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Systematics and phylogeography of *Acanthodactylus schreiberi* and its relationships with *Acanthodactylus boskianus* (Reptilia: Squamata: Lacertidae)

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Acanthodactylus is a widespread lacertid genus occurring from the Iberian Peninsula and western North Africa to western India including the Middle East, Cyprus, and the Arabian Peninsula. The genus is in dire need of a taxonomic revision, and the phylogenetic relationships amongst and within its species remain unclear. In particular, the taxonomy and relationship of the allopatric, narrow-ranged *Acanthodactylus schreiberi* and its close relative, the widespread *Acanthodactylus boskianus asper*, are poorly understood. We estimated the phylogenetic and phylogeographical structure of *A. schreiberi* across its distribution range, and evaluated its relationships to *A. b. asper*, using mitochondrial and nuclear data. The phylogenetic results indicate that both species are paraphyletic, with *A. schreiberi* nested within *A. b. asper*, and the subspecies *A. schreiberi syriacus* nested within a distinct lineage of *A. b. asper*. We suggest that the group is in need of a taxonomic revision because the identified lineages and genetic diversity are incongruent with the currently recognized taxonomy. We tentatively conclude that *A. schreiberi* is restricted to Cyprus and Turkey, reduced to a single form, and that the populations in Lebanon and Israel belong to *A. b. asper*.

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ADDITIONAL KEYWORDS: convergence – east Mediterranean – ecotype – haplotype network – molecular clock – mtDNA + nDNA lineages – taxonomy.

INTRODUCTION

The genus *Acanthodactylus* Fitzinger, 1834, is commonly known as the fringe-fingered lizards and is the largest genus in the family Lacertidae with over 40 described species (Uetz, 2013). Members of this genus are small- to medium-sized, diurnal, terrestrial, and oviparous species that inhabit semi-arid to desert ecosystems from the Iberian Peninsula, through North Africa, to the Middle East and west India, including Cyprus and the Arabian Peninsula (Salvador, 1982; Sindaco & Jeremčenko, 2008). Four fundamental studies constructed the systematic knowledge of *Acanthodactylus*, mainly based on external morphology, osteological characters, and the morphology of the hemipenes: Boulenger (1918), Salvador (1982), Arnold (1983), and Harris & Arnold (2000). The latter three studies divided the genus into species groups, a division that is commonly used today, although the assignment of some species to groups is debated (e.g. *Acanthodactylus blanfordii* Boulenger, 1918, and *Acanthodactylus masirae* Arnold, 1980; Harris & Arnold, 2000). The systematics of some species groups is unclear and unstable because of high intraspecific variability of some species and morphological convergence of similar

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species (e.g. the description of *Acanthodactylus mechriguensis* Nouira & Blanc, 1999; Fonseca *et al*., 2008). Even though it is fairly easy to assign species to species groups, the boundaries between species and relationships within species groups are often unclear and unresolved (Salvador, 1982; Arnold, 1983; Harris & Arnold, 2000; Crochet, Geniez & Ineich, 2003; Harris, Batista & Carretero, 2004; Fonseca *et al*., 2008, 2009). Thus, the most problematic and interesting issues in *Acanthodactylus* systematics are the relations amongst and within species groups, the taxonomy of the genus, and its biogeography.

The *Acanthodactylus boskianus* species group is a striking case of taxonomic uncertainty. Although it is a small group of only three species, its geographical range is the largest in the genus (Salvador, 1982; Sindaco & Jeremčenko, 2008). It includes *Acanthodactylus boskianus* (Daudin, 1802), *Acanthodactylus schreiberi* Boulenger, 1878 (Salvador, 1982; Arnold, 1983), and *Acanthodactylus nilsoni* Rastegar-Pouyani, 1998. *Acanthodactylus nilsoni* is known only from western Iran (Anderson, 1999). *Acanthodactylus boskianus* is the most widespread species of its genus (~8 000 000 km^2; S. Meiri, unpubl. data), ranging through North Africa and the Sahel, the whole Arabian Peninsula, eastwards to Iran, and northwards to Turkey (Salvador, 1982; Schleich, Kästle & Kabisch, 1996; Rastegar-Pouyani, 1999; Sindaco *et al*., 2000; Sindaco & Jeremčenko, 2008). *Acanthodactylus boskianus* has been divided into five subspecies: *A. boskianus boskianus* (Daudin, 1802) from the Nile delta and parts of Sinai, *A. boskianus asper* (Audouin, 1827) from much of the distribution range of the species, *A. boskianus euphraticus* Boulenger, 1919, from Iraq, *A. boskianus khattensis* Trape & Trape, 2012, from Mauritania, and *A. boskianus nigeriensis* Trape, Chirio & Geniez, 2012, from Niger.

Acanthodactylus schreiberi was described from Cyprus where it is the only representative of *Acanthodactylus*, and it also inhabits south-western Asia. This species has been divided into three allopatric subspecies. The nominate subspecies, *A. schreiberi schreiberi* Boulenger, 1878, is endemic to Cyprus. *Acanthodactylus schreiberi syriacus* Böttger, 1879, inhabits isolated patches of the Mediterranean coastal areas of Israel and southern Lebanon (although its terra typical is given as 'Syria', it does not occur in modern Syria. In the late 19th century 'Syria' included modern-day Syria, Lebanon, and parts of modern-day Israel). *Acanthodactylus schreiberi ataturi* Yalçinkaya & Göçmen, 2012, is known from a single coastal locality in southern Turkey. This population was originally referred to *A. s. schreiberi* by Franzen (1998) because of the morphological similarity to the Cypriot form, and it was later described as a new subspecies by Yalçinkaya & Göçmen (2012).

The huge geographical range of *A. boskianus* includes areas with very different climates (from sub-Mediterranean climate on the sea coasts of North Africa to the hyperarid climate of Central Sahara). This wide range leads to adaptations to different environments, with great geographical variation (Boulenger, 1921; Salvador, 1982; Arnold, 1983; Pincheira-Donoso & Meiri, 2013) and consequent taxonomic confusion. This problem is well known (Salvador, 1982; Arnold, 1983; Baha El Din, 2006) and has great effect when examining closely related species in an attempt to assess their systematic status. Arnold (1983) suggested that *A. boskianus* and *A. schreiberi* might be sister species as they share a relatively high number of primitive features. He also suggested that *A. schreiberi* may have originated as an isolate of *A. boskianus*. Previous morphological studies on the *A. boskianus* species group indicated that the relationship between *A. boskianus* and its sister taxon, *A. schreiberi*, is far from resolved (Salvador, 1982; Arnold, 1983). The most obvious morphological differences between the Cypriot *A. schreiberi schreiberi* and the continental *A. schreiberi syriacus* are the size and degree of keeling of the dorsal and temporal scales (Boulenger, 1918, 1921; Salvador, 1982; Arnold, 1983; Franzen, 1998). Boulenger (1921) decided to unite *A. schreiberi* and *A. syriacus*, until then considered different species, as this difference is not greater than those found in variants of other species. By contrast, Franzen (1998) implied that those intraspecific differences indicate specific distinctiveness. In addition, the great intraspecific morphological variation of *A. boskianus* means that these characters fail to firmly distinguish it from *A. sc. syriacus*. Salvador (1982) presented the geographical variation of *A. boskianus*, admitting that the differences between it and *A. schreiberi* are unresolved and unsatisfactory.

The systematics of many lacertid lizards have recently been re-evaluated using molecular data (e.g. Arnold, Arribas & Carranza, 2007; Kapli *et al*., 2008; Greenbaum *et al*., 2011; Ahmadzadeh *et al*., 2012, 2013). The only molecular phylogenetic study on the entire *Acanthodactylus* genus, however, was published by Harris & Arnold (2000), who suggested that the genus originated in south-west Asia and later dispersed westwards into Africa. This study also indicates that *A. boskianus* may be paraphyletic as samples from Arabia and Morocco formed successive basal branches (Harris & Arnold, 2000). Four additional molecular studies on *Acanthodactylus* were conducted, focusing on *Acanthodactylus erythrurus* and *Acanthodactylus pardalis* species groups, in an attempt to understand the within-group systematics and relationships (Harris *et al*., 2004; Fonseca *et al*., 2008, 2009; Carretero *et al*., 2011). To date, the only molecular study with samples of the *A. boskianus* species group was conducted by Poulakakis *et al*. (2013). They concluded that *A. s. schreiberi* is a

relatively recent colonist in Cyprus, arriving from the mainland through transmarine dispersal around 0.85 Mya. In that study, based solely on *16S rRNA* data, and including a single sample of *A. s. syriacus*, they found that the examined individual branched within the specimens of *A. boskianus asper*. In another study by Trape, Trape & Chirio (2012), also based solely on *16S rRNA* data, one sample of *A. schreiberi* formed a polytomy with the *A. boskianus* samples. These molecular results present an additional dimension to the already enigmatic taxonomic relationships between the populations of *A. schreiberi* and *A. boskianus*.

The present taxonomic status of *A. schreiberi* is therefore unresolved as the differentiation amongst its subspecies is debated (Boulenger, 1921; Franzen, 1998), and the relationship with its closest relative, *A. boskianus*, should be revised.

In order to clarify the systematics and to reveal the phylogenetic relationships between *A. schreiberi* and *A. boskianus* in the eastern Mediterranean, and to determine the role of geological barriers in the evolutionary history of these two species, fragments of two mitochondrial genes [*12S rRNA* (*12S*), *cytochrome b* (*Cytb*)] and three nuclear genes [*melano-cortin 1 receptor* (*MC1R*), *acetylcholinergic receptor Muscarinic 4* (*ACM4*), *oocyte maturation factor MOS* (*c-mos*)] were sequenced and analysed for genetic variation. We aimed to examine the genetic relationships between *A. schreiberi* and the geographically close taxon, the widespread *A. b. asper*, with emphasis on the relations amongst the *A. schreiberi* subspecies.

MATERIAL AND METHODS

DNA EXTRACTION, AMPLIFICATION, AND SEQUENCE ANALYSIS

Samples of the three known subspecies of *A. schreiberi*, from Cyprus, Turkey, Lebanon, and Israel, and samples of *A. b. asper* from North Africa, the Middle East, and Arabia were included in this study (Fig. 1). The localities, specimen codes, and GenBank accession numbers are listed in Table 1. The genus *Acanthodactylus* is divided into three clades (Harris & Arnold, 2000; Pyron, Burbrink & Wiens, 2013; K. Tamar, S. Carranza, R. Sindaco, J. Moravec, JF. Trape & S. Meriri, unpubl. data); hence, representatives of five species from the same clade as the *A. boskianus* species group were used as the closest outgroups (i.e. *Acanthodactylus blanfordii*, *Acanthodactylus cantoris*, *Acanthodactylus felicis*, *Acanthodactylus masirae*, and *Acanthodactylus opheodurus*). In addition, we used samples of *Acanthodactylus scutellatus*, from another clade, as the distant outgroup and used it to root the tree.

