



# 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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## 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<sup>2</sup>; 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.

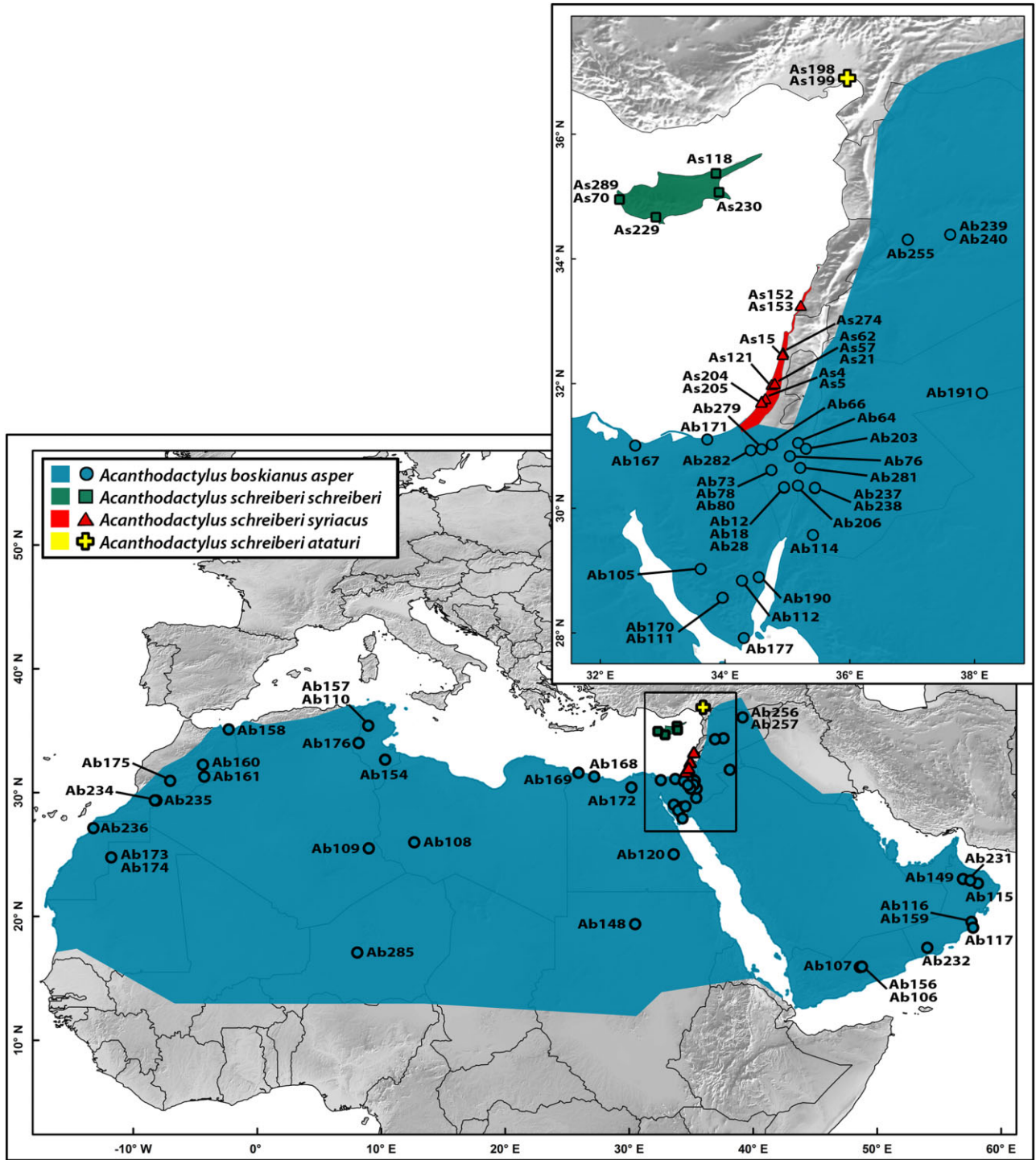
Chromatographs were checked manually, assembled and edited using GENEIOUS 5.3.6 (Biomatter Ltd). For the nuclear genes *MC1R*, *ACM4*, and *c-mos*, heterozygous individuals were identified and coded according to the International Union of Pure and Applied Chemistry (IUPAC) ambiguity codes. Coding gene 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 partition and 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*† | <i>Acanthodactylus boskianus</i> | MCCI-R471     | Algeria | Tassili 'n' Ajjer                                    | KJ567694 | KJ567812 | KJ548037 | KJ547885 | KJ547987 |
| Ab171   | <i>Acanthodactylus boskianus</i> |               | Egypt   | El Arish, Sinai                                      | KJ567676 | KJ567776 | KJ548044 | KJ547854 | KJ548003 |
| Ab167*† | <i>Acanthodactylus boskianus</i> |               | Egypt   | Baluz, Sinai   | KJ567727 | KJ567790 | KJ548063 | KJ547852 | KJ548014 |
| Ab105*  | <i>Acanthodactylus boskianus</i> | MCCI-R1566    | Egypt   | Between Serabit el Khadim and<br>Gebel Raqaba, Sinai | KJ567672 | KJ567768 | KJ548035 | KJ547849 | KJ547966 |
| Ab190*  | <i>Acanthodactylus boskianus</i> |               | Egypt   | 14 km SW of Nuweibaa, Sinai                          | KJ567693 | KJ567773 | KJ548057 | KJ547856 | KJ547951 |
| Ab112   | <i>Acanthodactylus boskianus</i> | MCCI-R1568    | Egypt   | Gebel Gunna, Sinai                                   | KJ567690 | KJ567771 | KJ548038 | KJ547851 | KJ547950 |
| Ab170*  | <i>Acanthodactylus boskianus</i> |               | Egypt   | St. Catherine, Sinai                                 | KJ567751 | KJ567772 | KJ548043 | KJ547853 | KJ547964 |
