the mesic clade of Moroccan <i>C. ocellatus</i>	2.4	3.9	1.2
Divergence between Moroccan and more eastern units of <i>C. ocellatus</i>	7.3	11.9	3.7
Divergence between Tunisian and more eastern units of <i>C. ocellatus</i>	5.2	6.1	2.4
Divergence between <i>C. o. humilis</i> and all the other units of <i>C. ocellatus</i>	7.1	11.3	3.2
Divergence between the main units of the Western clade of <i>Chalcides</i>	10.8	14.4	8.0
Divergence between <i>C. mionecton</i> and <i>C. manueli</i> + <i>C. polylepis</i> + <i>C. montanus</i>	9.3	11.9	7.4
Divergence between <i>C. m. mionecton</i> and <i>C. m. trisfasciatus</i>	4.5	6.6	2.9
Divergence between <i>C. manueli</i> and <i>C. polylepis</i> + <i>C. montanus</i>	4.8	6.2	3.3
Divergence between western Canary <i>Chalcides</i> and mainland relatives + <i>C. simonyi</i>	10.7	12.9	9.1
Divergence between <i>C. simonyi</i> of the eastern Canary Islands and mainland relatives	8.2	10.4	6.1
Divergence between the two subspecies of <i>C. sexlineatus</i> from Gran Canaria	3.5	5.5	2.1
Divergence between <i>Scincopus fasciatus</i> and <i>Eumeces algeriensis</i>	12.8	13.5	10.8
Divergence between <i>Scincopus</i> – <i>E. algeriensis</i> and <i>Scincus</i>	13.7	14.9	12.7
Divergence between North African and Arabian species of <i>Scincus</i>	8.1	11.8	6.8
Divergence between <i>S. m. mitranus</i> and <i>S. m. muscatensis</i>	1.8	2.8	0.7

Some members of this clade, also group together in an allozyme study by Caputo et al. (1999). The phylogenetic relationships of the six species of the Northern clade of *Chalcides* are largely unresolved in both ML and Bayesian analyses (see Fig. 3). This clade began to diversify 5–6 Ma, probably initially in northern Morocco where several representatives of the clade occur. The descendant populations in this region are largely allopatric, suggesting a vicariant origin. *C. parallelus* (Doumergue, 1901), which has one of

the smallest geographical distributions in *Chalcides* (Fig. 1B), and the mountain endemic *C. lanzai* Pasteur, 1967 form a well-supported clade, the molecular clock suggesting they diverged just 1.3 Ma. *C. ebneri*, which was not included in the present molecular study occurs allopatrically between the ranges of *C. colosii* and *C. lanzai* suggesting that it too may be a member of the Northern clade.

Most forms in the Northern clade are morphologically primitive, fairly mesic *Chalcides* with restricted distribu-

tions in Morocco, but *C. boulengeri* is adapted to loose sand in dry areas and extends eastwards in such habitats to around the longitude of Tripoli in northwest Libya (Fig. 1B). It is disjunctly replaced in northeast Libya, Egypt, and southern Israel by the similar *C. sepsoides*, which shares many derived morphological features and may be its sister taxon, although this is not apparent from the DNA phylogenies presented here (see Fig. 3). If so, this two-species clade has spread 2300 km from Morocco to the east. The two samples of *C. sepsoides*, which come from Egypt, show about the same genetic divergence as *C. parallelus* and *C. lanzai*, suggesting presence of the species in that region for over a million years.

The DNA investigation of Brandley et al. (2005) included a supposed *C. boulengeri* (under the name *Sphenops boulengeri*), a species belonging to the Northern clade, but its locality, on the border between Mauritania and Senegal, is hundreds of kilometers outside the known distribution of that species. The specimen is likely to be another member of the Western clade, either *C. sphenopsiformis* or *C. delislei*.

Chalcides bedriagai (Boscá, 1880) of the Iberian Peninsula also separated in the initial division of the Northern clade of *Chalcides*, and it may consequently have invaded this area very soon after that event. This date would fit with spread during the Messinian period, 5.3–5.9 Ma, during Mediterranean desiccation. Apparent fossils of *Chalcides* found on Menorca (Boulenger, 1918) may be Pliocene or later (Alcover et al., 1981) and the same may be true of remains from Granada in southern Spain (Barbadillo, 1989). As Menorca has had no land connection to mainland areas including the Iberian Peninsula since the Messinian, its apparent colonization by *Chalcides* also suggests that members of the genus may have reached Menorca by this time. This may have been one of the earliest incursions of skinks into Europe, as although there are abundant skink remains from the Middle Miocene in the Beni Mellal beds of Morocco (Rage, 1976), there are few in contemporaneous European deposits (Rage, 1976).

The samples of living populations of *C. bedriagai* investigated here, which nearly all come from the south of the Iberian Peninsula, appear to have started diverging around 2 Ma. There are at least six well-supported clades: *C. b. bedriagai* in the east, four assigned to the lowland *C. b. cobosii* Valverde, 1997 and one to the montane *C. b. pistaciae* Valverde, 1966 in the west. Among these, *C. b. pistaciae* appears to be the sister lineage of one of the *C. b. cobosii* clades. The samples used in the present study give no evidence of sympatry between the various clades, although the locality of the specimen assigned to *C. b. cobosii* from El Rincon, Huelva is only 40 km from that of a *C. b. pistaciae* from Lucena del Puerto in the same province. A further sample of *C. b. pistaciae* also occurs 600 km away, in northwest Spain, at Pontevedra. The genetic uniformity of *C. b. pistaciae* over such a large distance may indicate recent postglacial spread to the north, as with *C. striatus* of the Grass-swimming clade (Fig. 2).