Genomic DNA was isolated from ethanol-preserved tissue samples using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA). All individuals were sequenced for two mitochondrial gene fragments, *12S* and *Cytb*, and three nuclear gene fragments, *MC1R*, *ACM4*, and *c-mos*. Gene fragments were amplified and sequenced for both strands using published primers. The primers, references, and PCR conditions are listed in Table S1.

Chromatographs were checked manually, assembled and edited using GENEIOUS 5.3.6 (Biomatter Ltd). For the nuclear genes *MC1R*, *ACM4*, and *c-mos*, heterozygous individuals were identified and coded according to the International Union of Pure and Applied Chemistry (IUPAC) ambiguity codes. Coding gene fragments (*Cytb*, c-*mos*, *ACM4*, and *MC1R*) were translated into amino acids. No stop codons were observed, suggesting that the sequences were all functional. DNA sequences were aligned for each gene independently using the online version of MAFFT v. 6 (Katoh & Toh, 2008) with default parameters. In order to remove regions without specific conservation and poorly aligned positions of the 12S rRNA we used G-blocks (Castresana, 2000) with low stringency options (Talavera & Castresana, 2007). Inter- and intraspecific uncorrected *p*-distances and the number of variable and parsimony informative sites were calculated in MEGA v. 5 (Tamura *et al*., 2011).

PHYLOGENETIC ANALYSES AND HYPOTHESIS TESTING

Phylogenetic analyses were performed for the complete data set simultaneously both with partitions based on genes and partitions specified using PartitionFinder v. 1.1.0 (Lanfear *et al*., 2012). PartitionFinder was performed with the following parameters: linked branch length; all models; Bayesian information criterion (BIC) model selection; all schemes search; data blocks of the complete 12S and by codons for the other protein-coding genes (*Cytb*, *MC1R*, *ACM4*, *c-mos*). JModelTest v. 0.1.1 (Posada, 2008) was used to select the most appropriate model of sequence evolution under the Akaike information criterion (Akaike, 1973) for each partition. A summary of DNA partitions and relevant models is listed in Table 2.

Phylogenetic analyses were performed using maximum likelihood (ML) and Bayesian inference (BI) methods. ML analyses were performed with RAxML v. 7.4.2 (Stamatakis, 2006) using RAxMLGUI v. 1.3 (Silvestro & Michalak, 2012) with a general time-reversible + Gamma distribution (GTR + G) model of evolution, parameters estimated independently for each partition, and 100 addition replicates. Reliability of the ML tree was assessed by bootstrap analysis (Felsenstein, 1985) including 1000 replications. Bayesian analyses were performed with MrBayes v. 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) with the best-fitting models applied to each partitionand all

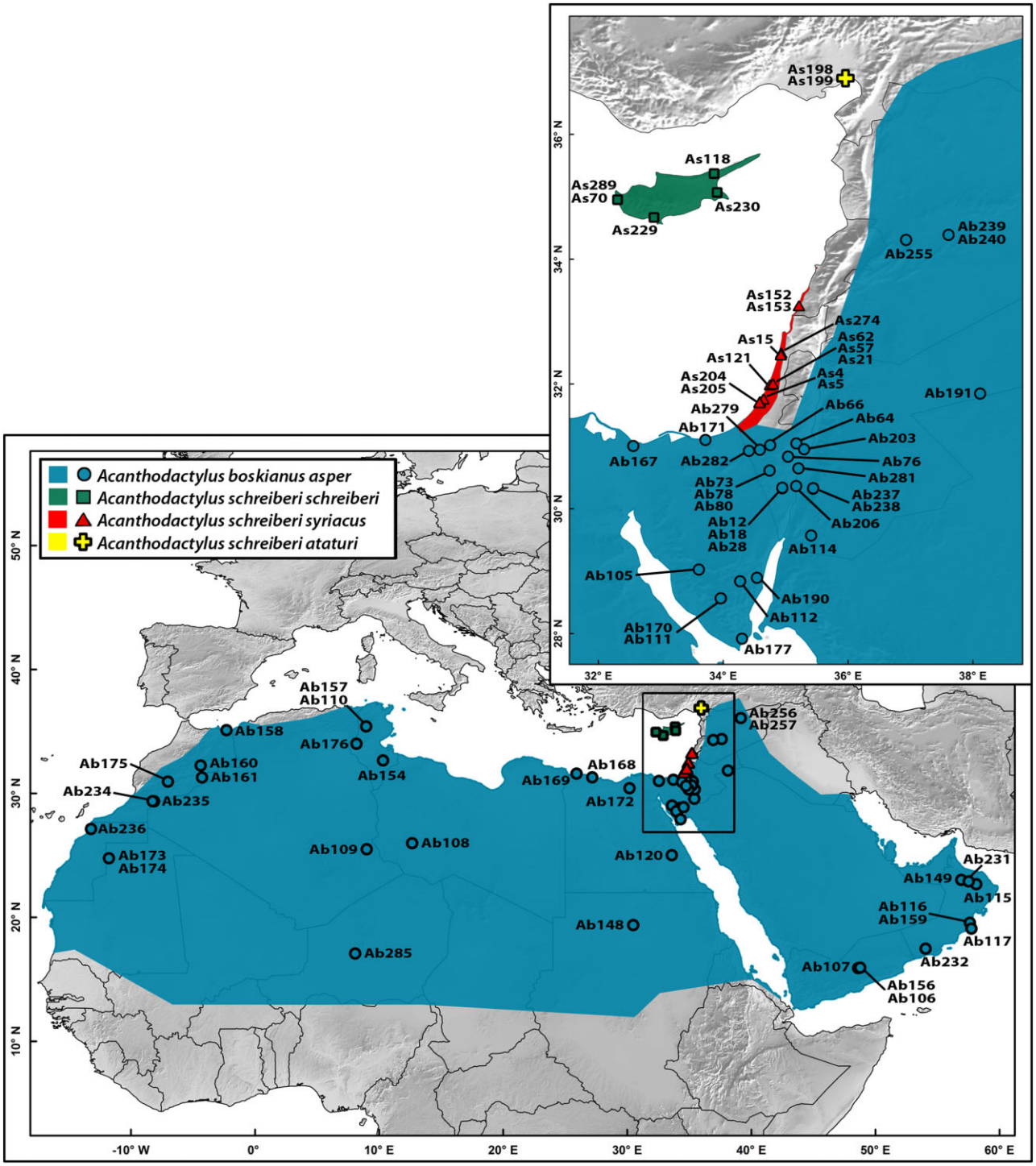

Figure 1. Sampling localities of the *Acanthodactylus schreiberi* and *Acanthodactylus boskianus* specimens used in this study, with the global distribution range of the species (data modified from Sindaco & Jeremčenko, 2008; IUCN, http://www.iucnredlist.org/). Locality codes and colours correlate to specimens in Table 1 and in Figures 2 and 3. (Colour version of figure available online.)

Table 1. Information on the specimens used and related GenBank accession numbers. Codes correspond to localities presented in Figure 1

Code	Species	Voucher	Country	Locality	*12S*	*Cytb*	*MC1R*	*ACM4*	*c-mos*
Ab109*†	*Acanthodactylus boskianus*	MCCI-R471	Algeria	Tassili 'n' Ajjer	KJ567694	KJ567812	KJ548037	KJ547885	KJ547987
Ab171	*Acanthodactylus boskianus*		Egypt	El Arish, Sinai	KJ567676	KJ567776	KJ548044	KJ547854	KJ548003
Ab167*†	*Acanthodactylus boskianus*		Egypt	Baluza, Sinai	KJ567727	KJ567790	KJ548063	KJ547852	KJ548014
Ab105*	*Acanthodactylus boskianus*	MCCI-R1566	Egypt	Between Serabit el Khadim and Gebel Raqaba, Sinai	KJ567672	KJ567768	KJ548035	KJ547849	KJ547966
Ab190*	*Acanthodactylus boskianus*		Egypt	14 km SW of Nuweibaa, Sinai	KJ567693	KJ567773	KJ548057	KJ547856	KJ547951
Ab112	*Acanthodactylus boskianus*	MCCI-R1568	Egypt	Gebel Gunna, Sinai	KJ567690	KJ567771	KJ548038	KJ547851	KJ547950
Ab170*	*Acanthodactylus boskianus*		Egypt	St. Catherine, Sinai	KJ567751	KJ567772	KJ548043	KJ547853	KJ547964
Ab111*	*Acanthodactylus boskianus*	MCCI-R1567	Egypt	Crossroad St. Catherine to Fox camp, Sinai	KJ567689	KJ567770	KJ548056	KJ547886	KJ547982
Ab177*	*Acanthodactylus boskianus*		Egypt	Sharm el Sheikh, Sinai	–	KJ567774	KJ548047	KJ547855	KJ547965
Ab168*†	*Acanthodactylus boskianus*		Egypt	Matruh	KJ567728	KJ567824	KJ548042	KJ547910	–
Ab169*†	*Acanthodactylus boskianus*		Egypt	Sidi Brani	KJ567729	KJ567813	KJ548068	KJ547872	KJ548015
Ab172*†	*Acanthodactylus boskianus*		Egypt	Wadi El Natrun	KJ567699	KJ567822	KJ548079	KJ547887	KJ547986
Ab120*	*Acanthodactylus boskianus*		Egypt	60 km E of Idfu	KJ567698	–	KJ548039	KJ547870	KJ547969
Ab279	*Acanthodactylus boskianus*	TAU-R.16058	Israel	Wadi Revivim	KJ567673	KJ567767	KJ548051	KJ547911	KJ547952
Ab66	*Acanthodactylus boskianus*	TAU-R.16160	Israel	Shivta junction	KJ567671	KJ567765	KJ548033	KJ547879	KJ547949
Ab282	*Acanthodactylus boskianus*	TAU-R.16295	Israel	Kmehin	KJ567682	KJ567780	KJ548053	KJ547932	KJ547954
Ab64*†	*Acanthodactylus boskianus*		Israel	Rotem plain	KJ567670	KJ567764	KJ548078	KJ547875	KJ547945
Ab203*	*Acanthodactylus boskianus*	HUJ-R-24055	Israel	S of Wadi Zafit	KJ567691	KJ567766	–	–	–
Ab76*	*Acanthodactylus boskianus*	TAU-R.16274	Israel	Mt Tzin	KJ567674	KJ567775	KJ548034	KJ547880	KJ547946
Ab73	*Acanthodactylus boskianus*	TAU-R.16013	Israel	Mitzpe Ramon	KJ567686	KJ567783	KJ548058	KJ547864	KJ547962
Ab78*	*Acanthodactylus boskianus*	TAU-R.16001	Israel	Mitzpe Ramon	KJ567692	KJ567784	KJ548061	KJ547874	KJ547963
Ab80	*Acanthodactylus boskianus*	TAU-R.16002	Israel	Mitzpe Ramon	KJ567687	KJ567785	KJ548060	KJ547865	KJ547957
Ab281	*Acanthodactylus boskianus*	TAU-R.16272	Israel	Wadi Nekarot	KJ567681	KJ567779	KJ548052	KJ547892	KJ547953
Ab206*	*Acanthodactylus boskianus*	HUJ-R-19646	Israel	Paran	KJ567677	KJ567787	–	–	–
Ab12	*Acanthodactylus boskianus*		Israel	Wadi Paran	KJ567683	KJ567781	KJ548059	KJ547861	KJ547955
Ab18	*Acanthodactylus boskianus*		Israel	Wadi Paran	KJ567684	KJ567782	KJ548064	KJ547884	KJ547956
Ab28*	*Acanthodactylus boskianus*		Israel	Wadi Paran	KJ567685	KJ567789	KJ548028	KJ547862	KJ547960
Ab191*†	*Acanthodactylus boskianus*		Jordan	Tell al Heber	KJ567733	KJ567830	KJ548067	KJ547883	KJ547974
Ab233	*Acanthodactylus boskianus*		Jordan	Petra	KJ567679	KJ567777	KJ548048	KJ547858	KJ547958
Ab237	*Acanthodactylus boskianus*	NMP6V 70481-2	Jordan	Petra	KJ567680	KJ567778	–	KJ547881	KJ547959
Ab238*	*Acanthodactylus boskianus*	NMP6V 70481-3	Jordan	Petra	KJ567688	KJ567788	–	KJ547908	KJ547961
Ab113*	*Acanthodactylus boskianus*	MCCI-R618	Jordan	Petra	KJ567675	KJ567786	–	–	–
Ab114*†	*Acanthodactylus boskianus*	MCCI-R621	Jordan	Wadi Ramm	KJ567730	KJ567826	–	KJ547876	KJ547988

