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Contribution to the study of *Acanthodactylus* (Sauria: Lacertidae) mtDNA diversity focusing on the *A. boskianus* species group

N. Psonis [a,b,*], P. Lymberakis [b], D. Poursanidis [c], N. Poulakakis [a,b]

[a] Biology Department, School of Sciences and Engineering, University of Crete, Vassilika Vouton, P.O. Box 2208, GR-70013 Heraklion, Crete, Greece
[b] Natural History Museum of Crete, School of Sciences and Engineering, University of Crete, Knossos Av., P.O. Box 2208, GR-71409, Heraklion, Crete, Greece
[c] Foundation for Research and Technology–Hellas (FORTH), Institute of Applied and Computational Mathematics, 100, N. Plastira St., Vassilika Vouton, 70013 Heraklion, Crete, Greece

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ABSTRACT

The *Acanthodactylus boskianus* species group includes three species (*A. boskianus*, *A. nilsoni*, and *A. schreiberi*) of unclear phylogeny and phylogeographic history. By sequencing fragments of two mtDNA genes and performing phylogenetic, demographic, and chronophylogenetic analyses, we aimed at identifying their phylogenetic relationships while unravelling their biogeographic history. The analyses indicated that *A. boskianus* is a species complex, while *A. s. schreiberi* and *A. s. ataturi* show, both, low intraspecific genetic diversity. From a biogeographic point of view, the ancestor of *A. s. schreiberi* colonized Cyprus from the Middle East through overseas dispersal during the Pleistocene, whereas *A. s. ataturi* is considered to be a relict of a previously wider distribution.

1. Introduction

The Old World's fringe-fingered lizards of the genus *Acanthodactylus* Fitzinger, 1834 belong to Lacertidae family and consist of 42 recognized morphological species (Uetz and Hošek, 2016). The genus originated in the Middle East and expanded into Africa during probably, the middle Miocene, when Africa and Asia were connected, evolving forms adapted to xeric environments (Arnold, 1989; Harris and Arnold, 2000). Nowadays, it is distributed from the Iberian Peninsula, through North Africa, to the Middle East and West India, including Cyprus and the Arabian Peninsula (Sindaco and Jeremcenko, 2008). The taxonomy at the species level is complex and it is continuously being revised (Arnold, 1983; Crochet et al., 2003; Harris and Arnold, 2000; Harris et al., 2004; Mellado and Olmedo, 1990; Moravec et al., 1999; Salvador, 1982), due to substantial intraspecific variability.

Initial studies on the genus were based on internal and external morphology, dividing the genus into several species groups (Arnold, 1983; Boulenger, 1918; Salvador, 1982). One of them, the *boskianus* species group, consists of three morphological species (Arnold, 1983; Heidari et al., 2014; Salvador, 1982); the Bosk's fringe-fingered lizard *A. boskianus* (Daudin, 1802), the Schreiber's fringe-fingered lizard *A. schreiberi* Boulenger, 1878, and the Nilson's spiny-toed lizard *A. nilsoni* Rastegar-Pouyani, 1998. The existence of a fourth undescribed species in Iran is also argued (Heidari et al., 2014). *Acanthodactylus boskianus* is one of the most widely distributed species of the genus, ranging from Morocco through North Africa to Iran (Fig. 1) (Sindaco and Jeremcenko, 2008; Uetz and Hošek, 2016). The other two species have substantially narrower distributions, with *A. schreiberi* occurring in Cyprus, Israel, Lebanon, and Turkey, while *A. nilsoni* being located only in western Iran (Sindaco and Jeremcenko, 2008; Uetz and Hošek, 2016).

Five morphological subspecies are currently recognized in Bosk's fringe-fingered lizard (Uetz and Hošek, 2016): *A. b. boskianus* (Daudin, 1802) from the Nile Delta and the Sinai Peninsula, *A. b. euphraticus* Boulenger, 1919 from Iraq and Iran, *A. b. asper* (Audouin, 1827) from the most of the species' distribution range, *A. b. khattensis* Trape & Trape, 2012 from Mauretania, and *A. b. nigeriensis* Trape, Chirio & Geniez, 2012 from Niger and Nigeria. However, the subspecific systematics of *A. boskianus* are still obscure despite the attempts of several studies do add clarity into that issue (e.g. Arnold, 1984; Modry et al., 2001; Salvador, 1982). In the case of *A. schreiberi* there are three geographically defined groups that correspond to the three subspecies (Uetz and Hošek, 2016); *A. s. schreiberi* Boulenger, 1878 from Cyprus, *A. s. syriacus* Böttger, 1879 from Lebanon and Israel and *A. s. ataturi* Yalçinkaya & Göçmen, 2012 from a single locality in South Turkey (Hatay Province).

The first molecular phylogenetic study focusing on *Acanthodavtylus* was conducted by Harris and Arnold (2000), who assigned the genus to eight species groups, suggesting a Southern-Western Asian origin of the genus while supporting the existence of three main clades: the

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* Corresponding author at: Biology Department, School of Sciences and Engineering, University of Crete, Vassilika Vouton, P.O. Box 2208, GR-70013, Heraklion, Crete, Greece.
E-mail address: nikos.psonis@gmail.com (N. Psonis).

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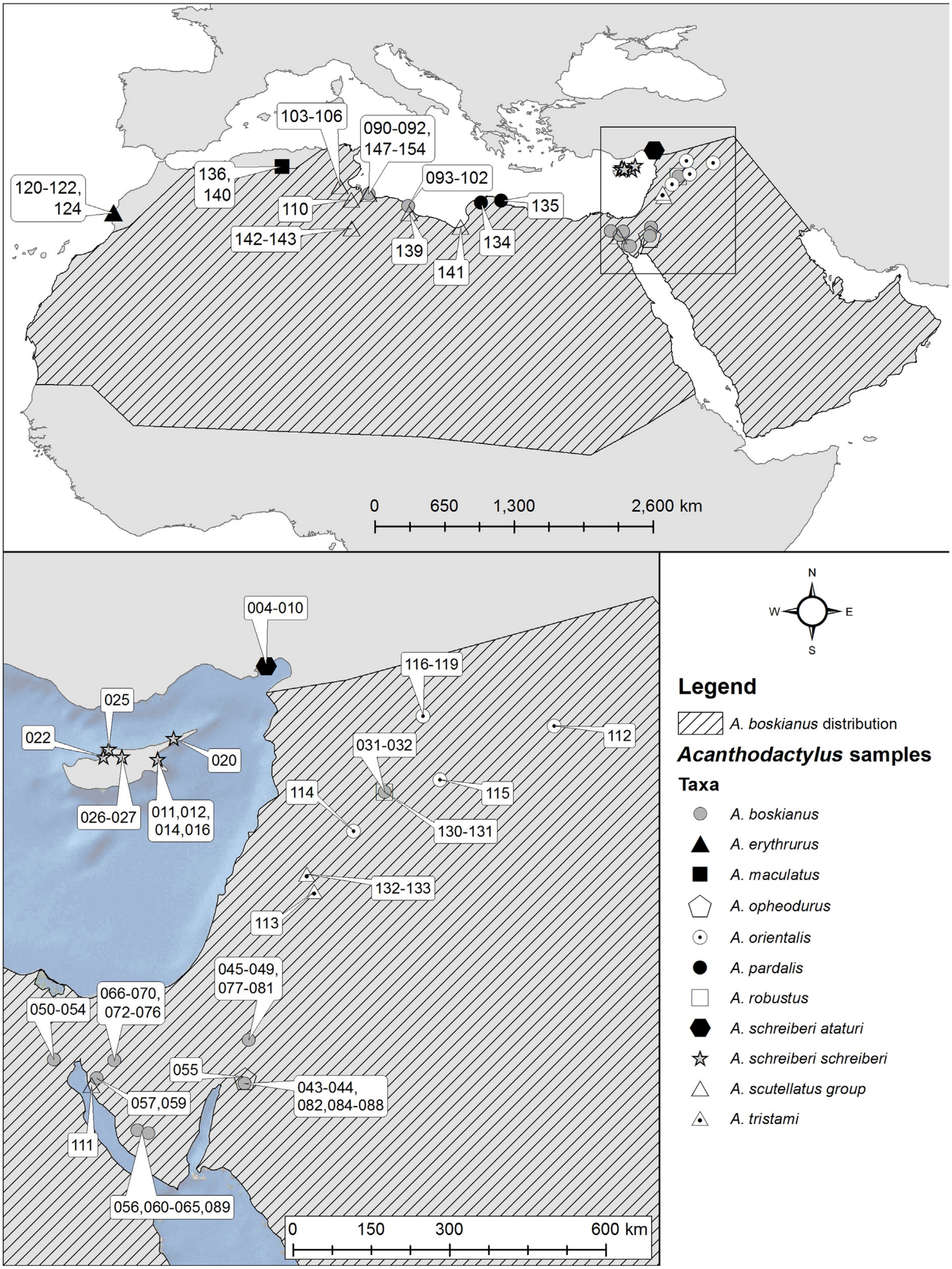


Fig. 1. Map showing the localities of the *Acanthodactylus* samples collected in the present study and the distribution of the *A. boskianus*. The numbers correspond to the sample codes of Appendix A.

Eastern, the *Western*, and the *scutellatus* clade. With regard to the *boskianus* species group the study at issue indicated that it might be paraphyletic. There were four other studies that dealt with the mitochondrial phylogeny of two species groups within *Acanthodactylus*; the *pardalis* species group (*A. beershebensis*, *A. busacki*, *A. guineensis*, *A. maculatus*, *A. mechriguensis*, *A. pardalis*, and *A. savignyi*) (Carretero et al., 2011; Fonseca et al., 2008) and the *erythrurus* species group (*A. erythrurus*, *A. blanci*, *A. guineensis*, *A. savignyi* and *A. lineomaculatus*)

(Fonseca et al., 2009; Harris et al., 2004). Their analyses supported the presence of several geographical clades in each species group and revealed several taxonomic inconsistencies (i.e., *A. mechriguensis* should be synonymized with *A. maculatus*). Moreover, four other studies used sequences that belonged to the *boskianus* species group (Heidari et al., 2014; Khannoon et al., 2013; Poulakakis et al., 2013; Tamar et al., 2014), revealing the low intraspecific diversity of *A. schreiberi* in Cyprus and Turkey, the genetic differentiation of the Eastern and the Western Egyptian populations of *A. boskianus*, and the sister taxon relationship of *A. boskianus* with *A. nilsoni*. Among them, the most recent study of Tamar et al. (2014) is the only one that has already looked into the molecular (mitochondrial and nuclear DNA data) phylogeny of the *boskianus* species group including samples of geographically distant populations of *A. b. asper*, as well as, individuals of the three *A. schreiberi* subspecies. Based on this study both species are paraphyletic, while it is suggested that; a) *A. schreiberi* is geographically restricted to Cyprus and Turkey, b) the populations from Lebanon and Israel belong to *A. b. asper*, and c) *A. s. ataturi* and *A. s. syriacus* are junior synonyms of *A. s. schreiberi* and *A. b. asper*, respectively.

From a phylogeographic point of view, there were two contradictory scenarios with regard to the evolutionary history of *A. schreiberi* (Poulakakis et al., 2013; Tamar et al., 2014). According to the first scenario, the ancestor of *A. s. schreiberi* colonized the island of Cyprus from the Middle East through overseas dispersal (probably in Pleistocene) and the Turkish populations of *A. s. ataturi*, thus being, possibly, a relict of a previously wider distribution (Poulakakis et al., 2013; Sindaco et al., 2000). The other scenario suggests that Cyprus was colonized by an ancestral form of *A. boskianus* during the Messinian Salinity Crisis (MSC) via land bridges connecting Cyprus with the surrounding area (Tamar et al., 2014). In accordance with this scenario, the Turkish population of *A. s. ataturi* is attributed to a very recent introduction event from Cyprus, highlighting its absence from other suitable habitats (coastal sand dunes) widespread in Southern-Eastern Turkey.

Species Distribution Modelling (SDM) can be used to answer past-historical and future biogeographical questions (Blois, 2012; Porfirio et al., 2014; Svenning et al., 2011; Wielstra et al., 2013), as well as to test niche similarity and overlap between current species and niche evolution across phylogenies (Ahmadzadeh et al., 2013a; Ahmadzadeh et al., 2013b; Rato et al., 2015). The process involves the rapprochement of the ecological needs of a species, based on the range of the environmental conditions experienced at known localities (Peterson, 2011). The designed model can be extrapolated on current climatic data (layers) to determine the species potential distribution. Afterwards, the model can be projected on climatological reconstructions of the past like the Last Glacial Maxima (LGM) or climatological reconstructions based on climate change scenarios (IPCC, 2014). Niches evolve over time, questioning the accuracy of predicting the past distributions based on present day models or the projection to the future. However, given the fact of the relatively short time period spanning the shift from the last glacial period to-day, niche conservatism is a realistic assumption (Peterson, 2011).

To sum up, our main objective is to contribute to the study of the *Acanthodactylus* mtDNA diversity focusing on the *boskianus* species group. More specifically, by both sequencing fragments of two mitochondrial DNA genes (the large subunit ribosomal RNA, 16S rRNA and the cytochrome *b*, cyt *b*) and using a SDM approach, the present study aims at evaluating the taxonomic status, identifying the phylogenetic relationships and unravelling the biogeographic history of the *boskianus* species group.