More samples from the north of the range of *C. bedriagai* are needed to confirm this. Similar genetic uniformity occurs in the northern part of the range of *C. chalcides*, in Italy (Giovannotti et al., 2007).

Endemic northern populations of widespread European species often retreated during the Pleistocene glaciations, and spread again from the south with the onset of warmer conditions (Hewitt, 2000; Taberlet et al., 1998; etc.). In some cases, all or most of the large northern range is now occupied by just one southern haplotype and sometimes minor variants of it, even though several others exist. This is true in Western Europe of such taxa as the Fire Salamander *Salamandra salamandra* (Linnaeus, 1758) (Escoriza et al., 2006), Natterjack toad *Bufo calamita* (Laurenti, 1768) (Rowe et al., 1998) and the Grass Snake *Natrix natrix* (Linnaeus, 1758) (Guicking et al., 2006). However, the lacertid lizards of the genus *Iberolacerta* Arribas, 1999; and the Smooth snake, *Coronella austriaca* Laurenti, 1768, did the reverse and seem to have colonized central and south Iberia from the north during the cold phases of the Pleistocene (Arribas and Carranza, 2004; Arribas et al., 2006; Santos et al., in press).

3.1.5. The *Chalcides ocellatus* clade (Fig. 4)

Species recognized: *Chalcides ocellatus* (Forskål, 1775).

Parsimony indicates that this clade, like the others within *Chalcides*, originated in Morocco, where *C. ocellatus* diverged 2.3 Ma into a southern xeric unit (assigned to *C. o. ocellatus*) and a more mesic northern one (assigned to *C. o. tiligugu* (Gmelin, 1787) and *C. o. subtypicus* Werner, 1909). The latter unit diversified 1.5 Ma and now extends at least as far east as Algiers. An older independent lineage spread eastwards into the Tunisian region about 4.6 Ma. The separation of this Tunisian lineage from Moroccan *C. ocellatus* is roughly synchronous with the similar divergence of *C. chalcides* and *C. mertensi* (Grass-swimming clade, Fig. 2) between the western and eastern Maghreb. The snakes *Malpolon monspessulanus* (Hermann, 1804) and *M. insignitus* (Geoffroy, 1827), may also have separated in this region around the same time (Carranza et al., 2006), and the same may be true of the three species of False smooth snake, *Macrotodon* Guichenot, 1850: *M. brevis* (Günther, 1862), *M. abubakeri* Wade, 2001 and *M. mauritanicus* Guichenot, 1846 (Carranza et al., 2004). This suggests there may have been a shared vicariant event in the area.

As in Morocco, there is a southern xeric clade in the Tunisian region and a more mesic northern one, which are currently assigned, respectively, to *C. o. ocellatus* and *C. o. tiligugu*. Members of the northern unit reached both Sardinia and Malta very recently, suggesting that, like *C. c. vittatus*, these populations may result from human introduction. The populations of *C. o. tiligugu* in Sicily and its offshore islands and in Lampedusa, Conigli and Linosa are morphologically similar to northern Tunisian animals and likely to be derived from this region, possibly by human introduction (Schneider, 1981).

Spread from Tunisia further east along the Mediterranean coastal region to northern Egypt appears to have happened around 3 Ma, followed by division into two subclades there around 1.4 Ma. One of these has also reached Israel, and the other Turkey and Cyprus. Interestingly, the Cyprus animals are genetically very similar to Egyptian ones, which may again suggest human introduction or very recent dispersal. Most other populations of the *C. ocellatus* group in southwest Asia, Iraq, Arabia and coastal Iran and Pakistan are morphologically similar to ones from North Egypt and so may ultimately stem from this region, but this has yet to be confirmed. *C. levitoni* Pasteur, 1981 of Southwest Arabia was not included in the present molecular analysis but may be part of the *C. ocellatus* clade.

The clade consisting of south Egyptian and Mauritanian samples of *C. ocellatus humilis* Boulenger, 1896 separated from other units in the *C. ocellatus* group about the time *C. ocellatus* from Morocco and more eastern animals became distinct, around 4.6 Ma. Despite the two samples of *C. o. humilis* being separated by about 4200 km, they are quite similar in the DNA fragments examined, suggesting a relatively recent spread, or that these populations have been in contact until very recently. *Chalcides o. humilis* now has a fragmented range which may be attributable to subsequent aridification. Parsimony suggests an eastward spread of *C. o. humilis* but, because there are only two widely separated DNA samples, there is no additional evidence of direction of spread based on the phylogenetic topology within this clade. Animals with the morphology of *C. o. humilis* also extend to the Red Sea coast of Sudan, and north Eritrea (Boulenger, 1896).

Nearly all populations of the *C. ocellatus* clade are presently assigned to a single species, but they exhibit as much phylogenetic differentiation as the other main groups of *Chalcides* in which six or more species are recognized. There are four holophyletic units within the *C. ocellatus* clade that show an uncorrected divergence of 6–8% from each other in *cytb* + 12S. The recognition of one of these as a full species, *C. humilis*, has already been advocated (Baha el Din, 2006) and, if this is accepted, it would be appropriate to give the others this status. The species name *C. tiligugu* is available for the Tunisian subclade but should not be applied to similar animals in the Moroccan region to which the name *C. subtypicus* Werner, 1909 is applicable.