Table 1. *Continued*

Code	Species	Voucher	Country	Locality	*12S*	*Cytb*	*MC1R*	*ACM4*	*c-mos*
Ab108*†	*Acanthodactylus boskianus*	MCCI-R1452(1)	Libya	Wadi Mathkendush	KJ567736	KJ567805	KJ548036	KJ547850	KJ547967
Ab173*	*Acanthodactylus boskianus*		Mauritania	Between Zouerat and Bir Moghrein	KJ567710	KJ567807	KJ548045	KJ547894	KJ547983
Ab174*	*Acanthodactylus boskianus*		Mauritania	Between Zouerat and Bir Moghrein	KJ567714	KJ567808	KJ548030	KJ547895	KJ547973
Ab158*†	*Acanthodactylus boskianus*	MCCI-R1088(4)	Morocco	Between Saidia and Moulouya	KJ567735	KJ567825	KJ548041	KJ547878	KJ547984
Ab160*	*Acanthodactylus boskianus*	NMP6V 74482	Morocco	Between Ait-Khoujman and Kerrandou	KJ567716	KJ567819	KJ548062	–	KJ548001
Ab161*†	*Acanthodactylus boskianus*	NMP6V 74483-1	Morocco	Rissani	KJ567717	KJ567818	KJ548054	KJ547882	KJ547985
Ab147	*Acanthodactylus boskianus*	NMP6V 74483-2	Morocco	Rissani	KJ567715	KJ567817	–	–	–
Ab175*	*Acanthodactylus boskianus*		Morocco	Ouarzazate	KJ567718	KJ567820	KJ548046	KJ547897	KJ548002
Ab234*	*Acanthodactylus boskianus*		Morocco	6.5 km E of Oum El-Alek	KJ567711	KJ567810	KJ548049	KJ547898	KJ547976
Ab235*†	*Acanthodactylus boskianus*		Morocco	Akka	KJ567713	KJ567811	KJ548069	KJ547899	KJ547977
Ab285*†	*Acanthodactylus boskianus*	MVZ:Herp-238925	Niger	Tafokin, 13 km NNE of Agadez	KJ567701	KJ567823	KJ548086	KJ547860	KJ548000
Ab115*†	*Acanthodactylus boskianus*		Oman	2 km S of Lizq	KJ567731	KJ567827	KJ548087	KJ547912	KJ547968
Ab231*†	*Acanthodactylus boskianus*		Oman	Nizwa	KJ567678	KJ567829	KJ548089	KJ547914	KJ547975
Ab149*	*Acanthodactylus boskianus*		Oman	10 km SE of Kubarah	KJ567732	KJ567828	KJ548088	KJ547913	KJ547971
Ab117	*Acanthodactylus boskianus*		Oman	16 km S of Duqm	KJ567707	KJ567802	KJ548091	KJ547905	KJ547990
Ab116*†	*Acanthodactylus boskianus*	MCCI-R1773(1)	Oman	Wadi Salit	KJ567706	KJ567801	KJ548090	KJ547903	KJ547989
Ab159	*Acanthodactylus boskianus*	MCCI-R1773(2)	Oman	Wadi Salit	KJ567708	KJ567803	KJ548092	KJ547906	KJ547991
Ab232*	*Acanthodactylus boskianus*		Oman	4 km N of Rawiyyah	KJ567709	KJ567804	KJ548093	KJ547904	KJ547992
Ab148*	*Acanthodactylus boskianus*		Sudan	N of El-Koin	KJ567700	KJ567821	KJ548040	KJ547877	KJ547970
Ab256*	*Acanthodactylus boskianus*	NMP6V 70450-2	Syria	Ar Raqqah	KJ567747	KJ567842	KJ548032	KJ547915	KJ548012
Ab257	*Acanthodactylus boskianus*	NMP6V 70470-1	Syria	Ar Raqqah	KJ567748	KJ567841	KJ548080	KJ547889	KJ548013
Ab239*†	*Acanthodactylus boskianus*	NMP6V 72502-1	Syria	Qasr al Hayr al Gharbi	KJ567744	KJ567838	KJ548031	KJ547890	KJ548009
Ab240	*Acanthodactylus boskianus*	NMP6V 72502-2	Syria	Qasr al Hayr al Gharbi	KJ567745	KJ567839	KJ548055	KJ547888	KJ548010
Ab255	*Acanthodactylus boskianus*	NMP6V 70443	Syria	Sadad	KJ567746	KJ567840	–	KJ547891	KJ548011
Ab110*	*Acanthodactylus boskianus*	MCCI-R1326(1)	Tunisia	NE slopes of Jebel Semmama	KJ567695	KJ567814	KJ548085	KJ547893	KJ548004
Ab157	*Acanthodactylus boskianus*	MCCI-R1326(2)	Tunisia	NE slopes of Jebel Semmama	KJ567696	KJ567815	–	–	–
Ab176*†	*Acanthodactylus boskianus*		Tunisia	Hammat al-Jarid	KJ567697	KJ567816	KJ548070	KJ547909	KJ548005
Ab154*†	*Acanthodactylus boskianus*	MCCI-R1346(2)	Tunisia	33 km S of Tataouine	KJ567702	KJ567806	KJ548081	KJ547907	KJ547972
Ab236*†	*Acanthodactylus boskianus*		Western Sahara	Laayoune	KJ567712	KJ567809	KJ548050	KJ547896	KJ547978
Ab156*	*Acanthodactylus boskianus*	MCCI-R823(3)	Yemen	Sa'yun oasis	KJ567705	KJ567800	KJ548066	KJ547902	KJ547981
Ab106*†	*Acanthodactylus boskianus*	MCCI-R823(4)	Yemen	Sa'yun oasis	KJ567703	KJ567799	KJ548065	KJ547900	KJ547980
Ab107*†	*Acanthodactylus boskianus*	MCCI-R824	Yemen	Dunes W of Shibam	KJ567704	KJ567769	–	KJ547901	KJ547979
As198*	*Acanthodactylus schreiberi ataturi*	MCCI-R1693(1)	Turkey	Botas	KJ567740	KJ567835	KJ548075	KJ547919	KJ547996

