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Quaternary range dynamics and taxonomy of the Mediterranean collared dwarf racer, *Platyceps collaris* (Squamata: Colubridae)

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The geological and geographical settings of the Eastern Mediterranean have resulted in complex patterns of intraspecific diversifications and phylogeographical histories that can be observed in squamates. In this study, we examined genetic differentiation of the Collared dwarf racer (*Platyceps collaris*) using a multilocus genetic dataset with a sampling that covered the entire range of the species. We developed distribution models in current and past climatic conditions to assess the dynamics of the species distribution through time. We sequenced a fragment of the cytochrome *b* mitochondrial gene of the holotype and eight paratypes of *Coluber rubriceps thracius*, which is considered a synonym of *Platyceps collaris*. Our results show that there are two distinct clades within *P. collaris*, one occupying the Balkans and western and southern Anatolia (termed the Balkan–Anatolian clade), the other in the Levant (termed the Levantine clade). All type specimens of *C. r. thracius* are genetically identical and cluster within the Balkan–Anatolian clade. Distribution models indicate the presence of two refugia during climatically challenging periods. One was in western Anatolia and served as a source for the colonization of the Balkans and southern Anatolia, and the other was in the northern Levant, from where *P. collaris* dispersed further south. According to our results, we revise the subspecific taxonomy of *P. collaris*.

ADDITIONAL KEYWORDS: biogeography – Colubroidea – Near East – reptiles – Serpentes – systematics.

INTRODUCTION

The Eastern Mediterranean is a region of complex geological and biological history. As a result of its position at the crossroads of Europe, Asia and Africa, the fauna of the Eastern Mediterranean shows a wide spectrum of biogeographical patterns and phylogeographical scenarios. Many recent studies have indicated that the diversity of squamates of the Eastern Mediterranean is underestimated and that

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traditionally recognized species described on the basis of morphological characters often encompass cryptic lineages (e.g. Bellati *et al.*, 2015; Jandzik *et al.*, 2018; Šmíd *et al.*, 2019). Although considerable interest has been attracted to the islands (Kyriazi *et al.*, 2013; Poulakakis *et al.*, 2013), differentiation and phylogeography of the fauna of coastal Mediterranean Turkey and the Levant are also being studied increasingly (Sindaco *et al.*, 2014; Kotsakiozi *et al.*, 2018; Kornilios *et al.*, 2019). However, little is known in this respect about some of the widespread and common taxa. The colubrid genus *Platyceps* Blyth, 1960 is one such example.

Racers of the genus *Platyceps* are medium-sized, slender and wary colubrid snakes. Their distribution spans from south-eastern Europe across the Middle East to central Asia and southwards across the Arabian Peninsula to north-eastern Africa (Sindaco *et al.*, 2013). South-eastern Europe, the Eastern Mediterranean and the Levant are occupied by two species, *Platyceps collaris* (Müller, 1878) and *Platyceps najadum* (Eichwald, 1831). Morphological data indicate that these two species are distinct from other congeners in having unpaired apical pits (Schätti *et al.*, 2014), and genetic evidence supports their sister relationship (Schätti & Utiger, 2001; Schätti, 2004).

The taxonomic history of *P. collaris* has been turbulent. It was originally described as a subspecies (originally a variety) of *Zamenis dahlii* (Fitzinger, 1826) (currently *Platyceps najadum*) in the late 19th century on the basis of two specimens, an adult male from Beirut, Lebanon [Naturhistorisches Museum, Basel, Switzerland (NHMB) 1166], and a juvenile from the vicinity of Tel Aviv, Israel (NHMB 1167; Müller, 1878; Schätti *et al.*, 2001). In the beginning of the 20th century, another subspecies (originally a variety), *Zamenis dahlii rubriceps* Venzmer, 1919, was described from the Taurus Mountains in southern Turkey [Venzmer, 1919; between Pozanti and Tarsus (37°12′N, 34°48′E) according to Schätti *et al.*, 2001]. The colubrid genera were later revised by Inger & Clark (1943), and the genus *Platyceps* was proposed for several Eurasian racers, including *Coluber najadum* (Eichwald, 1831). However, most subsequent authors did not follow this adjustment and applied the genus name *Coluber* Linnaeus, 1758 to most Eurasian racers (e.g. Mertens & Wermuth, 1960; Schätti, 1993). The trinomen *Coluber najadum rubriceps* was used until Baran (1976) elevated *Coluber rubriceps* to a full species. Rehák (1985) described specimens from the Black Sea coast of Bulgaria as a distinct subspecies, *Coluber rubriceps thracius* Rehák, 1985, which was characterized by its low number of ventral and subcaudal scales and a relatively short tail. The type series of 11 specimens was originally placed in the collection of the Department of Systematic Zoology (currently Department of Zoology), Charles University, Prague, Czech Republic (DZCHU). Owing to organizational changes at the department, the specimens were transferred to the herpetological collections of the National Museum in Prague (NMP) in 2014, where they are deposited under museum catalogue numbers NMP-P6V 75257 (holotype), NMP-P6V 75258, 75259/1–5 and 75260–62 (paratypes; Table 1). One paratype was sent to Bonn [Zoologisches Forschungsmuseum Alexander Koenig (ZFMK)], where it is catalogued under the number ZFMK 46091 (Böhme, 2014).