2. Materials and methods

2.1. Specimens, DNA extraction, amplification and sequencing

Total genomic DNA was isolated using a standard Ammonium acetate extraction protocol from muscle or liver of specimens preserved in absolute ethanol. The sampling localities are shown in Fig. 1, while the specimens are listed in Appendix A. All specimens were deposited in the Natural History Museum of Crete. The identification of species was based on external morphological characters following several authors (Anderson, 1999; Baha El Din, 2006; Crochet et al., 2003; Leviton et al., 1992; Salvador, 1982; Schleich et al., 1996). In total, 113 individuals were used, belonging to 12 species from 33 localities. Double-stranded PCR was used to amplify partial sequences of 16S rRNA and cyt *b*. Primers and conditions being used in PCR amplifications and cycle sequencing reactions are given in Table 1. Single stranded sequencing of the PCR product was performed using the Big-Dye Terminator (v3.1) Cycle Sequencing kit ® on an ABI377 automated sequencer following the manufacturer's protocol. Primers used in cycle-sequencing were the same with those used for PCR amplification. Sequences were viewed and edited using CodonCode Aligner v. 3.7.1 (Genecodes Corporation ®). The authenticity of the mtDNA sequences and homology to the targeted mtDNA genes were evaluated with a BLAST search in the NCBI genetic database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). All newly determined sequences have been deposited in GenBank (accession numbers will be provided; Appendix A).

2.2. Datasets, alignment and data analyses

Three datasets were formed so that all available sequences of *Acanthodactylus* deposited in GenBank are used. The first dataset included 211 sequences of 16S rRNA (105 produced in the present study). The second dataset consisted of 308 sequences of *cyt* b with 112 of them generated in the study at issue. The third dataset (132 specimens) was a concatenated dataset comprising only the specimens whose sequences are available for both genes. All phylogenetic analyses were separately performed for each dataset, as described below.

The alignment of the sequences was separately performed for each gene with MAFFT (Katoh and Standley, 2013) with default parameters, and FFT-NS-1 (cyt *b*)/Q-INS-i (16S rRNA) algorithms. Alignment gaps were inserted to resolve length differences between sequences. Cytochrome *b* sequences were translated into amino acids prior to analysis, and they did not show any stop codons. Sequence divergences were estimated based on Tamura and Nei's (1993) model of evolution (TrN) in MEGA (v.6; Tamura et al., 2013). The alignments used are available upon request.

2.3. Gene tree estimation

Phylogenetic trees for each of the three datasets were constructed using Neighbor Joining (NJ) (Saitou and Nei, 1987), Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BI). Neighbor Joining analyses were performed in MEGA applying the TrN model of evolution. Bootstrapping with 1000 pseudo-replicates was used to examine the robustness of clades in resulting trees (Felsenstein, 1985). Maximum Parsimony analyses were performed with PAUP* (v.4.0b10; Swofford, 2002) with heuristic searches using stepwise addition and performing tree-bisection-reconnection (TBR) branch swapping (Swofford et al., 1996). Confidence in the nodes was assessed by 1000 bootstrap replicates (100 for the cyt *b* dataset), with the random addition of taxa. Maximum Likelihood analyses were performed with RAxML (v.8; Stamatakis, 2014) under the Generalized time-reversible + gamma (G) model with 20 ML searches and 1000 rapid bootstrap replicates (using the GTRCAT model). Parameters were independently estimated for each partition (i.e., gene fragment) as well as each dataset. The evolutionary models used for the BI analyses [Generalized time-reversible (GTR; Rodriguez et al., 1990) + gamma (G) for both 16S and cyt *b* datasets] were selected using jModelTest (v0.1.1; Posada, 2008) based on the Bayesian information criterion (BIC; Schwarz, 1978), while ignoring the models that include both gamma distribution and invariable sites (Yang, 2006). Bayesian

Table 1
Primers and conditions used in PCR amplifications[a] and cycle sequencing reactions.

Gene	Primers	Sequence (5′–3′)	Size (bp)	Conditions	Reference
16S rRNA	16SAr-l	CGGCCGCCTGTTTATCAAAAACAT	~530	3 mM MgCl, 94 °C/1 min	Palumbi, 1996
	16SBr-h	GGAGCTCCGGTTTGAACTCAGATC		42–52.9 °C/1 min	
	Acanth_16SFint	GGTAGCATAATCACTTGTCC	~330	72 °C/1 min × 35 cycles	Present study
	Acanth_16SRint	GTCCTGATCCAACATCGAGG			
Cyt *b*	GLUDG	TGACTTGAARAACCAYCGTTG	~510	3 mM MgCl, 94 °C/1 min	Palumbi, 1996
	CB2	CCCTCAGAATGATATTTGTCCTCA		42–48.6 °C/1 min	
	L14841	AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA	~350	72 °C/1 min × 35 cycles	Kocher et al., 1989
	CB2	CCCTCAGAATGATATTTGTCCTCA			Palumbi, 1996
	Acant_cytb_Fint	CTGGACTATTYTTAGCCATRC	~400		Present study
	Acant_cytb_Rint	CAGGCTCCAACAACCCAACAG			

[a] Using single *Taq* DNA polymerase (Kappa ®).

Inferences were performed in MrBayes (Ronquist et al., 2012), with four runs and eight chains for each run for 6×10^6 (16S rRNA), 7.5×10^6 (cyt *b*), and 7.7×10^6 (concatenated dataset) generations, respectively, while the current trees were saved to files every 100 generations. In order to confirm that the chains had achieved stationarity, we evaluated "burn-in" by plotting log-likelihood scores and tree lengths against generation numbers using Tracer (v.1.5.0; Rambaut and Drummond, 2008). The –*ln*L stabilized after approximately 10^6 generations in all three datasets and the first 25% of the trees were discarded by default, as a conservative measure to avoid the possibility of including random sub-optimal trees. A majority rule consensus tree ("Bayesian" tree) was then produced from the posterior distribution of trees, and the posterior probabilities were calculated as the percentage of samples recovering any particular clade, where probabilities ≥95% indicate significant support.

2.4. Divergence times estimation

Mitochondrial genetic clusters representing "independently evolving" entities were selected using the concatenated data set and the Poisson Tree Processes (PTP) model (Zhang et al., 2013). One to four representatives of each independent PTP entities were used for the estimation of divergence times. Chronophylogenetic analysis was conducted under the Bayesian framework implemented in the BEAST software package (v.1.8.1; Drummond et al., 2012) using a fully partitioned, by gene fragment, dataset. This strategy permits the simultaneous estimation of divergence times, tree topology, and rates of molecular evolution. The input file was formatted with the BEAUti v. 1.8.1 utility included in the software package. Three "external" calibration age constraints, were used; a) the colonization of El Hierro Island (Canary Islands) (Guillou et al., 1996) by the endemic *Gallotia caesaris* from the neighboring La Gomera Island at 1.12 Mya (Cox et al., 2010), b) the divergence of *Podarcis peloponnesiacus* from *P. cretensis* and *P. levendis* at 5-5.5 Mya (Poulakakis et al., 2005a) that corresponds to the separation of Crete Island from Peloponnisos (Meulenkamp, 1985; Schule, 1993), and c) the divergence of *P. lilfordi* from *P. pityusensis* at ~5 Mya (Brown et al., 2008) when their respective island groups were separated (Terrasa et al., 2004). Normal prior distribution for each calibration point was used as follows: a) mean: 0.55, stdev: 0.3, b) mean: 5.3, stdev: 0.1, and c) mean: 5.25, stdev: 0.03. As for the priors, the Tree Prior category was set to Yule Process and the uncorrelated lognormal model was used to describe the relaxed clock. Model parameters were unlinked across partitions. The analysis was run for 10^8 generations with a 5000-step thinning. Results were analyzed in Tracer to assess convergence and effective sample sizes (ESSs) for all parameters (acceptable values >200). The –*ln*L was stabilized prior to 10^8, and the first 10% of the 20,000 sampled generations were discarded as recommended by Tracer. The final tree with divergence estimates was computed in TreeAnnotator v.1.8.1 (BEAST package). Trees were visualized using the software FigTree v.1.4.2 (BEAST package).

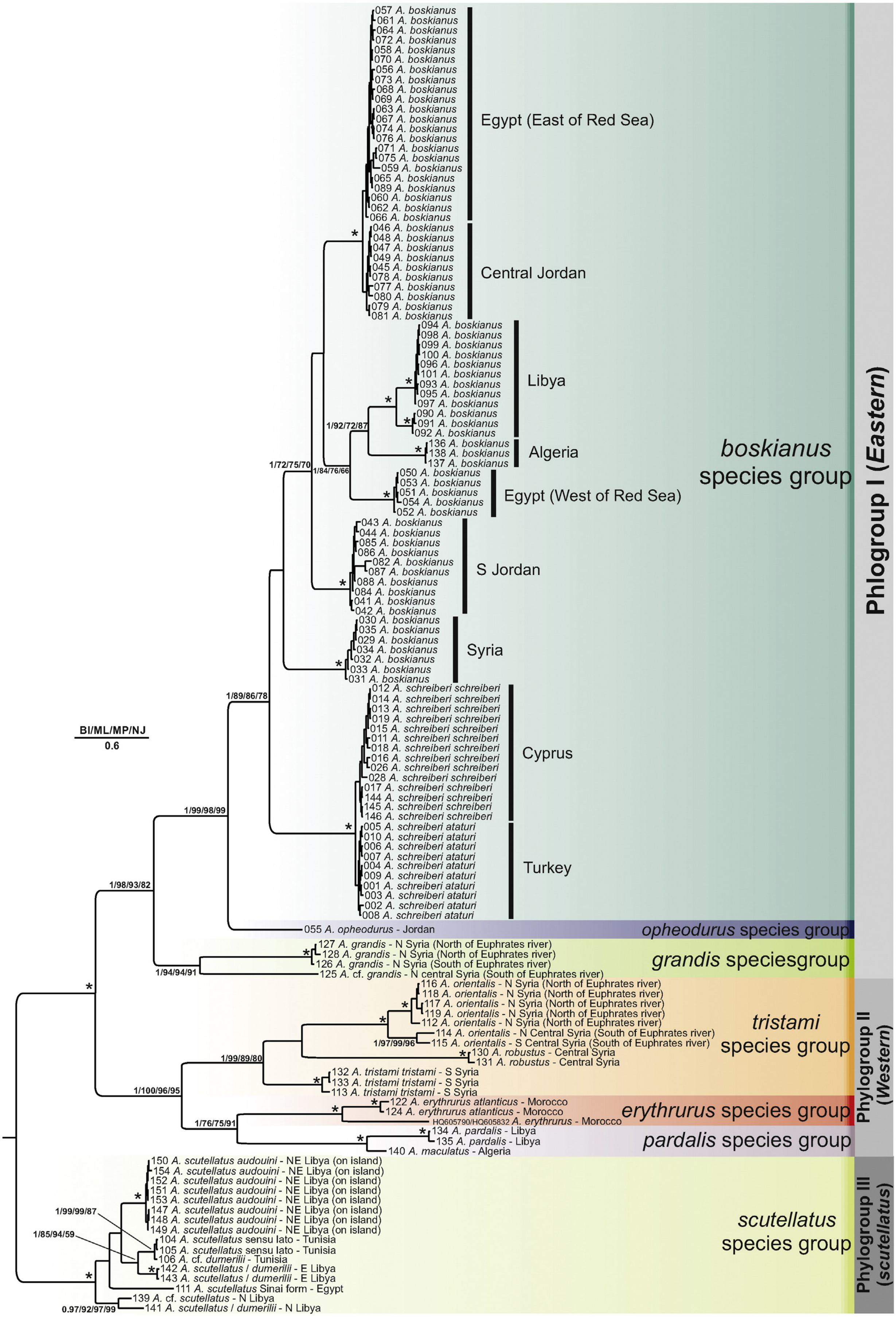
2.5. Biogeographic analyses

Algorithms and models, distribution data and geophysical variables establish complex mathematical relationships and, thus, SDMs can be used to estimate both the potential distribution of the studied species as well as the probability of presence under the studied conditions when these are projected into the geographical space (Soberón and Nakamura, 2009).

The MaxEnt (Phillips et al., 2006) approach, which is a presence-only data model, is one of the most popular niche model applications in species distribution. It uses the principle of maximum entropy on presence-only data in order to estimate a set of functions that correlate habitat suitability and environmental variables with the potential geographic distribution. MaxEnt is a machine-learning method that fits the probability distribution of maximum entropy for presences,

Table 2
Genetic distances (Tamura & Nei model of evolution) of the 16S rRNA (below diagonal-left) and cyt *b* (above diagonal-right) genes among the major clades, as revealed by the produced phylogeny based on the concatenated dataset. Values in diagonal (italics) are within lineages sequence divergence of each loci (16S rRNA/cyt *b*), whereas n.a. indicate inability to compute inter-lineage divergence due to unitary representative of the lineage.