As199	*Acanthodactylus schreiberi ataturi*	MCCI-R1693(1)	Turkey	Botas	KJ567741	KJ567836	KJ548076	KJ547918	KJ547997
As70*	*Acanthodactylus schreiberi schreiberi*	TAU-R. 16151	Cyprus	Lara bay, Akamas peninsula	KJ567737	KJ567831	KJ548071	KJ547916	KJ547993
As289	*Acanthodactylus schreiberi schreiberi*	TAU-R. 16150	Cyprus	Lara bay, Akamas peninsula	KJ567738	KJ567832	KJ548074	KJ547924	KJ547994
As118*	*Acanthodactylus schreiberi schreiberi*	NMP6V 74532	Cyprus	3 km S of Mersinlik, Famagusta	KJ567739	KJ567834	KJ548072	KJ547917	KJ547995
As229*†	*Acanthodactylus schreiberi schreiberi*		Cyprus	Episkopi	KJ567743	KJ567833	KJ548073	KJ547921	KJ547999
As230*	*Acanthodactylus schreiberi schreiberi*		Cyprus	Vrysoulles	KJ567742	KJ567837	KJ548077	KJ547920	KJ547998
As15*	*Acanthodactylus schreiberi syriacus*	HUJ-R-23653	Israel	Caesarea sands	KJ567719	KJ567791	KJ548025	KJ547866	KJ547937
As274	*Acanthodactylus schreiberi syriacus*	HUJ-R-23410	Israel	Hadera to Binyamina	KJ567722	KJ567792	–	KJ547859	KJ547947
As121*	*Acanthodactylus schreiberi syriacus*	TAU-R.16262	Israel	Rishon Le-Zion sands	KJ567723	KJ567795	KJ548026	KJ547867	KJ547938
As21*†	*Acanthodactylus schreiberi syriacus*	TAU-R.16398	Israel	Holon sands	KJ567724	KJ567798	KJ548027	KJ547873	KJ547942
As57*	*Acanthodactylus schreiberi syriacus*	TAU-R.16407	Israel	Holon sands	KJ567726	KJ567796	KJ548084	KJ547863	KJ547943
As62*	*Acanthodactylus schreiberi syriacus*	TAU-R.16412	Israel	Holon sands	KJ567725	KJ567797	KJ548029	KJ547871	KJ547944
As4*†	*Acanthodactylus schreiberi syriacus*	HUJ-R-23986	Israel	Nizzanim reserve	KJ567666	KJ567760	KJ548024	KJ547847	KJ547935
As5*	*Acanthodactylus schreiberi syriacus*	HUJ-R-23987	Israel	Nizzanim reserve	KJ567667	KJ567761	KJ548082	KJ547848	KJ547936
As204	*Acanthodactylus schreiberi syriacus*	HUJ-R-23321	Israel	Ashqelon	KJ567668	KJ567762	–	KJ547857	KJ547940
As205	*Acanthodactylus schreiberi syriacus*	HUJ-R-23331	Israel	Ashqelon	KJ567669	KJ567763	KJ548083	–	KJ547941
As152*	*Acanthodactylus schreiberi syriacus*	MCCI-R925(1)	Lebanon	Tyre	KJ567720	KJ567793	–	KJ547868	KJ547948
As153	*Acanthodactylus schreiberi syriacus*	MCCI-R925(2)	Lebanon	Tyre	KJ567721	KJ567794	–	KJ547869	KJ547939
Ab207	*Acanthodactylus blanfordii*	MVZ:Herp-234464	Iran	6 km NW of Bampur, Sistan va	KJ567754	KJ567846	KJ548101	KJ547929	KJ548020
Ab208	*Acanthodactylus blanfordii*	MVZ:Herp-246009	Iran	Sand dunes 7 km N of Bampur,	KJ567755	KJ567847	KJ548102	KJ547930	KJ548021
Ac286	*Acanthodactylus cantoris*	MVZ:Herp-248443	Pakistan	10 km S of Uthal, Baluchistan Province	KJ567756	KJ567850	KJ548096	KJ547922	KJ548018
Ac287	*Acanthodactylus cantoris*	MVZ:Herp-248447	Pakistan	45 km NW of Nagar Parkar	KJ567757	KJ567851	KJ548097	KJ547923	KJ548019
Af197	*Acanthodactylus felicis*	CAS 227596	Oman	23 km W of Ajdarawt	KJ567734	KJ567845	KJ548100	KJ547928	KJ548008
Am63	*Acanthodactylus masirae*	IBES7643	Oman	20 km E of Ras Madrakah	KJ567753	KJ567849	KJ548095	KJ547925	KJ548017
Am50	*Acanthodactylus masirae*		Oman	Masirae island	KJ567752	KJ567848	KJ548094	KJ547931	KJ548016
Ao25	*Acanthodactylus opheodurus*	HUJ-R-19189	Israel	Timna valley	KJ567750	KJ567844	KJ548099	KJ547927	KJ548007
Ao79	*Acanthodactylus opheodurus*	MCCI-R627	Jordan	Diseh	KJ567749	KJ567843	KJ548098	KJ547926	KJ548006
As11	*Acanthodactylus scutellatus*	TAU-R.16389	Israel	Bir Mashash sands	KJ567758	KJ567852	KJ548103	KJ547933	KJ548022
As44	*Acanthodactylus scutellatus*	TAU-R.16402	Israel	Holon sands	KJ567759	KJ567853	KJ548104	KJ547934	KJ548023

*Haplotypes ($N = 59$).

†Representatives used for the divergence time analysis ($N = 25$).

Gene abbreviations: *12S*, *12S rRNA*; *ACM4*, *acetylcholinergic receptor Muscarinic 4*; *c-mos*, *oocyte maturation factor MOS*; *Cytb*, *cytochrome b*; *MC1R*, *melano-cortin 1 receptor*.

Institutional abbreviations: CAS, California Academy of Sciences, USA; HUJ-R, Zoological Museum, Hebrew University of Jerusalem, Israel; IBES, Institute of Evolutionary Biology, Barcelona, Spain; MCCI-R, Museo Civico di Storia Naturale, Carmagnola (Torino), Italy; MVZ:Herp, Museum of Vertebrate Zoology (University of California, Berkeley), USA; NMP6V, National Museum (Natural History), Prague, Czech Republic; TAU-R, Zoological museum, Tel Aviv University, Israel.

Table 2. Information on the partitions used in the phylogenetic analyses with the different partition approaches (i.e. by gene and by PartitionFinder; C, codon) including the length, model of sequence evolution selected by JModelTest and PartitionFinder, and the results of the test of rate homogeneity (LRT) run in MEGA (see Material and methods)

Partition approach	Partition	Length (bp)	Model	LRT
By gene	*12S*	~387	GTR + I + G	Not rejected ($P < 0.7396$)
	Cytb	405	TrN + I + G	Rejected ($P < 2.1819E-7$)
	MC1R	663	GTR + I	Not rejected ($P < 1$)
	ACM4	429	HKY + I	Not rejected ($P < 1$)
	c-mos	522	TPM1uf + G	Not rejected ($P < 1$)
PartitionFinder – Concatenated	*12S*, *Cytb* (C1)	2406	GTR + I + G	
	c-mos(C1), *Cytb* (C2)		TrNef + I + G	
	Cytb (C3)		TrN + I + G	
	ACM4 (C1,2), *MC1R* (C1)		TrN	
	MC1R (C2)		F81	
	MC1R (C3)		HKY + G	
	ACM4 (C3), *c-mos*(C2, 3)		TrNef + I + G	
PartitionFinder – mtDNA	*12S*, *Cytb* (C1)	792	SYM + I + G	
	Cytb (C2)		TrN + I + G	
	Cytb (C3)		TrN + I + G	
PartitionFinder – nuclear DNA	*ACM4* (C1,2), *c-mos* (C1,2), *MC1R* (C1)	1614	HKY + I	
	MC1R (C2)		F81	
	MC1R (C3)		HKY + G	
	ACM4 (C3), *c-mos* (C3)		K80 + I	

Gene abbreviations: *12S*, *12S rRNA*; *ACM4*, *acetylcholinergic receptor Muscarinic 4*; *c-mos*, *oocyte maturation factor MOS*; *Cytb*, *cytochrome b*; *MC1R*, *melano-cortin 1 receptor*.
Model abbreviations: F81, Felsenstein 1981; GTR, general time-reversible; HKY, Hasegawa Kishino-Yano; K80, Kimura 1980; SYM, symmetrical model; TPM1uf, Kimura three-parameter model; TrN, Tamura-Nei. Any of these models can include invariable sites (+I), gamma distribution (+G), or both (+I+G).

parameters unlinked across partitions (Table 2). Two independent runs of 2×10^7 generations were carried out with a sampling frequency of every 1000 generations. After examining the standard deviation of the split frequencies between the two runs and the potential scale reduction factor diagnostic, burn-in was performed, discarding the first 25% trees of each run, and the remaining trees were combined in a majority consensus tree. In both ML and BI alignment gaps were treated as missing data and the nuclear gene sequences were not phased. Nodes were considered strongly supported if they received ML bootstrap values ≥ 70% and posterior probability (pp) support values ≥ 0.95 (Wilcox *et al.*, 2002; Huelsenbeck & Rannala, 2004).

A total of 59 haplotypes was identified amongst the *A. boskianus* species group using 792 bp of the concatenated *12S* and *Cytb* data set (see Table 1). Haplotype networks were constructed for the three nuclear genes *MC1R*, *ACM4*, and *c-mos* (only full-length sequences). SEQPHASE (Flot, 2010) was used to convert the input files, and the software PHASE v. 2.1.1 to resolve phased haplotypes (Stephens, Smith & Donnelly, 2001; Stephens & Scheet, 2005). Default settings of PHASE were used except for phase probabilities, which were set as ≥ 0.7. All polymorphic sites with a probability of < 0.7 were coded in both alleles with the appropriate IUPAC ambiguity code. The phased nuclear sequences were used to generate median-joining networks using NETWORKS v. 4.6.1.1 (Bandelt, Forster & Röhl, 1999).

In order to assess alternative topologies between *A. schreiberi* and *A. b. asper*, topological constraints that could be statistically rejected were constructed. We enforced alternative topologies by hand and compared with the unconstrained tree (best ML tree) using the approximately unbiased (AU; Shimodaira, 2002) and Shimodaira–Hasegawa (SH; Shimodaira & Hasegawa, 1999) tests. Per-site log likelihoods were estimated in using RAxMLGUI v. 1.3 (Silvestro & Michalak, 2012) and *P*-values were calculated using CONSEL (Shimodaira & Hasegawa, 2001).

Species delimitation

In order to reveal the main lineages with the concatenated analysis and as a prior for species groupings, a mitochondrial phylogeny of 59 haplotypes was performed with BEAST v. 1.6.2 (Drummond & Rambaut, 2007) without the outgroups. Three individual runs were

performed for 5×10^7 generations with a sampling frequency of 10 000. The results were combined to infer the ultrametric tree after discarding 10% of the samples from each run. Models and prior specifications applied were as follows (otherwise by default) for partitions by genes and by PartitionFinder. For gene partitions: GTR + I + G, strict clock (12S), Hasegawa-Kishino-Yano + Invariable sites + Gamma distribution (HKY + I + G), strict clock, molecular clock model (estimate, 0–1) (*Cytb*); coalescence: constant size process of speciation; random starting tree; alpha Uniform (0, 10); GTR Uniform. For partitions by PartitionFinder: GTR + I + G, strict clock (partition 1 = *12S* + *Cytb* codon 1 and 2), Tamura-Nei + Gamma distribution (TrN + G), strict clock (partition 2 = *Cytb* codon 3); coalescence: constant size process of speciation; random starting tree; alpha Uniform (0, 10). Parameter values both for clock and substitution models were unlinked across partitions. For all analyses implemented in BEAST, the three runs were analysed in TRACER v. 1.5 (Rambaut & Drummond, 2007) confirming convergence. The trees were combined in LogCombiner and TreeAnnotator (available in BEAST package) was used for the production of the final tree.

For estimating species limits directly from the Bayesian phylogenetic tree produced with the concatenated mitochondrial data, we used the independent generalized mixed Yule-coalescent (GMYC) method (Pons *et al*., 2006). The GMYC model estimated the number of phylogenetic clusters or 'species' by identifying the shifts between intraspecific (coalescence) and interspecific (diversification) branch rates (Pons *et al*., 2006). We performed the GMYC function in the R v.3.0.2 'splits' package (Ezard, Fujisawa & Barraclough, 2009). A likelihood-ratio test was used to determine if the GMYC model with a shift in the branching processes provided a better fit to the data than the null model with no shifts. We used a single threshold value (Monaghan *et al*., 2009), which has already been applied successfully to different groups of organisms (Pons *et al*., 2006; Fontaneto *et al*., 2007; Monaghan *et al*., 2009).