3.1.6. The Western clade of *Chalcides* (Fig. 5)

Species recognized: *C. manueli* Hediger, 1935; *C. montanus* Werner, 1931; *C. mionecton* (Boettger, 1873); *C. polylepis* Boulenger, 1890; *C. sexlineatus* Steindachner, 1891; *C. simonyi* Steindachner, 1891; *C. sphenopsiformis* (Duméril, 1856); *C. viridanus* (Gravenhorst, 1851); *C. coeruleopunctatus* stat. nov. Salvador, 1975.

The earliest bifurcation in the phylogeny of the Western clade of *Chalcides*, occurred around 7 Ma, and separated more northern forms from *C. sphenopsiformis*, which extends south from Morocco to Senegal. Here its range

approaches that of *C. delislei*, which was not included in the DNA analysis but shares several morphological synapomorphies with *C. sphenopsiformis* and is probably closely related to it. *Chalcides delislei* has spread east from here, through the more sandy parts of the Sahel as far as the Red Sea coast of Sudan 7000 km away (specimens from the Suakin region in the Natural History Museum, London: BMNH 97.10.28.474–478; BMNH 97.10.28.481–485). This long-distance Sahel dispersal parallels that of *C. o. humilis* (see left), and both fit with the very wide West–East distribution of ecological regions in this area (Burgess et al., 2004). The more northern species in the Western clade form a monophyletic group that bifurcated initially around 5.4 Ma. One branch is formed by *C. mionecton* (Boettger, 1873), a form that burrows in sandy soils and which divided into its two recognized subspecies, *C. m. mionecton* and *C. m. trifasciatus* (Chabanaud, 1917) around 3.2 Ma. The other branch comprises a more surface-dwelling lineage that divided 3.2 Ma into *C. manueli* with a restricted range on the Atlantic coast of southern Morocco (Fig. 1B), and two clades assigned to *C. polylepis*. This form is much more widespread, extending east into the High Atlas and Middle Atlas and as far north as Tangiers (Fig. 1B). *Chalcides mionecton*, *C. manueli* and *C. polylepis* are all sympatric in a small area close to the Atlantic coast of Morocco, in the vicinity of Agadir and Essaouira (Ph. Geniez, personal observation).

One clade of *C. polylepis* contains the single sample of the highland *C. montanus* included in phylogenetic analyses here, apparently making *C. polylepis* paraphyletic. Three additional specimens of *C. montanus* with the morphological characteristics of the species and from the same locality are identical in their sequence (Herpetological Collection of the Ecologie et Biogéographie des Vertébrés team of EPHE-UMR 5175, Université de Montpellier, France numbers BEV6024–BEV6026). All these animals exhibit only 1.5% uncorrected divergence in *cytb* + 12S genes from the genetically most similar specimen of *C. polylepis*, compared with a 7% divergence within *C. polylepis* as a whole. It might be thought from this that *C. montanus* is just a highland ecotype of *C. polylepis*, and the two do occur at different altitudes sometimes within a few kilometers of each other (Mateo et al., 1995; Bons and Geniez, 1996). However, *C. montanus* has previously been regarded, on morphological grounds, as most closely related to *C. lanzai* in the Northern clade of *Chalcides*. The two have adjacent ranges, are both montane, are morphologically quite similar, and have even been treated as subspecies (Mateo et al., 1995). One possibility is that *C. montanus* received mitochondrial DNA from *C. polylepis* through introgression, presumably involving male *C. montanus* breeding with female *C. polylepis*. But investigation of both mitochondrial and nuclear genes of animals assigned to *C. montanus*, *C. polylepis* and *C. lanzai* is required to establish their status and phylogenetic relationships. Such work is currently in progress.

There have probably been two independent transmarine colonizations of the Canary Islands by lineages of the Wes-

tern clade from the Atlantic coast of Morocco. Given the topology of the DNA phylogeny, it would be equally parsimonious, in number of events, to assume that there was a single invasion of the islands, followed by return to the mainland by the *C. mionecton*–*C. manueli*–*C. polylepis* clade, however, because oceanic currents, which would have transported the propagules of *Chalcides*, run strongly towards and through the Canary Islands (Guppy, 1917; Juan et al., 2000), a double invasion of these islands appears more likely. If this is accepted, the ancestor of *C. sexlineatus* and *C. viridanus* got to the central and western islands up to 7 Ma, while that of *C. simonyi* reached the eastern islands of Lanzarote and Fuerteventura as long as 5 Ma. In the first case, there appears to have been quite rapid spread 5–7 Ma, to Gran Canaria, Tenerife and La Gomera, islands that were all in existence at this time (Juan et al., 2000). Our results are largely congruent with those of Brown and Pestano (1998) and also suggest that spread further west to the more recent island of El Hierro was much later, after it rose from the sea around 1 Ma (Guillou et al., 1996). *Chalcides* skinks have evidently never reached La Palma, the next youngest island in the Canaries, which appeared around 2 Ma (Juan et al., 2000).

