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# A reinvestigation of phylogeny and divergence times of the *Ablepharus kitaibelii* species complex (Sauria, Scincidae) based on mtDNA and nuDNA genes



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## ABSTRACT

Morphological and DNA data support that the East Mediterranean snake-eyed skink *Ablepharus kitaibelii* represents a species complex that includes four species *A. kitaibelii*, *A. budaki*, *A. chernovi*, and *A. rueppellii*, highlighting the need of its taxonomic reevaluation. Here, we used Bayesian and Maximum Likelihood methods to estimate the phylogenetic relationships of all members of the complex based on two mitochondrial (cyt *b*, 16S rRNA) and two nuclear markers (MC1R, and NKTR) and using *Chalcides*, *Eumeces*, and *Eutropis* as outgroups. The biogeographic history of the complex was also investigated through the application of several phylogeographic (BEAST) and biogeographic (BBM) analyses. Paleogeographic and paleoclimatic data were used to support the inferred phylogeographic patterns. The *A. kitaibelii* species complex exhibits high genetic diversity, revealing cases of hidden diversity and cases of non-monophyletic species such as *A. kitaibelii* and *A. budaki*. Our results indicate that *A. pannonicus* branches off first and a group that comprises specimens of *A. kitaibelii* and *A. budaki* from Kastelorizo Island group (southeast Greece) and southwest Turkey, respectively is differentiated from the rest *A. kitaibelii* and *A. budaki* populations and may represent a new species. The estimated divergence times place the origin of the complex in the Middle Miocene (~16 Mya) and the divergence of most currently recognized species in the Late Miocene. The inferred ancestral distribution suggests that the complex originated in Anatolia, supposing that several vicariance and dispersal events that are related with the formation of the Mid-Aegean Trench, the Anatolian Diagonal and the orogenesis of the mountain chains in southern and eastern Anatolia have led to current distribution pattern of *A. kitaibelii* species complex in the Balkans and Middle East.

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## 1. Introduction

With 1598 species, skinks are the largest family of lizards (Uetz and Hošek, 2015). A still poorly known genera of the family is *Ablepharus* (Fitzinger, 1824), the diversity of which is vastly underestimated according to a recent study (Poulakakis et al., 2005a). It is

distributed from southeast Europe (Balkans, Hungary, Slovakia) to the Middle East, though most species are either exclusively or partially distributed in the Near East. More precisely, four out of ten species of the genus occur only in the Near East and southeastern Europe; *A. kitaibelii* (Bibron & Bory St-Vincent, 1833) in Turkey, Balkans, Hungary, and Slovakia, *A. budaki* Göçmen et al., 1996 in Turkey, Cyprus, Lebanon, Syria, *A. chernovi* Darevsky, 1953 in Turkey, Syria, and Armenia, and *A. rueppellii* (Gray, 1839) in Israel, Syria, Jordan, Lebanon and Sinai. Two other species expand their distribution to the Middle East; *A. pannonicus* (Fitzinger, 1824) that

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is found from Georgia to Jordan, Kyrgyzstan, northwest India as well as in Oman, and United Arab Emirates and *A. bivittatus* (Menetries, 1832) in east Turkey, Iran, Armenia, and Azerbaijan (Sindaco and Jeremčenko, 2008).

The taxonomic status of these species has drawn the attention of many taxonomists since the 1960s (i.e., Fuhn, 1969a,b, 1970). However, despite the large-scale morphological revision of this species group by Schmidtler (1997), it is still known as the *Ablepharus kitaibelii* species complex (Sindaco and Jeremčenko, 2008).

*Ablepharus kitaibelii* is the only species of the genus distributed in Europe, ranging from southern Slovakia and Hungary, the eastern parts of continental Croatia and Bosnia & Herzegovina, through most of Serbia, southern Romania, Bulgaria, FYROM, Albania (lowland areas), Greece (including many Ionian and Aegean islands), and Turkey (western and central) (Böhme et al., 2009; Gasc et al., 1997; Schmidtler, 1997). Its subspecific taxonomy has been revised several times with four subspecies of *A. kitaibelii* being currently recognized morphologically (Uetz and Hošek, 2015): *A. k. fitzingeri* Mertens, 1952 in Slovakia and Hungary, *A. k. kitaibelii* (Bibron & Bory, 1833) in Greece and in several Aegean islands, Turkey, *A. k. stepaneki* Fuhn, 1970 in Romania and Bulgaria, *A. k. fabichi* Stepanek, 1937 in Mikronisi islet of Crete, Karpathos, Kasos, and Armathia islands (Fuhn, 1969a, 1970; Schmidtler, 1997). Moreover, Schmidtler (1997) recognized the populations of *A. k. chernovi* and *A. k. budaki* as distinct species (*A. chernovi* and *A. budaki*) with four (*A. c. chernovi* Darevsky, 1953; *A. c. eiselti* Schmidtler, 1997; *A. c. isauriensis* Schmidtler, 1997, *A. c. resslii* Schmidtler, 1997) and two (*A. b. budaki* Göcmen, Kumlutas & Tosunoglu, 1996; *A. b. anatolicus* Schmidtler, 1997) subspecies, respectively (Uetz and Hošek, 2015). He also revalidated the former *Riopa rueppellii* (referred to as *A. k. kitaibelii* in Fuhn, 1969b) as *A. rueppellii* comprising two subspecies [*A. r. rueppellii* (Gray, 1839); *A. r. festae* Peracca, 1894] (Uetz and Hošek, 2015).

Most of the taxonomic studies on the genus *Ablepharus* have been based on morphology (Fuhn, 1969a,b, 1970; Göcmen et al., 1996; Ljubisavljevic et al., 2002; Lymberakis and Kalionzopoulou, 2003; Schmidtler, 1997). On the other hand, there are only two phylogenetic and phylogeographic studies that have included samples of the *A. kitaibelii* complex (Poulakakis et al., 2013, 2005a). However, in both studies, the number of samples was limited and collected mainly from the European part of the genus distribution. According to those studies, *A. kitaibelii* is paraphyletic and originated in the Late Miocene (~9.2 Mya), while it was indicated that its taxonomy had to be re-evaluated, including more sampling in the eastern Mediterranean in order to uncover further hidden diversity.

The region, including most of the *A. kitaibelii* species complex's range (Fig. 1), has a complicated geological history that has also left an imprint on the biogeography of many other vertebrate taxa, especially amphibians and reptiles (see Bilgin, 2011 for a review; Lymberakis and Poulakakis, 2010). This region belongs to the Mediterranean basin, which is recognized as one of the world's top 25 "biodiversity hotspots" (Myers et al., 2000). Located in the Alpine–Himalayan Mountain belt between Eurasia, Africa and Arabia, the geomorphology of this region is the result of the collision of the Arabian and African plates with the European plate, which promoted the closure of the Tethys Sea (Rögl, 1998). Due to its position and geological history, Anatolia and the Aegean have acted either as bridges or as barriers for species' dispersal between Asia, Europe, and the Ethiopian region, providing a natural pathway or acting as a vicariant agent (Tchernov, 1992). Two of the most important paleogeological changes in the structure of the Aegean and Anatolia that help understanding their faunal synthesis are the Mid-Aegean Trench (MAT) and the Anatolian Diagonal Line (ADL), respectively. The opening of the MAT initiated at the end of the Middle Miocene (12 Mya) by the splitting of Crete from

Kasos-Karpathos and Asia Minor. This initial split bifurcated, forming the MAT at a north south axis and a second split in a SE-NW axis separating Crete and Peloponnisos from the Cyclades Islands. It was fully accomplished in the Late Miocene (10–9 Mya), causing the separation of central Aegean from east Aegean islands (Dermitzakis, 1990; Dermitzakis and Papanikolaou, 1981; Meulenkamp, 1985; Meulenkamp and Sissingh, 2003; Steininger and Rögl, 1984). The ADL is a line of mountain ranges that run from the south of Gümüshane – Bayburt in the north, southwest across Turkey to the Taurus Mountains (Mutun, 2010). Both events, MAT and ADL, have been proposed as significant biogeographic barriers setting up the current fauna composition (Bilgin, 2011; Lymberakis and Poulakakis, 2010). Beyond the geomorphological events that were described above, the severe climatic oscillations in this region had also a significant role in the biogeographical history of animals inhabiting this area. The climatic alterations from wetter to drier conditions and *vice versa* have produced repeated habitat changes and periodic modifications of major biota (Douady et al., 2003; Prentice and Jolly, 2000).

Studying the processes responsible of the diversification of *A. kitaibelii* species complex could help to understand the patterns of speciation in this area and provide insights in the evolutionary history of other contemporary taxa. Here, we explore the phylogenetic relationships among the members of the *A. kitaibelii* species complex using three gene fragments from two mtDNA (the large ribosomal unit, 16S rRNA, and the cytochrome, *cyt b*) and two nuclear (the natural killer-tumor recognition, NKTR, and the melanocortin 1 receptor, MC1R) genes, to assess the taxonomy and biogeographic history of these taxa.

## 2. Materials and methods

### 2.1. Samples, DNA extraction, amplification and sequencing

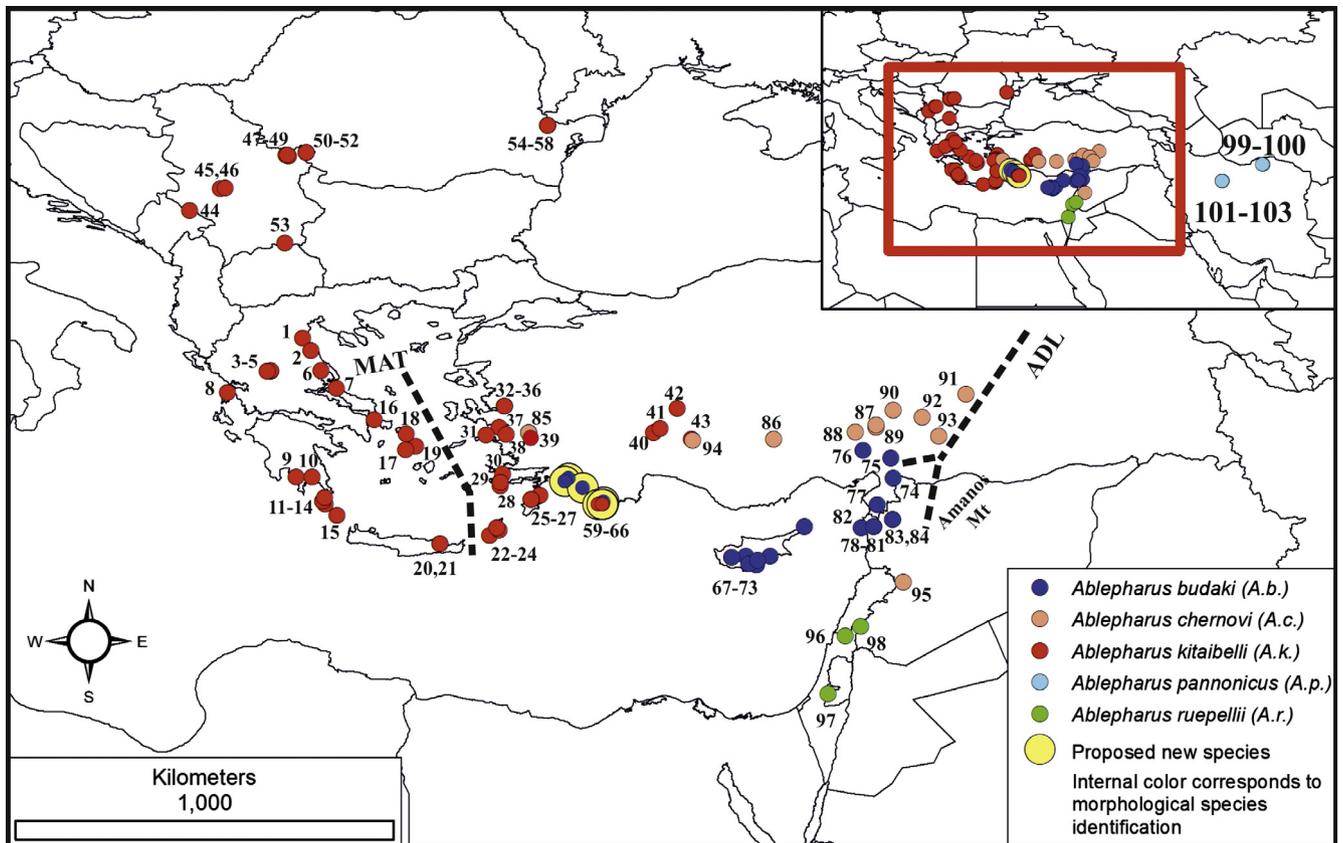
A total of 103 samples corresponding to all members of the *A. kitaibelii* species complex as well as *A. pannonicus* (Table 1) were used in this study. The geographic location of the samples is depicted in Fig. 1. Total genomic DNA was extracted from small tissue pieces (tail or liver) using a standard extraction method (Aljanabi and Martinez, 1997). Poorly preserved samples (preserved in formalin or 75% ethanol) were washed with 10 mM Tris-HCl (pH 8.0) as described in Austin and Melville (2006) before the extraction.