Clade	1	2	3	4	5	6	7	8	9	10	11	12	13
1. *A. boskianus*–East of Red Sea	*0.1/0.3*	10.3	7.3	12.3	12.1	13.6	24.5	24.1	25.5	23.7	29.4	27.8	21.9
2. *A. boskianus*–West of Red Sea	4.0	*2.5/6.0*	11.8	11.8	14.6	14.5	21.4	24.2	27.0	22.9	28.0	28.7	23.7
3. *A. boskianus*–South Jordan	3.2	4.4	*0.2/0.3*	12.1	13.2	12.4	23.8	24.8	25.4	23.1	28.2	26.2	23.2
4. *A. boskianus*–Syria	4.5	5.5	4.1	*0.1/0.7*	12.9	14.9	22.6	25.0	27.2	26.1	28.8	31.5	22.2
5. *A. schreiberi*	8.4	8.0	7.5	7.2	*0.2/0.6*	15.9	21.5	24.4	25.6	28.4	26.5	30.8	22.9
6. *A. opheodurus*	6.3	8.1	6.3	6.9	8.4	*n.a/n.a.*	20.5	27.2	25.3	24.6	28.8	28.2	22.8
7. *A. grandis*	10.1	11.6	11.3	13.4	12.4	10.9	*4.3/10.0*	24.2	23.9	25.8	29.4	29.3	21.7
8. *A. orientalis*	16.4	18.1	17.9	18.9	19.1	17.3	16.7	*2.0/3.7*	18.0	17.5	26.2	23.5	26.2
9. *A. robustus*	20.3	19.6	20.4	20.9	22.3	17.8	19.2	14.1	*0.0/0.9*	18.1	23.7	21.6	21.6
10. *A. tristami*	17.1	17.1	17.3	19.4	17.1	18.7	17.9	10.3	12.7	*0.6/0.8*	20.9	21.6	20.9
11. *A. erythrurus*	17.8	17.4	16.2	17.5	17.5	16.6	16.9	16.2	20.1	14.1	*4.0/9.4*	21.7	28.6
12. *A. pardalis*	16.4	15.9	16.6	15.7	16.8	16.1	16.3	14.3	18.9	12.0	13.1	*2.2/5.8*	25.6
13. *scutellatus* species group	16.9	14.6	14.9	17.8	18.5	15.2	15.3	18.6	22.3	16.8	15.5	15.3	*2.1/5.2*


057 A. boskianus
061 A. boskianus
064 A. boskianus
072 A. boskianus
058 A. boskianus
070 A. boskianus
056 A. boskianus
073 A. boskianus
068 A. boskianus
069 A. boskianus
063 A. boskianus
067 A. boskianus
074 A. boskianus
076 A. boskianus
071 A. boskianus
075 A. boskianus
059 A. boskianus
065 A. boskianus
089 A. boskianus
060 A. boskianus
062 A. boskianus
066 A. boskianus
Egypt (East of Red Sea)
046 A. boskianus
048 A. boskianus
047 A. boskianus
049 A. boskianus
045 A. boskianus
078 A. boskianus
077 A. boskianus
080 A. boskianus
079 A. boskianus
081 A. boskianus
Central Jordan
094 A. boskianus
098 A. boskianus
099 A. boskianus
100 A. boskianus
096 A. boskianus
101 A. boskianus
093 A. boskianus
095 A. boskianus
097 A. boskianus
090 A. boskianus
091 A. boskianus
092 A. boskianus
Libya
136 A. boskianus
138 A. boskianus
137 A. boskianus
Algeria
050 A. boskianus
053 A. boskianus
051 A. boskianus
054 A. boskianus
052 A. boskianus
Egypt (West of Red Sea)
043 A. boskianus
044 A. boskianus
085 A. boskianus
086 A. boskianus
082 A. boskianus
087 A. boskianus
088 A. boskianus
084 A. boskianus
041 A. boskianus
042 A. boskianus
S Jordan
030 A. boskianus
035 A. boskianus
029 A. boskianus
034 A. boskianus
032 A. boskianus
033 A. boskianus
031 A. boskianus
Syria
012 A. schreiberi schreiberi
014 A. schreiberi schreiberi
013 A. schreiberi schreiberi
019 A. schreiberi schreiberi
015 A. schreiberi schreiberi
011 A. schreiberi schreiberi
018 A. schreiberi schreiberi
016 A. schreiberi schreiberi
026 A. schreiberi schreiberi
028 A. schreiberi schreiberi
017 A. schreiberi schreiberi
144 A. schreiberi schreiberi
145 A. schreiberi schreiberi
146 A. schreiberi schreiberi
Cyprus
005 A. schreiberi ataturi
010 A. schreiberi ataturi
006 A. schreiberi ataturi
007 A. schreiberi ataturi
004 A. schreiberi ataturi
009 A. schreiberi ataturi
001 A. schreiberi ataturi
003 A. schreiberi ataturi
002 A. schreiberi ataturi
008 A. schreiberi ataturi
Turkey
boskianus species group
Phlogroup I (Eastern)
055 A. opheodurus - Jordan
opheodurus species group
127 A. grandis - N Syria (North of Euphrates river)
128 A. grandis - N Syria (North of Euphrates river)
126 A. grandis - N Syria (South of Euphrates river)
125 A. cf. grandis - N central Syria (South of Euphrates river)
grandis speciesgroup
116 A. orientalis - N Syria (North of Euphrates river)
118 A. orientalis - N Syria (North of Euphrates river)
117 A. orientalis - N Syria (North of Euphrates river)
119 A. orientalis - N Syria (North of Euphrates river)
112 A. orientalis - N Syria (North of Euphrates river)
114 A. orientalis - N Central Syria (South of Euphrates river)
115 A. orientalis - S Central Syria (South of Euphrates river)
130 A. robustus - Central Syria
131 A. robustus - Central Syria
132 A. tristami tristami - S Syria
133 A. tristami tristami - S Syria
113 A. tristami tristami - S Syria
tristami species group
122 A. erythrurus atlanticus - Morocco
124 A. erythrurus atlanticus - Morocco
HQ605790/HQ605832 A. erythrurus - Morocco
erythrurus species group
134 A. pardalis - Libya
135 A. pardalis - Libya
140 A. maculatus - Algeria
pardalis species group
Phylogroup II (Western)
150 A. scutellatus audouini - NE Libya (on island)
154 A. scutellatus audouini - NE Libya (on island)
152 A. scutellatus audouini - NE Libya (on island)
151 A. scutellatus audouini - NE Libya (on island)
153 A. scutellatus audouini - NE Libya (on island)
147 A. scutellatus audouini - NE Libya (on island)
148 A. scutellatus audouini - NE Libya (on island)
149 A. scutellatus audouini - NE Libya (on island)
104 A. scutellatus sensu lato - Tunisia
105 A. scutellatus sensu lato - Tunisia
106 A. cf. dumerilii - Tunisia
142 A. scutellatus / dumerilii - E Libya
143 A. scutellatus / dumerilii - E Libya
111 A. scutellatus Sinai form - Egypt
139 A. cf. scutellatus - N Libya
141 A. scutellatus / dumerilii - N Libya
scutellatus species group
Phylogroup III (scutellatus)
BI/ML/MP/NJ
0.6
1/92/72/87
1/84/76/66
1/72/75/70
1/89/86/78
1/99/98/99
1/98/93/82
1/94/94/91
1/97/99/96
1/99/89/80
1/100/96/95
1/76/75/91
1/99/99/87
1/85/94/59
0.97/92/97/99
*

constrained by the values of the pixels where the species has been found. The convergence to the optimal probability is guaranteed by the deterministic algorithms (Elith et al., 2011; Phillips et al., 2006).

Our data has been retrieved from two different sources: (a) previously published studies on the focal species (Heidari et al., 2014; Khannoon et al., 2013; Tamar et al., 2014), by extracting location information through geocoding, and (b) the Natural History Museum of Crete (NHMC) collections. Thirty-nine presence points have been used for the model construction of *A. schreiberi* and 78 for *A. boskianus*. WorldClim database (Hijmans et al., 2005; http://www.worldclim.org/) has been used for the environmental data at a 2.5 arc-minutes resolution. SDMs were projected into Last Glacial Maximum (LGM; 21 ky BP) and future (2070 CE, average for 2061–2080). All projections (LGM and 2070 CE) were downloaded from the WorldClim database (http://www.worldclim.org/past & http://www.worldclim.org/CMIP5). MaxEnt 3.3.3 k (http://www.cs.princeton.edu/~schapire/maxent) was run with a 10-fold cross-validation as our running method. Occurrence thinner has been applied so that we have one presence point per pixel. The importance and effects of each bioclimatic variable were evaluated by exploring percent contribution, permutation importance and response curves generated by MaxEnt. Model results were tested with receiver operating characteristics (ROC) plots. ROC curves plot true-positive rate against false-positive rate (Phillips et al., 2004) and the area under the curve (AUC) was used as a measure of the overall fit of the model. AUC values higher than 0.7 show as good model performance (Araújo and Guisan, 2006). We used a set of 12 bioclimatic factors from the WORLCLIM database (bio1,2,3,4,6,9,12,14,15,18,19 & elevation) that were selected to describe habitat variability and species preferences based on literature (Carretero, 2004; Perry and Dmi'el, 1994).

2.6. Demographic analyses

In order to investigate and evaluate the two proposed scenarios regarding the evolutionary history of *A. schreiberi*, a series of demographic analyses were carried out, concerning the specific taxon and its focal subspecies, by using the concatenated dataset of each examined taxon. The demographic history of each taxon was inferred by the distribution analysis of the number of site differences between pairs of sequences (mismatch distribution) using ARLEQUIN (v.3.5.2.1; Excoffier and Lischer, 2010). The observed values were calculated and plotted against expected values for a model of constant population size. Unimodal distributions indicate a rapid demographic growth of the population in the recent past, whereas multimodal distributions indicate demographic equilibrium (Rogers and Harpending, 1992). The same applies for the spatial expansion. Harpending's (1994) raggedness index (*r*) and the sum of squared deviations (SSD), as implemented in ARLEQUIN, were used to evaluate the Rogers' (1995) sudden expansion model that fits to a unimodal mismatch distribution (Rogers and Harpending, 1992). To further test population expansion two additional tests were computed: Fu's (1997) *Fs* test by using ARLEQUIN; and Ramos-Onsins & Rozas' (Ramos-Onsins and Rozas, 2002) R_2 test in DNASP. Tajima's (1989) *D* was also estimated by means of ARLEQUIN. The statistical support of the above metrics and tests (except R_2 test) was assessed by 1000 bootstrap replicates.

3. Results

3.1. 16S rRNA and cyt b datasets

The alignment of the 16S rRNA dataset contained 539 base pairs with 202 (37.5%) alignment sites being variable and 175 (32.5%) parsimony informative. Similarly, the cyt *b* dataset included 777 base pairs, with 344 (44.3%) alignment sites being variable and 309 (39.8%) parsimony informative.

Pairwise genetic distances ranged from 0 to 27.0% and from 0 to 38.7% for the 16S rRNA and the cyt *b* dataset, respectively (Supplementary Table S1).

All phylogenetic analyses (NJ, MP, ML, and BI) produced trees with very similar topologies for both datasets. Unweighted MP analyses produced >10,000 equally parsimonious trees with a length of 1055 steps (consistency index, CI = 0.324 retention index, RI = 0.887) for 16S rRNA, and of 2336 steps (consistency index, CI = 0.234 retention index, RI = 0.873) for cyt *b*. The large number of equally parsimonious solutions was largely due to terminal branch swapping. Maximum likelihood analysis for the 16S rRNA dataset resulted in a topology with lnL = −5119.03. For the cyt *b* dataset ML analysis resulted in a topology with lnL = −9997.04. Bayesian inferences resulted in a topology with mean lnL = −1285.82 (16S rRNA) and lnL = −10,613.79 (cyt *b*). For both datasets, identical topologies were recovered for each of the four runs with the combined dataset, and the 50% majority-rule consensus trees of the 75×10^3 trees remaining after burn-in are presented in Supplementary Figs. S1 and S2, for 16S rRNA and cyt *b*, respectively.

According to the 16S rRNA tree (Supplementary Fig. S1) that includes eight out of nine species groups, three major phylogroups are formed (I, II, and III). Phylogroup I contains the species groups of *boskianus*, *cantoris*, *opheodurus*, and *grandis* with unresolved inter-group relationships. Phylogroup II comprises the species groups of *tristami*, *erythrurus*, and *pardalis* with unresolved phylogenetic relationships among them. Finally, phylogroup III includes only the species group of *scutellatus*. The topology of the tree based on the cyt *b* dataset (Supplementary Fig. S2) that incorporates all species groups shows almost the same basic phylogenetic pattern compared to the 16S rRNA tree. Considering the focal *boskianus* species group, several monophyletic clusters are recognized in both trees, yet the relationships among them are mostly unresolved.

3.2. Concatenated mtDNA dataset and species tree estimation

A total of 1148 base pairs (16S rRNA 535 bp and cyt *b* 613 bp) were aligned, with 419 (36.5%) alignment sites being variable and 384 (33.4%) parsimony informative.

Pairwise genetic distances ranged from 0 to 26.4% and from 0 to 34.3% for the 16S rRNA and the cyt *b* dataset, respectively (Supplementary Table 1). Table 2 shows the genetic distances among and within the major lineages revealed by the phylogenetic analyses, for the concatenated dataset.

All phylogenetic analyses (NJ, MP, ML, and BI) produced trees with very similar topologies that are in agreement with the trees of the other two datasets. Unweighted MP analysis produced >10,000 equally parsimonious trees with a length of 1424 steps (consistency index, CI = 0.440 retention index, RI = 0.902). The large number of equally parsimonious solutions was largely due to terminal branch swapping. Maximum likelihood analysis resulted in a topology with lnL = −7699.76 (final parameter estimates for 16S rRNA: base frequencies A = 0.32, C = 0.25, G = 0.19, *T* = 0.26, shape value (a) = 0.18, A/C = 1.35, A/G = 3.11, A/T = 0.97, C/G = 0.18, C/T = 6.26 and G/T = 1 and for COI: A = 0.27, C = 0.29, G = 0.15, T = 0.30, shape value (a) = 0.23, A/C = 1.84, A/G = 6.80, A/T = 0.85, C/G = 0.53, C/T = 11.33 and G/T = 1). Bayesian inference resulted in a topology with mean lnL = −1317.18. Identical topologies were recovered for each of the four runs with the combined dataset, and the 50% majority-rule consensus tree of the 75×10^3 trees remaining after burn-in is presented in Fig. 2.

According to this tree, the specimens of the genus *Acanthodactylus* are clustered into three major phylogroups (I, II, and III) that are

Fig. 2. Phylogenetic tree constructed using the Bayesian Inference method from the concatenated dataset. The posterior probabilities (>0.95) and bootstrap support (>50%) for each major lineage are given above the branches (NJ/MP/ML/BI). Asterisks indicate absolute support for all the methods, whereas the lack of values stands for very low support.