Until now, *Chalcides* from the more western Canary islands were all referred to *C. viridanus*; however animals from La Gomera and El Hierro, often named *C. v. coeruleopunctatus*, are genetically very different from those on Tenerife (10% uncorrected genetic divergence in the *cytb* + 12S genes) and may possibly be more closely related to *C. sexlineatus* of Gran Canaria. The *Chalcides* from the islands of La Gomera and El Hierro are consequently recognized here as a full species, *C. coeruleopunctatus* stat. nov. Genetic divergence within *C. viridanus* on Tenerife suggests that the population from Anaga is distinct from an individual from Teno and two individuals from La Laguna, in northeast Tenerife (Brown et al., 2000).

The present study indicates that *C. sexlineatus* on Gran Canaria divided into northern and southern units around 2.2 Ma. This is in agreement with a previous investigation, using 384 bp of 12S rRNA sequence and 96 individuals from Gran Canaria (Pestano and Brown, 1999). Separation of the two units was attributed by these authors to volcanic activity that commenced around 2.8 Ma.

3.1.7. Sub-Saharan *Chalcides* and the early history of the genus

Five species of *Chalcides* occur south of the Sahara but no DNA samples of these were available for inclusion in the present analysis. They are: *C. bottegi* Boulenger, 1898, of Ethiopia, Kenya and Sudan; *C. ragazzii* Boulenger, 1890, of Djibouti, Eritrea, Ethiopia and northwestern Somalia; *C. pulchellus* Mocquard, 1906 of western Guinea to southeast Senegal and southeastern Mali and *C. thierryi* (Tornier, 1901) of northern Ghana to eastern Nigeria; and *C. armitagei* Boulenger, 1922; of Senegal. The morphological systematics of the first four, which are geographically closer to populations confirmed as members of the *C. ocell-*

atus group, have recently been discussed (Greenbaum, 2005; Greenbaum et al., 2006). It has been suggested that the Senegalese *C. armitagei* is closer to *C. mionecton* (Pasteur, 1981), which would mean that it was a member of the Western clade of *Chalcides*, but this hypothesis needs to be tested using DNA sequence.

The large assemblage of *Chalcides* species investigated here that originated in Morocco must once have been geographically continuous with other more southern members of the larger African-Madagascan clade of non-lygosomine skinks (Brandley et al., 2005), or at least sequentially so. One possibility is that *Chalcides* initially had a more southern distribution, now represented by some or all of the sub-Saharan species in the genus, and the ancestor of the initially Moroccan clade spread northwards. If such an interpretation is correct, the latter clade would be expected to be the sister taxon of the sub-Saharan forms, or nested within a clade otherwise consisting of these.

3.1.8. *Eumeces*, *Scincopus* and *Scincus* (Figs. 1A and 6)

Relationships of the *E. schneideri* group, *Scincopus* and *Scincus* are largely corroborated by another recent DNA study (Schmitz et al., 2004). On our phylogeny, the *E. schneideri* group does not form a clade and falls into two separate units that are geographically disjunct: *E. schneideri* s. lat. from the Middle East to the Maghreb and *E. algeriensis* Peters, 1864 in the west of the latter region. The distinctness of these units is also supported by morphology (Arnold and Leviton, 1977; Caputo et al., 1993a,b,c) and karyology (Caputo et al., 1993a,b,c).

Scincopus is the sister taxon of *E. algeriensis* and *Scincus* groups with this clade with weak bootstrap support (66–70%) and Bayesian posterior probability values below 95%. Within *Scincus*, there are four main units: *S. albifasciatus* Boulenger, 1890, *S. conirostris* Blanford, 1881, *S. scincus* (Linnaeus, 1758), and *S. m. mitranus* Anderson, 1871 + *S. m. muscatensis* Murray, 1886.

The phylogeny suggests that sand-dwelling *Scincopus* and *Scincus* are independently derived from more mesic forms similar to *E. algeriensis*. Grouping of *Scincopus* with the Moroccan *E. algeriensis*, rather than with the more eastern *E. schneideri*, indicates that it entered desert conditions in Africa up to 9.6 Ma, concordant with its present exclusively African range paralleling the invasions of the Sahara by the two lineages of *Chalcides* that gave rise to pairs of species previously placed in *Sphenops* (Fig. 1B). The same may be true of *Scincus*, which diverged from its more mesic relatives 11.7 Ma, but the case is not as strong, because its relationship to *E. algeriensis* and *Scincopus* is less well supported. The basal tetrachotomy in *Scincus* suggests relatively rapid spread over its large North African and Arabian range around 6 Ma. A much later division occurred around 1 Ma between *S. mitranus muscatensis* of east and south Oman and the allopatric *S. mitranus mitranus*, which is widespread in a much larger area of southern and eastern Arabia. This result fits a frequent pattern of vicariance in

reptiles between Oman and more western parts of the Arabian Peninsula, although the estimated dates of separation of the units concerned are very variable.