Estimation of divergence times

The lack of internal calibration points in *Acanthodactylus* (no fossils are known) prevents the direct estimation of time in our phylogeny. Therefore, we used the mean substitution rates and their standard error of the same *12S* and *Cytb* mitochondrial regions extracted from a fully calibrated phylogeny of another lacertid group, the lizards of the genus *Gallotia* endemic to the Canary Islands (Cox, Carranza & Brown, 2010; as was implemented in Carranza & Arnold, 2012). The inferred calibration rate was estimated using the age of El Hierro Island (Canary Islands), estimated at 1.12 Mya (Guillou *et al*., 1996). They assumed colonization of the island by members of the lacertid genus *Gallotia* (*Gallotia caesaris caesaris*, endemic to El Hierro Island) immediately after its formation from the neighbouring La Gomera Island (inhabited by the endemic *Gallotia caesaris gomerae*). These two subspecies are monophyletic sister taxa with low intraspecific variability (Maca-Meyer *et al*., 2003; Cox *et al* 2010) and thus suitable for calibration.

For the estimation of divergence times one representative of each independent GMYC lineage was used from the ultrametric tree (for the representatives see Table 1). We used a likelihood-ratio test implemented in MEGA 5.2 (Tamura *et al*., 2011) to test if the different partitions (by genes) included in the dating analysis were evolving in a clock-like fashion (Table 2). This information was used to choose between the strict clock and the relaxed uncorrelated lognormal clock priors implemented in BEAST (Monaghan *et al*., 2009). The data set included one representative from each lineage from the GMYC analysis using sequences from all five partitions (nuclear genes unphased). Three individual runs were performed for 5×10^7 generations with a sampling frequency of 10 000 and the results were combined to infer the ultrametric tree after discarding 10% of the samples from each run. Models and prior specifications applied were as follows (otherwise by default): GTR + I + G, relaxed uncorrelated lognormal clock, molecular clock model (estimate) (*12S*, *Cytb*), HKY, strict clock (*MC1R*, *c-mos*), and TrN + I, strict clock (*ACM4*); Yule process of speciation; random starting tree; yule.birthRate (0, 1000); alpha Uniform (0, 10); ucld.mean of *12S* Normal (initial value: 0.00553, mean: 0.00553, SD: 0.00128); ucld.mean of *Cytb* Normal (initial value: 0.0164, mean: 0.0164, SD: 0.00317). Parameter values both for clock and substitution models were unlinked across partitions.

RESULTS

The data set of this study is comprised of 19 samples of *A. schreiberi*, 65 samples of *A. b. asper*, and 11 outgroup samples (Table 1; Fig. 1). The data set included mitochondrial DNA (mtDNA) gene fragments of *12S* (~387 bp) and *Cytb* (405 bp), and nuclear DNA (nDNA) gene fragments of *MC1R* (663 bp), *ACM4* (429 bp), and *c-mos* (522 bp) totalling to ~2406 bp. The number of variable (V) and parsimony-informative (Pi) sites for the ingroup are listed in Table S1. The two partition approaches (i.e. by gene and by PartitionFinder) gave similar results for both the ML and BI analyses. The results of the phylogenetic analyses of the complete concatenated data set using ML and BI methods produced very similar topologies but differed, to some extent, at the less supported nodes at the intraspecific level (Fig. 2). Separated analyses of the nuclear data sets are presented in Figure S1.

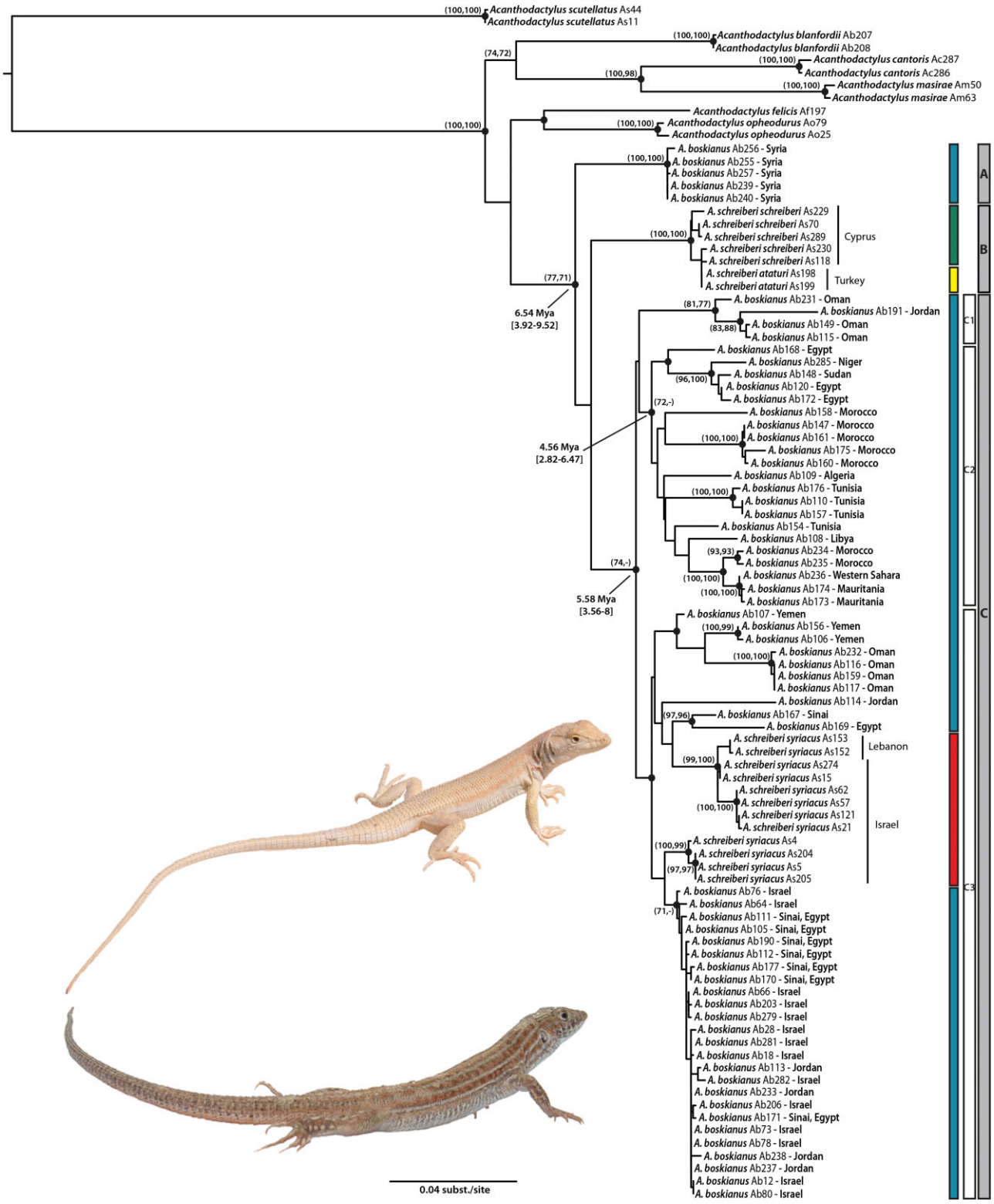
(100,100) Acanthodactylus scutellatus As44
Acanthodactylus scutellatus As11
(100,100) Acanthodactylus blanfordii Ab207
Acanthodactylus blanfordii Ab208
(74,72)
(100,100) Acanthodactylus cantoris Ac287
(100,98) Acanthodactylus cantoris Ac286
(100,100) Acanthodactylus masirae Am50
Acanthodactylus masirae Am63
Acanthodactylus felicis Af197
(100,100) Acanthodactylus opheodurus Ao79
Acanthodactylus opheodurus Ao25
A. boskianus Ab256 - Syria
(100,100) A. boskianus Ab255 - Syria
A. boskianus Ab257 - Syria
A. boskianus Ab239 - Syria
A. boskianus Ab240 - Syria
A. schreiberi schreiberi As229
A. schreiberi schreiberi As70
(100,100) A. schreiberi schreiberi As289 Cyprus
A. schreiberi schreiberi As230
A. schreiberi schreiberi As118
A. schreiberi ataturi As198 Turkey
(77,71)
A. schreiberi ataturi As199
6.54 Mya
[3.92-9.52]
(81,77) A. boskianus Ab231 - Oman
A. boskianus Ab191 - Jordan
(83,88) A. boskianus Ab149 - Oman
A. boskianus Ab115 - Oman
A. boskianus Ab168 - Egypt
A. boskianus Ab285 - Niger
(96,100) A. boskianus Ab148 - Sudan
A. boskianus Ab120 - Egypt
A. boskianus Ab172 - Egypt
(72,-)
A. boskianus Ab158 - Morocco
A. boskianus Ab147 - Morocco
(100,100) A. boskianus Ab161 - Morocco
A. boskianus Ab175 - Morocco
A. boskianus Ab160 - Morocco
4.56 Mya
[2.82-6.47]
A. boskianus Ab109 - Algeria
A. boskianus Ab176 - Tunisia
(100,100) A. boskianus Ab110 - Tunisia
A. boskianus Ab157 - Tunisia
A. boskianus Ab154 - Tunisia
A. boskianus Ab108 - Libya
(93,93) A. boskianus Ab234 - Morocco
A. boskianus Ab235 - Morocco
(74,-)
(100,100) A. boskianus Ab236 - Western Sahara
(100,100) A. boskianus Ab174 - Mauritania
A. boskianus Ab173 - Mauritania
5.58 Mya
[3.56-8]
A. boskianus Ab107 - Yemen
(100,99) A. boskianus Ab156 - Yemen
A. boskianus Ab106 - Yemen
(100,100) A. boskianus Ab232 - Oman
A. boskianus Ab116 - Oman
A. boskianus Ab159 - Oman
A. boskianus Ab117 - Oman
A. boskianus Ab114 - Jordan
(97,96) A. boskianus Ab167 - Sinai
A. boskianus Ab169 - Egypt
A. schreiberi syriacus As153
A. schreiberi syriacus As152 Lebanon
(99,100) A. schreiberi syriacus As274
A. schreiberi syriacus As15
A. schreiberi syriacus As62
(100,100) A. schreiberi syriacus As57
A. schreiberi syriacus As121 Israel
A. schreiberi syriacus As21
(100,99) A. schreiberi syriacus As4
A. schreiberi syriacus As204
(97,97) A. schreiberi syriacus As5
A. schreiberi syriacus As205
A. boskianus Ab76 - Israel
(71,-) A. boskianus Ab64 - Israel
A. boskianus Ab111 - Sinai, Egypt
A. boskianus Ab105 - Sinai, Egypt
A. boskianus Ab190 - Sinai, Egypt
A. boskianus Ab112 - Sinai, Egypt
A. boskianus Ab177 - Sinai, Egypt
A. boskianus Ab170 - Sinai, Egypt
A. boskianus Ab66 - Israel
A. boskianus Ab203 - Israel
A. boskianus Ab279 - Israel
A. boskianus Ab28 - Israel
A. boskianus Ab281 - Israel
A. boskianus Ab18 - Israel
A. boskianus Ab113 - Jordan
A. boskianus Ab282 - Israel
A. boskianus Ab233 - Jordan
A. boskianus Ab206 - Israel
A. boskianus Ab171 - Sinai, Egypt
A. boskianus Ab73 - Israel
A. boskianus Ab78 - Israel
A. boskianus Ab238 - Jordan
A. boskianus Ab237 - Jordan
A. boskianus Ab12 - Israel
A. boskianus Ab80 - Israel
A
B
C
C1
C2
C3
0.04 subst./site