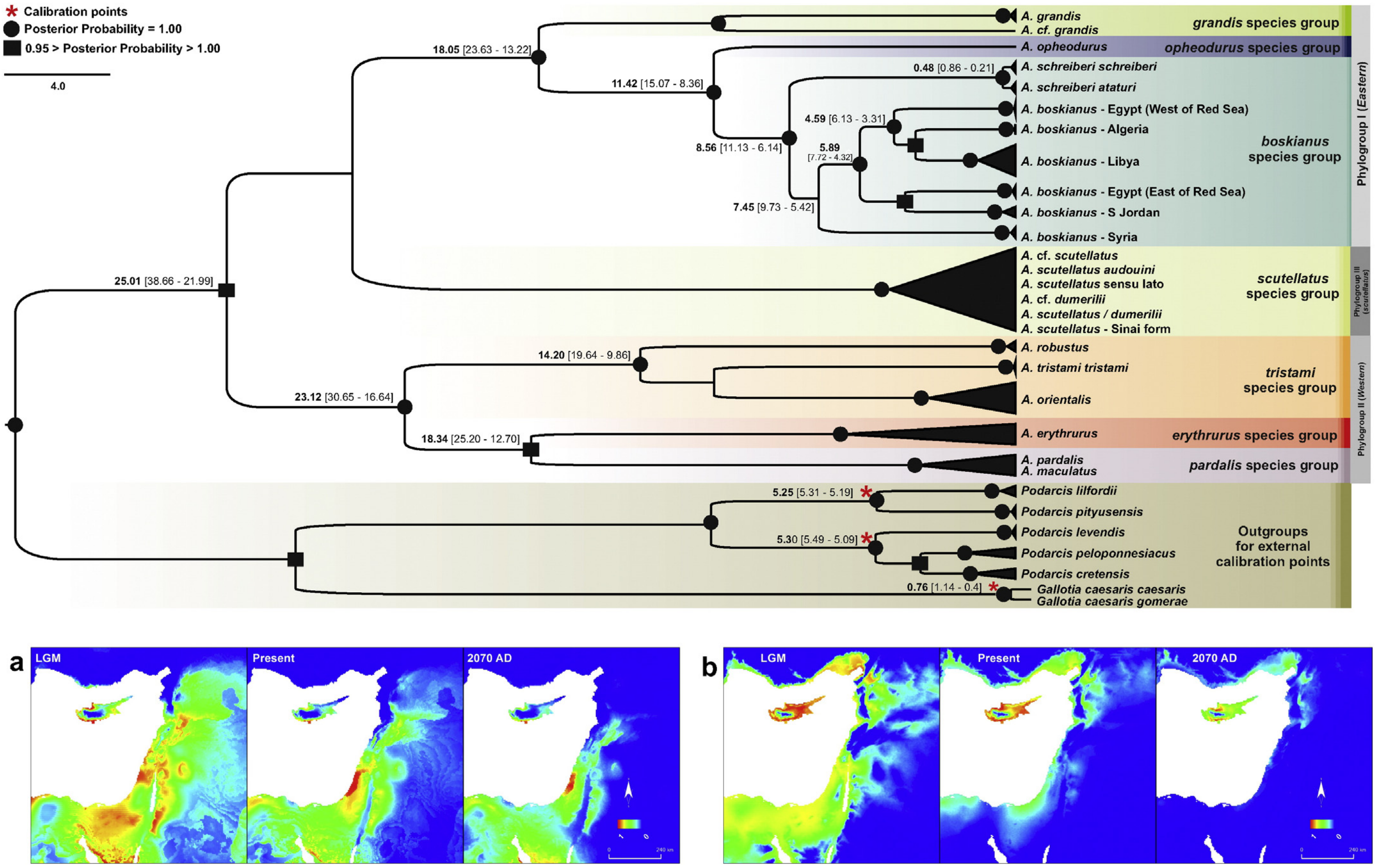


Fig. 3. Molecular timescale for *Acanthodactylus*. Ultrametric collapsed phylogenetic tree constructed by BEAST. The numbers on branches, given only for selected lineages of interest, are the estimated times of divergences (in Mya), whereas 95% highest posterior densities (HPD) are given in parenthesis. Individuals of one subclade or species are collapsed into one terminal branch. The maps under the tree correspond to the distribution maps under LGM, Present and 2070 CE climate conditions, for (a) *A. boskianus* and (b) *A. schreiberi*, respectively.

identical to the ones of the single locus trees (see above), while being divided into seven clades. In the *boskianus* species group, *A. schreiberi* is monophyletic with two, statistically unsupported, clusters that correspond to the two, geographically distinct, subspecies (*A. s. schreiberi* and *A. s. ataturi*). Although the monophyly of *A. boskianus* is not statistically supported, six major subclades are recognized, which could be grouped into four geographic regions a) Syria, b) South Jordan, c) Central Jordan and Egypt (to the east of the Red Sea), and d) to the west of the Red Sea. Within the latter that includes lineages from Egypt, Algeria and Libya, the populations from Algeria have a sister-lineage relationship with Libya.

3.3. Divergence times on mtDNA

In PTP analysis 26 distinct evolutionary entities were identified. While estimating divergence times, high effective sample sizes were observed for all parameters in the BEAST analysis (posterior ESS values >952), and convergence in the chosen chronophylogenetic analyses was reached prior to 10^8 generations ($\ln L = -8094.225$). According to the inferred dates (Fig. 3) the genus of *Acanthodactylus* started to diversify in the upper Miocene (~25.01 Mya), whereas the diversification of the *Eastern* clade (Phylogroup I) to its species groups is estimated to have occurred during the middle Miocene (~18.05 to 11.42 Mya). With respect to the focal *boskianus* species group, its divergence from the *opheodurus* species group seems to have taken place in the Middle Miocene (~11.42 Mya), whereas its diversification into different species were estimated to have happen during the Lower Miocene (~8.56 Mya). The inner clade of *A. boskianus* that includes all its subclades except for the one from Syria is estimated to have been diversified at the Lower Miocene (~5.89 Mya), whereas the diversification of the subclades from the area to the west of the Red Sea (Egypt, Algeria, and Libya) dates back to Middle Pliocene (~4.59 Mya) following the end of the Messinian Salinity Crisis (MSC) at 5.33 Mya. The split between the two focal subspecies of *A. schreiberi* is estimated during the Late Pleistocene (~480 Kya).

3.4. Biogeographic analysis

The analyses of SDM provide an insight on the past and future evolution of the spatial distribution of both species. ROC curves have shown a good model performance with a mean AUC of 0.84 for *A. boskianus* and 0.93 for *A. schreiberi* (Fig. 3a & b), having a wider distribution during LGM than the current. On present conditions the distribution of *A. schreiberi* has a significant reduction in comparison to *A. boskianus*, while under the climate change scenario, *A. schreiberi* will be restricted only in the island of Cyprus, and *A. boskianus*, although with broader distribution than *A. schreiberi*, will also be reduced in comparison to the present one. From the environmental variables that have been used, the most contributed to *A. boskianus* are BIO6 (Min Temperature of Coldest Month), BIO12 (Annual Precipitation) and BIO14 (Precipitation of Driest Month) while to *A. schreiberi* BIO6 (Min Temperature of Coldest Month), BIO18 (Precipitation of Warmest Quarter) and Elevation (alt). In Table 3 the percentage contribution and the permutation importance of the climatic variables are provided.

3.5. Historical demography

The observed demographic and spatial mismatch distributions in *A. schreiberi* did not significantly deviate from the expected curve, forming unimodal distributions (Table 4, but also see Supplementary

Table 3
Percent contribution & permutation importance of the variables used in *A. boskianus* & *A. schreiberi* models.

Variable	*Acanthodactylus boskianus*		*Acanthodactylus schreiberi*	
	Percent contribution	Permutation importance	Percent contribution	Permutation importance
alt	0.5	2	41.4	24.6
bio1	0.2	0.5	6.9	19.7
bio2	16.3	4.1	3.5	3.4
bio3	8.8	12.2	4.8	0
bio5	17.2	6.7	0.1	0
bio6	9.4	29.3	7.5	26.6
bio9	4.2	0	0.5	2.7
bio12	1.2	13.6	11.5	0
bio14	28.1	24.4	0.1	0
bio15	7	0.2	0.3	0.9
bio18	6.8	0.2	18.2	21.4
bio19	0.4	6.6	5.2	0.8

Fig. S3). The demographic SSD values were not noteworthy in all cases, with the exception of *A. s. ataturi*. On the other hand, the later taxon was the only one showing non-significant spatial SSD values. All *r* values exhibited a lack of statistical significance. Fu's *Fs* neutrality test gave valid negative values suggesting demographic expansions.

4. Discussion

4.1. Phylogenetic relationships and taxonomic implications

Based on the produced trees, several observations could be made regarding the phylogenetic relationships within the *boskianus* species group, contributing to the current taxonomy of the genus. The three phylogroups (I, II and III) correspond to the three clades described by Harris and Arnold (2000). The species group of *boskianus* belongs to the *Eastern* one (Phylogroup I) alongside with four other species groups (*blanfordii*, *grandis*, *micropholis*, and *opheodurus*).

The monophyly of *A. boskianus* species group (Fig. 2) is in agreement with previous studies (Poulakakis et al., 2013; Tamar et al., 2014). Within this clade, the two focal species form several distinct subclades, yet the unresolved phylogenetic relationships among many of them do not allow for safe conclusions. Within *A. boskianus,* four distinct subclades are currently recognized [Syria, South Jordan, Central Jordan and Egypt (to the east of the Red Sea), and to the west of the Red Sea]. The latter diversifies into three more lineage groups. The high number of distinct groups of lineages within *A. boskianus* is more apparent in the cyt *b* tree (Supplementary Fig. S2), in which >10 ones are depicted. The mean genetic distances among the four subclades ranges from 3.2% to 5.5% for the 16S rRNA and from 7.3% to 12.3% for the cyt *b* (Table 2). These values are comparable to the distances among species of other lacertids with wide distribution range and multiple distinct lineages. More specifically, the genetic differentiation among the subclades of *Ophisops elegans* are 4.4–10% and 6.3–12.9% for 16S rRNA and cyt *b*, respectively (Kyriazi et al., 2008), whereas in *Mesalina guttulata* the corresponding values are 2.8–7.2% and 4.6–15.6% (Kapli et al., 2008). Both studies proposed that these species should be considered as species complexes. Consequently, all the above indicate that A. *boskianus* should be also considered as a species complex, which is congruent with the data of nuclear markers of Tamar et al. (2014). On the other hand, the status of *A. schreiberi* is completely different. The absence of genetic divergence between *A. s. schreiberi* and *A. s. ataturi* indicates that this taxon does not merit a subspecific rank, a view also supported by Tamar et al. (2014). *Acanthodactylus schreiberi syriacus* is not included in the concatenated dataset (Fig. 2), but according to the cyt *b* gene tree (Supplementary Fig. S2) that is based on all the published sequences, it is nested topologically into the *A. boskianus*, although with low statistical support. In the paper of Tamar et al. (2014) this topology is statistically well-supported leading the authors to propose that *A. s. syriacus* belong in fact to *A. b. asper*. The phylogenetic relationships of all the *Acanthodactylus* species known in Iran have been recently examined, thus revealing the presence of two new species (*Acanthodactylus* sp$_1$ and *Acanthodactylus* sp$_2$) that clustered within the *blanfordii* and *boskianus* species groups, respectively (Heidari et al., 2014). Although the phylogenetic position of the first species is well supported (present study; Heidari et al., 2014), the position of the second one is being questioned due to low statistical support [in *boskianus* species group in Heidari et al. (2014) and in *blanfordii* species group in the present study; Supplementary Fig. S2). *Acanthodactylus nilsoni* is absent from the concatenated dataset (Fig. 2) of the present study. According to the cyt *b* gene tree (Supplementary Fig. S2) this species is topologically the root taxon of the *A. boskianus* species group, but with low statistical support. In the paper of Heidari et al. (2014) in which *A. schreiberi* is not included, *A. nilsoni* is the sister taxon to *A. boskianus* (well supported relationship), with *Acanthodactylus* sp$_2$. being the root taxon (low statistical support) of the species group. Given the uncertainty of both phylogenetic inferences the phylogenetic position of *A. nilsoni* remains unclear.

4.2. Phylogeography and divergence times

The diversification of *Acanthodactylus* started in the late Late Oligocene (~25 Mya), and the *Eastern* (Phylogroup I) and *Western* (Phylogroup II) clades were diversified into their species groups during the Early and Middle Miocene (~18–11 Mya) for the *Eastern* clade and during the Early Miocene (~23–18 Mya) for the *Western* clade, respectively.

Regarding the *boskianus* species group, the focal species started to diverge in Late Miocene (Fig. 3; 8.56 Mya), almost two million years earlier than the time estimated by Tamar et al. (2014), but within their HPD 95% confidence interval (3.92–9.52 Mya). Given its ancestor's eastern origin (Fig. 3), we supposed that either the dispersal of *A. boskianus* to the Northern Africa (to the west of the Red Sea), or a vicariance event at 5.89 Mya (Messinian) led to two recognized groups of lineages in *A. boskianus*; the one to the east and the other one to the west of the Red Sea. This time is very close to the time estimated by Tamar et al. (2014), which is 5.58 Mya. The separation between the two aforementioned subclades is also inferred by Khannoon et al. (2013), considering the river of Nile playing the role of a biogeographic barrier. The flooding

Table 4
Sum of squared differences (SSD) and Harpending's (1994) raggedness index (*r*) in demographic and spatial mismatch analysis, Fu's (1997) *Fs* and Ramos-Onsins and Rozas' (2002) R_2 statistics in samples of *A. schreiberi* and its subspecies. Indications of an expansion event are considered the non-significant SSD and *r* values (non-significance $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$), the significantly negative Fu's F_S values (significant threshold * $P < 0.02$), the significant negative Tajima's *D* values and the unimodal mismatch distribution. The significant positive Tajima's *D* and Fu's F_S values indicate bottleneck effect.