3.2. *The age of the Sahara Desert*

Direct evidence of the age of the Sahara suggests that the recent hyperarid phase was a comparatively recent phenomenon, perhaps 1–2 My old (Goudie, 2003). However aeolian sand and dust in deep sea cores, taken in the Atlantic Ocean off the northwest African coast, suggest earlier periods of dryness. Fossil sand dunes about 7 My old have also been found deep in the present Sahara, in Chad, interspersed with more mesic strata (Schuster et al., 2006). The estimated dates derived from the present investigation for origin and divergence of desert clades in *Chalcides*, *Scincus* and *Scincopus* agree with this recent evidence of a relatively early origin of the Sahara. The *C. sepsoides*–*boulengeri* clade originated and diverged around 5 Ma; the *C. sphenopsiformis*–*delislei* clade originated about 7 Ma; *Scincus* originated 12 Ma and diverged 6 Ma; and *Scincopus* originated 10 Ma. Desert dwelling *Tarentola* geckos also appear to have evolved around 6 Ma (Carranza et al., 2002) as well as *Hemorrhhois algirus* (Jan, 1863) and *Malpolon moilensis* (Reuss, 1834), the North African xeric adapted sister taxa to, respectively, the more mesic *Hemorrhhois hippocrepis* and *Malpolon monspessulanus* + *M. insignitus* (Carranza et al., 2006). The most psammophilous of all North African snakes, the Sand viper *Cerastes vipera* (Linnaeus, 1758) also appears to have evolved a long time ago (Carranza and Arnold, unpublished). Fragmentation of a more mesic clade, of *Pristurus* geckos Rüppel, 1835, along the southern edge of the desert, and perhaps caused by its appearance, is also estimated at about 7 Ma (Geniez and Arnold, 2006).

3.3. *Morphology and evolution of Chalcides*

3.3.1. *Morphology in relation to environment*

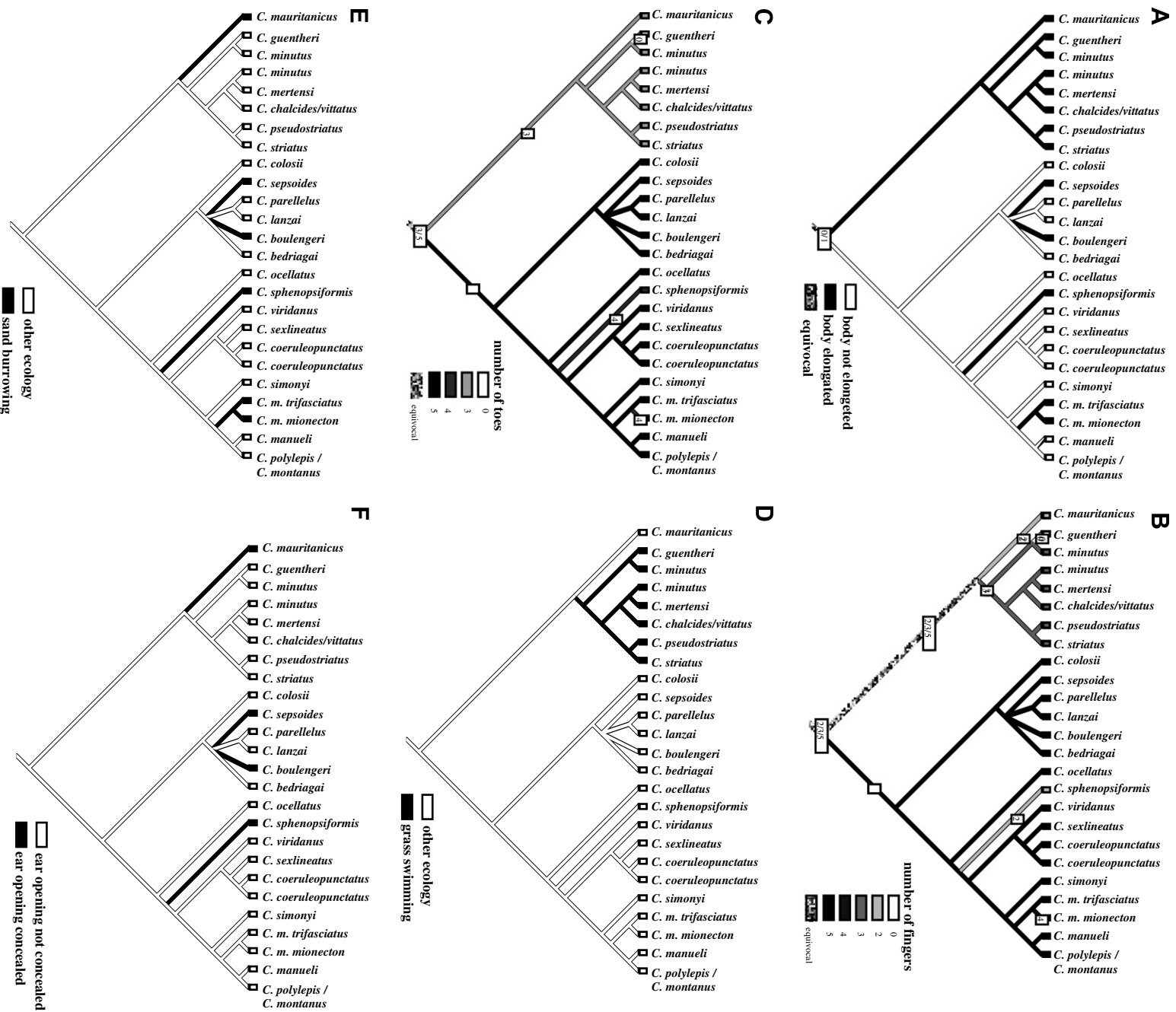
Chalcides appears to be primitively an interface form, spending much time in the topmost loose layers of soil and in litter and often intermittently dense vegetation immediately above it. Probably in connection with the locomotory problems of such habitats, many species have relatively elongate bodies with 34–40 presacral vertebrae, compared with an apparent primitive number in skinks and other lizards of 26 (Caputo et al., 1995). The limbs are quite small in these forms, although nearly always without much reduction of the digits, apart from universal loss of one phalanx of toe 5 of the pes, and another of toe 4 of the manus (Caputo et al., 1995; Greer et al., 1998). This last reduction occurs in some other members of the African and Madagascan group (Brandley et al., 2005) that have generally unreduced legs, and is also found more widely among skinks (Greer and Shea, 2000). The snout tends to be quite pointed in *Chalcides* and, as with many burrowing lizards, the rostral scale is large, with the nostrils bordering its hind margin. Ear openings are well defined but not very large,