| Ab111*  | <i>Acanthodactylus boskianus</i> | MCCI-R1567    | Egypt   | Crossroad St. Catherine to<br>Fox camp, Sinai        | KJ567689 | KJ567770 | KJ548056 | KJ547886 | KJ547982 |
| Ab177*  | <i>Acanthodactylus boskianus</i> |               | Egypt   | Sharm el Sheikh, Sinai                               | –        | KJ567774 | KJ548047 | KJ547855 | KJ547965 |
| Ab168*† | <i>Acanthodactylus boskianus</i> |               | Egypt   | Matruh   | KJ567728 | KJ567824 | KJ548042 | KJ547910 | –        |
| Ab169*† | <i>Acanthodactylus boskianus</i> |               | Egypt   | Sidi Brani   | KJ567729 | KJ567813 | KJ548068 | KJ547872 | KJ548015 |
| Ab172*† | <i>Acanthodactylus boskianus</i> |               | Egypt   | Wadi El Natrun                                       | KJ567699 | KJ567822 | KJ548079 | KJ547887 | KJ547986 |
| Ab120*  | <i>Acanthodactylus boskianus</i> |               | Egypt   | 60 km E of Idfu                                      | KJ567698 | –        | KJ548039 | KJ547870 | KJ547969 |
| Ab279   | <i>Acanthodactylus boskianus</i> | TAU-R.16058   | Israel  | Wadi Revivim   | KJ567673 | KJ567767 | KJ548051 | KJ547911 | KJ547952 |
| Ab66    | <i>Acanthodactylus boskianus</i> | TAU-R.16160   | Israel  | Shivita junction                                     | KJ567671 | KJ567765 | KJ548033 | KJ547879 | KJ547949 |
| Ab282   | <i>Acanthodactylus boskianus</i> | TAU-R.16295   | Israel  | Kmehin   | KJ567682 | KJ567780 | KJ548053 | KJ547932 | KJ547954 |
| Ab64*†  | <i>Acanthodactylus boskianus</i> |               | Israel  | Rotem plain  | KJ567670 | KJ567764 | KJ548078 | KJ547875 | KJ547945 |
| Ab203*  | <i>Acanthodactylus boskianus</i> | HUJ-R-24055   | Israel  | S of Wadi Zafit                                      | KJ567691 | KJ567766 | –        | –        | –        |
| Ab76*   | <i>Acanthodactylus boskianus</i> | TAU-R.16274   | Israel  | Mt Tzin  | KJ567674 | KJ567775 | KJ548034 | KJ547880 | KJ547946 |