In a detailed taxonomic treatment, Schätti *et al.* (2001) revised the taxonomy of *Coluber collaris* (Müller, 1878) and *C. rubriceps*. They recognized these two taxa to be conspecific, which resulted in the relegation of the name *C. rubriceps* to a junior synonym of *C. collaris*. The authors also designated the adult male specimen from Beirut as a lectotype of the species. With respect to the subspecies *C. r. thracius*, Schätti *et al.* (2001) pointed out that Rehák (1985) based his comparisons on published data instead of examining the specimens himself. Also, the variance of the diagnostic characters of *C. r. thracius* falls within the range of Asian populations according to Schätti *et al.* (2001). Based on these grounds, they synonymized the subspecies *C. r. thracius* with the nominotypical one, which resulted in the species becoming monotypic. This has been followed by most recent authors (Sindaco *et al.*, 2013; Wallach *et al.*, 2014; Geniez, 2018), although some still use the subspecific designation (Stojanov *et al.*, 2011).

The last nomenclatural adjustment of the species had stemmed from the phylogenetic results of Schätti & Utiger (2001), who confirmed earlier findings of Inger & Clark (1943) and placed the Eurasian racers, including *C. collaris* and *C. najadum*, in the genus *Platyceps*.

The distribution of *P. collaris*, as currently recognized, spans from south-eastern Bulgaria along the entire Mediterranean coast of Turkey through the Mediterranean part of Syria and Lebanon to Israel and western Jordan (Sindaco *et al.*, 2013; Geniez, 2018). Isolated populations have been recorded from central and southern Anatolia (Baran, 1982; İğci *et al.*, 2015), but the identification of these specimens needs to be verified because the localities are geographically disparate from the rest of the range and lie well within the range of the closely related and morphologically similar *P. najadum*.

In this paper, we explore the genetic diversity and phylogeography of *P. collaris* across its entire range using a multilocus dataset of three mitochondrial and three nuclear markers. Using a new approach of multistep polymerase chain reaction (PCR) and Illumina amplicon sequencing, we sequence a fragment of the cytochrome *b* (*cytb*) mitochondrial gene of the holotype

and eight paratypes of the nominal taxon *Coluber rubriceps thracius*. We conduct a species distribution modelling analysis for the present climatic conditions and conditions during the last 3 Myr to assess the limits of the current potential distribution for the species, its historical range dynamics and location of potential refugia.

MATERIAL AND METHODS

Sampling, DNA extraction and amplification of recent material

For the phylogenetic analysis, we assembled a dataset of 35 samples of *P. collaris* from 26 localities in Bulgaria, Israel, Syria and Turkey, covering the entire range of the species (Table 1). We supplemented this dataset with GenBank sequences of three individuals, two from Israel and one from Jordan (Schätti & Utiger, 2001; Nagy *et al.*, 2004).