and the tympanum is deeply situated. Although primitive *Chalcides* skinks spend a lot of time hidden and some may be active at night, members of the genus appear to be partly heliothermic and bask in the sun at times. They all possess a transparent window in their lower eyelids, usually comprising a single large scale, a feature that enables them to bask with the eyes shut while still retaining vision, something that appears to reduce water loss in dry situations with high evaporation levels (Arnold, 1973; Greer, 1983).

In contrast to this relatively primitive morphology, some *Chalcides* have a much more elongate and slender body with 45–65 presacral vertebrae and very small limbs (especially the front ones) that often show digital reduction (Fig. 1B). Parsimony reconstruction based on the DNA phylogeny (Fig. 7A) indicates that marked elongation has occurred at least four times in *Chalcides*, assuming that the ancestor of the genus was not elongated. Evolution in parallel is also supported by constraint analysis (Table 3), which rejects a single common origin for elongation. *C. armitagei* may represent a fifth case, but this possibility cannot be tested until it is included in a DNA phylogeny of *Chalcides*.

Details of losses of digits associated with reduction in limb size, and of phalanges in those that persist, are given in Fig. 1B (mainly based on observations by Caputo et al. (1995) and Greer et al. (1998)). Parsimony reconstruction (Fig. 7B and C) indicates that a degree of reduction in digit number has evolved independently at least three times. In two of the units involved some species underwent further reduction in the digits of the forelimb, and in one unit this also happened to the digits of the hind limb. Constraint analysis (Table 3) also indicates that digital loss occurred more than once. In apparent contrast to the South American gymnophthalmid genus *Bachia* (Kohlsdorf and Wagner, 2006), there is no evidence for reversal of loss of digits in *Chalcides*.

Very marked elongation of the body and limb reduction occur in two different environmental situations: it occurs in forms that live among grass and other vegetation and move rapidly using serpentine locomotion (grass swimming), and in ones that burrow in loose sandy soil or wind-blown sand, and spend much time below its surface (sand burrowing). The first spatial niche is occupied by most members of the Grass-swimming clade (see Fig. 2), while the second occurs in *C. mauritanicus*, *C. mionecton*, and the species previously placed in *Sphenops*. Parsimony reconstructions of these two life modes (Fig. 7D and E) indicate that while grass swimming has appeared just once, sand-burrowing has evolved a minimum of four times. As with body elongation, the unsampled *C. armitagei* may possibly represent a fifth case of independent origin of the sand burrowing. While grass swimming does not involve reduction of the ear opening, this occurs in sand-burrowing forms in which the aperture is often concealed. Parsimony reconstruction indicates that it has disappeared three times independently (Fig. 7F) and constraint analysis (Table 3) rejects the hypothesis of only a single origin for the condition.



The two-species pairs of *Chalchicomula*, previously considered part of the genus *Sphenops* (*C. sepsoides* + *C. boutergeri*, and *C. sphenopsiformis* + *C. delislei*) exhibit numerous parallel adaptations to life in loose aeolian sand, in addition to body elongation and limb reduction. These include

a very streamlined head profile; a particularly extensive rostral scale that has an uncurred posterior dorsal margin reaching back to the level of the postnasal scales or beyond, rostral and upper labial scales that are sharp-edged and strongly projecting, a countersunk lower jaw, ear openings

Table 3
Statistical support for alternative hypotheses on *Chalcides* phylogeny

Tree	–loglikelihood	Δ – loglikelihood	SH <i>P</i>
Unconstrained ML tree	8565.11189	(Best)	
Monophyletic origin for elongated body	8665.60338	100.49149	0.000
Monophyletic origin for digit loss	8700.36451	135.25262	0.000
Monophyletic origin for loss of ear opening	8672.77641	107.66452	0.000
Monophyletic origin for: <i>C. sepsoides</i> , <i>C. sphenopsiformis</i> , <i>C. boulengeri</i>	8606.24675	82.26962	0.019

P < 0.05 suggests that the constrained and unconstrained solutions are significantly different. SH, Shimodaira and Hasegawa (1999) test; ML, Maximum likelihood.

largely covered by scales, prominent grooves present in the anterior lateral body wall that house the forelimbs when these are laid backwards during serpentine locomotion, and a body with more or less vertical sides which are separated from the flat belly by a lateroventral ridge on each side. These features either facilitate locomotion in loose sand or help keep sand grains out of body openings (Arnold, 1984). In addition, breathing when buried in the sand may be facilitated by shifting respiratory movements from the sides of the thorax to its ventral surface, a feature found in many other lizards that burrow in this habitat (Pough, 1969; Arnold, 1984, 1990, 1995). Similar adaptations occur in other lizards that burrow in aeolian sand, such as *Scincus* (Arnold, 1984, 1990, 1995). *C. mionecton* usually lives in sandy situations that are less extreme than those favored by the species just discussed, but has evolved some of the distinctive traits found in these forms, although usually to a less marked extent. Included are a partly hidden ear opening, an extensive rostral scale and grooves that houses the forelegs when they are turned back. *C. mauritanicus* is less modified still.