Partial segments of two mtDNA (16S, and *cyt b*) and two nuclear (MC1R, and NKTR) genes were selected for the phylogenetic analyses. Primers and conditions used in PCR amplifications and in the cycle sequencing reactions are shown in Table S1. PCR products were purified with the Nucleospin PCR purification kit (Macherey-Nagey). Double stranded sequencing of the purified PCR products was performed using a Big-Dye Terminator Cycle sequencing Kit (v.3.1) on ABI 3730XL automated sequencer.

Three samples of the genus *Chalcides*, three more from the genus *Eutropis*, one of the genus *Eumeces*, and 13 of the genus *Podarcis* were retrieved from GenBank and used as outgroup taxa and age constraints (Table 1). All newly produced sequences have been deposited in GenBank (accession numbers are given in Table 1).

### 2.2. Alignment and model selection

Chromatographs were checked and sequences were corrected using the CodonCode Aligner v. 3.7.1 CodonCode Corporation. *Cyt b* and NKTR gene fragments were translated into amino acids and no stop codons were observed. DNA sequences were aligned using MAFFT v.6 (Katoh et al., 2002) with auto (for *cyt b*, NKTR, and



**Fig. 1.** Map showing the sampling localities of the specimens examined. Numbers refer to specimen codes given in Table 1. Dashed lines indicate geographical barriers (MAT: Mid-Aegean Trench, ADL: Anatolian Diagonal Line, Amanos Mt).

MC1R) and Q-INS-i (for 16S rRNA) strategies. The haplotype phase of the nuclear genes was inferred using Phase v. 2.1 (Stephens and Donnelly, 2003; Stephens et al., 2001) implemented in DnaSP v. 5.10.01 (Librado and Rozas, 2009).

### 2.3. Sequence divergence

Sequence divergences were estimated in MEGA v.6.06 (Tamura et al., 2013), using the Tamura & Nei (TrN) (Tamura and Nei, 1993) model of evolution for the current taxonomic units of *Ablepharus*, and the main lineages produced through the phylogenetic analyses.

### 2.4. Phylogenetic analyses on the complete dataset

DNA sequences of all target genes were concatenated in a single dataset (110 specimens; 103 ingroup and 7 outgroup). The genetic data were partitioned into 10 distinct data blocks including 9 blocks for the 1st, 2nd, and 3rd codon positions for each of the three protein-coding genes (e.g., *cyt b*, *NKTR*, *MC1R*) and one block for the non-coding 16S rRNA gene. The optimal partitioning scheme for the 10 data blocks, and the best-fit nucleotide substitution model for each partition were identified using the PartitionFinder (PF) v.1.1.1 (Lanfear et al., 2012). We ran PartitionFinder twice with the models of molecular evolution restricted to those that are available in either MrBayes or RAxML. All the analyses used the greedy search algorithm, linked branch lengths in calculations of likelihood scores, and the Bayesian Information Criterion (BIC) for selecting among alternative partitioning strategies. We did not evaluate models that include both a param-

eter for among-site rate heterogeneity (G) and a parameter for invariant sites (I), because the adding of a proportion of invariable sites creates a strong correlation, making it impossible to estimate both parameters reliably correlation (Yang, 2006).

The best partitioning schemes were used for maximum likelihood (ML) and Bayesian phylogeny inferences (BI).

Maximum likelihood analyses were conducted with RAxML (v. 8.1.21) (Stamatakis, 2014) using RAxMLGUI v.1.5 (Silvestro and Michalak, 2011) under the GTR + G model of evolution (see the results of PF) and parameters estimated independently for each partition. The best ML tree was selected from 500 iterations and the confidence of the branches of the best ML tree was assessed based on 1000 thorough bootstrap replicates.

Bayesian Inference was performed in MrBayes v.3.2.6 (Ronquist et al., 2012). All nucleotide substitution model parameters were unlinked across partitions and the different partitions were allowed to evolve at different rates using the “prset ratepr = variable” command. We ran eight concurrent chains (one cold and seven heated) for  $5 \times 10^7$  generations and recorded samples every 5000 generations. The first 25% of the samples were discarded as burn-in, and the remaining samples were used to summarize the posterior probability distributions for parameters ( $\geq 95\%$  indicate significant support) (Huelsenbeck and Ronquist, 2001). Results were analyzed in Tracer v1.6 (Drummond and Rambaut, 2007) to assess convergence and effective sample sizes (ESS) for all parameters. We checked (i) the average standard deviation of split frequencies between chains (based on the MrBayes manual: keep adding generations until the value falls below 0.01 or 0.05 (may be adequate) if you are interested mainly in the well-supported parts of the tree), (ii) the potential scale reduction factor (PSRF)

**Table 1**  
Specimens used in this study. Sample codes, species names, sampling localities, collection/museum numbers, accession numbers and references (NHMC: Natural History Museum of Crete). n.a. refers to not available sequences. f.a. = failed to amplify . PS. present study. # indicates the specimens that were selected from PTP analysis for the estimation of divergence times.

Code	Species	Locality	Museum no. (NHMC)	Acc. No. Cyt b/16S rRNA//MC1R/NKTR	References
1	<i>A. kitaibelii</i> <i>kitaibelii</i> #	Greece (Olympos)	80.3.82.2	AY561326/AY561380// KX591313/KX591479	Poulakakis et al. (2005a) and PS
2	<i>A. k. kitaibelii</i> #	Greece (Larissa)	80.3.82.87	AY561327/AY561381// KX591314/KX591480	Poulakakis et al. (2005a) and PS
3	<i>A. k. kitaibelii</i>	Greece (Kazarma)	80.3.82.70	AY561328/AY561382// KX591315/KX591481	Poulakakis et al. (2005a) and PS
4	<i>A. k. kitaibelii</i> #	Greece (Plastira)	80.3.82.64	AY561329/AY561383// KX591316/KX591482	Poulakakis et al. (2005a) and PS
5	<i>A. k. kitaibelii</i>	Greece (Plastira)	80.3.82.65	AY561330/AY561384// KX591317/KX591483	Poulakakis et al. (2005a) and PS
6	<i>A. k. kitaibelii</i>	Greece (Volos)	80.3.82.115	AY561331/AY561385// KX591318/KX591484	Poulakakis et al. (2005a) and PS
7	<i>A. k. kitaibelii</i> #	Greece (Evoia)	80.3.82.63	AY561332/AY561386// KX591319/KX591485	Poulakakis et al. (2005a) and PS
8	<i>A. k. kitaibelii</i> #	Greece (Leukada isl.)	80.3.82.30	AY561333/AY561387/ f.a./KX591486	Poulakakis et al. (2005a) and PS
9	<i>A. k. kitaibelii</i> #	Greece (Peloponnisos)	80.3.82.33	AY561334/AY561388// KX591320/KX591487	Poulakakis et al. (2005a) and PS
10	<i>A. k. kitaibelii</i>	Greece (Peloponnisos)	80.3.82.4	AY561335/AY561389// KX591321/KX591488	Poulakakis et al. (2005a) and PS
11	<i>A. k. kitaibelii</i> #	Greece (Kythira isl.)	80.3.82.55	AY561336/AY561390// KX591322/KX591489	Poulakakis et al. (2005a) and PS
12	<i>A. k. kitaibelii</i>	Greece (Kythira isl.)	80.3.82.56	AY561337/AY561391// KX591323/KX591490	Poulakakis et al. (2005a) and PS
13	<i>A. k. kitaibelii</i>	Greece (Kythira isl.)	80.3.82.57	AY561338/AY561392// KX591324/KX591491	Poulakakis et al. (2005a) and PS
14	<i>A. k. kitaibelii</i>	Greece (Kythira isl.)	80.3.82.59	AY561339/AY561393// KX591325/KX591492	Poulakakis et al. (2005a) and PS
15	<i>A. k. kitaibelii</i>	Greece (Antikythira isl.)	80.3.82.43	AY561340/AY561394// KX591326/KX591493	Poulakakis et al. (2005a) and PS
16	<i>A. k. kitaibelii</i> #	Greece (Stouronisia isl.)	80.3.82.119	AY561341/AY561395// f.a./KX591494	Poulakakis et al. (2005a) and PS
17	<i>A. k. kitaibelii</i> #	Greece (Syros isl.)	80.3.82.78	AY561342/AY561396// KX591327/KX591495	Poulakakis et al. (2005a) and PS
18	<i>A. k. kitaibelii</i> #	Greece (Andros isl.)	80.3.82.88	AY561343/AY561397// KX591328/f.a.	Poulakakis et al. (2005a) and PS
19	<i>A. k. kitaibelii</i>	Greece (Tinos isl.)	80.3.82.3	AY561344/AY561398// KX591329/KX591496	Poulakakis et al. (2005a) and PS
20	<i>A. kitaibelii fabichi</i> #	Greece (Crete isl.)	80.3.82.5	AY561345/AY561399// KX591330/KX591497	Poulakakis et al. (2005a) and PS
21	<i>A. k. fabichi</i>	Greece (Crete isl.)	80.3.82.6	AY561346/AY561400// KX591331/KX591498	Poulakakis et al. (2005a) and PS
22	<i>A. k. fabichi</i> #	Greece (Kasos isl.)	80.3.82.112	AY561347/AY561401//f.a./f.a.	Poulakakis et al. (2005a) and PS
23	<i>A. k. fabichi</i>	Greece (Karpachos isl.)	80.3.82.47	AY561348/AY561402// KX591332/KX591499	Poulakakis et al. (2005a) and PS
24	<i>A. k. fabichi</i>	Greece (Karpachos isl.)	80.3.82.72	AY561349/AY561403// KX591333/KX591500	Poulakakis et al. (2005a) and PS
25	<i>A. k. kitaibelii</i> #	Greece (Rhodes isl.)	80.3.82.1	AY561350/AY561404/ KX591334/KX591501	Poulakakis et al. (2005a) and PS
26	<i>A. k. kitaibelii</i>	Greece (Rhodes isl.)	80.3.82.61	AY561351/AY561405// KX591335/KX591502	Poulakakis et al. (2005a) and PS
27	<i>A. k. kitaibelii</i>	Greece (Rhodes isl.)	80.3.82.62	AY561352/AY561406// KX591336/KX591503	Poulakakis et al. (2005a) and PS
28	<i>A. k. kitaibelii</i>	Greece (Nisyros isl.)	80.3.82.113	AY561353/AY561407// KX591337/KX591504	Poulakakis et al. (2005a) and PS
29	<i>A. k. kitaibelii</i>	Greece (Strogylo isl.)	80.3.82.89	AY561354/AY561408// KX591338/KX591505	Poulakakis et al. (2005a) and PS
30	<i>A. k. kitaibelii</i>	Greece (Kos isl.)	80.3.82.25	AY561355/AY561409// KX591339/KX591506	Poulakakis et al. (2005a) and PS
31	<i>A. k. kitaibelii</i>	Greece (Samos isl.)	80.3.82.54	AY561356/AY561410// KX591340/KX591507	Poulakakis et al. (2005a) and PS
32	<i>A. k. kitaibelii</i>	W Turkey (İzmir)	80.3.82.101	AY561361/AY561415// KX591341/KX591508	Poulakakis et al. (2005a) and PS
33	<i>A. k. kitaibelii</i>	W Turkey (İzmir)	80.3.82.102	AY561362/AY561416// KX591342/KX591509	Poulakakis et al. (2005a) and PS
34	<i>A. k. kitaibelii</i>	W Turkey (İzmir)	80.3.82.103	AY561363/AY561417// KX591343/KX591510	Poulakakis et al. (2005a) and PS
35	<i>A. k. kitaibelii</i>	W Turkey (İzmir)	80.3.82.104	AY561364/AY561418// KX591344/KX591511	Poulakakis et al. (2005a) and PS
36	<i>A. k. kitaibelii</i>	W Turkey (İzmir)	80.3.82.105	AY561365/AY561419// KX591345/KX591512	Poulakakis et al. (2005a) and PS