Genomic DNA was extracted from 96% ethanol-preserved tissue samples using an Invisorb Spin Tissue Kit (STRATEC), following the manufacturer's instructions. We PCR-amplified six genetic markers: three from the mitochondrial DNA [12S ribosomal RNA (12S), cytochrome *b* (*cytb*) and cytochrome *c* oxidase I (*coi*)] and three from the nuclear DNA [oocyte maturation factor MOS (*cmos*), neurotrophin-3 (*nt3*) and recombination activating gene 1 (*rag1*)]. We used the following primer pairs and annealing temperatures for the amplifications: for 12S, the primers were 12S268 and 12S916 (Schätti & Utiger, 2001), and the annealing temperature was 65 °C; for *cytb*, L14910 and H16064 (Burbrink *et al.*, 2000) and 46 °C; for *coi*, COIb and COIbdeg (Schätti & Utiger, 2001; Utiger *et al.*, 2002) and 56 °C; for *cmos*, S77 and S78 (Lawson *et al.*, 2005) and 53 °C; for *nt3*, F3 and R4 (Noonan & Chippindale, 2006) and 50 °C; and for *rag1*, R13 and R18 (Groth & Barrowclough, 1999) and 58 °C. The PCRs contained 0.2 µM of each primer, 7.5 µL of 2× QIAGEN Multiplex PCR Kit, 1.5 µL of genomic DNA (concentration ~30 ng/µL) and double-distilled H_2O to a total volume of 15 µL. The PCR products were purified with calf intestine alkaline phosphatase and exonuclease I (New England Biolabs, Ipswich, MA, USA) and Sanger sequenced along both strands using the PCR primers in Macrogen Europe (The Netherlands). Sequences were checked and contigs assembled in GENEIOUS v.11 (Kearse *et al.*, 2012).

Sampling, DNA extraction and amplification of the type specimens of *Coluber rubriceps thracius*

We sampled ten individuals from the type series of *Coluber rubriceps thracius*, the holotype and nine paratypes (Table 1). The museum samples were handled in a specialized non-invasive laboratory designed for work with rare DNA to prevent contamination (Institute of Vertebrate Biology of the Academy of Sciences, Studenec, Czech Republic). DNA was extracted using Invisorb Spin Forensic Kit (STRATEC). Concentration of samples was measured on a Qubit 1.0 fluorometer (Thermo Fisher Scientific) using the Qubit dsDNA HS Assay kit. To obtain DNA sequences of the type specimens, we modified the rodent-based DNA mini-barcoding protocol for the Illumina platform that targets a short *cytb* fragment (Galan *et al.*, 2012; Bryja *et al.*, 2014). The mini-barcode is a fragment of 148 bp, a length short enough to amplify in samples with fragmented or degraded DNA (Galan *et al.* 2012). For the library preparation, we used a three-step PCR. All PCRs contained 0.4 µM of each primer, 5 µL (7.5 µL for the third PCR) of 2× QIAGEN Multiplex PCR Kit, 1.0–1.5 µL of DNA (concentration 0.01–3.00 ng/µL) and double-distilled H_2O to a total volume of 10 µL (for the first and second PCR) and 15 µL (for the third PCR). The PCRs were initiated by a denaturation step of 94 °C for 15 min, followed by 15–25 cycles of denaturation at 94 °C for 30 s, annealing at 45 °C for 45 s and an extension at 72 °C for 30 s, followed by a final extension step at 72 °C for 10 min. All PCRs were prepared in duplicates. In the first PCR of 25 cycles, we amplified the target region with short specific primers, L15411_modif (GAYAAAATYYCHTTYCACCC) and H15553_modif (GTAGGCRAAYAGGAARTATCA). The product of the first PCR was then used as a template for the second PCR of 20 cycles, for which the primers were elongated at the 5′ end: L15411_modif_F_nextera (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAYAAAATYYCHTTYCACCC) and H15553_modif_R_nextera (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTAGGCRAAYAGGAARTATCA). The template for the third PCR of 15 cycles was the product of the second PCR. Primers for this reaction contained Illumina adaptors with a unique combination of barcodes/tags for each sample. After the three-step amplification, we visualized the products on a 1.5% agarose gel and measured the gel band intensity with GenoSoft software (VWR International, Belgium). The concentration of all samples was measured with a fluorometer (Qubit 1.0) and purity with a spectrophotometer (DS-11; DeNovix Inc.). All samples were then diluted to be of an equimolar concentration (~100 ng/µL). This library was purified using Agencourt SPRIselect (Beckman Coulter) and sequenced using MiSeq Illumina at CEITEC (Masaryk University, Brno, Czech Republic). Sequences were demultiplexed based on the unique tags. Primers were cut off and low-quality reads filtered out as follows: sequences