| Ab73    | <i>Acanthodactylus boskianus</i> | TAU-R.16013   | Israel  | Mitzpe Ramon   | KJ567686 | KJ567783 | KJ548058 | KJ547864 | KJ547962 |
| Ab78*   | <i>Acanthodactylus boskianus</i> | TAU-R.16001   | Israel  | Mitzpe Ramon   | KJ567692 | KJ567784 | KJ548061 | KJ547874 | KJ547963 |
| Ab80    | <i>Acanthodactylus boskianus</i> | TAU-R.16002   | Israel  | Mitzpe Ramon   | KJ567687 | KJ567785 | KJ548060 | KJ547865 | KJ547957 |
| Ab281   | <i>Acanthodactylus boskianus</i> | TAU-R.16272   | Israel  | Wadi Nekarot   | KJ567681 | KJ567779 | KJ548052 | KJ547892 | KJ547953 |
| Ab206*  | <i>Acanthodactylus boskianus</i> | HUJ-R-19646   | Israel  | Paran  | KJ567677 | KJ567787 | –        | –        | –        |
| Ab12    | <i>Acanthodactylus boskianus</i> |               | Israel  | Wadi Paran   | KJ567683 | KJ567781 | KJ548059 | KJ547861 | KJ547955 |
| Ab18    | <i>Acanthodactylus boskianus</i> |               | Israel  | Wadi Paran   | KJ567684 | KJ567782 | KJ548064 | KJ547884 | KJ547956 |
| Ab28*   | <i>Acanthodactylus boskianus</i> |               | Israel  | Wadi Paran   | KJ567685 | KJ567789 | KJ548028 | KJ547862 | KJ547960 |
| Ab191*† | <i>Acanthodactylus boskianus</i> |               | Jordan  | Tell al Heber  | KJ567733 | KJ567830 | KJ548067 | KJ547883 | KJ547974 |
| Ab233   | <i>Acanthodactylus boskianus</i> |               | Jordan  | Petra  | KJ567679 | KJ567777 | KJ548048 | KJ547858 | KJ547958 |
| Ab237   | <i>Acanthodactylus boskianus</i> | NMP6V 70481-2 | Jordan  | Petra  | KJ567680 | KJ567778 | –        | KJ547881 | KJ547959 |
| Ab238*  | <i>Acanthodactylus boskianus</i> | NMP6V 70481-3 | Jordan  | Petra  | KJ567688 | KJ567788 | –        | KJ547908 | KJ547961 |
| Ab113*  | <i>Acanthodactylus boskianus</i> | MCCI-R618     | Jordan  | Petra  | KJ567675 | KJ567786 | –        | –        | –        |
| Ab114*† | <i>Acanthodactylus boskianus</i> | MCCI-R621     | Jordan  | Wadi Ramm  | KJ567730 | KJ567826 | –        | KJ547876 | KJ547988 |