Table 1 (continued)

Code	Species	Locality	Museum no. (NHMC)	Acc. No. Cyt b/16S rRNA/MC1R/NKTR	References
37	<i>A. k. kitaibelii</i>	W Turkey (Claros, Aydın)	80.3.82.175	KX591402/KX591443// KX591346/KX591513	PS
38	<i>A. k. kitaibelii</i>	W Turkey (Kuşadası, Aydın)	80.3.82.176	KX591403/KX591444// KX591347/KX591514	PS
39	<i>A. k. kitaibelii</i>	W Turkey (Kepez, Aydın)	80.3.82.178	KX591404/KX591445// KX591348/KX591515	PS
40	<i>A. k. kitaibelii</i> #	C Turkey (Eğirdir, Isparta)	80.3.82.180	KX591405/KX591446// KX591349/KX591516	PS
41	<i>A. k. kitaibelii</i>	C Turkey (Eğirdir & Gelendost, Isparta)	80.3.82.183	KX591406/KX591447// KX591350/KX591517	PS
42	<i>A. k. kitaibelii</i>	C Turkey (Akşehir, Konya)	80.3.82.184	KX591407/KX591448// KX591351/KX591518	PS
43	<i>A. k. kitaibelii</i>	C Turkey (Beyşehir, Konya)	80.3.82.186	KX591408/KX591449// KX591352/KX591519	PS
44	<i>A. kitaibelii fitzingeri</i>	Serbia (Vrbnica)	80.3.82.204	KX591409/KX591450// KX591353/KX591520	PS
45	<i>A. k. fitzingeri</i>	Serbia (Lopatnica)	80.3.82.205	KX591410/KX591451// KX591354/KX591521	PS
46	<i>A. k. fitzingeri</i>	Serbia (Lojanik)	80.3.82.206	KX591411/KX591452// KX591355/KX591522	PS
47	<i>A. k. fitzingeri</i>	Serbia (Djerdap)	80.3.82.207	KX591412/KX591453// KX591356/KX591523	PS
48	<i>A. k. fitzingeri</i>	Serbia (Djerdap)	80.3.82.208	KX591413/KX591454// KX591357/KX591524	PS
49	<i>A. k. fitzingeri</i>	Serbia (Djerdap)	80.3.82.209	KX591414/KX591455// KX591358/KX591525	PS
50	<i>A. k. fitzingeri</i>	Serbia (Velesnica)	80.3.82.210	KX591415/f.a./KX591359/ KX591526	PS
51	<i>A. k. fitzingeri</i>	Serbia (Velesnica)	80.3.82.211	KX591416/KX591456// KX591360/KX591527	PS
52	<i>A. k. fitzingeri</i>	Serbia (Velesnica)	80.3.82.212	KX591417/KX591457// KX591361/KX591528	PS
53	<i>A. k. fitzingeri</i>	Serbia (Trgoviste)	80.3.82.213	KX591418/KX591458//f.a./f.a.	PS
54	<i>A. kitaibelii stepanaki</i> #	Romania	80.3.82.226	KX591419/KX591459//f.a./f.a.	PS
55	<i>A. k. stepanaki</i>	Romania	80.3.82.227	KX591420/f.a./f.a./f.a.	PS
56	<i>A. k. stepanaki</i>	Romania	80.3.82.228	KX591421/KX591460//f.a./f.a.	PS
57	<i>A. k. stepanaki</i>	Romania	80.3.82.230	KX591422/f.a./f.a./f.a.	PS
58	<i>A. k. stepanaki</i>	Romania	80.3.82.231	KX591423/f.a./f.a./f.a.	PS
59	<i>A. k. kitaibelii</i> * #	Greece (Kastelorizo isl.)	80.3.82.20	AY561357/AY561411// KX591362/f.a.	Poulakakis et al. (2005a) and PS
60	<i>A. k. kitaibelii</i> *	Greece (Ag. Georgios isl.)	80.3.82.18	AY561358/AY561412// KX591363/KX591529	Poulakakis et al. (2005a) and PS
61	<i>A. k. kitaibelii</i> *	Greece (Ro isl.)	80.3.82.19	AY561359/AY561413// KX591364/KX591530	Poulakakis et al. (2005a) and PS
62	<i>A. k. kitaibelii</i> *	Greece (Psoradia isl.)	80.3.82.16	AY561360/AY561414// KX591365/KX591531	Poulakakis et al. (2005a) and PS
63	<i>A. budaki</i> #	S Turkey (Kaş, Antalya)	80.3.131.51	KX591424/KX591461// KX591366/KX591532	PS
64	<i>A. budaki</i> #	S Turkey (Dalaman, Muğla)	80.3.131.57	KX591425/KX591462// KX591367/KX591533	PS
65	<i>A. budaki</i>	S Turkey (Dalaman, Mugla)	80.3.131.58	KX591426/KX591463// KX591368/KX591534	PS
66	<i>A. budaki</i> #	S Turkey (Babadağ, Fethiye, Muğla)	80.3.131.60	KX591427/f.a./KX591369/ KX591535	PS
67	<i>A. budaki</i> #	Cyprus (Agros)	80.3.131.1	AY561366/AY561420// KX591370/f.a.	Poulakakis et al. (2005a) and PS
68	<i>A. budaki</i>	Cyprus (Kivernitis)	80.3.131.2	AY561367/AY561421// KX591371/KX591536	Poulakakis et al. (2005a) and PS
69	<i>A. budaki</i>	Cyprus (Kamares)	80.3.131.3	AY561368/AY561422// KX591372/KX591537	Poulakakis et al. (2005a) and PS
70	<i>A. budaki</i>	Cyprus (Germasogeia)	80.3.131.4	AY561369/AY561423// KX591373/KX591538	Poulakakis et al. (2005a) and PS
71	<i>A. budaki</i>	Cyprus (Vavla)	80.3.131.5	AY561370/AY561424// KX591374/KX591539	Poulakakis et al. (2005a) and PS
72	<i>A. budaki</i>	Cyprus (Roudia bridge)	80.3.131.6	AY561371/AY561425// KX591375/KX591540	Poulakakis et al. (2005a) and PS
73	<i>A. budaki</i>	Cyprus (Rizocarpaso)	80.3.131.44	JX847566/JX847527// KX591376/KX591541	Poulakakis et al. (2013) and PS
74	<i>A. budaki</i> #	S Turkey (Çardak Plateau, Hassa, Hatay)	80.3.131.46	JX847562/JX847528// KX591377/KX591542	Poulakakis et al. (2013) and PS
75	<i>A. budaki</i> #	S Turkey (Dumanlı Plateau, Düziçi, Osmaniye)	80.3.131.47	JX847563/JX847529// KX591378/KX591543	Poulakakis et al. (2013) and PS

(continued on next page)

Table 1 (continued)