Table 1. Material used for the genetic analysis

				mtDNA			nDNA					
Taxon	Voucher	Sample	Specimen status	12S	*cytb*	*coi*	*cmos*	*nt3*	*rag1*	Locality	Latitude	Longitude
Platyceps collaris	–	DJ7739		MT856882	MT862657	MT862569	MT862592	MT862617	MT862640	Bulgaria, Sveti Vlas (1)	42.725	27.766
P. collaris	–	DJ1405		MT856879	MT862654	MT862566	MT862589	MT862614	MT862638	Bulgaria, Sozopol (2)	42.397	27.703
P. collaris	–	DJ6132		MT856881	MT862656	MT862568	MT862591	MT862616	–	Bulgaria, Sozopol (3)	42.395	27.689
P. collaris	–	DJ607		MT856883	MT862658	MT862570	MT862593	MT862618	MT862641	Bulgaria, Ropotamo (5)	42.305	27.728
P. collaris	–	DJ1417		MT856880	MT862655	MT862567	MT862590	MT862615	MT862639	Bulgaria, Sinemorec (7)	42.071	27.963
P. collaris	–	DJ2478		MT856884	MT862659	MT862571	MT862594	MT862619	MT862642	Israel, Hermon (22)	33.296	35.762
P. collaris	MCCI R-0649	R649		MT856885	–	MT862572	MT862595	MT862620	–	Israel, Haifa (23)	32.806	35.007
P. collaris	MHNG 2447.74	Schatti1		AY039133	–	AY039171	–	–	–	Israel, Tel Aviv (25)	32.327	34.855
P. collaris	MHNG 2447.75	Schatti2		AY039157	–	AY039195	–	–	–	Israel, Tel Aviv (25)	32.327	34.855
P. collaris	TAU.R15955	TAU. R15955		MT856886	–	–	–	–	–	Israel, Yizre'el Valley (24)	32.72	35.14
P. collaris	TAU.R16921	TAU. R16921		MT856887	MT862660	–	MT862596	MT862621	–	Israel, Nezer Sereni (26)	31.922	34.822
P. collaris	TAU.R16927	TAU. R16927		MT856888	MT862661	–	MT862597	MT862622	–	Israel, Shomeron (28)	32.517	35.382
P. collaris	HLMD J14	Nagy		–	AY486922	–	AY486946	–	–	Jordan (27)		
P. collaris	NMP-P6V 70502	JIR187		MT856889	MT862662	MT862573	MT862598	MT862623	–	Syria, 2 km N of Seydnaya (20)	33.717	36.372
P. collaris	NMP-P6V 70503	JIR188		MT856890	MT862663	MT862574	MT862599	MT862624	–	Syria, E of Utaibeh (21)	33.506	36.627
P. collaris	–	DJ8209		MT856903	MT862685	MT862587	MT862612	MT862636	–	Turkey, Bozdağ-Ödemiş (8)	38.367	28.103
P. collaris	–	DJ8198		MT856892	MT862674	MT862576	MT862601	MT862626	MT862644	Turkey, 10 km N of Saimbeyli (15)	38.061	36.149
P. collaris	–	DJ8202		MT856896	MT862678	MT862580	MT862605	MT862630	MT862648	Turkey, Temürağa (16)	38.046	36.586