Table 1. Continued

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

|         |  |                 |          |                                |          |          |          |          |          |
|---------|--|-----------------|----------|--------------------------------|----------|----------|----------|----------|----------|
| As199   | <i>Acanthodactylus schreiberi ataturi</i>    | MCCI-R1693(1)   | Turkey   | Botas                          | KJ567741 | KJ567886 | KJ548076 | KJ547918 | KJ547997 |
| As70*   | <i>Acanthodactylus schreiberi schreiberi</i> | TAU-R. 16151    | Cyprus   | Lara bay, Akamas peninsula     | KJ567737 | KJ567881 | KJ548071 | KJ547916 | KJ547998 |
| As289   | <i>Acanthodactylus schreiberi schreiberi</i> | TAU-R. 16150    | Cyprus   | Lara bay, Akamas peninsula     | KJ567738 | KJ567882 | KJ548074 | KJ547924 | KJ547994 |
| As118*  | <i>Acanthodactylus schreiberi schreiberi</i> | NMP6V 74532     | Cyprus   | 3 km S of Mersinlik, Famagusta | KJ567739 | KJ567884 | KJ548072 | KJ547917 | KJ547995 |
| As229*† | <i>Acanthodactylus schreiberi schreiberi</i> |                 | Cyprus   | Episkopi                       | KJ567743 | KJ567883 | KJ548073 | KJ547921 | KJ547999 |
| As230*  | <i>Acanthodactylus schreiberi schreiberi</i> |                 | Cyprus   | Vrysoules                      | KJ567742 | KJ567887 | KJ548077 | KJ547920 | KJ547998 |
| As15*   | <i>Acanthodactylus schreiberi syriacus</i>   | HUJ-R-23653     | Israel   | Caesarea sands                 | KJ567719 | KJ567791 | KJ548025 | KJ547866 | KJ547937 |
| As274   | <i>Acanthodactylus schreiberi syriacus</i>   | HUJ-R-23410     | Israel   | Hadera to Binyamina            | KJ567722 | KJ567792 | -        | KJ547859 | KJ547947 |
| As121*  | <i>Acanthodactylus schreiberi syriacus</i>   | TAU-R.16262     | Israel   | Rishon Le-Zion sands           | KJ567723 | KJ567795 | KJ548026 | KJ547867 | KJ547938 |
| As21*†  | <i>Acanthodactylus schreiberi syriacus</i>   | TAU-R.16398     | Israel   | Holon sands                    | KJ567724 | KJ567798 | KJ548027 | KJ547873 | KJ547942 |
| As57*   | <i>Acanthodactylus schreiberi syriacus</i>   | TAU-R.16407     | Israel   | Holon sands                    | KJ567726 | KJ567796 | KJ548084 | KJ547863 | KJ547943 |
| As62*   | <i>Acanthodactylus schreiberi syriacus</i>   | TAU-R.16412     | Israel   | Holon sands                    | KJ567725 | KJ567797 | KJ548029 | KJ547871 | KJ547944 |
| As4*†   | <i>Acanthodactylus schreiberi syriacus</i>   | HUJ-R-23986     | Israel   | Nizzanim reserve               | KJ567666 | KJ567760 | KJ548024 | KJ547847 | KJ547935 |
| As5*    | <i>Acanthodactylus schreiberi syriacus</i>   | HUJ-R-23987     | Israel   | Nizzanim reserve               | KJ567667 | KJ567761 | KJ548082 | KJ547848 | KJ547936 |
| As204   | <i>Acanthodactylus schreiberi syriacus</i>   | HUJ-R-23321     | Israel   | Ashqelon                       | KJ567668 | KJ567762 | -        | KJ547857 | KJ547940 |
| As205   | <i>Acanthodactylus schreiberi syriacus</i>   | HUJ-R-23331     | Israel   | Ashqelon                       | KJ567669 | KJ567763 | KJ548083 | -        | KJ547941 |
| As152*  | <i>Acanthodactylus schreiberi syriacus</i>   | MCCI-R925(1)    | Lebanon  | Tyre                           | KJ567720 | KJ567793 | -        | KJ547868 | KJ547938 |
| As153   | <i>Acanthodactylus schreiberi syriacus</i>   | MCCI-R925(2)    | Lebanon  | Tyre                           | KJ567721 | KJ567794 | -        | KJ547869 | KJ547939 |
| Ab207   | <i>Acanthodactylus blanfordii</i>            | MVZ:Herp-234464 | Iran     | 6 km NW of Bampur, Sistan va   | KJ567754 | KJ567846 | KJ548101 | KJ547929 | KJ548020 |
| Ab208   | <i>Acanthodactylus blanfordii</i>            | MVZ:Herp-246009 | Iran     | Sand dunes 7 km N of Bampur,   | KJ567755 | KJ567847 | KJ548102 | KJ547930 | KJ548021 |
| Ac286   | <i>Acanthodactylus cantoris</i>              | MVZ:Herp-248443 | Pakistan | 10 km S of Uthal,              | KJ567756 | KJ567850 | KJ548096 | KJ547922 | KJ548018 |
|         |  |                 |          | Baluchistan Province           |          |          |          |          |          |