Code	Species	Locality	Museum no. (NHMC)	Acc. No. Cyt b/16S rRNA/MC1R/NKTR	References
76	<i>A. budaki</i> #	S Turkey (Kozan, Adana)	80.3.131.48	JX847564/JX847530//KX591379/KX591544	Poulakakis et al. (2013) and PS
77	<i>A. budaki</i> #	S Turkey (Harbiye, Hatay)	80.3.131.56	JX847565/JX847531//KX591380/KX591545	Poulakakis et al. (2013) and PS
78	<i>A. budaki</i> #	Syria (Alawit mt)	80.3.131.7	AY561372/AY561426//KX591381/f.a.	Poulakakis et al. (2005a) and PS
79	<i>A. budaki</i>	Syria (Alawit mt)	80.3.131.8	AY561373/AY561427//KX591382/KX591546	Poulakakis et al. (2005a) and PS
80	<i>A. budaki</i>	Syria (Alawit mt)	80.3.131.9	AY561374/AY561428//KX591383/KX591547	Poulakakis et al. (2005a) and PS
81	<i>A. budaki</i>	Syria (Alawit mt)	80.3.131.10	AY561375/AY561429//KX591384/KX591548	Poulakakis et al. (2005a) and PS
82	<i>A. budaki</i> #	Syria (Lattakia)	80.3.131.11	AY561376/AY561430//KX591385/KX591549	Poulakakis et al. (2005a) and PS
83	<i>A. budaki</i> #	Syria (Allepo)	80.3.131.12	AY561377/AY561431//KX591386/KX591550	Poulakakis et al. (2005a) and PS
84	<i>A. budaki</i>	Syria (Allepo)	80.3.131.13	AY561378/AY561432//KX591387/KX591551	Poulakakis et al. (2005a) and PS
85	<i>A. chernovi</i> #	W Turkey (Aydın)	80.3.82.177	KX591428/KX591464//f.a./KX591552	PS
86	<i>A. chernovi</i> #	C Turkey (Karapınar, Konya)	80.3.79.21	KX591429/KX591465//KX591388/f.a.	PS
87	<i>A. chernovi</i> #	S Turkey (Saimbeyli, Adana)	80.3.79.23	KX591430/KX591466//KX591389/KX591553	PS
88	<i>A. chernovi</i> #	S Turkey (Mansurlu, Adana)	80.3.79.24	KX591431/KX591467//KX591390/KX591554	PS
89	<i>A. chernovi</i> #	S Turkey (north of Saimbeyli, Adana)	80.3.79.25	JX847568/JX847533//KX591391/KX591555	Poulakakis et al. (2013) and PS
90	<i>A. chernovi</i>	C Turkey (Tarlaköy, Sarız, Kayseri)	80.3.79.28	KX591432/KX591468//KX591392/KX591556	PS
91	<i>A. chernovi</i>	E Turkey (Yoncalı, Arguvan, Malatya)	80.3.79. 31	JX847567/JX847532//KX591393/KX591557	Poulakakis et al. (2013) and PS
92	<i>A. chernovi</i>	S Turkey (Elbistan, Kahramanmaraş)	80.3.79. 32	KX591433/KX591469//KX591394/KX591558	PS
93	<i>A. chernovi</i>	S Turkey (Gölbaşı, Adıyaman)	80.3.79. 34	KX591434/KX591470//KX591395/KX591559	PS
94	<i>A. chernovi</i>	C Anatolia (Beyşehir, Konya)	80.3.79. 35	JX847569/JX847534//KX591396/KX591560	Poulakakis et al. (2013) and PS
95	<i>A. chernovi</i> #	Syria (Homs)	80.3.79.1	AY561379/AY561423//f.a./KX591561	Poulakakis et al. (2005a) and PS
96	<i>A. rueppellii</i> #	Israel (Meron)	80.3.169.5	KX591435/KX591471//KX591397/f.a.	PS
97	<i>A. rueppellii</i> #	Israel (Buregin)	80.3.169.6	KX591436/KX591472//KX591398/KX591562	PS
98	<i>A. rueppellii</i> #	Israel (Nimrod)	80.3.169.7	KX591437/KX591473//f.a./KX591563	PS
99	<i>A. pannonicus</i> #	Iran (Khorasan)	80.3.173.1	KX591438/KX591474//KX591399/KX591564	PS
100	<i>A. pannonicus</i> #	Iran (Khorasan)	80.3.173.2	KX591439/KX591475//KX591400/KX591565	PS
101	<i>A. pannonicus</i>	Iran (Tehran)	80.3.173.3	KX591440/KX591476//KX591401/KX591566	PS
102	<i>A. pannonicus</i>	Iran (Tehran)	80.3.173.4	KX591441/KX591477//f.a./KX591567	PS
103	<i>A. pannonicus</i>	Iran (Tehran)	80.3.173.6	KX591442/KX591478//f.a./KX591568	PS
104	<i>Eutropis multifasciata</i>			AY151513/JQ767964//KJ574548/n.a.	Barley et al. (2015) and Datta-Roy et al. (2012)
105	<i>Eutropis macularia</i>			DQ239136/JQ767961//KJ574630/n.a.	Barley et al. (2015), Datta-Roy et al. (2012) and Whiting et al. (2006)
106	<i>Mabuya rudis</i>			DQ239135/AB028790//KJ574628/n.a.	Barley et al. (2015), Honda et al. (2000) and Whiting et al. (2006)
107	<i>Eumeces algeriensis</i>			EU278253/EU278086//n.a./n.a	Carranza et al. (2008)
108	<i>Chalcides sphenopsiformis</i>			EU278107/EU278131//n.a./n.a	Carranza et al. (2008)
109	<i>C. viridanus</i>			EU278117/EU278036//n.a./n.a	Carranza et al. (2008)
110	<i>C. ocellatus</i>			JQ344286/JQ344265//n.a./n.a	Lavin and Papenfuss (2012)
111	<i>Podarcis cretensis</i> 1	Argyroupoli, Crete	Greece	AF486216/AY896153//n.a./n.a	Poulakakis et al. (2003, 2005b)
112	<i>P. cretensis</i> 2	Lafonisi, Crete	Greece	AF486193/AY896157//n.a./n.a	Poulakakis et al. (2003, 2005b)
113	<i>P. cretensis</i> 3	Samaria, Crete	Greece	AF486204/AY896161//n.a./n.a	Poulakakis et al. (2003, 2005b)
114	<i>P. cretensis</i> 4	Chrisi isl, Crete	Greece	AF486212/AY896148//n.a./n.a	Poulakakis et al. (2003, 2005b)
115	<i>P. peloponnesiacus</i> 1	Kalavritra	Greece	AY896121/AY896177//n.a./n.a	Poulakakis et al. (2003, 2005b)
116	<i>P. peloponnesiacus</i> 2	Feneos	Greece	AY896116/AY896173//n.a./n.a	Poulakakis et al. (2003, 2005b)

Table 1 (continued)

Code	Species	Locality	Museum no. (NHMC)	Acc. No. Cyt b/16S rRNA/MC1R/NKTR	References
117	<i>P. bocagei</i> (Gpb6)	Malpica, Galicia	Spain	AF469426/DQ081075//n.a./n.a	Pinho et al. (2006)
118	<i>P. carbonelli</i> (Albc1)	La Alberca, Castilla y León	Spain	DQ081142/DQ081080//n.a./n.a	Pinho et al. (2006)
119	<i>P. carbonelli</i> (PR1)	Playa del Rompeculos	Spain	DQ081141/DQ081079//n.a./n.a	Pinho et al. (2006)
120	<i>P. hispanica</i> (Mon8)	Montesinho	Portugal	AF469447/DQ081086//n.a./n.a	Pinho et al. (2006)
121	<i>P. hispanica</i> (Trj1)	Trujillo Extremadura	Spain	AF469451/DQ081088//n.a./n.a	Pinho et al. (2006)
122	<i>P. hispanica</i> (And1)	Benatae, Andalucía	Spain	DQ081143/DQ081084//n.a./n.a	Pinho et al. (2006)
123	<i>P. hispanica</i> (Phv4)	Beja	Portugal	AF469455/DQ081083//n.a./n.a	Pinho et al. (2006)

\* After Poulakakis et al. (2005a,b).

of all the estimated parameters approached values of  $\sim 1$ , and (iii) the plot of the generation versus the log probability of the data (the log likelihood values). Maximum likelihood bootstrap values and Bayesian posterior probability values were joined and mapped to the Bayesian tree (i.e., the 50% majority-rule consensus tree calculated from the posterior distribution of trees).

To test alternative phylogenetic hypotheses and relationships, we compared the best resulting ML topology to topologies constrained to fit alternative hypotheses using the approximately unbiased test (AU) (Shimodaira, 2002), Shimodaira–Hasegawa (SH) test, Kishino – Hasegawa test and the weighted SH (wSH) and KH (wKH) in the program CONSEL (Shimodaira and Hasegawa, 2001). Constraint topologies were estimated in RAxML with the  $-g$  constraint option using the best scoring tree for topology tests estimated from 200 ML searches starting from random tree topologies. Topological constraints: (i) *A. kitaibelii* and *A. budaki* are monophyletic at the same tree, (ii) *A. kitaibelii* is monophyletic, ignoring the phylogenetic status of *A. budaki*, (iii) *A. budaki* is monophyletic, ignoring the phylogenetic status of *A. kitaibelii*, (iv) all the specimens of the mixed clade [*A. kitaibelii* from Kastelorizo island group (specimens 59, 60, 61, and 62) and *A. budaki* from southwest Turkey (63, 64, 65, and 66)] are *A. budaki*, and (v) all the specimens of the mixed clade are *A. kitaibelii*. In all hypothesis testing a  $p$ -value  $< 0.05$  was considered a statistically significant result.

## 2.5. Divergence times estimation on mt tree - Ancestral Area Reconstruction

Due to the fact that the extrapolation of rates across different timescales (i.e. across the population-species boundary) might result in non-valid dates (Ho et al., 2011), the sequence variation was divided into intra- and interspecies groups using the Poisson Tree Processes (PTP) model for delimiting species on a rooted phylogenetic tree (Zhang et al., 2013). This method identifies the location of the switches from speciation to coalescent nodes on a tree and delimits independently entities. In PTP, speciation or branching events are modeled in terms of number of substitutions (represented by branch lengths), considering that the number of substitutions between species is significantly higher than the number of substitutions within species. Analyses were conducted on the web server for PTP (<http://species.h-its.org/ptp/>) using the produced BI phylogenetic tree (see above).

Divergence times of *Ablepharus* were estimated using BEAST v1.8.2 (Drummond and Rambaut, 2007) on the new dataset that comprised of mtDNA sequences and using one representative per PTP entity. The reason that we used the mtDNA genes only was that the nuclear genes were not available in GenBank for the outgroup taxa, which were used as external calibration points. As in the cases of ML and BI, the optimum partitioning scheme and nucleotide substitution models were estimated according to the BIC and the option beast in PF. The analysis was run for  $2 * 10^8$  gen-

erations with a sampling frequency of 1 per 5000 trees from which 10% were discarded as burn-in. Prior specifications applied were as follows (otherwise by default): Relaxed Uncorrelated Lognormal Clock (estimate); Yule process of speciation. Results were analyzed in Tracer to assess effective sample sizes (ESSs) for all parameters. The  $-\ln L$  was stabilized prior to  $10^8$ , and the first 10% of the sampled generations were discarded. The final tree with divergence estimates and their 95% highest posterior densities (HPD) was computed in TreeAnnotator v1.8.2. Trees were visualized using the software FigTree v1.3.1 (Rambaut, 2006–2009).

The divergence times for the phylogenetic clades of the studied species were estimated by using four (three external and one internal) calibration age constraints (Table 2); (a) the separation of the island of Crete from Peloponnisos (reflecting the separation of *P. cretensis* from *P. peloponnesiacus*) at 5.3 Mya (normal distribution with mean value 5.3 and standard deviation 0.1) (Poulakakis et al., 2003), (b) the divergence of two Iberian *Podarcis* species (*P. bocagei* from *P. hispanica*) at 5.6 Mya (normal distribution with mean value 5.6 and standard deviation 0.1) (Kaliontzopoulou et al., 2011), (c) the separation of *C. viridanus* and *C. sphenopsiformis* at 7.2 Mya (normal distribution with mean value 5.25 and standard deviation 0.1) (Carranza et al., 2008), and (d) the geographic isolation of the island group of Kasos/Karpathos from Rhodes at early Pliocene (normal distribution with mean value 3.5 and standard

Table 2

Calibration points (given in bold) and estimated divergence times with confidence intervals (95%) for the outgroup clades and the main nodes (A–K) of the tree presented in Fig. 3.