Table 1. Continued

Taxon	Voucher	Sample	Specimen status	mtDNA			nDNA			Locality	Latitude	Longitude
				12S	*cytb*	*coi*	*cmos*	*nt3*	*rag1*			
P. collaris	–	DJ8203		MT856897	MT862679	MT862581	MT862606	MT862631	MT862649	Turkey, Temürağa (16)	38.046	36.586
P. collaris	–	DJ8197		MT856891	MT862673	MT862575	MT862600	MT862625	MT862643	Turkey, Gürümze (14)	38.042	35.811
P. collaris	–	DJ8206		MT856900	MT862682	MT862584	MT862609	MT862634	MT862652	Turkey, Suna Plateau (13)	37.993	35.397
P. collaris	–	DJ8207		MT856901	MT862683	MT862585	MT862610			Turkey, Işıklı (9)	37.832	27.799
P. collaris	–	DJ8200		MT856894	MT862676	MT862578	MT862603	MT862628	MT862646	Turkey, Ömerli (12)	37.535	34.875
P. collaris	–	DJ8205		MT856899	MT862681	MT862583	MT862608	MT862633	MT862651	Turkey, Ceyhan (18)	36.895	35.916
P. collaris	–	DJ8199		MT856893	MT862675	MT862577	MT862602	MT862627	MT862645	Turkey, Küplüce (19)	36.757	37.236
P. collaris	–	DJ8201		MT856895	MT862677	MT862579	MT862604	MT862629	MT862647	Turkey, Kaldırım (17)	36.674	35.525
P. collaris	–	DJ8208		MT856902	MT862684	MT862586	MT862611	MT862635	MT862653	Turkey, Erdemli (11)	36.639	34.340
P. collaris	–	DJ8204		MT856898	MT862680	MT862582	MT862607	MT862632	MT862650	Turkey, Bozyazı-Anamur (10)	36.097	33.042
P. collaris	NMP-P6V 75259/1 (DZCHU 435)	CRT01	Paratype of *Coluber rubriceps thracius*	–	MT862664	–	–	–	–	Bulgaria, Arkutino (4)	42.323	27.738
P. collaris	NMP-P6V 75259/2 (DZCHU 436)	CRT02	Paratype of *C. r. thracius*	–	MT862665	–	–	–	–	Bulgaria, Arkutino (4)	42.323	27.738
P. collaris	NMP-P6V 75257 (DZCHU 433)	CRT04	Holotype of *C. r. thracius*	–	MT862666	–	–	–	–	Bulgaria, Arkutino (4)	42.323	27.738
P. collaris	NMP-P6V 75259/5 (DZCHU 437)	CRT05	Paratype of *C. r. thracius*	–	–	–	–	–	–	Bulgaria, Arkutino (4)	42.323	27.738
P. collaris	NMP-P6V 75259/4 (DZCHU 438)	CRT06	Paratype of *C. r. thracius*	–	MT862667	–	–	–	–	Bulgaria, Arkutino (4)	42.323	27.738

Table 1. Continued

				mtDNA			nDNA					
Taxon	Voucher	Sample	Specimen status	12S	*cytb*	*coi*	*cmos*	*nt3*	*rag1*	Locality	Latitude	Longitude
P. collaris	NMP-P6V 75258 (DZCHU 441)	CRT12	Paratype of *C. r. thracius*	–	MT862669	–	–	–	–	Bulgaria, Arkutino (4)	42.323	27.738
P. collaris	NMP-P6V 75259/3 (DZCHU 439)	CRT13	Paratype of *C. r. thracius*	–	MT862670	–	–	–	–	Bulgaria, Arkutino (4)	42.323	27.738
P. collaris	NMP-P6V 75262 (DZCHU 440)	CRT14	Paratype of *C. r. thracius*	–	MT862671	–	–	–	–	Bulgaria, Arkutino (4)	42.323	27.738
P. collaris	NMP-P6V 75260 (DZCHU 442)	CRT15	Paratype of *C. r. thracius*	–	MT862672	–	–	–	–	Bulgaria, Arkutino (4)	42.323	27.738
P. collaris	NMP-P6V 75261 (DZCHU 443)	CRT11	Paratype of *C. r. thracius*	–	MT862668	–	–	–	–	Bulgaria, Ahtopol (6)	42.098	27.917
Platyceps najadum	MCCI R-1399	R1399		MT856904	MT862686	MT862588	MT862613	MT862637	–	Armenia, Khosrov Reserve	39.968	44.951
Telescopus dhara	–	CN10774		MK372075	MK373064	–	MK373173	MK373244	MK373213	Oman, Wadi Ayoun	17.253	53.894
Telescopus fallax	ZMHRU 2012_103	2012_103		MK372088	MK373073	–	MK373186	MK373253	MK373222	Turkey, Şanlıurfa	37.212	37.969
Telescopus obtusus	TMHC841	TMHC841		MK372104	MK373090	–	MK373202	MK373269	MK373237	Somalia, 9 km SE of Boorama	9.86	43.244
Telescopus pulcher	NMP-P6V 75609	TMHC843		MK372105	MK373092	–	MK373204	MK373271	MK373239	Somalia, 15 km SE of Sheikh	9.825	45.29

The sample column refers to codes shown in the Supporting Information (Figs S1–S4). Numbers in parentheses in the locality column show locality numbers as given in Figures 1 and 2. Collection acronyms are as follows: DZCHU, Department of Zoology, Charles University, Prague, Czech Republic; HLMD, Hessisches Landesmuseum, Darmstadt, Germany; MCCI, Museo Civico di Storia Naturale di Carmagnola, Torino, Italy; MHNG, Muséum d'histoire naturelle, Genève, Switzerland; NMP-P6V, National Museum in Prague, Czech Republic; TAU.R, Steinhardt Museum of Natural History, Tel Aviv, Israel; TMHC, Tomas Mazuch herpetological collection (private), Dřítež, Czech Republic; ZMHRU, Zoology Museum of Harran University, Sanliurfa, Turkey. GenBank accessions of sequences newly produced for this study are those starting with 'MT'.

containing more than two expected sequencing errors were eliminated; then we used the dada2 denoising algorithm (https://benjjneb.github.io/dada2/) for correction of sequencing errors in the quality-filtered dataset. Only variants with a minimum coverage of four sequences per variant and those that occurred in both duplicates were used for a BLAST analysis.