| Ac287   | <i>Acanthodactylus cantoris</i>              | MVZ:Herp-248447 | Pakistan | 45 km NW of Nagar Parkar       | KJ567757 | KJ567851 | KJ548097 | KJ547923 | KJ548019 |
| Af197   | <i>Acanthodactylus felcis</i>                | CAS 227596      | Oman     | 23 km W of Ajdarawt            | KJ567734 | KJ567845 | KJ548100 | KJ547928 | KJ548008 |
| Am63    | <i>Acanthodactylus masirae</i>               | IBES7643        | Oman     | 20 km E of Ras Madrakah        | KJ567753 | KJ567849 | KJ548095 | KJ547925 | KJ548017 |
| Am50    | <i>Acanthodactylus masirae</i>               |                 | Oman     | Masirae island                 | KJ567752 | KJ567848 | KJ548094 | KJ547931 | KJ548016 |
| Ao25    | <i>Acanthodactylus ophiodurus</i>            | HUJ-R-19189     | Israel   | Timna valley                   | KJ567750 | KJ567844 | KJ548099 | KJ547927 | KJ548007 |
| Ao79    | <i>Acanthodactylus ophiodurus</i>            | MCCI-R627       | Jordan   | Diseh                          | KJ567749 | KJ567843 | KJ548098 | KJ547926 | KJ548006 |
| As11    | <i>Acanthodactylus scutellatus</i>           | TAU-R.16389     | Israel   | Bir Mashash sands              | KJ567758 | KJ567852 | KJ548103 | KJ547933 | KJ548022 |
| As44    | <i>Acanthodactylus scutellatus</i>           | 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                           | <i>12S</i>   | ~387        | GTR + I + G   | Not rejected ( $P < 0.7396$ ) |
|                                   | <i>Cytb</i>  | 405         | TrN + I + G   | Rejected ( $P < 2.1819E-7$ )  |
|                                   | <i>MC1R</i>  | 663         | GTR + I       | Not rejected ( $P < 1$ )      |
|                                   | <i>ACM4</i>  | 429         | HKY + I       | Not rejected ( $P < 1$ )      |
|                                   | <i>c-mos</i>   | 522         | TPM1uf + G    | Not rejected ( $P < 1$ )      |
| PartitionFinder –<br>Concatenated | <i>12S</i> , <i>Cytb</i> (C1)                                | 2406        | GTR + I + G   |                               |
|                                   | <i>c-mos</i> (C1), <i>Cytb</i> (C2)                          |             | TrNef + I + G |                               |
|                                   | <i>Cytb</i> (C3)   |             | TrN + I + G   |                               |
|                                   | <i>ACM4</i> (C1,2), <i>MC1R</i> (C1)                         |             | TrN           |                               |
|                                   | <i>MC1R</i> (C2)   |             | F81           |                               |
|                                   | <i>MC1R</i> (C3)   |             | HKY + G       |                               |
|                                   | <i>ACM4</i> (C3), <i>c-mos</i> (C2, 3)                       |             | TrNef + I + G |                               |
| PartitionFinder –<br>mtDNA        | <i>12S</i> , <i>Cytb</i> (C1)                                | 792         | SYM + I + G   |                               |
|                                   | <i>Cytb</i> (C2)   |             | TrN + I + G   |                               |
|                                   | <i>Cytb</i> (C3)   |             | TrN + I + G   |                               |
| PartitionFinder –<br>nuclear DNA  | <i>ACM4</i> (C1,2), <i>c-mos</i> (C1,2),<br><i>MC1R</i> (C1) | 1614        | HKY + I       |                               |
|                                   | <i>MC1R</i> (C2)   |             | F81           |                               |
|                                   | <i>MC1R</i> (C3)   |             | HKY + G       |                               |
|                                   | <i>ACM4</i> (C3), <i>c-mos</i> (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  $2 \times 10^7$  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  $\geq 70\%$  and posterior probability (pp) support values  $\geq 0.95$  (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  $\geq 0.7$ .