Taxa	Number of samples	Mismatch distribution (demographic & spatial)	Demographic expansion		Fu's F_S	Tajima's *D*	R_2	Spatial expansion	
			SSD	*r*				SSD	*r*
A. s. schreiberi	14	Unimodal	$0.033^{n.s.}$	$0.195^{n.s}$	-4.740^{*}	$0.005^{n.s}$	0.159	0.081^{*}	$0.195^{n.s}$
A. s. ataturi	10	Unimodal	0.006^{***}	$0.182^{n.s}$	-2.880^{*}	-1.401^{*}	0.200	$0.006^{n.s}$	$0.182^{n.s}$
A. schreiberi	24	Unimodal	$0.009^{n.s}$	$0.113^{n.s}$	-14.910^{*}	$-1.292^{n.s}$	0.089	0.010^{*}	$0.113^{n.s}$

of the Nile in early Pliocene, due to sea-level uplift of the Mediterranean Sea (Goudie, 2005), could have been indeed the driving force of the *A. boskianus* splitting into the above subclades. However, synchronous climatic changes in the circum-Mediterranean (Elewa, 2005) could have also been the driving force of this splitting.

The estimated divergence times (Fig. 3) coupled with the results from the historical demographic analyses (Table 4 and Supplementary Fig. S3) and the SDM (Fig. 3) could be used to evaluate the two suggested phylogeographic scenarios concerning the evolutionary history of *A. schreiberi*. The first scenario (Tamar et al., 2014) suggested that Cyprus was colonized during the MSC through potential land bridges connecting Cyprus to Turkey/Syria. The ancestor of *A. schreiberi* was a mainland *A. boskianus,* whereas the Turkish populations of *A. s. ataturi* were attributed to a very recent introduction event from Cyprus to Turkey. Arguments supporting this scenario are as follows: a) the potential existence of emerged sea-mountains forming land bridges in the area during the MSC, b) the restricted distribution of *A. s. ataturi* in a single locality in Hatay Province, despite the existence of suitable habitats nearby, and c) their estimated divergence time of the splitting of *A. schreiberi* at ~6 Mya. According to the second scenario (Poulakakis et al., 2013), the ancestor of *A. s. schreiberi* colonized the island of Cyprus from the Middle East through overseas dispersal during the Pleistocene with the Turkish *A. s. ataturi* considered as a relict of a previously wider distribution. The latter is also proposed by Sindaco et al. (2000) on the grounds that such a distribution has also been reported from other reptiles, such as *Phoenicolacerta laevis* and *Ablepharus budaki*. It is worth noting that the low genetic distance between the two subspecies used by Tamar et al. (2014) to support their scenario cannot be used as an argument, since both scenarios are able to explain this low value. According to our results, the estimated divergence time of the isolation of *A. schreiberi* from the rest of the *A. boskianus* group lineages is depicted in the Tortonian (~8.5 Mya) as a result of a dispersal event. This date does not fit with the first scenario that is based on the MSC event, yet it can be explained on the basis of the second one, on condition that the divergence of *A. schreiberi* took place in the mainland at that time, and Cyprus was colonized afterwards during the Pleistocene. Given the fact that there is no evidence that Cyprus reconnected with the mainland at that time (Jolivet et al., 2006; Kenneth et al., 1977; Steininger and Rögl, 1984), a dispersal event is more likely to have occurred. One more piece of evidence that enhance the second scenario and the recent colonization of Cyprus from Middle East is the extremely low intraspecific genetic diversification of *A. s. schreiberi*. Moreover, the mean genetic distance (Tamura-Nei model in cyt *b*) between *A. schreiberi* and *A. boskianus* is 14.2% (*A. s. syriacus* was considered as member to the *A. boskianus* clade in this calculation). They diverged either in 6 Mya (Tamar et al., 2014) or 8.56 Mya (present study), which is translated into an evolutionary rate of 2.33% or 1.56% my^{-1}, respectively (or 1.165% or 0.78% per my and per lineage). Based on the first scenario, the ancestor of *A. schreiberi* colonized Cyprus at 6 Mya. Taking into account that the sampling effort is quite enough covering a large part of its distribution on the island, someone will have expecting a differentiation within the Cypriot *A. schreiberi* to be close to 6.99% (6 my $*$ 1.165% my^{-1}) or 4.68% (6 my $*$ 0.78% my^{-1}), respectively. However, the estimated intra-Cyprus genetic diversification does not exceed 0.3%. This means that either the taxon does not have a very old history on the island as Tamar et al. (2014) argue or that it has experienced a very intense bottleneck effect that due to the amazing change in the diversity it will have been obvious in the tests' results (statistically significant positive Tajima's D or Fu's F_S). An analogous example is the one of *Podarcis cretensis* that was colonized the island of Crete in MSC (~5 my ago). In that case the within Crete genetic distances in cyt *b* ranged from 5 to 6% and the estimated evolutionary rate was 1.45–1.59% per million years (Poulakakis et al., 2003; Poulakakis et al., 2005b). According to the Species Distribution Modelling (SDM) that revealed a reduction of the distribution of *A. schreiberi* since GLM with its possible restriction solely on the island of Cyprus in the future, the distribution of *A. schreiberi* in Turkey seems to be a relict, thus supporting the scenario of the island of Cyprus being colonized from the Middle East through overseas dispersal during the Pleistocene and *A. s. ataturi* being a relict of a previously wider distribution.

5. Conclusions

According to the phylogenetic trees, the *boskianus* species group is monophyletic and there are at least four distinct subclades within *A. boskianus*, thus indicating that this taxon should be considered as a species complex. On the other hand, *A. s. schreiberi* and *A. s. ataturi* display, both, low intra-genetic diversification. The chronophylogenetic analyses revealed that the focal species started to diverge during the late Miocene (8.56 Mya), whereas either a dispersal or a vicariance event at 5.89 Mya led to the two groups of lineages in *A. boskianus* (to the east and west of the Red Sea). Moreover, according to the demographic analyses and the species distribution modelling, the ancestor of *A. s. schreiberi* colonized the island of Cyprus from the Middle East through overseas dispersal during the Pleistocene, and the Turkish population (*A. s. ataturi*) is considered as a relict of a previously wider distribution.

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Appendix A. Alphabetically ordered by taxon name list of specimens examined in the present study and sequences retrieved from NCBI (under the horizontal bar). The provided information include unique sample codes (only for NHMC specimens), taxon names, NHMC voucher numbers, locality names (detailed only where available), studies that their sequences were generated, and their accession numbers in GenBank. Asterisks indicate the selected samples representing the entities that have been identified by the PTP analysis

Sample code	Taxon name	NHMC voucher number	Country & locality	Study	Accession numbers (16S/cyt *b*)
004	*A. schreiberi ataturi*	80.3.11.4	Turkey – Yumurtalik – Adana	At issue	KX528701/KX528806
005	*A. schreiberi ataturi*	80.3.11.5	Turkey – Yumurtalik – Adana	At issue	KX528702/KX528807
006	*A. schreiberi ataturi*	80.3.11.6	Turkey – Yumurtalik – Adana	At issue	KX528703/KX528808
007	*A. schreiberi ataturi*	80.3.11.7	Turkey – Yumurtalik – Adana	At issue	KX528704/KX528809

(*continued*)

Sample code	Taxon name	NHMC voucher number	Country & locality	Study	Accession numbers (16S/cyt *b*)
008	*A. schreiberi ataturi*	80.3.11.8	Turkey – Yumurtalik – Adana	At issue	KX528705/KX528810
009	*A. schreiberi ataturi*	80.3.11.9	Turkey – Yumurtalik – Adana	At issue	KX528706/KX528811
010	*A. schreiberi ataturi*	80.3.11.10	Turkey – Yumurtalik – Adana	At issue	KX528707/KX528812
*011	*A. schreiberi*	80.3.11.11	Cyprus – Ammochostos – Karpasia	At issue	KX528708/KX528813
*012	*A. schreiberi schreiberi*	80.3.11.12	Cyprus – Ammochostos – Karpasia	At issue	KX528709/KX528814
014	*A. schreiberi schreiberi*	80.3.11.14	Cyprus – Ammochostos – Karpasia	At issue	KX528710/KX528815
016	*A. schreiberi schreiberi*	80.3.11.16	Cyprus – Ammochostos – Karpasia	At issue	KX528711/KX528816
020	*A. schreiberi schreiberi*	80.3.11.20	Cyprus – Gialousa – Karpasia	At issue	–/KX528817
022	*A. schreiberi schreiberi*	80.3.11.22	Cyprus – Morfou	At issue	–/KX528818
025	*A. schreiberi schreiberi*	80.3.11.25	Cyprus – Panagra – Kerinia	At issue	–/KX528819
026	*A. schreiberi schreiberi*	80.3.11.26	Cyprus – Goneli – Nicosia	At issue	KX528712/KX528820
027	*A. schreiberi schreiberi*	80.3.11.27	Cyprus – Goneli – Nicosia	At issue	–/KX528821
031	*A. boskianus*	80.3.76.3	Syria – Steppe from Palmyra to Hamah	At issue	KX528713/KX528822
032	*A. boskianus*	80.3.76.4	Syria – Steppe from Palmyra to Hamah	At issue	KX528714/KX528823
043	*A. boskianus*	80.3.76.15	Jordan – Wadi Rum spring	At issue	KX528715/KX528824
044	*A. boskianus*	80.3.76.16	Jordan – Wadi Rum spring	At issue	KX528716/KX528825
045	*A. boskianus*	80.3.76.17	Jordan – Petra	At issue	KX528717/KX528826
046	*A. boskianus*	80.3.76.18	Jordan – Petra	At issue	KX528718/KX528827
047	*A. boskianus*	80.3.76.19	Jordan – Petra	At issue	KX528719/KX528828
048	*A. boskianus*	80.3.76.20	Jordan – Petra	At issue	KX528720/KX528829
049	*A. boskianus*	80.3.76.21	Jordan – Petra	At issue	KX528721/KX5288230
*050	*A. boskianus*	80.3.76.22	Egypt – Ata' mountain	At issue	KX528722/KX528831
*051	*A. boskianus*	80.3.76.23	Egypt – Ata' mountain	At issue	KX528723/KX528832
052	*A. boskianus*	80.3.76.24	Egypt – Ata' mountain	At issue	KX528724/KX528833
053	*A. boskianus*	80.3.76.25	Egypt – Ata' mountain	At issue	KX528725/KX528834
054	*A. boskianus*	80.3.76.26	Egypt – Ata' mountain	At issue	KX528726/KX528835
*055	*A. opheodurus*	80.3.180.1	Jordan – Wadi Rum	At issue	KX528727/KX528836
056	*A. boskianus*	80.3.76.28	Egypt – Wadi el Sheikh (Feiran oasis)	At issue	KX528728/KX528837
057	*A. boskianus*	80.3.76.29	Egypt – Wadi Sudr – Qa'lat el Jundi	At issue	KX528729/KX528838
059	*A. boskianus*	80.3.76.31	Egypt – Wadi Sudr – Qa'lat el Jundi	At issue	KX528730/KX528839
060	*A. boskianus*	80.3.76.32	Egypt – Wadi Feiran – Feiran oasis to Ras Sharatib	At issue	KX528731/KX528840
061	*A. boskianus*	80.3.76.33	Egypt – Wadi Feiran – Feiran oasis to Ras Sharatib	At issue	KX528732/KX528841
062	*A. boskianus*	80.3.76.34	Egypt – Wadi Feiran – Feiran oasis to Ras Sharatib	At issue	KX528733/KX528842
063	*A. boskianus*	80.3.76.35	Egypt – Wadi Feiran – Feiran oasis to Ras Sharatib	At issue	KX528734/KX528843
064	*A. boskianus*	80.3.76.36	Egypt – Wadi Feiran – Feiran oasis to Ras Sharatib	At issue	KX528735/KX528844
065	*A. boskianus*	80.3.76.37	Egypt – Wadi Feiran – Feiran oasis to Ras Sharatib	At issue	KX528736/KX528845
*066	*A. boskianus*	80.3.76.38	Egypt – Qa'lat el Jundi	At issue	KX528737/KX528846
067	*A. boskianus*	80.3.76.39	Egypt – Qa'lat el Jundi	At issue	KX528738/KX528847
068	*A. boskianus*	80.3.76.40	Egypt – Qa'lat el Jundi	At issue	KX528739/KX528848
069	*A. boskianus*	80.3.76.41	Egypt – Qa'lat el Jundi	At issue	KX528740/KX528849
070	*A. boskianus*	80.3.76.42	Egypt – Qa'lat el Jundi	At issue	KX528741/KX528850
072	*A. boskianus*	80.3.76.44	Egypt – Qa'lat el Jundi	At issue	KX528742/KX528851
*073	*A. boskianus*	80.3.76.45	Egypt – Qa'lat el Jundi	At issue	KX528743/KX528852
074	*A. boskianus*	80.3.76.46	Egypt – Qa'lat el Jundi	At issue	KX528744/KX528853
075	*A. boskianus*	80.3.76.47	Egypt – Qa'lat el Jundi	At issue	KX528745/KX528854
076	*A. boskianus*	80.3.76.48	Egypt – Qa'lat el Jundi	At issue	KX528746/KX528855
077	*A. boskianus*	80.3.76.49	Jordan – Petra	At issue	KX528747/KX528856
078	*A. boskianus*	80.3.76.50	Jordan – Petra	At issue	KX528748/KX528857
079	*A. boskianus*	80.3.76.51	Jordan – Petra	At issue	KX528749/KX528858
080	*A. boskianus*	80.3.76.52	Jordan – Petra	At issue	KX528750/KX528859
081	*A. boskianus*	80.3.76.53	Jordan – Petra	At issue	KX528751/KX528860
082	*A. boskianus*	80.3.76.54	Jordan – Wadi Rum spring	At issue	KX528752/KX528861
084	*A. boskianus*	80.3.76.56	Jordan – Wadi Rum spring	At issue	KX528753/KX528862
085	*A. boskianus*	80.3.76.57	Jordan – Wadi Rum spring	At issue	KX528754/KX528863
086	*A. boskianus*	80.3.76.58	Jordan – Wadi Rum spring	At issue	KX528755/KX528864
*087	*A. boskianus*	80.3.76.59	Jordan – Wadi Rum spring	At issue	KX528756/KX528865
*088	*A. boskianus*	80.3.76.60	Jordan – Wadi Rum spring	At issue	KX528757/KX528866
089	*A. boskianus*	80.3.76.61	Egypt – Wadi el Sheikh (Feiran oasis)	At issue	KX528758/KX528867
090	*A. boskianus*	80.3.76.62	Libya – Farwa, on the island	At issue	KX528758/KX528868
*091	*A. boskianus*	80.3.76.63	Libya – Farwa, on the island	At issue	KX528760/KX528869
*092	*A. boskianus*	80.3.76.64	Libya – Farwa, on the island	At issue	KX528761/KX528870
093	*A. boskianus*	80.3.76.65	Libya – Sirte to Misirata	At issue	KX528762/KX528871
094	*A. boskianus*	80.3.76.66	Libya – Sirte to Misirata	At issue	KX528763/KX528872
*095	*A. boskianus*	80.3.76.67	Libya – Sirte to Misirata	At issue	KX528764/KX528873
096	*A. boskianus*	80.3.76.68	Libya – Sirte to Misirata	At issue	KX528765/KX528874
*097	*A. boskianus*	80.3.76.69	Libya – Sirte to Misirata	At issue	KX528766/KX528875
098	*A. boskianus*	80.3.76.70	Libya – Sirte to Misirata	At issue	KX528767/KX528876
099	*A. boskianus*	80.3.76.71	Libya – Sirte to Misirata	At issue	KX528768/KX528877