Taxon/clade	Divergence times in my	References
<i>C. sphenopsiformis</i> - <i>C. viridanus</i>	<b>7.2 (7.02–7.41)</b>	Carranza et al. (2008)
<i>P. cretensis</i> - <i>P. peloponnesiacus</i>	<b>5.3 (5.03–5.42)</b>	Poulakakis et al. (2003)
<i>P. hispanica</i> I - <i>P. bocagei</i>	<b>5.6 (5.39–5.78)</b>	Kaliontzopoulou et al. (2011)
A	16.51 (12.2–21.6)	
B	12.87 (9.78–16.39)	
C	11.24 (8.46–14.40)	
D	10.08 (7.49–13.03)	
E	9.2 (6.58–12.3)	
F	5.15 (3.26–7.34)	
G	5.74 (4.01–7.85)	
H	5.13 (3.48–7.06)	
I	4.17 (2.81–5.81)	
J	5.51 (3.96–7.46)	
K	3.14 (2.15–4.33)	
Isolation of Karpathos/ Kasos	<b>3.5 (3.30–3.69)</b>	Böger and Dermitzakis (1987) and Daams and Van Der Weerd (1980)

deviation 0.1) (Böger and Dermitzakis, 1987; Daams and Van Der Weerd, 1980).

We used the recently developed Bayesian Binary MCMC (BBM) method implemented in RASP v.3.0 (Yu et al., 2014) to reconstruct the possible ancestral ranges of *A. kitaibelii* species complex on the phylogenetic trees. To account for uncertainties in phylogeny, we used 40,000 trees from the output of BEAST. The number of maximum areas was kept as 2. The MCMC chains were run simultaneously for  $5 \times 10^6$  generations. The state was sampled every 100 generations and the fixed JC + G (Jukes-Cantor + Gamma) model were used with null root distribution. Distribution ranges of sequences (haplotypes) instead of species were used (see Fernandez-Mazuco and Vargas, 2011). All outgroup taxa were trimmed, while the distribution range of *Ablepharus* was divided into nine main distributional areas; (A) Anatolia and East Aegean Islands, (B) Syria and Israel, (C) Iran, (D) Cyprus Island, (E) Cyclades Islands, (F) Balkans (including continental Greece and Peloponnisos), (G) Kythira Island group, (H) Crete Island, and (I) Kasos and Karpathos Islands.

### 2.6. Phylogenetic analyses on the nuclear dataset

The genealogical relationships among the nuclear DNA sequences (MC1R and NKTR) were estimated based on maximum parsimony (MP) analysis performed in PAUP\* (v.4.0b10) (Swofford, 2002). In MP, the shortest trees were obtained by means of heuristic searches with 100 random taxon addition replicates and tree-bisection-reconnection (TBR) branch swapping and saving all shortest trees per replicate. Bootstrap analysis with 1000 replicates was assessed to provide confidence estimate in the nodes (Felsenstein, 1985).

## 3. Results

### 3.1. Alignment and genetic distances

A total of 2156 base pairs (bp) of concatenated DNA sequences (*cyt b* 420 bp, *16S rRNA* 529 bp, *MC1R* 634 bp, and *NKTR* 573 bp) were obtained. The concatenated alignment of the ingroup sequences revealed 68 haplotypes and contained 397 variable and 346 parsimony informative sites [158 variable (138

parsimony) in *cyt b*, 121 (111) in *16S rRNA*, 53 (46) in *MC1R*, and 65 (50) in *NKTR*]. Unfortunately, we failed to amplify 5 *16S rRNA* sequences (specimens 50, 55, 57, 58, and 66 in Table 1), 14 *MC1R* sequences (specimens 8, 16, 22, 53–58, 85, 95, 98, 102, and 103 in Table 1) and 13 *NKTR* sequences (specimens 18, 22, 53–59, 67, 78, 86, and 96 in Table 1).

The pairwise TrN genetic distances in *A. kitaibelii* species complex varied from 0 to 21.2% in *cyt b*, 0–13.1% in *16S rRNA*, 0–4.0% in *MC1R*, and 0–4.7% in *NKTR*. The mean genetic distances between the main lineages and the species of the *A. kitaibelii* complex are given in Table 3.

### 3.2. Phylogenetic trees from the complete dataset

The best-fit partitioning scheme for the 10 data blocks and the nucleotide substitution models selected for each data partition are provided in Table 4. Maximum Likelihood ( $-\ln L = 10837.88$ ) and BI (arithmetic mean:  $-\ln L = 10965.00$ ) analyses of the concatenated dataset produced similar topologies (Fig. 2). Considering the BI, the MCMC convergence diagnostics indicated convergence and stationarity.

Six clades were recovered from these analyses, corresponding to the recognized species of *Ablepharus* included in this study. Five of them correspond to *A. kitaibelii*, *A. budaki*, *A. rueppellii*, *A. chernovi*, and *A. pannonicus*. The sixth includes specimens of *A. kitaibelii*\* (Poulakakis et al., 2005a) and *A. budaki* from southeast Greece (Kastelorizo Island group) and southwest Turkey, rendering both *A. kitaibelii* and *A. budaki* polyphyletic species. Results of the AU, KH, and wKH tests rejected all the alternative hypotheses ( $p < 0.05$ ), indicating that *A. kitaibelii* and *A. budaki* are polyphyletic species. The SH test did not reject the hypotheses 4 and 5, but the wSH, which is recommended by the authors of CONSEL when you must be very cautious with the results, supports their rejection (Table 5).

### 3.3. Divergence times estimation on mt tree and Ancestral Area Reconstruction

The number and composition of putative mitochondrial lineages identified by the PTP test were 38. One specimen from each PTP entity was selected for BEAST analysis. In the estimation of the

**Table 3**  
Sequence divergences (%) among the main lineages of *Ablepharus* for the *cyt b*/16S (below diagonal) and MC1R/NKTR (above diagonal) based on Tamura & Nei model of evolution. Values in diagonal (bold) are within lineages sequence divergences [*cyt b*/16S/MC1R/NKTR].

Clade – species	1	2	3	4	5	6	7	8	9	10
1 West subclade - <i>A. kitaibelii</i>	<b>3.3/1.1/ 0.4/0.3</b>	0.7/1.6	1.1/1.8	1.6/2.1	1.7/2.1	1.5/3.0	1.8/3.8	1.3/2.3	2.4/2.7	2.7/1.9
2 East subclade - <i>A. kitaibelii</i>	6.8/3.9	<b>1.5/0.6/ 0.8/0.2</b>	1.2/0.6	1.6/2.1	1.7/2.1	1.6/3.0	1.7/3.7	1.4/2.2	2.4/2.7	2.6/2.0
3 Kasos/Karpathos group - <i>A. kitaibelii</i>	7.6/3.7	6.9/2.1	<b>0.7/0.3/ 0.4/0.1</b>	1.5/2.1	1.6/2.2	1.4/3.0	1.5/3.4	1.2/2.0	2.2/2.8	2.5/2.1
4 Cyprus - <i>A. budaki</i>	12.5/6.9	13.5/7.3	12.5/8.3	<b>0.9/0.2/ 0.1/0.1</b>	0.3/0.3	0.1/1.3	1.3/3.5	1.3/1.8	0.9/1.2	2.1/1.7
5 Syria - <i>A. budaki</i>	14.0/8.2	15.2/8.3	15.1/8.5	9.7/5.0	<b>1.5/1.1/ 0.3/0.2</b>	0.2/1.3	1.4/3.7	1.2/2.0	1.0/1.2	2.1/1.8
6 Turkey - <i>A. budaki</i>	14.1/7.5	15.5/7.7	14.5/7.7	7.8/4.2	7.6/5.3	<b>4.4/1.5/ 0.1/0.9</b>	1.3/2.4	1.1/2.7	0.8/2.0	2.0/2.5
7 SW Turkey and Kastelorizo	13.9/7.3	14.2/8.0	16.9/8.1	16.7/6.4	17.7/8.0	17.6/6.3	<b>1.4/1.1/ 0.4/0.5</b>	1.5/3.4	2.0/4.1	2.4/3.5
8 Turkey - <i>A. chernovi</i>	13.0/6.5	12.1/4.5	13.3/4.4	14.4/8.9	16.6/9.8	16.5/8.7	15.7/8.7	<b>5.7/1.6/ 0.1/0.9</b>	2.0/2.5	2.6/2.0
9 Israel - <i>A. rueppellii</i>	17.9/6.5	18.3/6.2	18.1/6.1	14.5/6.2	15.1/6.9	15.7/5.2	17.4/6.7	16.5/7.0	<b>8.5/2.3/ 0.3/0.4</b>	2.8/2.1
10 Iran - <i>A. pannonicus</i>	15.8/9.9	17.0/9.8	17.4/9.3	17.7/10.6	18.4/12.3	17.8/8.8	16.2/11.0	18.1/10.4	19.0/8.8	<b>1.0/0.2/ 0.0/0.7</b>

**Table 4**

Best-fit models of sequence evolution and partitioning schemes selected in PartitionFinder (PF) for phylogenetic reconstructions using Maximum Likelihood (RAxML) and Bayesian Inference (MrBayes), and divergence dating using BEAST. Codon position is denoted by Cp1–Cp3.

Analysis	Dataset	Partition scheme	Gene*		Length (bp)
RAxML	Concatenated 103 inroup taxa and 7 outgroup taxa	1	GTR + I + G	Cyt <i>b</i> – Cp1, 16S rRNA	140 + 529
		2	GTR + I + G	Cyt <i>b</i> – Cp2, MC1R – Cp2	140 + 211
		3	GTR + I + G	Cyt <i>b</i> – Cp3	141
		4	GTR + I + G	MC1R – Cp1, NKTR – Cp1, NKTR – Cp2, NKTR – Cp3	212 + 191 + 191 + 191
		6	GTR + G	MC1R – Cp3	211
		MrBayes	Concatenated 103 inroup taxa and 7 outgroup taxa	1	K80 + G
2	K80 + I	Cyt <i>b</i> – Cp2, MC1R – Cp1	140 + 212		
3	GTR + I + G	Cyt <i>b</i> – Cp3	140		
4	GTR + G	16S rRNA	529		
5	F81 + I + G	MC1R – Cp2	211		
6	GTR + G	MC1R – Cp3	211		
7	K80 + I	NKTR – Cp1, NKTR – Cp2, NKTR – Cp3	191 + 191 + 191		
BEAST	MtDNA 38 ingroup taxa and 17 outgroup taxa	1	GTR + I + G	Cyt <i>b</i> – Cp1, 16S rRNA	140 + 529
		2	HKY + I	Cyt <i>b</i> – Cp2,	140
		3	GTR + I + G	Cyt <i>b</i> – Cp3	140

\* The parameter I was ignored in the models with I + G (see Section 2).

divergence times, high effective sample sizes were observed for all parameters in all BEAST analysis ( $-\ln L = -7387.54$ ; ESS values  $> 200$ ) and assessment of convergence statistics in Tracer indicated that all analyses had converged. According to the inferred dates (Fig. 3 and Table 2), the diversification of *Ablepharus* lineages dates back to Early and Middle Miocene with the splitting of the clade of *A. pannonicus* at 16.51 Mya and the mixed clade of *A. kitaibelii*\* and *A. budaki* from Kastelorizo island group and southwest Turkey from the rest of *Ablepharus* lineages at 12.87 Mya. Within the latter group the diversification in species level seems to have occurred in Middle to Upper Miocene (11.24–9.2 Mya).

The results of the BBM analysis for the major nodes of the tree are presented in Fig. 3. The two runs produced identical results (with a 0.0003 distance between them). These data suggest that the *A. kitaibelii* species complex began to diversify somewhere in Anatolia.