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  $5 \times 10^7$  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

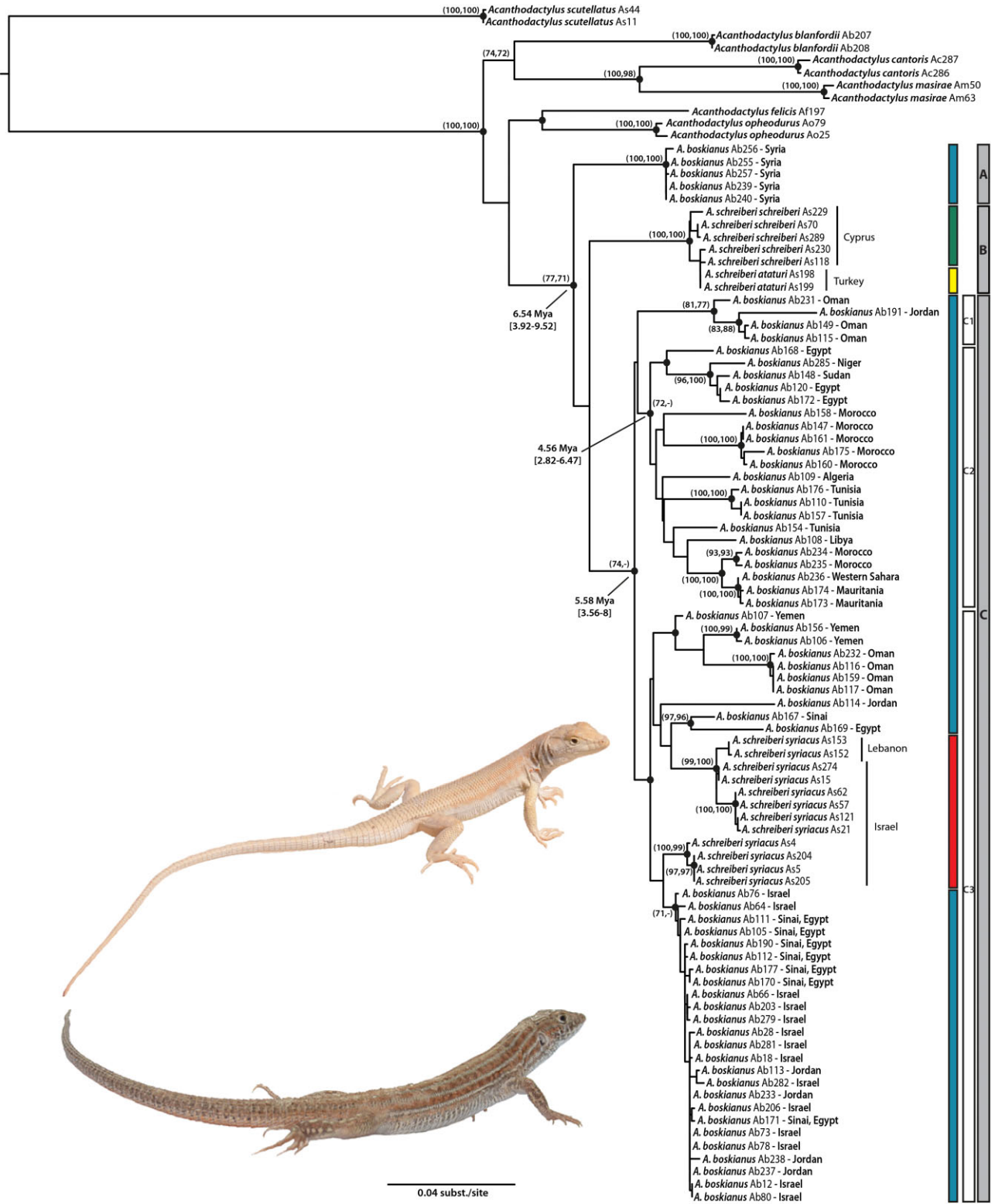
The lack of internal calibration points in *Acanthodactylus* (no fossils are known) prevents the direct estimation of time in our phylogeny. Therefore, we used the mean substitution rates and their standard error of the same 12S and *Cytb* mitochondrial regions extracted from a fully calibrated phylogeny 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 coloni-

zation 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  $5 \times 10^7$  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); ucl.d.mean of 12S Normal (initial value: 0.00553, mean: 0.00553, SD: 0.00128); ucl.d.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.



**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  $\geq 0.95$  shown, for both gene partitions and partitions by PartitionFinder (PF)], and ML bootstrap support values are indicated in parentheses (values  $\geq 70\%$  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).