The suite of features that have evolved independently in *Chalcides* living in desert sand constitutes an ecomorph (Williams, 1972, 1983), a pattern of morphology that has been acquired independently by ecological analogues living in similar niches. Other ecomorphic resemblances in *Chalcides* include the evolution of large, heavily marked animals with pale dorsolateral stripes and high numbers of scales around the mid-body in relatively mesic situations. These populations have long been placed in a single subspecies, *C. ocellatus tiligugu* but, as shown here, this ecomorph has originated independently in two separate allopatric lineages of the *C. ocellatus* clade (Fig. 4). In the Canary Islands, aspects of color pattern also show parallel correlations with environment, in *C. sexlineatus* on Gran Canaria and *C. viridanus* on Tenerife (Brown et al., 1991).

3.3.2. Reproductive concomitants of elongation

In at least the Grass-swimming clade of *Chalcides* (Fig. 2), elongation of the body is associated with changes in reproductive strategy (Caputo et al., 2000). These forms are viviparous, like other *Chalcides*, but their eggs have a small diameter and mass compared with those of species with more normal body proportions, such as *C. ocellatus*. This may be related to the narrowness of the abdominal

cavity. Perhaps as an evolutionary response to the restricted vitelline resources in small eggs, a nutritional, elliptic placenta or placentome develops during gestation, something that is not found in *Chalcides* with more primitive body morphology. Most members of the Grass-swimming clade have also become sexually dimorphic in adult size, females being larger than males and so capable of accommodating the large litters of young they bear. It is unknown if similar changes occur in other elongate *Chalcides*, which, unlike the Grass-swimming clade, live in desert conditions.

3.3.3. Morphology and sympatry

As noted by Mateo et al. (1995), sympatry of Moroccan species of *Chalcides* usually involves forms with different gross morphology, and this applies to other geographical regions as well. Most commonly, taxa with a primitive body form occur alongside more elongate ones. Within these two main body types, species usually exhibit a largely allopatric pattern of distribution, suggesting that mutual exclusion by the species concerned may be occurring. The initial divergence in the genus about 10 Ma, led to clades with different body forms in the north Maghreb, where the Grass-swimming clade (Fig. 2) occurs alongside forms with relatively primitive morphology in the Northern clade (Fig. 3) and the *C. ocellatus* clade (Fig. 4). Sympatry of species with the two different body types occurs in the Iberian Peninsula, where invasion 5 Ma by the ancestor of *C. bedriagai* (Fig. 3), which had primitive morphology, was followed 2 Ma by colonization by the long-bodied *C. striatus* (Fig. 2). A similar two-species community was formed on Sardinia, where *C. ocellatus tiligugu* (Fig. 4) and *C. c. vittatus* (Fig. 2) have both arrived recently. On the Atlantic coast of Morocco, *C. polylepis* and *C. manueli* with primitive body shapes occur alongside their more elongate sister taxon, *C. mionecton*. Outside Morocco, members of the *C. ocellatus* group may live close to the elongate sand forms *C. sepsoides* and *C. boulengeri* (Fig. 3) and *C. delislei*. Within the same gross body form, coexistence occasionally occurs but usually involves forms with markedly different adult sizes. Cases include: *C. manueli* with *C. polylepis* on the Atlantic coast of Morocco; *C. parallelus* and *C. colosii* with *C. ocellatus* in north Morocco; *C. minutus* s. lat with *C. pseudostriatus* in this area; and may be *C. minutus* s. lat and *C. mertensi* in western Algeria (see Mateo et al., 1995).

for other cases). Areas of sympatry between species with similar body shapes but different body sizes are usually relatively small.

3.4. Why has *Chalcides* been so successful?

If evolutionary success of a clade is judged by high species number, wide geographical range and adaptive radiation involving marked morphological change, *Chalcides* is certainly successful. It has a total of about 39 deep independent lineages (Figs. 2–5), and has a very large geographical distribution, having made several extensive dispersals (Fig. 8). *Chalcides* probably reached the Canary Islands twice and southern Europe at least five times, and there have been at least five long-distance dispersals across North Africa, one of them extending far into southwest Asia. Adaptive shifts have also occurred: into grass swimming and burrowing in a variety of sandy soils. These have been accompanied by large changes in morphology functionally related to such factors as locomotion and reproductive strategy.

The success of *Chalcides* is likely to have stemmed partly from the inherent properties of the lizards themselves. They have effective anti-predator strategies, often a generalized diet, and are sometimes voracious predators (Schleich et al., 1996; Greenbaum and Rassmussen, 2004). Geophysical and climatic events with which they have interacted have also been important. The Moroccan region has appar-

ently given ample opportunities for speciation, perhaps partly related to fragmentation of habitat produced by the orogenic folding that has occurred in the region over the past 20 My or so, producing the Atlas and Rif Mountains and their outliers (Brown et al., 2002). The rock outcropping involved is likely to have fragmented the areas of light soil and vegetation with which *Chalcides* is often associated, and formed barriers that would be difficult for these essentially non-climbing lizards to cross. Also, during the last 10 My, climatic oscillations in North Africa have produced repeated changes in habitat, from heavily vegetated land to desert and back again, promoting rapid changes in distribution and speciation by vicariance (Prentice and Jolly, 2000; Douady et al., 2003; Schuster et al., 2006).