### 3.4. Phylogenetic analyses on the nuclear dataset

The MP tree (score 244, Homoplasy Index = 0.44) on the two nuclear genes (MC1R and NKTR) produced a tree with similar topology (considering the major clades and subclades) to the tree produced from the complete dataset and the Bayesian tree generated by the BEAST analysis (Fig. S1), but with low statistical support.

## 4. Discussion

### 4.1. Systematics

A more comprehensive and robust assessment of the phylogenetic relationships within the *Ablepharus kitaibelii* species complex is presented here, based on both mitochondrial and nuclear data. The inferred phylogeny shows high genetic diversity, revealing several major incongruences with the currently accepted taxonomy (polyphyly of *A. kitaibelii* and *A. budaki*) for both mtDNA and nuclear datasets.

The traditional phylogenetic reconstructions (BI and ML) highlights the presence of six major clades, showing high statistical support for each clade (pp 1.00 and bs  $> 89$ ). Five of them match the main morphological species of *A. kitaibelii* species complex (*A. kitaibelii*, *A. chernovi*, *A. budaki*, *A. pannonicus*, *A. rueppellii*), increasing our confidence that the recovered topology represents the true evolutionary history of this complex. However, an asterisk could be added in the case of *A. rueppellii* for which the clustering

of specimen 98 with the other two *A. rueppellii* specimens (96 and 97) was not strongly supported. This indicates a high intraspecific variation in *A. rueppellii*. Although only three specimens of *A. rueppellii* were used, the intraspecific differentiation is higher than any other species in the *A. kitaibelii* species complex (see Table 3; 8.5% in *cyt b*). All these raise suspicions with regards to the taxonomic status of *A. rueppellii* and increase the necessity for analysis of many more samples from the whole distribution area of this species in order to evaluate its taxonomy.

The clades of *A. kitaibelii*, *A. chernovi*, *A. budaki*, and *A. rueppellii* are clustered into two groups. The first group includes *A. rueppellii* from Israel and *A. budaki* from Turkey, Syria, and Cyprus, all originating from Middle East. The latter species is further subdivided into three monophyletic lineages that include specimens from different geographic regions (Cyprus, Syria, and Turkey) with relatively low diversification among lineages (7.6–9.7% in *cyt b* and 4.2–5.3% in 16S rRNA) compared to the between species divergence (12.7–19.0% in *cyt b* and 5.6–11.0% in 16S rRNA).

The second group comprises the species *A. kitaibelii* and *A. chernovi*. Within *A. kitaibelii*, the addition of specimens from Turkey, Serbia and Romania led to the same topology produced in Poulakakis et al. (2005a) with two distinct subclades (western and eastern) that are in accordance with the geographical origin of the specimens. The western subclade includes populations of morphologically defined subspecies (i) *A. k. kitaibelii* from continental Greece, Peloponnisos, Ionian Islands, Cyclades and Kythira island group, (ii) *A. k. stepaneki* from Romania, and (iii) *A. k. fitzingeri* from Serbia. The eastern subclade consists of populations of *A. k. kitaibelii* from East Aegean islands and Turkey and populations of *A. k. fabichi* from Mikronisi islet of Crete, and the island group of Karpathos (Kasos and Karpathos). The phylogenetic relationships within these subclades could be considered as unresolved due to the low bootstrap support in all the phylogenetic analyses. According to the presented topology and the corresponding statistical support, the morphological subspecies of *A. kitaibelii* are not monophyletic. This brings into question the practice of subspecies recognition and subsequent assignment of local populations into these subspecies on the basis of an exclusive or limited collection of characters, be that morphological, behavioral or molecular. The inability to distinguish morphologically the western *A. k. kitaibelii* from the eastern ones that might indicate morphological stasis, the polyphyly of *A. k. kitaibelii*, and the lack of genetic differentiation of *A. k. fitzingeri* highlight the need for a taxonomic revision of *A. kitaibelii* at the subspecific level.

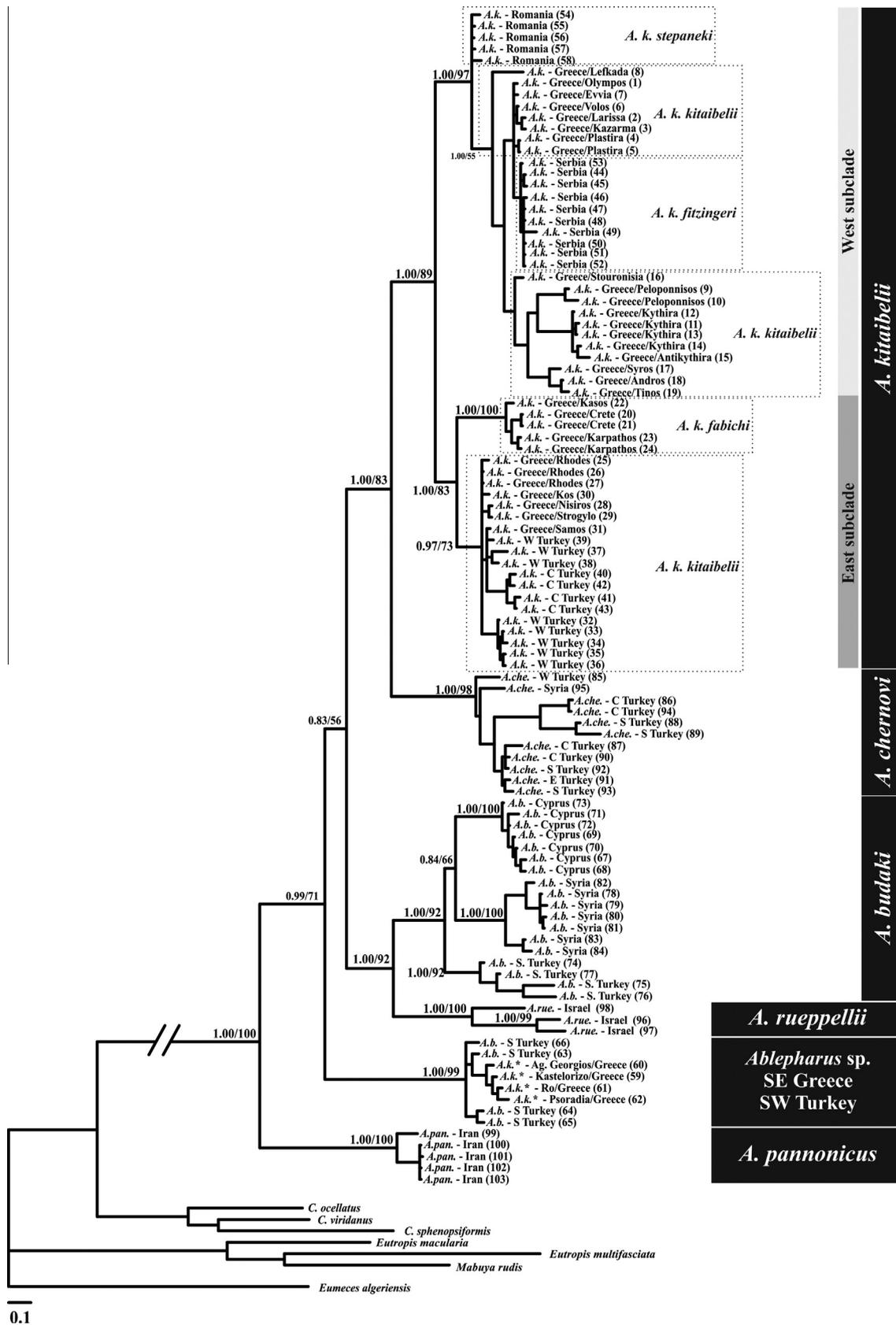


Fig. 2. Bayesian Inference (BI) tree reconstructed from the complete dataset. Numbers on branches indicate posterior probabilities and bootstrap supports (BI/ML).

The status of *A. chernovi* as a distinct species has been uncertain (originally described as a subspecies of *A. kitaibelii*; Fuhn, 1969a,b, 1970). It has been recognized by Schmidtler (1997) based on

morphometric data. In a previous study (Poulakakis et al., 2005a), the authors stated that although only one individual of *A. chernovi* was used, its high divergence from *A. kitaibelii* and

**Table 5**Approximately Unbiased (AU) and Shimodaira Hasegawa (SH) tests results for alternative hypotheses of *Ablepharus* relationships.

Tree	Hypothesis tested	Likelihood	AU	KH	SH	wKH	wSH
	Un-constrained tree	−10837.88	0.970	0.973	1.000	0.973	1.000
1	<i>A. kitaibelii</i> and <i>A. budaki</i> are monophyletic at the same tree	−11097.62	<0.000	0	0	0	0
2	<i>A. kitaibelii</i> is monophyletic, ignoring the phylogenetic status of <i>A. budaki</i>	−11059.40	<0.000	0	0	0	0
3	<i>A. budaki</i> is monophyletic, ignoring the phylogenetic status of <i>A. kitaibelii</i>	−11067.58	<0.000	0	0	0	0
4	The clade that branches off first belongs to <i>A. budaki</i>	−10860.44	0.03	0.027	0.201	0.027	0.043
5	The clade that branches off first belongs to <i>A. kitaibelii</i>	−10891.21	<0.000	0	0.064	0	0

*A. budaki* supported its recognition as a separate species. Here, the increased number of samples and genetic data reinforce this statement and reveal its relationship with *A. kitaibelii*.

The phylogenetic position of the mixed clade (*Ablepharus* sp.) that includes specimens of *A. kitaibelii* and *A. budaki*, represents an unexpected and interesting problem for the taxonomy of these species. This clade is totally supported (pp = 1.00, b.s. = 99%), and the individuals forming it were collected from southwest Turkey and the island group of Kastelorizo, which is situated opposite the town of Kas (Antalya) at the southwest coast of Turkey (Fig. 1). All tests reject the hypothesis of the monophyly of *A. kitaibelii* and *A. budaki*. Notably, the nuclear DNA tree (Fig. S1), although with no statistical support, suggests the non-monophyly of *A. budaki* and *A. kitaibelii* as well. The only remarkable difference is that the mixed clade appears topologically closer to the group of *A. rueppellii* and *A. budaki*, but as we said before with no statistical support. We suppose that the addition of more samples from the wider area of Turkey and more genes will shed more light on the phylogenetic position of the mixed clade in order to conclude if (a) the mixed clade is member of the group of *A. budaki* and *A. rueppellii* or (b) the mixed clade is the basal lineage of a wider group that includes *A. kitaibelii*, *A. chernovi*, *A. budaki*, and *A. rueppellii*. The diversity in mitochondrial DNA found among the lineages of *Ablepharus* specimens from these regions and the rest of *A. kitaibelii* and *A. budaki* specimens from Turkey, Cyprus, Syria, and Greece is very high, reaching 17.7% in *cyt b* and 8.1% in 16S rRNA. This differentiation is similar and, in some cases, higher than the divergence found between the *Ablepharus* species included in this study (i.e., the genetic distance of *A. kitaibelii* from *A. chernovi* is 12.8% in *cyt b* and 5.1% in 16S rRNA) or in other lizard species [in *cyt b*: 17.7% in *Ophiomorus* spp. (Scincidae), 3.1–8.2% in *Anatololacerta* spp. (Lacertidae), 7.4–8.2% in *Iberolacerta* spp. (Lacertidae), ~15% in *Timon* spp. (Lacertidae), 17–20% in *Mesalina* spp. (Lacertidae), and up to 20.7% in *Apathya* spp. (Lacertidae)] (Ahmadzadeh et al., 2012; Bellati et al., 2015; Crochet et al., 2004; Kapli et al., 2013, 2008; Poulakakis et al., 2008).