(*continued on next page*)

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Sample code	Taxon name	NHMC voucher number	Country & locality	Study	Accession numbers (16S/cyt *b*)
100	*A. boskianus*	80.3.76.72	Libya – Sirte to Misirata	At issue	KX528769/KX528878
101	*A. boskianus*	80.3.76.73	Libya – Sirte to Misirata	At issue	KX528770/KX528879
102	*A. boskianus*	80.3.76.74	Libya – Sirte to Misirata	At issue	–/KX528880
103	*A. scutellatus* sensu lato	80.3.110.1	Tunisia – Oued el Mahred	At issue	–/KX528881
104	*A. scutellatus* sensu lato	80.3.110.2	Tunisia – Oued el Mahred	At issue	KX528771/KX528882
*105	*A. scutellatus* sensu lato	80.3.110.3	Tunisia – Oued el Mahred	At issue	KX528772/KX528883
*106	*A.* cf. *dumerilii*	80.3.179.1	Tunisia – Oued el Mahred	At issue	KX528773/KX528884
110	*A. scutellatus audouini*	80.3.110.8	Tunisia – Remanda	At issue	–/KX528885
*111	*A. scutellatus* Sinai form	80.3.110.9	Egypt – Ras el Sud	At issue	KX528774/KX528886
112	*A. orientalis*	80.3.75.2	Syria – As Suwar	At issue	KX528775/KX528887
113	*A. tristami*	80.3.100.3	Syria – As Suwayda	At issue	KX528776/KX528888
*114	*A. orientalis*	80.3.75.4	Syria – Al Badiah desert	At issue	KX528777/KX528889
*115	*A. orientalis*	80.3.75.5	Syria – As Sukhnah	At issue	KX528778/KX528890
*116	*A. orientalis*	80.3.75.6	Syria – Al Asad lake	At issue	KX528779/KX528891
117	*A. orientalis*	80.3.75.7	Syria – Al Asad lake	At issue	KX528780/KX528892
*118	*A. orientalis*	80.3.75.8	Syria – Al Asad lake	At issue	KX528781/KX528893
119	*A. orientalis*	80.3.75.9	Syria – Al Asad lake	At issue	KX528782/KX528894
120	*A. erythrurus atlanticus*	80.3.145.1	Morocco – Essaouira	At issue	KX528783/–
121	*A. erythrurus atlanticus*	80.3.145.2	Morocco – Essaouira	At issue	–/KX528895
*122	*A. erythrurus atlanticus*	80.3.145.3	Morocco – Essaouira	At issue	KX528784/KX528896
*124	*A. erythrurus atlanticus*	80.3.145.5	Morocco – Essaouira	At issue	KX528785/KX528897
*130	*A. robustus*	80.3.74.2	Syria – Steppe from Palmyra to Hamah	At issue	KX528786/KX528898
*131	*A. robustus*	80.3.74.3	Syria – Steppe from Palmyra to Hamah	At issue	KX528787/KX528899
*132	*A. tristami*	80.3.100.1	Syria – Buraq	At issue	KX528788/KX528900
*133	*A. tristami*	80.3.100.2	Syria – Buraq	At issue	KX528789/KX528901
*134	*A. pardalis*	80.3.73.1	Libya – Igdeida semidesert	At issue	KX528790/KX528902
*135	*A. pardalis*	80.3.73.2	Libya – Om Arazam	At issue	KX528791/KX528903
*136	*A. boskianus*	80.3.76.75	Algeria – Natura Reserve of Mergheb	At issue	KX528792/KX528904
*139	*A.* cf. *scutellatus*	80.3.110.4	Libya – Sirte to Misirata	At issue	KX528793/KX528905
*140	*A. maculatus*	80.3.146.1	Algeria – Natura Reserve of Mergheb	At issue	KX528794/KX528906
*141	*A.* cf. *scutellatus***	80.3.110.18	Libya – Ajdabiya towards Sirte	At issue	KX528795/KX528907
*142	*A.*cf. *scutellatus***	80.3.110.19	Libya – Derj oasis	At issue	KX528796/KX528908
*143	*A.* cf. *scutellatus***	80.3.110.20	Libya – Derj oasis	At issue	KX528797/KX528909
147	*A. scutellatus audouini*	80.3.110.10	Libya – Farwa, on the island	At issue	KX528798/KX528910
148	*A. scutellatus audouini*	80.3.110.11	Libya – Farwa, on the island	At issue	KX528799/KX528911
149	*A. scutellatus audouini*	80.3.110.12	Libya – Farwa, on the island	At issue	KX528800/KX528912
150	*A. scutellatus audouini*	80.3.110.13	Libya – Farwa, on the island	At issue	KX528801/KX528913
151	*A. scutellatus audouini*	80.3.110.14	Libya – Farwa, on the island	At issue	KX528802/KX528914
*152	*A. scutellatus audouini*	80.3.110.15	Libya – Farwa, on the island	At issue	KX528803/KX528915
153	*A. scutellatus audouini*	80.3.110.16	Libya – Farwa, on the island	At issue	KX528804/KX528916
*154	*A. scutellatus audouini*	80.3.110.17	Libya – Farwa, on the island	At issue	KX528805/KX528917
–	*A. aureus*	–	Unknown	Harris and Arnold, 2000)	AF197486/–
–	*A. bedriagai*	–	Unknown	Harris and Arnold, 2000)	AF197506/–
–	*A. bedriagai*		Morocco – Taroudent	Harris et al., 2004)	AY633438/–
–	*A. bedriagai*	–	Morocco – Agadir – Tiznit	Harris et al., 2004)	AY633439/–
–	*A. beershebensis*	–	Israel – Nequev desert	Carretero et al., 2011)	JF912448/–
–	*A. blanci*	–	Tunisia – Bou Chebka	Harris et al., 2004)	AY633430/–
–	*A. blanci*	–	Tunisia – Bou Chebka	Harris et al., 2004)	AY633431/–
–	*A. blanfordii*	–	Unknown	Harris and Arnold, 2000)	AF197482/–
–	*A. blanfordii*	–	Iran – Qeshm Island	Heidari et al., 2014)	–/KJ652710
–	*A. blanfordii*	–	Iran – Qeshm Island	Heidari et al., 2014)	–/KJ652711
–	*A. blanfordii*	–	Iran – Qeshm Island	Heidari et al., 2014)	–/KJ652712
–	*A. blanfordii*	–	Iran – Qeshm Island	Heidari et al., 2014)	–/KJ652713
–	*A. blanfordii*	–	Iran – Chabahar-Beriis	Heidari et al., 2014)	–/KJ652714
–	*A. blanfordii*	–	Iran – Chabahar-Tiskoopan	Heidari et al., 2014)	–/KJ652715
–	*A. blanfordii*	–	Iran – Hormozgan-Minab	Heidari et al., 2014)	–/KJ652716
–	*A. blanfordii*	–	Iran – Hormozgan-Minab	Heidari et al., 2014)	–/KJ652717
–	*A. blanfordii*	–	Iran – Hormozgan-Minab	Heidari et al., 2014)	–/KJ652718
–	*A. blanfordii*	–	Iran – Hormozgan-Minab	Heidari et al., 2014)	–/KJ652719
–	*A. blanfordii*	–	Iran – Chabahar-Tiskoopan	Heidari et al., 2014)	–/KJ652720
–	*A. blanfordii*	–	Iran – Chabahar-Govater	Heidari et al., 2014)	–/KJ652721
–	*A. blanfordii*	–	Iran – Chabahar-Govater	Heidari et al., 2014)	–/KJ652722
–	*A. blanfordii*	–	Iran – Bampoor	Heidari et al., 2014)	–/KJ652723
–	*A. blanfordii*	–	Iran – Chabahar-Beriis	Heidari et al., 2014)	–/KJ652724
–	*A. blanfordii*	–	Iran – Chabahar-Beriis	Heidari et al., 2014)	–/KJ652725
–	*A. blanfordii*	–	Iran – Chabahar-Lipar	Heidari et al., 2014)	–/KJ652726
–	*A. blanfordii*	–	Iran – Bampur, Sistan va	Tamar et al., 2014)	–/KJ567846
–	*A. blanfordii*	–	Iran – Bampur	Tamar et al., 2014)	–/KJ567847
*029	*A. boskianus*	80.3.76.1	Syria – Steppe from Palmyra to Hamah	Poulakakis et al., 2013)	JX847509/JX847544
*030	*A. boskianus*	80.3.76.2	Syria – Steppe from Palmyra to Hamah	Poulakakis et al., 2013)	JX847510/JX847545
033	*A. boskianus*	80.3.76.5	Syria – Steppe from Palmyra to Hamah	Poulakakis et al., 2013)	JX847511/JX847546
034	*A. boskianus*	80.3.76.6	Syria – Steppe from Palmyra to Hamah	Poulakakis et al., 2013)	JX847512/JX847547
035	*A. boskianus*	80.3.76.7	Syria – Steppe from Palmyra to Hamah	Poulakakis et al., 2013)	JX847513/JX847548
041	*A. boskianus*	80.3.76.13	Jordan – Wadi Rum spring	Poulakakis et al., 2013)	JX847514/JX847549
042	*A. boskianus*	80.3.76.14	Jordan – Wadi Rum spring	Poulakakis et al., 2013)	JX847515/JX847550

(*continued*)