Dispersal of *Chalcides* to areas now separated by sea was facilitated by the regions concerned, like the Canary Islands and southern Europe, being geographically close, so transmarine colonization was relatively easy. It was probably enhanced by the ability of skinks in general to disperse in this way (Carranza and Arnold, 2003; Carranza et al., 2001). The spread of *C. bedriagai* into southwest Europe may also have been aided by the Messinian desiccation of the Mediterranean (see Carranza et al., 2006 for a review).

With the exception of the Grass-swimming clade, most of the overland dispersals in North Africa appear to have followed the development of widespread arid habitats in this region, which bordered the Moroccan centre of origin

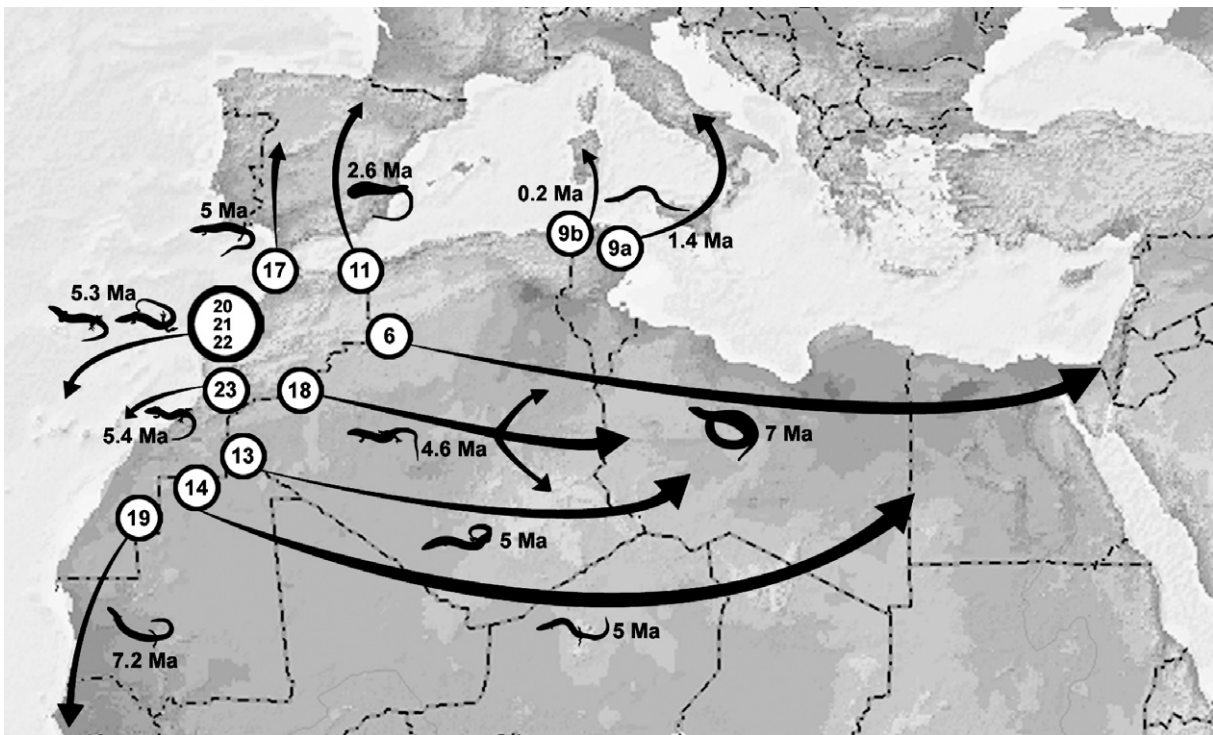


Fig. 8. Main dispersals of *Chalcides* from its Moroccan source region. (Lines do not represent exact dispersal routes, or circles precise areas of origin. Numbers indicate taxa: 6, *C. guentheri*; 9a, *C. c. chalcides*; 9b, *C. c. vittatus*; 11, *C. striatus*; 13, *C. boulengeri*; 14, *C. sepsoides*; 17, *C. bedriagai*; 19, *S. sphenopsiformis*; 20, 21, 22, ancestor of *C. viridanus*, *S. coeruleopunctatus* and *C. sexlineatus*; 23, *C. simonyi*. See Fig. 1 for further information. Taxa 13 and 14 may share an ancestral lineage and constitute a single dispersal. *C. o. humilis* and *C. delislei* may also have spread eastwards along the southern edge of the Sahara.

and to which some *Chalcides* were already adapted. The opportunities for speciation in the Moroccan region may have facilitated the evolution of such adapted forms, after which they could have dispersed through the habitats concerned, either immediately or as the habitats became more extensive. Another favorable environmental factor in the dispersal of *Chalcides* is the absence of other taxa adapted to the kinds of niches it occupied, something that applies as much to the Canaries and Europe as to North Africa. As we have seen, in some cases, dispersal may also have been inadvertently mediated by people; populations of the *C. ocellatus* clade frequently live in cultivated regions and are sometimes accidentally transported with crops.

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