Figure 2. Maximum likelihood (ML) tree of the *Acanthodactylus boskianus* and *Acanthodactylus schreiberi* specimens inferred using *12S rRNA*, *cytochrome b* mtDNA and *melano-cortin 1 receptor*, *acetylcholinergic receptor M4*, and *oocyte maturation factor MOS* nuclear gene fragments. Posterior probability in the Bayesian analysis is indicated by black dots on the nodes [values ≥ 0.95 shown, for both gene partitions and partitions by PartitionFinder (PF)], and ML bootstrap support values are indicated in parentheses (values ≥ 70% shown; ML, ML-PF). Age estimates with BEAST are indicated near the relevant nodes and include the mean and, in brackets, the HPD 95% confidence interval. Sample codes relate to specimens in Table 1 and in Figures 1 and 3. Colours: blue, *Acanthodactylus boskianus asper*; yellow, *Acanthodactylus schreiberi ataturi*; red, *Acanthodactylus schreiberi syriacus*; green, *Acanthodactylus schreiberi schreiberi*. (Colour version of figure available online.)

Together, *A. b. asper* and *A. schreiberi* form a monophyletic group within *Acanthodactylus* (Fig. 2). Within the group, however, both taxa are paraphyletic, with *A. schreiberi* as a whole nested within *A. b. asper*. Our analyses distinguish three major clades: (1) clade A, formed by *A. b. asper* from Syria; (2) clade B, includes the two subspecies, *A. sc. ataturi* from Turkey together with *A. sc. schreiberi* from Cyprus; (3) clade C, which includes specimens of *A. b. asper* from the remaining localities in its distribution range together with *A. sc. syriacus* from Israel and Lebanon. Clade A is very well supported and includes specimens of *A. b. asper* from central and northern Syria (Fig. 1), splitting from other specimens at the basal node of the group is estimated to have occurred *c.* 6.54 Mya [95% highest posterior density (HPD): 3.92–9.52 Mya]. The level of genetic differentiation
(*p*-distance) between these specimens and the remaining *A. b. asper* and all *A. schreiberi* specimens is 3.7–4.6% for *12S* and 10.7–11.9% for *Cytb*. Clade B is also very well supported and includes two of the three nominal subspecies of *A. schreiberi*: *A. s. schreiberi* the nominotypical subspecies endemic to Cyprus, and *A. s. ataturi* from Turkey. The Turkish subspecies is nested within the Cypriot specimens and the two forms have low genetic distances from each other (*12S*: 0.16%; *Cytb*: 1.23%). This clade is nested between the two *A. b. asper* clades (clades A and C) in both the concatenated and the nuclear tree although the nodes are not well supported. Clade C is not very well supported. It includes a cluster of *A. b. asper* and *A. s. syriacus.* This clade includes two inner clades that split around 5.58 Mya (95% HPD: 3.56–8 Mya) and divided into three poorly supported geographical inner groups (Fig. 2): northern Jordan and northern Oman (group C1), North Africa (group C2), and samples from the Middle East (Egypt, south Israel, and south Jordan) with samples from Yemen and southern Oman (group C3) – the latter including all specimens of the subspecies *A. s. syriacus*. The diversification within the North African group is estimated to have started around 4.56 Mya (95% HPD: 2.82–6.47 Mya). The Israel–Lebanon endemic subspecies *A. s. syriacus* is genetically highly distinct from *A. s. schreiberi* and *A. s. ataturi*, making *A. schreiberi* paraphyletic (*p*-distance: *12S*: 4.31, 4.16%; *Cytb*: 11.8, 12.02%, respectively).

The networks constructed for the phased haplotypes of the full length nuclear markers (*MC1R*, *ACM4*, and *c-mos*) are presented in Figure 3. The nuclear network analyses show similar results for each of the three genes and closely agree with the phylogenetic tree. The Cypriot *A. s. schreiberi* and Turkish *A. s. ataturi* subspecies share alleles for all three genes, and both are distinct from the third subspecies *A. s. syriacus*. *Acanthodactylus schreiberi syriacus* shares no alleles with the other subspecies of *A. schreiberi*, but does share alleles with *A. b. asper* for each of the genes. *Acanthodactylus schreiberi syriacus* shares *MC1R* alleles with *A. b. asper* specimens from Tunisia, Syria, and Israel, *ACM4* alleles with Egyptian, Israeli, Jordanian, and North African specimens, and *c-mos* alleles only with Israeli *A. b. asper* specimens. Syrian *A. b. asper* samples share one allele with *A. s. syriacus* and two with other *A. b. asper* specimens from Egypt, Israel, and North Africa in the *MC1R*, one allele with an Egyptian *A. b. asper* in the *ACM4*, and none in the *c-mos* gene.

In order to better understand the relationships between *A. schreiberi* and *A. boskianus*, we performed three topology tests in which we forced monophyletic groupings: (1) monophyly of *A. schreiberi* (all three subspecies together); (2) monophyly of *A. b. asper*; (3) monophyly of *A. b. asper* and of *A. schreiberi*. The results of the topological tests indicate that our data set cannot reject the alternative hypotheses of monophyly of *A. schreiberi* (AU: $P = 0.091$, SH: $P = 0.062$) and that of *A. b. asper* (AU: $P = 0.11$, SH: $P = 0.072$) if we allow *A. schreiberi* to nest within *A. b. asper* or a monophyletic *A. b. asper* nesting within *A. schreiberi*. When forcing monophyly of both *A. schreiberi* and of *A. b. asper* together in the same tree, the results are inconclusive (AU: $P = 0.046$, SH: $P = 0.051$).

The single-threshold model in GMYC yield a topology that is clearly different from the known taxonomy. The GMYC results present a total of 25 and 24 ML independent lineages from the Bayesian haplotype mitochondrial phylogeny of the two species for the two

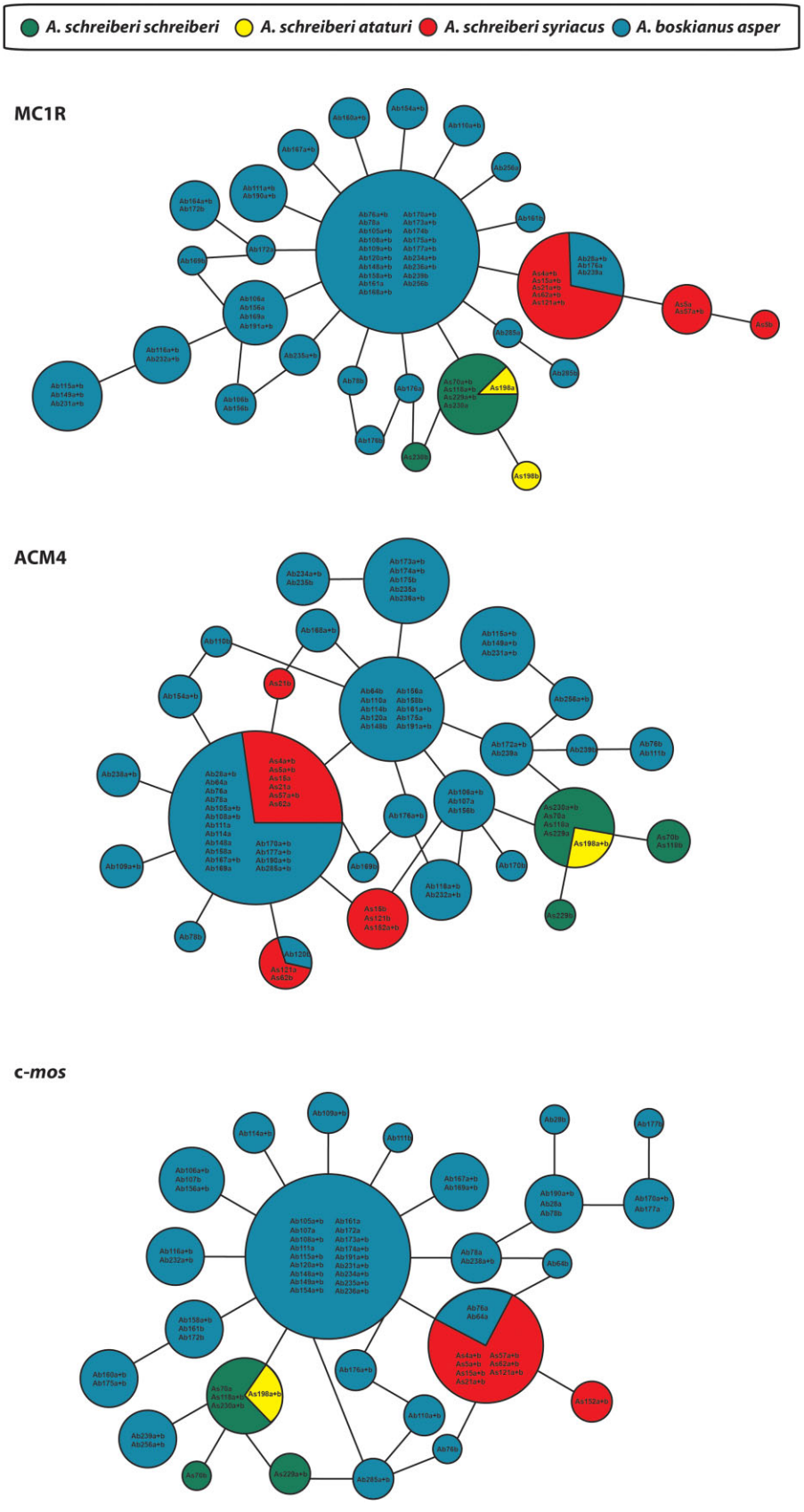

Figure 3. Haplotype networks of the nuclear gene fragments *melano-cortin 1 receptor* (*MC1R*), *acetylcholinergic receptor M4* (*ACM4*), and *oocyte maturation factor MOS* (*c-mos*) with colours corresponding to Figures 1 and 2. Codes correlate to the two alleles (i.e. a and b) of specimens in Table 1. Circle sizes are proportional to the number of alleles. (Colour version of figure available online.)

partition approaches (i.e. by gene and by PartitionFinder, Figs S2, S3, respectively). The two partition approaches gave similar clusters, but at the less supported nodes they differed at the positions of several lineages. The single threshold GMYC result is indicated for a single line at 0.0037 Mya for the gene partitions and at 0.02 Mya for PartitionFinder (vertical lines in Figs S2, S3). The topology and clusters revealed in this analysis correspond to the lineages from the phylogeny of the ML and BI methods, both for the paraphyly of the two species and the geographical groupings within *A. b. asper*. The GMYC results mainly differ from the ML and BI methods in the position of *A. schreiberi* from Cyprus and Turkey as a sister clade to the Syrian *A. b. asper*.