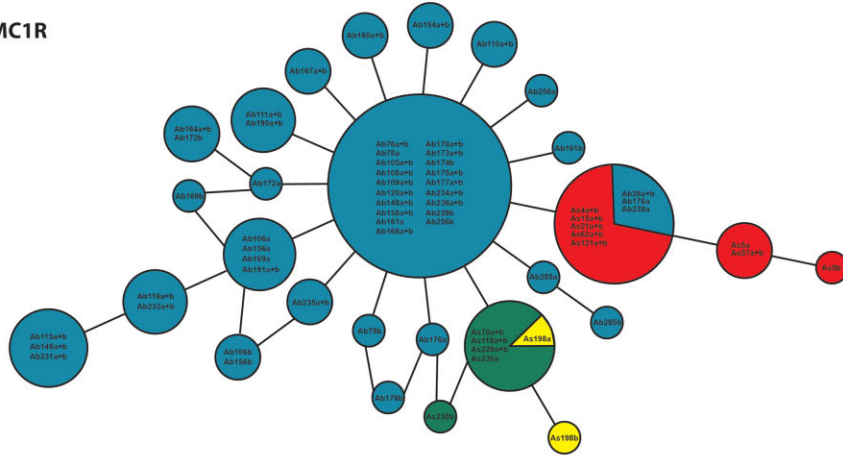
The networks constructed for the phased haplotypes of the full length nuclear markers (*MC1R*, *ACM4*, and *c-mos*) are presented in Figure 3. The nuclear network analyses show similar results for each of the three genes and closely agree with the phylogenetic tree. The Cypriot *A. s. schreiberi* and 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:  $P = 0.051$ ).

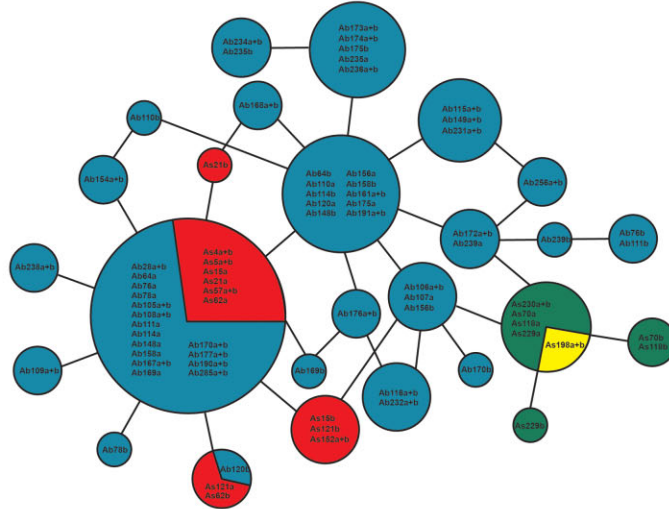
The single-threshold model in GMYC yield a topology that is clearly different from the known taxonomy. The GMYC results present a total of 25 and 24 ML independent lineages from the Bayesian haplotype mitochondrial phylogeny of the two species for the two



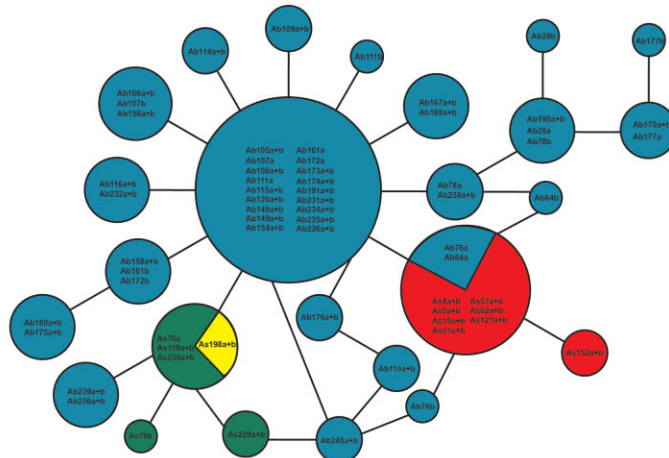
MC1R



ACM4



c-mos





**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  $\geq 0.95$  shown) and maximum likelihood bootstrap support values are indicated in parentheses (values  $\geq 70\%$  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.