Phylogenetic analyses

The six genetic markers were aligned independently with MAFFT v.7 (Katoh *et al.*, 2019) using the default settings. No stop codons were found in the alignments of the protein-coding genes (*cytb*, *coi*, *cmos*, *nt3* and *rag1*), indicating that no pseudogenes were amplified. The final alignments had the following lengths: 12S, 633 bp; *cytb*, 1072 bp; *coi*, 594 bp; *cmos*, 567 bp; *nt3*, 486 bp; and *rag1*, 1009 bp. We concatenated all markers in an alignment of a total length of 4361 bp. *Platyceps najadum* and four species of *Telescopus* Wagler, 1830 were used as outgroup taxa based on published evidence (Table 1; Zheng & Wiens, 2016).

Phylogenetic analyses were conducted by two means: maximum likelihood (ML) and Bayesian inference (BI). For the ML analysis, we used RAxML v.7.3 (Stamatakis, 2006). The concatenated alignment was partitioned by gene, and the GTRGAMMA model was used for all partitions. A heuristic search included 100 random addition replicates and 1000 thorough bootstrap pseudo-replicates. Identical haplotypes were retained in the analysis to assess intraspecific variability. The Bayesian analysis ran in BEAST v.2.5.2 (Bouckaert *et al.*, 2014). The dataset was partitioned by gene, and heterozygous positions in the nuclear markers were coded using International Union of Pure and Applied Chemistry (IUPAC) ambiguity codes. The best evolutionary substitution models were estimated using the reversible-jump algorithm (RB; Bouckaert *et al.*, 2013) with four exponentially distributed rate categories (+Γ; mean = 1) and the shape parameter estimated. The RB algorithm estimates substitution models alongside the phylogeny without the need for a priori selection. The parameter for the proportion of invariable sites (+I) was not estimated given its correlation with the +Γ parameter, resulting in inadequate models when both +Γ and +I are included (Sullivan *et al.*, 1999; Mayrose *et al.*, 2005). Given that the dataset focused on intraspecific diversification, we did not assume the substitution rate to vary substantially throughout the tree and, as a result, we used a strict clock model with lognormally distributed priors (mean = 1, SD = 1.25) for all partitions and a coalescent constant population tree prior. The analysis ran three times for 5×10^7 Markov chain Monte Carlo generations sampled every 25 000 generations, producing a set of 2000 posterior trees. We discarded 10% of trees as burn-in after determining that the effective sample size (ESS) values of all parameters exceeded the recommended value of 200 using TRACER v.1.6 (Rambaut *et al.*, 2014), and ensuring that convergence and stationarity had been reached by assuring that parameter values in the independent runs plateaued at similar values. Tree files from the three runs were combined using LogCombiner, and a maximum clade credibility tree was identified using TreeAnnotator (both programs are from the BEAST package). Nodes were considered supported when they received a Bayesian posterior probability (pp) ≥ 0.95 and ML bootstrap ≥ 70. All phylogenetic analyses ran through the CIPRES Science Gateway (Miller *et al.*, 2010). To inspect the influence of the fast-evolving mitochondrial markers on the tree topology visually, we ran the same analyses as described above (both ML and BI) with a concatenated dataset of the three mitochondrial genes. Together with the haplotype networks (see two paragraphs below), this was meant to assess the extent to which the variation in the nuclear markers contributes to the topology of the tree.