Both the genetic differentiation of this clade and its phylogenetic position outside a clade containing *A. rueppellii*, *A. chernovi* and all other populations of *A. kitaibelii* and *A. budaki* (Fig. 2) reveal a case of hidden diversity (cryptic species) and raise questions regarding its taxonomic status. Cryptic species are almost evenly distributed among major metazoan taxa, and can be found in all sorts of habitats and biogeographic zones (Bickford et al., 2007; Pfenninger and Schwenk, 2007; Trontelj and Fiser, 2009). Although the literature is replete with definitions of cryptic species, we followed the definition of Bickford et al. (2007) who considers that two or more species are “cryptic” when they are erroneously classified (and hidden) under one species name because they are at least superficially morphologically indistinguishable. This means that morphological change might not be correlated with species boundaries or might not be useful in discriminating species (Bickford et al., 2007), since speciation and morphological evolution are two independent processes that usually occur synchronously but may proceed independently (Sturmbauer and Meyer, 1992).

From a morphological point of view, the specimens from Kastelorizo fit very well both with the description of *A. k. kitaibelii* (Göçmen et al., 1996) and *A. b. anatolicus* (Schmidtler, 1997) from southern Turkey (for more details see Poulakakis et al., 2005a). Even if we consider that all the specimens of the mixed clade belong to *A. kitaibelii* or *A. budaki* the problem of polyphyly still remains, but it is restricted in one of these species (in *A. kitaibelii* in the first case and in *A. budaki* in the second case). In order to avoid the polyphyly in *A. kitaibelii* species complex, someone could suggest the elevation of this clade to species level, considering the populations from Kastelorizo Island group and southwestern Turkey as distinct species (*Ablepharus* sp.). For this, the name *A. anatolicus* is available. This is not uncommon for this region, as several recently published molecular studies of the Middle East herpetofauna have revealed cases of cryptic species (Ahmadzadeh et al., 2012, 2013; Bellati et al., 2015; Kapli et al., 2015, 2008; Kornilios et al., 2011, 2012; Kyriazi et al., 2008; Sagonas et al., 2014). Though the para- or poly-phyletic problems could be “easily” resolved by merging or splitting species, the most important question that emerges and have to be answered is what evolutionary and ecological processes lead to genetic divergence and reproductive isolation in the absence of morphological differentiation. Studies using other than morphological characters to discriminate indistinguishable species are rapidly published, giving the opportunity through the discovering of new cryptic species to study important mechanisms of speciation, possible interactions in the relevant contact zones, mate recognition and conservation management.

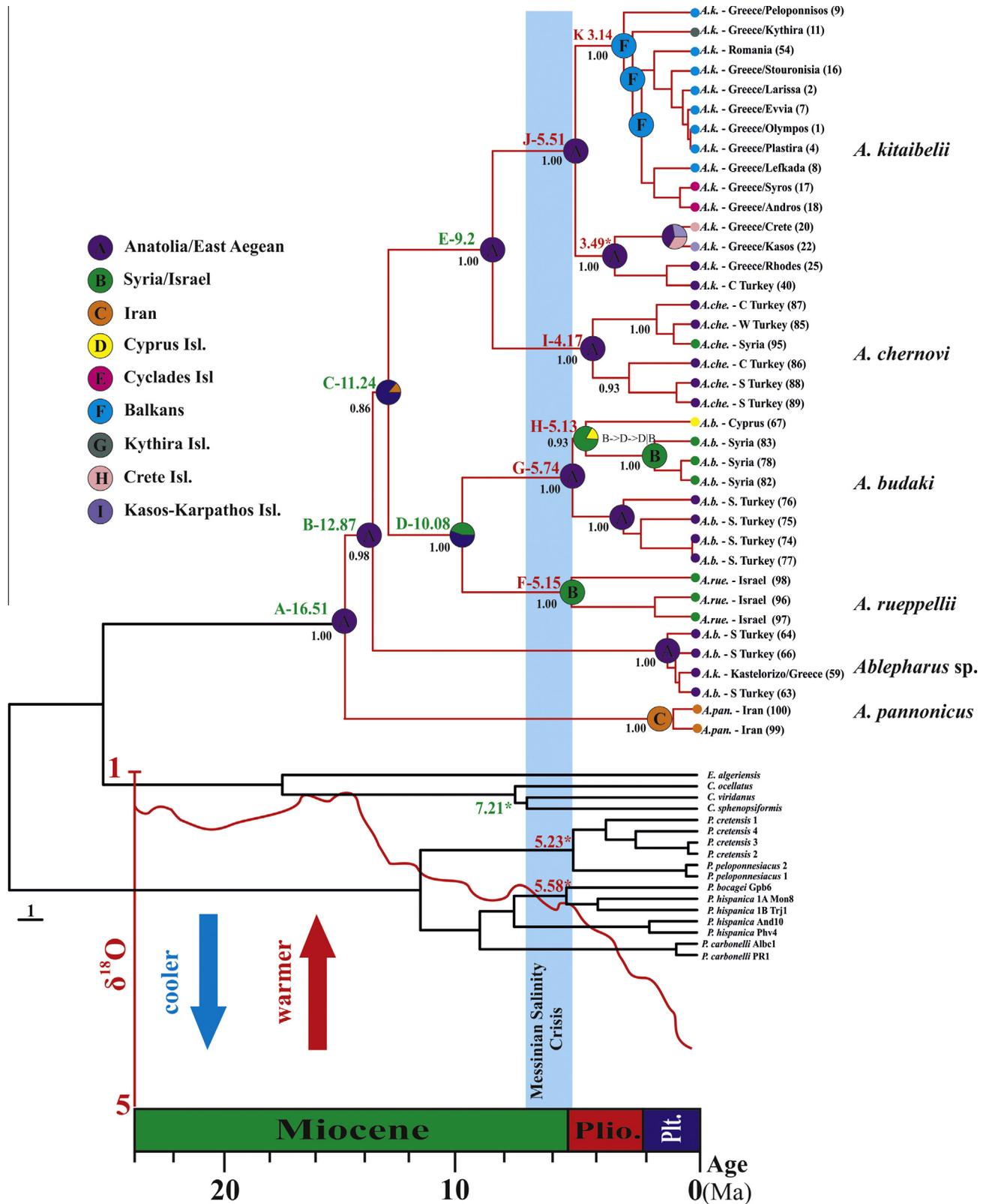
From another point of view, why the phenomenon of cryptic diversity is so abundant in this region (southwestern Anatolia)? Which are the environmental conditions that might impose stabilizing selection on morphology (morphological stasis), reducing or eliminating morphological change that can accompany speciation? Clearly, more research on hidden species and on this region is urgently needed.

#### 4.2. Phylogeography

The palaeogeographic history of the eastern Mediterranean repeatedly caused numerous vicariant and/or dispersal events on the ancestral stocks, producing various distribution patterns in this region based on the dispersal ability and speciation rate of the organisms (e.g., Poulakakis et al., 2015).

Fossil findings indicate that the genus *Ablepharus* has an Asiatic origin (southeastern Asia) and that its former distribution was much wider (Fuhrn, 1969a). Our hypothesis is that the ancestor of the *A. kitaibelii* species complex colonized the area of southwestern Asia (Anatolia and Asiatic adjacent regions) from the east (south or southeastern Asia) in Early Miocene. However, the absence of data from SE Asia doesn't permit to reach any robust conclusion. The inclusion of specimens of *Ablepharus* from south Asia (*A. grayanus*, *A. deserti*, *A. darvazi*, and *A. bivittatus*) will shed more light on the origin of the *A. kitaibelii* species complex in southwestern Asia.

The first splitting within the *A. kitaibelii* species complex (Figs. 3 and 4) occurred in latest Burdigalian (Early Miocene; 16.51 Mya)



**Fig. 3.** Divergence time estimation and ancestral area reconstruction of *A. kitaibelii* species complex using the mtDNA data. The chronogram is obtained from molecular clock analysis using BEAST. Pie chart in each node indicates the possible ancestral distributions inferred from Bayesian Binary MCMC analysis (BBM) implemented in RASP. Numbers above branches represent mean estimates of divergence time in my. Numbers below branches indicate posterior probabilities (BEAST Bayesian analysis). Asterisks on the branches indicate the calibration points. At the bottom of the figure, a graphical representation of temperature fluctuations during the past 25 My, as depicted from deep-sea oxygen isotope records (redraw after Zachos et al. (2001)).

with the separation of *A. pannonicus* from all other clades of the complex. At that time severe tectonic and climatic changes occurred (Popov et al., 2004; Rögl, 1998, 1999; Zachos et al.,

2001). Although there is no a direct evidence to explain this splitting, during Burdigalian the continental area that corresponds to the present-day southwestern Asia (Anatolia and Asiatic adjacent

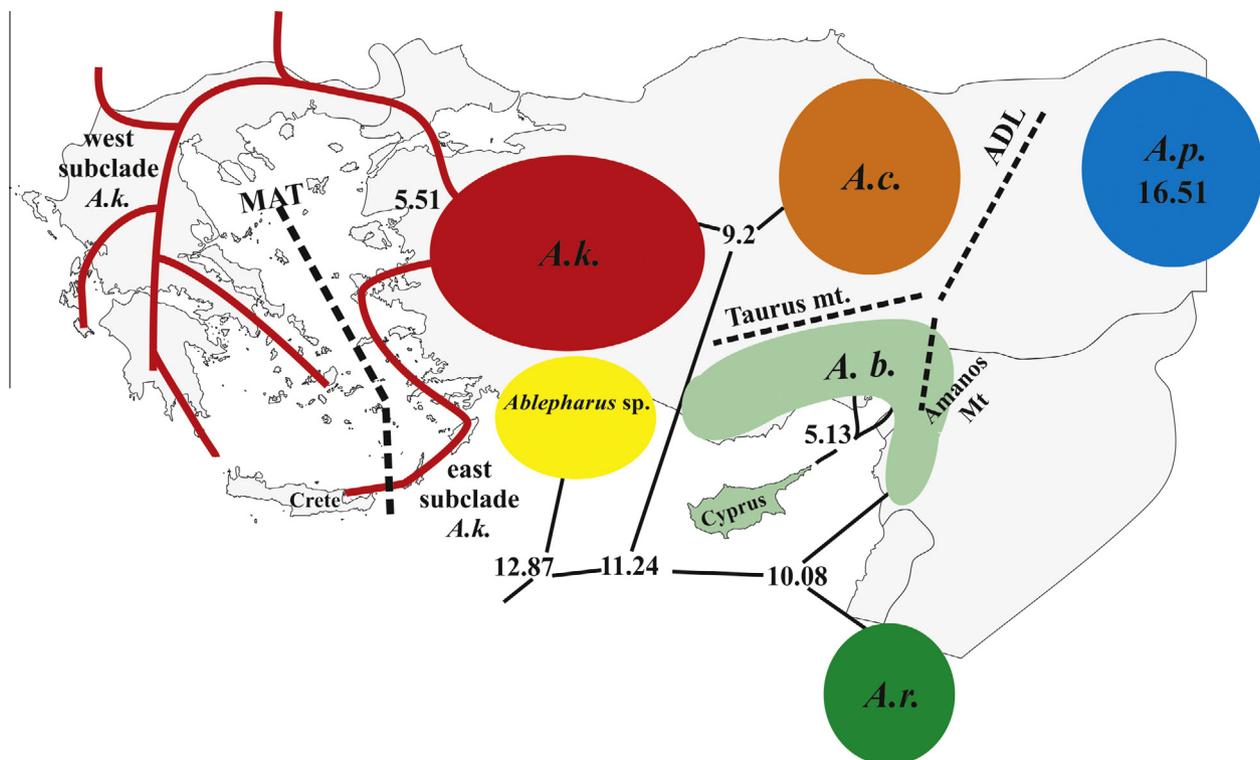
regions) connected to the east with south Asia (see Fig. 7 of Rögl, 1999) and the climate underwent a transition from the Mid-Miocene Climatic Optimum (~15–17 Mya; Figs. 3 and 4) to a drastic decrease of temperature (Zachos et al., 2001). In the same period, the Aegeid plate (Anatolia, Aegean archipelago, mainland Greece and part of the Balkans) was fully isolated from all other continental plates and became surrounded by the Tethys/Paratethys ocean (16.8–16 Mya) (Steininger and Rögl, 1984).