DISCUSSION

We have provided a comprehensive and thorough assessment of the intraspecific phylogenetic relationships within *A. schreiberi* and its closest relative *A. b. asper.* Our results, based on mitochondrial and nuclear DNA data from 84 specimens across the entire distribution range of *A. schreiberi* and most of the distribution range of *A. b. asper*, reveal that *A. schreiberi* is paraphyletic and nested entirely within the *A. boskianus* subspecies.

Historical biogeography

Acanthodactylus schreiberi is thought to comprise three subspecies, corresponding to three allopatric populations in Cyprus, Turkey, and Israel–Lebanon. The Cypriot endemic, nominotypical, subspecies, *A. sc. schreiberi*, and the Turkish subspecies, *A. sc. ataturi*, cluster together (to form clade B; Fig. 2), nesting between *A. b. asper* clades. This lineage is sister to a clade of *A. b. asper* including *A. sc. syriacus* (clade C; Fig. 2). We estimate the divergence time of the Cypriot–Turkish lineage of *A. schreiberi* to have been during the late Miocene around 6 Mya, although there is no support for this split in the tree. In other analyses using the whole genus, this split is well supported in Bayesian analyses (K. Tamar, S. Carranza, R. Sindaco, J. Moravec, JF. Trape & S. Meriri, unpubl. data). Based on mitochondrial data Poulakakis *et al*. (2013) found that both the Cypriot and Turkish subspecies are monophyletic, and diverged from each other 0.85 Mya (0.38–1.56 Mya). According to our results this date corresponds to an inner divergence of the *A. sc. schreiberi* lineage rather than to the date at which *A. sc. schreiberi* colonized Cyprus.

The discrepancy in the phylogenetic relationship of *A. sc. schreiberi* raises questions regarding the arrival on Cyprus. Cyprus originated with the raising of the Troodos Massif during the upper Cretaceous, *c.* 91 to 88 Mya (Clube & Robertson, 1986; Mukasa & Ludden, 1987). During the middle to late Miocene only a small proportion of Cyprus was exposed above the Mediterranean (McCallum & Robertson, 1990; Robertson, 1990). Towards the end of the Miocene ~5.96 Mya, with the closing of the passage between the Atlantic Ocean and the Mediterranean basin, the Messinian salinity crisis began (Krijgsman *et al*., 1999). This resulted in the drying up of much of the Mediterranean Sea and high sea-mounts emerged to form land bridges with the surrounding land (Hsü *et al*., 1977). By the end of the Miocene and early Pliocene, ~5.33 Mya, the passage with the Atlantic Ocean reopened and the Mediterranean basin was refilled (Krijgsman *et al*., 1999). Resulting from compressions, raising, and uplifting of the surrounding areas, towards and during the Pleistocene, Cyprus was a complete emergent island (McCallum & Robertson, 1990). The possible connection of Cyprus to the mainland (i.e. to Turkey/Syria) during the Messinian is debated, as are suggestions of a land connection at later periods (Steininger & Rögl, 1984; Jolivet *et al*., 2006; Bache *et al*., 2012). Such a connection, if it existed, could have provided access for terrestrial organisms with poor overseas dispersal ability, such as lizards, to colonize the island. Several studies argue that post-Messinian sea level changes are unlikely to have formed connections between Cyprus and the mainland (Steininger & Rögl, 1984; Jolivet *et al*., 2006). Thus, our dating of the split between the Cypriot *A. schreiberi* and *A. b. asper* at *c.* 6 Mya leads us to suggest that the ancestor of *A. s. schreiberi* colonized Cyprus from the mainland through a land bridge connection at the beginning of the Messinian crisis, rather than by a much later/more recent transmarine dispersal as suggested by Poulakakis *et al*. (2013). Owing to its close relations with *A. b. asper*, the ancestor of *A. schreiberi* was, presumably, mainland *A. boskianus*, and the cladogenesis leading to *A. schreiberi* thus rendered *A. b. asper* paraphyletic.

The Turkish subspecies, *A schreiberi ataturi*, was recorded for the first time by Franzen (1998) at a very restricted area, of around 15 km of coastal strip (between Botas and Yukarı Burnaz, Hatay Province). Owing to the remarkable morphological similarity between

A. sc. ataturi and the Cypriot population, the specimens were initially identified as *A. sc. schreiberi* (Franzen, 1998). Yalçinkaya & Göçmen (2012), however, described this population as a new distinct subspecies, *A. s. ataturi*, presenting several differences between the two, in both morphology and blood-serum proteins. The origin of *A. s. ataturi* remains uncertain, as it is debated whether the newly discovered Turkish population is a relict or an introduction from Cyprus. Franzen (1998) described this population as a possible introduction from Cyprus through the harbour of Botas, but Sindaco *et al*. (2000) suggested that it might be a relict of a previously larger population because its present distribution is similar to that of some insects and lizards [*Archaeolacerta* (*Phoenicolacerta*) *laevis* and *Ablepharus budaki*]. Yalçinkaya & Göçmen (2012) proposed that *A. sc. ataturi* arrived in Turkey from the nominate population in Cyprus during the Messinian crisis. The phylogenetic results, haplotype networks, and low levels of genetic divergence we found suggest that the two subspecies from Cyprus and Turkey have not been genetically isolated for a long period of time (i.e. they share alleles in all three nuclear genes and *A. s. ataturi* is nested within *A. s. schreiberi* in the phylogeny; Figs 2, 3). Our results therefore contrast with the two latter scenarios of a relict population or a Messinian dispersal. Both divergence time and the genetic similarity of the two subspecies agree with the original suggestion of Franzen (1998) that these animals were introduced into Turkey from Cyprus. Further support for this hypothesis is that *A. s. ataturi* is restricted to the vicinity of the Botas-Adana harbour and is absent in other suitable habitats (coastal sand dunes) widespread in south-eastern Turkey. Its close morphological features to *A. s. schreiberi* (Franzen, 1998) likewise support an introduction scenario.

The third subspecies, *A. s. syriacus*, is nested within *A. b. asper* in the concatenated, mtDNA and nDNA trees and is clearly genetically distinct from the Cyprus and Turkey *A. schreiberi* lineage. The close relations of *A. s. syriacus* with *A. boskianus* may shed light on the origin of the former. *Acanthodactylus schreiberi syriacus* is distributed on stable sands of the coastal plain of the eastern Mediterranean in Israel and southern Lebanon (Salvador, 1982; Hraoui-Bloquet *et al*., 2002; Bar & Haimovitch, 2011), habitats resembling those of *A. s. schreiberi* from Cyprus (Baier, Sparrow & Wiedl, 2009). The oldest divergence of the *A. b. asper* clade that includes *A. s. syriacus* is estimated to have occurred during the late Miocene around 5.58 Mya, but no further dates are available for the grouping of *A. s. syriacus*, as a result of low support values. The coastal plain of the eastern Mediterranean was submerged during the late Miocene, and re-emerged only toward the Pliocene (Nir, 1970; Horowitz, 1979). The sands of the coastal plain, where *A. s. syriacus* occurs (Salvador, 1982; Arnold, 1983; K. Tamar & S. Meiri, pers. observ.), were repeatedly submerged and re-emerged during the Pleistocene sea-level changes (during interglacial and glacial periods, respectively). A possible scenario for *A. sc. syriacus*'s origin includes several waves of dispersal of Middle Eastern *A. boskianus*, which occurs on coarse substrates (Amitai & Bouskila, 2001; Disi *et al*., 2001; Baha El Din, 2006; pers observ.) toward the Mediterranean shore. *Acanthodactylus boskianus asper* is absent from Mediterranean climate habitats in Lebanon and Israel. It occupies only xeric zones, suggesting an invasion to the coastal plain when sandy habitats allowed desert flora and fauna to migrate northwards (Yom-Tov, 1988). These populations adapted to sandy soils and evolved morphological features that distinguish them from the desert hard substrate forms of *A. b. asper*. We view this as the most likely scenario given the biogeography, the phylogenetic results, and the habitat preferences and adaptations of these lizards. An alternative scenario, according to which the ancestor of *A. schreiberi* originated in Cyprus and dispersed to the shores of Israel and Lebanon (or originated in the coastal plain of the Eastern Mediterranean and dispersed to Cyprus), we regard as far less likely. Such a scenario requires much closer genetic relationships between these two forms, and is further weakened by the close relationship between *A. s. syriacus* and the geographically adjacent *A. b. asper* populations.