Sample code	Taxon name	NHMC voucher number	Country & locality	Study	Accession numbers (16S/cyt *b*)
058	*A. boskianus*	80.3.76.30	Egypt – Wadi Sudr – Qa'lat el Jundi	Poulakakis et al., 2013)	JX847517/JX847552
071	*A. boskianus*	80.3.76.43	Egypt – Qa'lat el Jundi	Poulakakis et al., 2013)	JX847516/JX847551
*137	*A. boskianus*	80.3.76.76	Algeria – Natura Reserve of Mergheb	Poulakakis et al., 2013)	JX847522/JX847557
138	*A. boskianus*	80.3.76.77	Algeria – Natura Reserve of Mergheb	Poulakakis et al., 2013)	JX847523/JX847558
–	*A. boskianus*	–	Tunisia – Bou Ghanem	Harris et al., 2004)	AY633440/–
–	*A. boskianus*	–	Tunisia – Bou Ghanem	Harris et al., 2004)	AY633441/–
–	*A. boskianus*	–	Israel	Tikochinski et al. (Unpublished)	GU433284/–
–	*A. boskianus*	–	Israel	Tikochinski et al. (Unpublished)	GU433285/–
–	*A. boskianus*	–	Israel	Tikochinski et al. (Unpublished)	GU433286/–
–	*A. boskianus*	–	Israel	Tikochinski et al. (Unpublished)	GU433287/–
–	*A. boskianus*	–	Israel	Tikochinski et al. (Unpublished)	GU433288/–
–	*A. boskianus*	–	Israel	Tikochinski et al. (Unpublished)	GU433289/–
–	*A. boskianus*	–	Israel	Tikochinski et al. (Unpublished)	GU433290/–
–	*A. boskianus*	–	Israel	Tikochinski et al. (Unpublished)	GU433291/–
–	*A. boskianus*	–	Egypt – Sinai	Khannoon et al., 2013)	–/HM778103
–	*A. boskianus*	–	Egypt – Sinai	Khannoon et al., 2013)	–/HM778104
–	*A. boskianus*	–	Egypt – El Sheikh	Khannoon et al., 2013)	–/HM778105
–	*A. boskianus*	–	Egypt – Sharm El Sheikh	Khannoon et al., 2013)	–/HM778106
–	*A. boskianus*	–	Egypt – Siwa	Khannoon et al., 2013)	–/HM778107
–	*A. boskianus*	–	Egypt – Siwa	Khannoon et al., 2013)	–/HM778108
–	*A. boskianus*	–	Iran – Dehloran-Zarrinabad	Heidari et al., 2014)	–/KJ652758
–	*A. boskianus*	–	Iran – Dehloran-Zarrinabad	Heidari et al., 2014)	–/KJ652759
–	*A. boskianus*	–	Iran – Dehloran-Golan	Heidari et al., 2014)	–/KJ652760
–	*A. boskianus*	–	Iran – Dehloran-Golan	Heidari et al., 2014)	–/KJ652761
–	*A. boskianus*	–	Iran – Dehloran-Golan	Heidari et al., 2014)	–/KJ652762
–	*A. boskianus*	–	Iran – Dehloran-Golan	Heidari et al., 2014)	–/KJ652763
–	*A. boskianus*	–	Iran – Kermanshah, Harsin	Heidari et al., 2014)	–/KJ652764
–	*A. boskianus*	–	Iran – Kermanshah, Harsin	Heidari et al., 2014)	–/KJ652765
–	*A. boskianus*	–	Iran – Kermanshah, Harsin	Heidari et al., 2014)	–/KJ652766
–	*A. boskianus*	–	Iran – Ilam, Darre Shahr	Heidari et al., 2014)	–/KJ652767
–	*A. boskianus*	–	Iran – Ilam, Darre Shahr	Heidari et al., 2014)	–/KJ652768
–	*A. boskianus*	–	Algeria – Tassili 'n' Ajjer	Tamar et al., 2014)	–/KJ567812
–	*A. boskianus*	–	Egypt – El Arish, Sinai	Tamar et al., 2014)	–/KJ567776
–	*A. boskianus*	–	Egypt – Baluza, Sinai	Tamar et al., 2014)	–/KJ567790
–	*A. boskianus*	–	Egypt – Between Serabit el Khadim and Gebel Raqaba, Sinai	Tamar et al., 2014)	–/KJ567768
–	*A. boskianus*	–	Egypt – Nuweibaa, Sinai	Tamar et al., 2014)	–/KJ567773
–	*A. boskianus*	–	Egypt – Gebel Gunna, Sinai	Tamar et al., 2014)	–/KJ567771
–	*A. boskianus*	–	Egypt – St. Catherine, Sinai	Tamar et al., 2014)	–/KJ567772
–	*A. boskianus*	–	Egypt – St. Catherine to Fox camp, Sinai	Tamar et al., 2014)	–/KJ567770
–	*A. boskianus*	–	Egypt – Sharm el Sheikh, Sinai	Tamar et al., 2014)	–/KJ567774
–	*A. boskianus*	–	Egypt – Matruh	Tamar et al., 2014)	–/KJ567824
–	*A. boskianus*	–	Egypt – Sidi Brani	Tamar et al., 2014)	–/KJ567813
–	*A. boskianus*	–	Egypt – Wadi El Natrun	Tamar et al., 2014)	–/KJ567822
–	*A. boskianus*	–	Israel – Wadi Revivim	Tamar et al., 2014)	–/KJ567767
–	*A. boskianus*	–	Israel – Shivta junction	Tamar et al., 2014)	–/KJ567765
–	*A. boskianus*	–	Israel – Kmehin	Tamar et al., 2014)	–/KJ567780
–	*A. boskianus*	–	Israel – Rotem plain	Tamar et al., 2014)	–/KJ567764
–	*A. boskianus*	–	Israel – Wadi Zafit	Tamar et al., 2014)	–/KJ567766
–	*A. boskianus*	–	Israel – Mt. Tzin	Tamar et al., 2014)	–/KJ567775
–	*A. boskianus*	–	Israel – Mitzpe Ramon	Tamar et al., 2014)	–/KJ567783
–	*A. boskianus*	–	Israel – Mitzpe Ramon	Tamar et al., 2014)	–/KJ567784
–	*A. boskianus*	–	Israel – Mitzpe Ramon	Tamar et al., 2014)	–/KJ567785
–	*A. boskianus*	–	Israel – Wadi Nekarot	Tamar et al., 2014)	–/KJ567779
–	*A. boskianus*	–	Israel – Paran	Tamar et al., 2014)	–/KJ567787
–	*A. boskianus*	–	Israel – Wadi Paran	Tamar et al., 2014)	–/KJ567781
–	*A. boskianus*	–	Israel – Wadi Paran	Tamar et al., 2014)	–/KJ567782
–	*A. boskianus*	–	Israel – Wadi Paran	Tamar et al., 2014)	–/KJ567789
–	*A. boskianus*	–	Jordan – Tell al Heber	Tamar et al., 2014)	–/KJ567830
–	*A. boskianus*	–	Jordan – Petra	Tamar et al., 2014)	–/KJ567777
–	*A. boskianus*	–	Jordan – Petra	Tamar et al., 2014)	–/KJ567778
–	*A. boskianus*	–	Jordan – Petra	Tamar et al., 2014)	–/KJ567788
–	*A. boskianus*	–	Jordan – Petra	Tamar et al., 2014)	–/KJ567786
–	*A. boskianus*	–	Jordan – Wadi Ramm	Tamar et al., 2014)	–/KJ567826
–	*A. boskianus*	–	Libya – Wadi Mathkendush	Tamar et al., 2014)	–/KJ567805
–	*A. boskianus*	–	Mauritania – Between Zouerat and Bir Moghrein	Tamar et al., 2014)	–/KJ567807
–	*A. boskianus*	–	Mauritania – Between Zouerat and Bir Moghrein	Tamar et al., 2014)	–/KJ567808
–	*A. boskianus*	–	Morocco – Between Saidia and Moulouya	Tamar et al., 2014)	–/KJ567825
–	*A. boskianus*	–	Morocco – Between Ait–Khoujman and Kerrandou	Tamar et al., 2014)	–/KJ567819
–	*A. boskianus*	–	Morocco – Rissani	Tamar et al., 2014)	–/KJ567818
–	*A. boskianus*	–	Morocco – Rissani	Tamar et al., 2014)	–/KJ567817

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Sample code	Taxon name	NHMC voucher number	Country & locality	Study	Accession numbers (16S/cyt *b*)
–	*A. boskianus*	–	Morocco – Ouarzazate	Tamar et al., 2014)	–/KJ567820
–	*A. boskianus*	–	Morocco – Oum El-Alek	Tamar et al., 2014)	–/KJ567810
–	*A. boskianus*	–	Morocco – Akka	Tamar et al., 2014)	–/KJ567811
–	*A. boskianus*	–	Niger – Tafokin	Tamar et al., 2014)	–/KJ567823
–	*A. boskianus*	–	Oman – Lizq	Tamar et al., 2014)	–/KJ567827
–	*A. boskianus*	–	Oman – Nizwa	Tamar et al., 2014)	–/KJ567829
–	*A. boskianus*	–	Oman – Kubarah	Tamar et al., 2014)	–/KJ567828
–	*A. boskianus*	–	Oman – Duqm	Tamar et al., 2014)	–/KJ567802
–	*A. boskianus*	–	Oman – Wadi Salit	Tamar et al., 2014)	–/KJ567801
–	*A. boskianus*	–	Oman – Wadi Salit	Tamar et al., 2014)	–/KJ567803
–	*A. boskianus*	–	Oman – Rawiyyah	Tamar et al., 2014)	–/KJ567804
–	*A. boskianus*	–	Sudan – El–Koin	Tamar et al., 2014)	–/KJ567821
–	*A. boskianus*	–	Syria – Ar Raqqah	Tamar et al., 2014)	–/KJ567842
–	*A. boskianus*	–	Syria – Ar Raqqah	Tamar et al., 2014)	–/KJ567841
–	*A. boskianus*	–	Syria – Qasr al Hayr al Gharbi	Tamar et al., 2014)	–/KJ567838
–	*A. boskianus*	–	Syria – Qasr al Hayr al Gharbi	Tamar et al., 2014)	–/KJ567839
–	*A. boskianus*	–	Syria – Sadad	Tamar et al., 2014)	–/KJ567840
–	*A. boskianus*	–	Tunisia – Jebel Semmama	Tamar et al., 2014)	–/KJ567814
–	*A. boskianus*	–	Tunisia – Jebel Semmama	Tamar et al., 2014)	–/KJ567815
–	*A. boskianus*	–	Tunisia – Hammat al-Jarid	Tamar et al., 2014)	–/KJ567816
–	*A. boskianus*	–	Tunisia –33 km S of Tataouine	Tamar et al., 2014)	–/KJ567806
–	*A. boskianus*	–	Western Sahara – Laayoune	Tamar et al., 2014)	–/KJ567809
–	*A. boskianus*	–	Yemen – Sa'yun oasis	Tamar et al., 2014)	–/KJ567800
–	*A. boskianus*	–	Yemen – Sa'yun oasis	Tamar et al., 2014)	–/KJ567799
–	*A. boskianus*	–	Yemen – Shibam	Tamar et al., 2014)	–/KJ567769
–	*A. boskianus* Arabian strain	–	Unknown	Harris and Arnold, 2000	AF197500/–
–	*A. boskianus* Moroccan strain	–	Unknown	Harris and Arnold, 2000	AF197484/–
–	*A. busacki*	–	Western Sahara – sebkha Oum Dba	Fonseca et al., 2008	EU086896/–
–	*A. busacki*	–	Morocco – Oued Massa	Fonseca et al., 2008	EU086903/–
–	*A. cantoris*	–	Unknown	Harris and Arnold, 2000	AF080346/–
–	*A. cantoris*	–	Pakistan – Uthal, Baluchistan Province	Tamar et al., 2014	–/KJ567850
–	*A. cantoris*	–	Pakistan – Nagar Parkar	Tamar et al., 2014	–/KJ567851
125	*A.* cf. *grandis*	80.3.77.1	Syria – As Sukhnah	Poulakakis et al., 2013	JX847518/JX847553
–	*A. erythrurus*	–	Unknown	Harris and Arnold, 2000	AF197498/–
–	*A. erythrurus*	–	Spain – Cadiz – Punta Paloma	Fu, 2000	AF206607/–
*–	*A. erythrurus*	–	Morocco – Foret de Cedres (Azrou)	Greenbaum et al., 2011	HQ605790/HQ605832
–	*A. erythrurus atlanticus*	–	Morocco – Ida-ou-bouzia	Harris et al., 2004	AY633436/–
–	*A. erythrurus belli*	–	Morocco – Bab Taza	Harris et al., 2004	AY633424/–
–	*A. erythrurus belli*	–	Morocco – Bab Taza	Harris et al., 2004	AY633425/–
–	*A. erythrurus belli*	–	Morocco – Taza	Harris et al., 2004	AY633426/–
–	*A. erythrurus belli*	–	Morocco – Taza	Harris et al., 2004	AY633427/–
–	*A. erythrurus belli*	–	Morocco – Debdou	Harris et al., 2004	AY633428/–
–	*A. erythrurus belli*	–	Morocco – Debdou	Harris et al., 2004	AY633429/–
–	*A. erythrurus belli*	–	Morocco – Debdou	Harris et al., 2004	AY633432/–
–	*A. erythrurus belli*	–	Morocco – Debdou	Harris et al., 2004	AY633433/–
–	*A. erythrurus belli*	–	Morocco – Debdou	Harris et al., 2004	AY633434/–
–	*A. erythrurus belli*	–	Morocco – Debdou	Harris et al., 2004	AY633435/–
–	*A. erythrurus belli*	–	Morocco – Taza	Harris et al., 2004	AY633443/–
–	*A. erythrugus*	–	Portugal – Picote	Harris et al., 2004	AY633420/–
–	*A. erythrurus erythrurus*	–	Portugal – Picote	Harris et al., 2004	AY633421/–
–	*A. erythrurus erythrurus*	–	Spain – Torredambarra	Harris et al., 2004	AY633422/–
–	*A. erythrurus erythrurus*	–	Spain – Valencia	Harris et al., 2004	AY633423/–
–	*A. erythrurus lineomaculatus*	–	Morocco – Kenitra	Harris et al., 2004	AY633437/–
–	*A. erythrurus lineomaculatus*	–	Morocco – Moussa	Harris et al., 2004	AY633442/–
–	*A. felicis*	–	Oman – Ajdarawt	Tamar et al., 2014	–/KJ567845
–	*A. gongrorhynchatus*	–	Unknown	Harris and Arnold, 2000	AF080343/–
126	*A. grandis*	80.3.77.2	Syria – Dayr az Zawr	Poulakakis et al., 2013	JX847519/JX847554
*127	*A. grandis*	80.3.77.3	Syria – Sapkha	Poulakakis et al., 2013	JX847520/JX847555
*128	*A. grandis*	80.3.77.4	Syria – Sapkha	Poulakakis et al., 2013	JX847521/JX847556
–	*A. grandis*	–	Iran – Bushehr – Alichangii	Heidari et al., 2014	–/KJ652751
–	*A. grandis*	–	Iran – Bushehr – Alichangii	Heidari et al., 2014	–/KJ652752
–	*A. khamirensis*	–	Iran – Khamir Port	Heidari et al., 2014	–/KJ652702
–	*A. khamirensis*	–	Iran – Khamir Port	Heidari et al., 2014	–/KJ652703
–	*A. khamirensis*	–	Iran – Khamir Port	Heidari et al., 2014	–/KJ652704
–	*A. khamirensis*	–	Iran – Khamir Port	Heidari et al., 2014	–/KJ652705
–	*A. khamirensis*	–	Iran – Khamir Port	Heidari et al., 2014	–/KJ652706
–	*A. khamirensis*	–	Iran – Hormozgan, Parsian	Heidari et al., 2014	–/KJ652707
–	*A. khamirensis*	–	Iran – Khamir Port	Heidari et al., 2014	–/KJ652708
–	*A. khamirensis*	–	Iran – Hormozgan, Parsian	Heidari et al., 2014	–/KJ652709
–	*A. longipes*	–	Unknown	Harris and Arnold, 2000	AF197490/–
–	*A. maculatus*	–	Unknown	Harris and Arnold, 2000	AF197496/–
–	*A. maculatus*	–	Morocco – Saka	Fonseca et al., 2008	EU086881/–
–	*A. maculatus*	–	Morocco – El Aioun	Fonseca et al., 2008	EU086882/–
–	*A. maculatus*	–	Tunisia – Haidra – Thala	Fonseca et al., 2008	EU086886/–
–	*A. maculatus*	–	Tunisia – Haidra – Thala	Fonseca et al., 2008	EU086887/–