The next cladogenetic event is timed within a period known as the Middle Miocene disruption, which is characterized by severe effects on the distribution and regional diversity of ectothermic vertebrates (migration and extinction) due to a global cooling (Böhme, 2003). This split (12.87 Mya) was observed between the mixed clade of *Ablepharus* sp. and all the other clades of *A. kitaibelii* species complex. Shortly thereafter (11.24 Mya) the group (hereinafter group A) that includes *A. chernovi* and *A. kitaibelii* was separated from the group (hereinafter group B) that contains (*A. budaki*, and *A. rueppellii*). Subsequently (10.08–9.2 Mya), two more cladogenetic events occurred. The first was observed between *A. budaki* and *A. rueppellii* and the second between *A. chernovi* and *A. kitaibelii*. These splits are timed within a period which is characterized by pronounced aridification (Zachos et al., 2001) and the fragmentation of the Aegeid plate (Çiplak et al., 2010). We hypothesize that the above could have contributed to the isolation of several *Ablepharus* lineages. Due to its position, Anatolia has acted either as a bridge or as a barrier for species' dispersal between Asia and Europe, providing either a natural pathway or acting as a vicariant agent (Tchernov, 1992). The Anatolian mountain chains (i.e., the Anatolian Diagonal, the Taurus and the Black Sea Mountains) as well as the Central Anatolian Plateau and the Central Anatolian Lake system have acted as significant barriers (Eronen et al., 2009; Nilson et al., 1990; Popov et al., 2006; Rögl, 1999), affecting the distribution patterns of several taxa (Bilgin, 2011).

Returning to the second group (B), the allopatric distribution of *A. budaki* and *A. rueppellii* could be explained by the presence of the ADL; *A. budaki* west and south of ADL and north of Amanos Mt., and *A. rueppellii* south of the ADL and Amanos Mt. As we mentioned in the beginning, ADL has shaped the current composition of various species across Turkey by dividing species/lineage distribution into east and west (Bilgin, 2011; Çiplak et al., 1993; Mutun, 2010; Rokas et al., 2003; Sengor et al., 2003), from the beginning of its formation in Miocene-Pliocene (Bilgin, 2011).

Similarly, in the group A, the present day distributions of *A. kitaibelii* and *A. chernovi* are almost complementary. The first occurs in central and western Turkey and Balkans and the second in central and eastern Turkey. These areas are separated by the Taurus Mountains, the formation of which can be tracked back to the Tertiary (Bellati et al., 2015). However, the case of *A. chernovi* is uncertain, since this species is also found in the easternmost part of Turkey and Armenia (see Tok et al., 2009), east of the ADL. Unfortunately, we didn't have specimens from these regions. Thus, the biogeographic pattern of *A. chernovi* is not clear and the inclusion of specimens from eastern Turkey and Armenia will shed more light on its phylogeography.

*Ablepharus budaki* diverged from *A. rueppellii* during Upper Miocene (10.08 Mya) and diversified into the three subclades of Cyprus, Syria and Turkey in Late Miocene/Early Pliocene (5.74–5.13 Mya), which coincides with the Messinian Salinity Crisis (MSC; 5.96–5.33 Mya) (Krijgsman et al., 1999). The two continental subclades (Syria and Turkey) are geographically separated by the Amanos Mountain range of 2300 m high, which started to form in Pliocene (Seyrek et al., 2008). The third subclade is found on the island of Cyprus, an oceanic island that raised from the seabed (Hadjikyriakou and Hadjisterkotis, 2002). There is a debate among the scientists in regards to the connection of Cyprus with the neighboring mainland (Anatolia or Syria) (Hadjisterkotis et al.,



**Fig. 4.** Simplified illustration of the proposed biogeographic scenario for *Ablepharus*. Dashed lines indicate geographical (biogeographic) barriers (MAT: Mid-Aegean Trench, ADL: Anatolian Diagonal Line, Taurus Mt, Amanos Mt). The numbers represent the time of divergences in my. A.k.: *A. kitaibelii*, A.b.: *A. budaki*, A.c.: *A. chernovi*, A.r.: *A. rueppellii*, and A.p.: *A. pannonicus*.

2000 and references therein). One side supports that Cyprus was never connected to the neighboring mainland, while the second suggest that it was, and the alleged connection has probably persisted long enough to allow the penetration of biota (Zohary, 1973). The latter aspect could be supported by the paleogeographic representations of the eastern Mediterranean region that showed the connection of the island of Cyprus with the continental areas of Turkey and Syria (Bache et al., 2012; Jolivet et al., 2006). However, the high number of Cypriote endemics points to a long separation of the island from the mainland (Hadjisterkotis et al., 2000). It is unequivocally accepted that Cyprus never joined by a land bridge to the mainland shoreline in Pliocene and Pleistocene (Hadjisterkotis et al., 2000). The existence of a land bridge in the Late Miocene fits very well with the estimated time of divergence of the Cypriote *A. budaki*, which isolated to Cyprus at end of MSC. Based on this, it seems that the ancestral lineage of Cypriote *A. budaki* colonized the island of Cyprus through a land bridge that connected Cyprus with the nearby mainland (geodispersal), a scenario that has been already supported in the case of the Eurasian blind snake (*Typhlops vermicularis*) for which the isolation of Cyprus' lineage from the mainland were estimated at 5.2 Mya (Kornilios et al., 2012).

On the other hand, the biogeographic analyses suggest the origin of *A. kitaibelii* somewhere in Anatolia where it started to diversify at the end of the MSC (5.51 Mya) (Fig. 3), producing the two major subclades that we described above (see Systematics). Based on this we suppose that *A. kitaibelii* invaded the Balkans probably from Anatolia after the formation of MAT, via two distinct dispersal routes. The first followed the path of Asia Minor – northern Greece, and evolved in the Balkans, producing the present western subclade. The second route of invasion was from Anatolia to the East Aegean islands, and evolved in this area to produce the eastern subclade.

Within each subclade, several vicariant and dispersal events have produced the present day distribution of the evolutionary lineages of *A. kitaibelii*, e.g., the isolation of Antikythira and Kythira islands during the Pliocene, the isolation of Kasos/Karpathos islands from Rhodes during the Pliocene, the dispersion of *Ablepharus* in Nisyros and Stroglylo (volcanic islands never connected to continental areas) from the neighbor mainland and in the islet of Mikronisi (Crete) from Kasos/Karpathos, which is the reason that although Crete is west of the Mid-Aegean trench, phylogenetically it belongs to the east subclade.

Though *A. kitaibelii*, like most other terrestrial lizards, is a species with poor overseas dispersal abilities, its presence in Nisyros and the neighboring Stroglylo suggests a transmarine dispersal. Moreover, the existence of *A. kitaibelii* on Mikronisi consists of one of the most peculiar features of its present distribution. The island of Crete has never been connected to the east islands (Kasos, Karpathos) since the opening of MAT (12–9 Mya) (Dermitzakis, 1990; Dermitzakis and Papanikolaou, 1981). The phylogenetic analyses of two specimens from Mikronisi showed that they have a sister-group relationship with the lineages from the islands of Kasos and Karpathos (Fig. 2), which were still connected with the mainland in the east until the Middle or Late Pliocene (Böger and Dermitzakis, 1987; Daams and Van Der Weerd, 1980). The time of divergence of Mikronisi lineage from Kasos and Karpathos was very recent (Fig. 3). All the above indicate that the lineage of Mikronisi originated from the lineages of Kasos/Karpathos and that *A. kitaibelii* crossed the MAT by transmarine dispersal. It is worth noticing here that even though the most likely interpretation involves rafting, it is extremely bizarre how the rafts hit a single “tree” (Mikronisi) missing the “forest” (Crete) (Lymberakis and Poulakakis, 2010). However, this is not the only case of terrestrial animals that indeed crossed the MAT via dispersal. In a recent review on the phylogeography of animal taxa in the Aegean,

Poulakakis et al. (2015) claimed 7 similar cases and characterized all these animals as ‘naughty’, highlighting the question if these animals are actually ‘naughty’ or is the infringement of the ‘MAT law’ simply a matter of time?

## 5. Conclusions

In summary, building on the *A. kitaibelii* molecular dataset of Poulakakis et al. (2005a), we provide new information about the phylogenetic relationships of the species belong to *A. kitaibelii* species complex. Although several phylogenetic studies on reptiles in Anatolia and Middle East have indicated the presence of cryptic diversity (Ahmadzadeh et al., 2013; Bellati et al., 2015; Kapli et al., 2013, 2015, 2008; Kornilios et al., 2012; Kyriazi et al., 2008; Sagonas et al., 2014), the high level of mitochondrial divergence among the major clades is almost exceptional, ranging from 0 to 21.2% in *cyt b*. This raises the question about the taxonomic status of the clade from southwestern Turkey (*Ablepharus* sp.) and the island group of Kastelorizo. Phylogenetic information can now be added to the knowledge of their morphology and distribution, revisiting and raising this clade to species level.

From a phylogeographical point of view, the estimated diversification dates (from Early Miocene to Pleistocene) and the respective palaeogeographical events that roughly occurred during these time-periods, provide clues to resolve the phylogeographic history of the complex in this region. They indicate that several vicariance and dispersal events that are related with the formation of MAT, Anatolian Diagonal and the orogenesis of the mountain chains in southern and eastern Anatolia led to current distribution pattern of *A. kitaibelii* species complex in the Balkans and Middle East. Adding more samples of *Ablepharus* sp. from southwestern Turkey and Kastelorizo as well as populations of other species of the snake-eyed skinks from Asia (*A. grayanus*, *A. deserti*, *A. darvazi*, and *A. bivittatus*) would improve our understanding of the evolutionary history of the whole genus.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2016.07.005>.

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