Acanthodactylus boskianus asper is highly variable, both morphologically (Salvador, 1982; Arnold, 1983) and genetically (this study). The subspecies is paraphyletic, as *A. schreiberi* is nested within it. The topology of the *A. b. asper* tree shows four different geographical groupings: Syria (clade A), north Jordan plus north Oman, North Africa, and Middle East plus south Arabia (groups C1, C2, C3, respectively). The different groups in this subspecies are estimated to have first diverged during the late Miocene approximately 6.5 Mya with the split of the Syrian population. The Syrian lineage is genetically distant from *A. schreiberi* and the other *A. b. asper* specimens. The nuclear networks indicate that this group is closer to the other *A. b. asper* samples rather than to *A. schreiberi*. The geographical splits in the rest of the *A. b. asper* range (clade C) are estimated to have started around 5.58 Mya. These groups are supported as a distinct clade, but are closely related to each other in both the concatenated and nuclear trees (Figs 2, S1, respectively). The diversification within this clade is estimated to have occurred during the late Miocene to early Pliocene, when *A. b. asper* dispersed widely, west to North Africa and in Arabia. The divergence within the North African group (group C2) is estimated to have occurred during the Pliocene, approximately 4.56 Mya, with the Egyptian, Nigerian, and Sudanese populations later dispersing west and north

in Africa. This diversification correlates to the arid climate starting in southern Sahara during the early-mid Pliocene and later in northern Africa between the Pliocene and the Pleistocene (Le Houérou, 1997), as has been suggested for the dispersal of *Mesalina guttulata* in Africa (Kapli *et al*., 2008). Other evidence relates dry climate in North Africa to an earlier period around 7 Mya (Schuster *et al*., 2006) as has been suggested for the genus *Chalcides* and other reptiles (Carranza *et al*., 2008; Metallinou *et al*., 2012 and reference therein). The aridification of North Africa has most likely contributed for the successful dispersal of *A. b. asper* west from south-west Asia into Africa. Morphological studies of *A. boskianus* show relatively uniform populations in North Africa, suggesting recent migration (Salvador, 1982; Arnold, 1983). The other two geographical groupings of *A. b. asper* from the Middle East and Arabia (groups C1 and C3) are located in two distinct inner clades, but their location within each inner clade is poorly supported. The topology of the concatenated tree (Fig. 2) shows that the group from northern Jordan and northern Oman (group C1) is closer to the North African one than to the geographically close Middle-Eastern and south Arabian group (group C3). The taxonomic separation between north and south Oman has been recognized in other species of reptiles and supported by the topography of Oman (e.g. *Echis coloratus* and *Echis omanensis*; Arnold, Robinson & Carranza, 2009). In the nuclear tree (Fig. S1) these two groups are closer to one another, and with the North African group form clade C. Therefore, the low support values amongst these groupings prevent an appropriate and thorough analysis of this subspecies. The close relationship amongst the geographical groups may reflect close phylogenetic relationships amongst these populations, suggesting recent migration, divergence, and ongoing gene flow.

Systematics and taxonomic implications

The relationships within the *A. boskianus* species group conflict with the current known taxonomy of *A. schreiberi* and *A. b. asper* (samples of the other subspecies of *A. boskianus* and of *A. nilsoni* were unavailable for this study). Both species have been found to be closely related and paraphyletic. The constrained topology tests exemplify the close entangled relationship between the two species as the separate monophyly of the two species was not rejected, and the enforced monophyly of them both together was inconclusive.

Several causes can be responsible for paraphyly in species such in the *A. boskianus* species group (Funk & Omland, 2003 and references therein): (1) inadequate phylogenetic information; (2) imperfect taxonomy (incorrect/inaccurate species limits) derived from misidentifying intraspecific variation; (3) interspecific gene flow – hybridization through interspecific mating and the subsequent backcrossing of hybrids into the parental populations; (4) incomplete lineage sorting because of recent speciation events; (5) unrecognized paralogy. We suggest that the relationships between *A. schreiberi* and *A. b. asper*, based on mitochondrial and nuclear data, are most likely explained by incorrect taxonomy, probably because of the great variability of the latter species, and to convergence. As was the case in the molecular studies of the *A. pardalis* and *A. erythrurus* species groups (Harris *et al*., 2004; Fonseca *et al*., 2008, 2009; Carretero *et al*., 2011 and reference therein), there are many problems with the current taxonomic status of several species groups within *Acanthodactylus*.

Taking the molecular results of our study into account, there are several systematic approaches to classifying the *A. schreiberi*–*A. b. asper* clade. The Cypriot and Turkish populations of *A. schreiberi* are very closely related, with the latter nested in the former, and the two subspecies share nuclear alleles (Fig. 3). Furthermore, the low uncorrected *p*-distance is positively correlated with subspecies-level distances within other lacertid species (i.e. 1.6% of *Cytb* in *Lacerta bilineata chloronota*; Godinho *et al*., 2005). We therefore conclude that Cypriot animals were recently introduced to Turkey, and that the Turkish population does not merit a subspecific rank. We suggest that *A. s. ataturi* Yalçinkaya & Göçmen 2012 is a junior synonym of *A. s. schreiberi* Boulenger, 1878.

Regarding the relationships between *A. schreiberi* and *A. b. asper*, a few scenarios are possible. One is to sink *A. schreiberi* within *A. boskianus* to create one species (*A. boskianus*) with high genetic and morphological variability ranging over a broad distribution. Another is for the two taxa be regarded as a species complex (the *A. boskianus-schreiberi* complex) until further investigation on the subject. However, although *A. schreiberi* is nested within *A. b. asper*, the populations from Cyprus and Turkey represent a distinct evolutionary lineage with distinct genetic and morphological features, and thus it is logical to retain the specific status. Two other solutions are possible. The first is to re-evaluate the Syrian populations and to consider elevating them, as well as the more divergent lineages (and subspecies) of *A. boskianus* to specific status. This would necessitate an examination of the phylogeny and morphology of the other four subspecies of *A. boskianus* (*A. b. boskianus*, *A. b. euphraticus*, *A. b. khattensis*, and *A. b. nigeriensis*), and the identification of distinctive phenotypic features in the Syrian lizards. Another solution is to recognize the maintenance of gene flow amongst mainland populations of *A. b. asper* after the divergence of the insular endemic *A. schreiberi*, and thus the evolutionary cohesion of the paraphyletic *A. b. asper*. Arnold (1983) noted that *A. schreiberi* may

have originated as an isolate from *A. boskianus* because of their shared morphology and hemipenis features. Our results support this scenario, which includes the dispersal of *A. schreiberi* to Cyprus from a mainland population that was most probably *A. boskianus*. It may be assumed that the ancestor of the Cypriot *A. schreiberi*, after arriving on Cyprus, remained isolated for a long period of time and thus evolved to the modern form of *A. schreiberi*. Meanwhile, the same ancestral continental populations, not isolated from each other, continued to exchange genes to varying degrees, remaining *A. boskianus*.

The Israeli–Lebanese subspecies *A. sc. syriacus* is only distantly related to the nominate form *A. sc. schreiberi*. This subspecies is highly phylogenetically divergent from the Cypriot and Turkish populations, having higher *p*-distances (*12S*: 4%; *Cytb*: 11–12%) than those found between other lacertid species (e.g. 7.4–8.2% of *Cytb* amongst *Iberolacerta aranica*, *Iberolacerta aurelioi*, and *Iberolacerta bonnali*, and 4.1–5.8% of *Cytb* between *Lacerta bilineata* and *Lacerta viridis*; Crochet *et al*., 2004; Godinho *et al*., 2005, respectively). The nuclear haplotype networks further show that Lebanese and Israeli populations share alleles only with *A. b. asper*, but not with the nominotypical, Cypriot, form. Arnold (1983) suggested that the geographical variation of *A. boskianus* reflects niche differences, with animals from xeric areas with dense, rigid, and spiny vegetation having larger dorsal scales than animals from more mesic areas. As was assumed for *A. schreiberi*, we suggest that other mainland populations of *A. b. asper* were the ancestors of the Lebanese–Israeli Coastal plain forms. We suggest that *A. s. syriacus* is an ecomorph of *A. b. asper* that dispersed from the usual xeric habitats of the species and adapted to the new, more mesic environment of the stable sands of the coastal plains of the eastern Mediterranean. As a consequence, this ecomorph converged on the morphology of *A. s. schreiberi*, which inhabits the coastal sands of Cyprus (Baier *et al*., 2009), but still maintains differentiating features by having coarser dorsal scales and sharp keels (Salvador, 1982; Arnold, 1983; Franzen, 1998). This convergence led to the description of *A. s. syriacus* as a member of *A. schreiberi*. The morphological assessment and the close morphological similarities between *A. b. asper* and *A. s. syriacus* may explain the wrong classification. A similar, erroneous, reasoning led Reed & Marx (1959) to identify specimens with fine scales from Iraq as *A. schreiberi.* Salvador (1982) re-examined these specimens and assigned them to *A. boskianus*. The morphological differences between the two forms are less prominent, especially where the two forms occur in close geographical proximity, in the southern coastal plain and north-western Negev Desert of Israel (Bar & Haimovitch, 2011). According to our results, *A. s. syriacus* actually belongs to *A. b. asper*, being a coastal-dune ecomorph, convergent with, but evolutionarily distinct from, *A. schreiberi*. Thus, our preferred scenario is to treat the name *Acanthodactylus schreiberi syriacus* Böttger, 1879 (which was originally described as *A. boskianus* var. *syriacus* by Böttger, 1879) as a junior synonym of the name *Acanthodactylus boskianus asper* (Audouin, 1827).

Recognizing *A. s. syriacus* as a junior synonym of *A. b. asper* may have important implications for the conservation of this coastal sand dune form, which is classified as critically endangered in Israel (Dolev & Pervolutzki, 2004). However, as the Israeli and Lebanese coastal dune ecosystem has probably developed only very recently during the Quaternary (Nir, 1970; Horowitz, 1979), this form represents a remarkable case of rapid evolutionary change. It is also a remarkable case of convergent evolution (with the Cypriot *A. sc. schreiberi*). Thus, we feel that these populations are unique evolutionary entities that merit special conservation efforts.

The use of nuclear genes is a valuable method for estimating species divergence and lineage sorting, and helps evaluate isolated lineages and evolutionary history. The incorporation of mitochondrial and nuclear data provides thorough topologies, informative networks, and divergence times that reveal useful information for a problematic taxonomy such as that of the *A. boskianus* species group. We have shown that phylogenetic approaches to the confusing taxonomy of two closely related, and morphologically similar, species can shed light on their unclear relationships, resolve between homoplasy and shared ancestry, and identify patterns of species evolutionary history and biogeography.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. Bayesian inference tree of the *Acanthodactylus boskianus* and *Acanthodactylus schreiberi* specimens inferred using *melano-cortin 1 receptor* (*MC1R*), *acetylcholinergic receptor Muscarinic 4* (*ACM4*), and *oocyte maturation factor MOS* (*c-mos*) nuclear gene fragments. Posterior probability in the Bayesian analysis is indicated by black dots on the nodes (values ≥ 0.95 shown) and maximum likelihood bootstrap support values are indicated in parentheses (values ≥ 70% shown). Sample codes and colours correlate to specimens in Table 1 and in Figures 1–3.

Figure S2. Phylogenetic tree of the generalized mixed Yule-coalescent model based on the Bayesian mtDNA haplotype data with a single threshold model for the partitions by genes. The threshold between intra- vs. interspecific variation is indicated by a vertical red line.

Figure S3. Phylogenetic tree of the generalized mixed Yule-coalescent model based on the Bayesian mtDNA haplotype data with a single threshold model for the partitions based on PartitionFinder. The threshold between intra- vs. interspecific variation is indicated by a vertical red line.

Table S1. Information on the length and primers used (orientation, reference, and PCR conditions) for all genes in this study and the number of variable (V) and parsimony-informative (Pi) sites in the alignment calculated for the ingroup only.