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Sample code	Taxon name	NHMC voucher number	Country & locality	Study	Accession numbers (16S/cyt *b*)
–	*A. maculatus*	–	Libya – Hamadath al Hamrah	Fonseca et al., 2008	EU086889/–
–	*A. maculatus*	–	Libya – Hamadath al Hamrah	Fonseca et al., 2008	EU086890/–
–	*A. maculatus*	–	Tunisia – Jugurtha Table	Fonseca et al., 2008	EU086894/–
–	*A. maculatus*	–	Tunisia – Kairouan	Fonseca et al., 2008	EU086895/–
–	*A. maculatus*	–	Tunisia – Kairouan	Fonseca et al., 2008	EU086897/–
–	*A. maculatus*	–	Morocco – Outat-Oulad-El-Haj	Fonseca et al., 2008	EU086898/–
–	*A. maculatus*	–	Morocco – Outat-Oulad-El-Haj	Fonseca et al., 2008	EU086899/–
–	*A. maculatus*	–	Morocco – Outat-Oulad-El-Haj	Fonseca et al., 2008	EU086900/–
–	*A. maculatus*	–	Morocco – Alnif	Fonseca et al., 2008	EU086901/–
–	*A. maculatus*	–	Morocco – Alnif	Fonseca et al., 2008	EU086902/–
–	*A. maculatus*	–	Algeria – Ain Naga	Fonseca et al., 2008	EU086906/–
–	*A. maculatus*	–	Algeria – Ain Naga	Fonseca et al., 2008	EU086907/–
–	*A. maculatus* (uploaded in ncbi as *mechriguensis*)	–	Tunisia – Cape Serrat beach	Fonseca et al., 2008	EU086883/–
–	*A. maculatus* (uploaded in ncbi as *mechriguensis*)	–	Tunisia – el Berrak dam – Nefza	Fonseca et al., 2008	EU086884/–
–	*A. maculatus* (uploaded in ncbi as *mechriguensis*)	–	Tunisia – el Berrak dam – Nefza	Fonseca et al., 2008	EU086885/–
–	*A. maculatus* (uploaded in ncbi as *mechriguensis*)	–	Tunisia – Cape Serrat beach	Fonseca et al., 2008	EU086888/–
–	*A. maculatus* (uploaded in ncbi as *mechriguensis*)	–	Tunisia – Sidi Mechrig	Fonseca et al., 2008	EU086891/–
–	*A. maculatus* (uploaded in ncbi as *mechriguensis*)	–	Tunisia – Sidi Mechrig	Fonseca et al., 2008	EU086892/–
–	*A. maculatus* (uploaded in ncbi as *mechriguensis*)	–	Tunisia – Sidi Mechrig	Fonseca et al., 2008	EU086893/–
–	*A. masirae*	–	Unknown	Harris and Arnold, 2000	AF197504/–
–	*A. masirae*	–	Oman – Ras Madrakah	Tamar et al., 2014	–/KJ567849
–	*A. masirae*	–	Oman – Masirae island	Tamar et al., 2014	–/KJ567848
–	*A. micropholis*	–	Iran – Zahedan-Mianbazar	Heidari et al., 2014	–/KJ652737
–	*A. micropholis*	–	Iran – Zahedan-Mianbazar	Heidari et al., 2014	–/KJ652738
–	*A. micropholis*	–	Iran – Chabahar-Tiskoopan	Heidari et al., 2014	–/KJ652739
–	*A. micropholis*	–	Iran – Chabahar-Tiskoopan	Heidari et al., 2014	–/KJ652740
–	*A. micropholis*	–	Iran – Chabahar-Tiskoopan	Heidari et al., 2014	–/KJ652741
–	*A. micropholis*	–	Iran – Chabahar-Tiskoopan	Heidari et al., 2014	–/KJ652742
–	*A. micropholis*	–	Iran – Chabahar-Tiskoopan	Heidari et al., 2014	–/KJ652743
–	*A. micropholis*	–	Iran – Chabahar-Tiskoopan	Heidari et al., 2014	–/KJ652744
–	*A. micropholis*	–	Iran – Zahedan-Mianbazar	Heidari et al., 2014	–/KJ652745
–	*A. micropholis*	–	Iran – Zahedan-Mianbazar	Heidari et al., 2014	–/KJ652746
–	*A. micropholis*	–	Iran – Zahedan-Mianbazar	Heidari et al., 2014	–/KJ652747
–	*A. nilsoni*	–	Iran – Qasr-e-Shirin	Heidari et al., 2014	–/KJ652732
–	*A. nilsoni*	–	Iran – Qasr-e-Shirin	Heidari et al., 2014	–/KJ652733
–	*A. nilsoni*	–	Iran – Qasr-e-Shirin	Heidari et al., 2014	–/KJ652734
–	*A. nilsoni*	–	Iran – Qasr-e-Shirin	Heidari et al., 2014	–/KJ652735
–	*A. nilsoni*	–	Iran – Qasr-e-Shirin	Heidari et al., 2014	–/KJ652736
–	*A. opheodurus*	–	Unknown	Harris and Arnold, 2000	AF197502/–
–	*A. opheodurus*	–	Israel – Timna valley	Tamar et al., 2014	–/KJ567844
–	*A. opheodurus*	–	Jordan – Diseh	Tamar et al., 2014	–/KJ567843
–	*A. orientalis*	–	Unknown	Harris and Arnold, 2000	AF197492/–
–	*A. pardalis*	–	Libya – Igdeida semidesert	Fonseca et al., 2008	EU086904/–
–	*A. pardalis*	–	Libya – Om Arazam	Fonseca et al., 2008	EU086905/–
–	*A. schmidti*	–	Unknown	Harris and Arnold, 2000	AF080377/–
–	*A. schmidti*	–	Iran – N Ahwaz, Albaji	Heidari et al., 2014	–/KJ652753
–	*A. schmidti*	–	Iran – N Ahwaz, Albaji	Heidari et al., 2014	–/KJ652754
–	*A. schmidti*	–	Iran – N Ahwaz, Albaji	Heidari et al., 2014	–/KJ652755
–	*A. schmidti*	–	Iran – Southern Ilam	Heidari et al., 2014	–/KJ652756
–	*A. schmidti*	–	Iran – Southern Ilam	Heidari et al., 2014	–/KJ652757
–	*A. schreiberi*	–	Israel	Tikochinski et al. Unpublished	GU433292/–
*001	*A. schreiberi ataturi*	80.3.11.1	Turkey – Yumurtalik – Adana	Poulakakis et al., 2013	JX847500/JX847535
*002	*A. schreiberi ataturi*	80.3.11.2	Turkey – Yumurtalik – Adana	Poulakakis et al., 2013	JX847501/JX847536
003	*A. schreiberi ataturi*	80.3.11.3	Turkey – Yumurtalik – Adana	Poulakakis et al., 2013	JX847502/JX847537
–	*A. schreiberi ataturi*	–	Turkey – Botas	Tamar et al., 2014	–/KJ567835
–	*A. schreiberi ataturi*	–	Turkey – Botas	Tamar et al., 2014	–/KJ567836
013	*A. schreiberi schreiberi*	80.3.11.13	Cyprus – Ammochostos – Karpasia	Poulakakis et al., 2013	JX847503/JX847538
015	*A. schreiberi schreiberi*	80.3.11.15	Cyprus – Ammochostos – Karpasia	Poulakakis et al., 2013	JX847504/JX847539
017	*A. schreiberi schreiberi*	80.3.11.17	Cyprus – Derinia – Larnaca	Poulakakis et al., 2013	JX847505/JX847540
018	*A. schreiberi schreiberi*	80.3.11.18	Cyprus – Nicosia	Poulakakis et al., 2013	JX847506/JX847541
019	*A. schreiberi schreiberi*	80.3.11.19	Cyprus – Salamina – Ammochostos	Poulakakis et al., 2013	JX847507/JX847542
028	*A. schreiberi schreiberi*	80.3.11.28	Cyprus – Goneli – Nicosia	Poulakakis et al., 2013	JX847508/JX847543
144	*A. schreiberi*	80.3.11.29	Cyprus – Kato Moni	Poulakakis et al., 2013	JX847524/JX847559
145	*A. schreiberi schreiberi*	80.3.11.30	Cyprus – Kato Moni	Poulakakis et al., 2013	JX847525/JX847560
146	*A. schreiberi schreiberi*	80.3.11.31	Cyprus – Kato Moni	Poulakakis et al., 2013	JX847526/JX847561
–	*A. schreiberi schreiberi*	–	Cyprus – Lara bay, Akamas peninsula	Tamar et al., 2014	–/KJ567831
–	*A. schreiberi schreiberi*	–	Cyprus – Lara bay, Akamas peninsula	Tamar et al., 2014	–/KJ567832

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Sample code	Taxon name	NHMC voucher number	Country & locality	Study	Accession numbers (16S/cyt *b*)
–	*A. schreiberi schreiberi*	–	Cyprus – Mersinlik, Famagusta	Tamar et al., 2014	–/KJ567834
–	*A. schreiberi schreiberi*	–	Cyprus – Episkopi	Tamar et al., 2014	–/KJ567833
–	*A. schreiberi schreiberi*	–	Cyprus – Vrysoulles	Tamar et al., 2014	–/KJ567837
–	*A. schreiberi syriacus*	–	Israel – Caesarea sands	Tamar et al., 2014	–/KJ567791
–	*A. schreiberi syriacus*	–	Israel – Hadera to Binyamina	Tamar et al., 2014	–/KJ567792
–	*A. schreiberi syriacus*	–	Israel – Rishon Le–Zion sands	Tamar et al., 2014	–/KJ567795
–	*A. schreiberi syriacus*	–	Israel – Holon sands	Tamar et al., 2014	–/KJ567798
–	*A. schreiberi syriacus*	–	Israel – Holon sands	Tamar et al., 2014	–/KJ567796
–	*A. schreiberi syriacus*	–	Israel – Holon sands	Tamar et al., 2014	–/KJ567797
–	*A. schreiberi syriacus*	–	Israel – Nizzanim reserve	Tamar et al., 2014	–/KJ567760
–	*A. schreiberi syriacus*	–	Israel – Nizzanim reserve	Tamar et al., 2014	–/KJ567761
–	*A. schreiberi syriacus*	–	Israel – Ashqelon	Tamar et al., 2014	–/KJ567762
–	*A. schreiberi syriacus*	–	Israel – Ashqelon	Tamar et al., 2014	–/KJ567763
–	*A. schreiberi syriacus*	–	Lebanon – Tyre	Tamar et al., 2014	–/KJ567793
–	*A. schreiberi syriacus*	–	Lebanon – Tyre	Tamar et al., 2014	–/KJ567794
–	*A. scutellatus*	–	Unknown	Harris and Arnold, 2000	AF197488/–
–	*A. scutellatus*	–	Israel – Bir Mashash sands	Tamar et al., 2014	–/KJ567852
–	*A. scutellatus*	–	Isreal – Holon sands	Tamar et al., 2014	–/KJ567853
–	*A. sp*$_1$	–	Iran – Bampoor to Bazman	Heidari et al., 2014	–/KJ652727
–	*A. sp*$_1$	–	Iran – Bampoor to Bazman	Heidari et al., 2014	–/KJ652728
–	*A. sp*$_1$	–	Iran – Bampoor to Bazman	Heidari et al., 2014	–/KJ652729
–	*A. sp*$_1$	–	Iran – Bampoor to Bazman	Heidari et al., 2014	–/KJ652730
–	*A. sp*$_1$	–	Iran – Bampoor to Bazman	Heidari et al., 2014	–/KJ652731
–	*A. sp*$_2$	–	Iran – Parsian	Heidari et al., 2014	–/KJ652748
–	*A. sp*$_2$	–	Iran – Parsian	Heidari et al., 2014	–/KJ652749
–	*A. sp*$_2$	–	Iran – Parsian	Heidari et al., 2014	–/KJ652750
–	*A. tristami*	–	Unknown	Harris and Arnold, 2000	AF197494/–
BEAST calibration	*Gallotia caesaris*	–	Spain – El Hierro Isl., Canary Islands	Carranza et al., 2004; Carranza et al., 2006	DQ298684/AY151843
BEAST calibration	*Gallotia caesaris gomerae*	–	Spain – La Gomera Isl., Canary Islands	Carranza et al., 2004; Carranza et al., 2006	DQ298683/AY151842
BEAST calibration	*Podarcis cretensis*	80.3.51.176-177	Greece – Samaria, Crete	Poulakakis et al., 2003	AY896161/AF486202
BEAST calibration	*Podarcis cretensis*	80.3.51.327	Greece – Koufonisi islet, Crete	Poulakakis et al., 2003; Poulakakis et al., 2005b	AY896147/AF486213
BEAST calibration	*Podarcis levendis*	80.3.51.279	Greece – Pori islet	Poulakakis et al., 2003; Poulakakis et al., 2005b	AY896170/AF486221
BEAST calibration	*Podarcis levendis*	80.3.51.288	Greece – Pori islet	Poulakakis et al., 2003; Poulakakis et al., 2005b	AY896171/AF486222
BEAST calibration	*Podarcis lilfordi*	80.3.59.2	Spain – Mallorca Isl.	At issue & Kapli et al., 2013	To be provided/KF003361
BEAST calibration	*Podarcis lilfordi*	80.3.59.3	Spain – Mallorca Isl.	At issue & Kapli et al., 2013	To be provided/KF003362
BEAST calibration	*Podarcis peloponnesiacus*	80.3.54.19	Greece – Lafka, Peloponnisos	Poulakakis et al., 2005b	AY896178/AY896122
BEAST calibration	*Podarcis peloponnesiacus*	80.3.54.9	Greece – Stoupa, Peloponnisos	Poulakakis et al., 2005b	AY896179/AY896124
BEAST calibration	*Podarcis pityusensis*	80.3.176.1	Spain – Palma, Mallorca Isl.	At issue & Kapli et al., 2013	To be provided/KF003363
BEAST calibration	*Podarcis pityusensis*	80.3.176.2	Spain – Palma, Mallorca Isl.	At issue & Kapli et al., 2013	To be provided/KF003364

**These samples (141–143) morphologically belong either to *A. scutellatus* or to *A. dumerilii*.

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