We also constructed a phylogenetic network among the *P. collaris* samples using SplitsTree v.4.15.1 (Huson & Bryant, 2006), with the Neighbor-Net algorithm (Bryant & Moulton, 2004). Phylogenetic networks are useful in that they can help to visualize reticulate relationships among samples and detect hybrid individuals. We used the alignment of all six markers concatenated as an input. The nuclear loci were phased as described below, and the mitochondrial markers were duplicated to match the phased nuclear samples. Outgroups were not included in this analysis, nor were the short *cytb* sequences of the *C. r. thracius* type specimens. We used 1000 bootstrap replicates to assess support for the network groups. We tested individual nuclear loci for recombination using the φ statistic implemented in SplitsTree (Bruen *et al.*, 2006).

Intraspecific relationships at the level of individual genes were assessed by reconstructing haplotype networks. Heterozygous positions present in the alignments of the three nuclear loci were resolved by phasing. We used SeqPHASE (Flot, 2010) to convert input files and PHASE (Stephens *et al.*, 2001) to reconstruct the gametic phases, with the probability threshold set to 0.7 (Harrigan *et al.*, 2008). Networks were constructed with the TCS algorithm (Clement *et al.*, 2000) as implemented in POPART (Leigh & Bryant, 2015). For the *cytb* gene network, we used the 148-bp-long alignment that included sequences of the *C. r. thracius* types.

Uncorrected genetic distances (*p*-distances) for the mitochondrial genes were calculated in MEGA7 (Kumar *et al.*, 2016).

Modelling the potential species distribution in the present and in the past

We compiled a dataset of distribution records by searching published sources, museum catalogues, public biodiversity databases and from our own fieldwork. The final set totalled 539 georeferenced records that covered the known range of the species densely. However, the records varied considerably in their geographical accuracy (0.005–55 km), and the less accurate ones might affect model performance. We therefore selected only records with an accuracy of ≤ 2 km. For this reason, the outlying localities in central Anatolia and by the Syrian border could not be included. Also, we removed duplicates and included only records collected after 1980 (Supporting Information, Table S1).

As a background for modelling in present conditions, we selected an area within 200 km of the species range, as presented by Roll *et al.* (2017), from which we cropped countries where the species has never been recorded: Cyprus, Egypt, Iran, Romania, Saudi Arabia and the Greek Aegean Sea islands. To avoid model bias caused by high densities of records in certain regions, we thinned the dataset using the *spThin* R package (Aiello-Lammens *et al.*, 2015). We tested three minimum distance radii to separate any two records: 5, 20 and 50 km. They all produced comparable models, and we show results only for the 5 km model. The thinning ran ten times, and each run produced a different dataset of 90 records. These were used as independent random replicates. We did not develop independent models for the two clades found within *P. collaris* because their genetic differentiation is shallow, especially when compared with the genetic differentiation between *P. collaris* and its sister species, *P. najadum* (see Results below); thus, we assumed their ecological niches to be similar.

As input variables, we used the 19 BioClim variables (CHELSA; Karger *et al.*, 2017), elevation slope and land cover (GlobCover 2009 2.3; European Space Agency; http://due.esrin.esa.int/page_globcover.php) and tested them for collinearity using Pearson's *r* coefficient in ENMTools (Warren *et al.*, 2010). Based on the correlation matrix, we selected only the uncorrelated ($r < 0.75$) and more biologically meaningful variables. The final set of variables was as follows: elevation, slope, land cover, Bio2 (mean diurnal temperature range), Bio4 (temperature seasonality), Bio8 (mean temperature of wettest quarter), Bio9 (mean temperature of driest quarter), Bio16 (precipitation of wettest quarter) and Bio17 (precipitation of driest quarter).

We used the maximum entropy approach implemented in MaxEnt v.3.3 (Phillips *et al.*, 2006) to develop the model and to assess the importance of each input variable. MaxEnt ran with the following settings (other than default): random seed, replicated run type = cross validate and maximum iterations = 5000. We ran ten replicates for each of the ten thinned datasets, thus producing 100 models. In each run, 10% of records were randomly selected as test points.

The area under the receiver-operating characteristic curve (AUC) value of each model was taken as a measure of the model accuracy. Models with AUC > 0.8 were considered to perform well (Araújo *et al.*, 2005). To test whether the predictive models performed better than random, we generated 100 null models based on 100 sets of 90 records randomly distributed in the background of the model. The probabilistic models were converted to binary maps of potential presence/absence using two thresholds, a ten-percentile training presence threshold and maximum training sensitivity plus specificity threshold (Jiménez-Valverde & Lobo, 2007; Worth *et al.*, 2014). The resulting areas of the species potential presence were largely overlapping for the two thresholds, and we show results for the latter. All spatial analyses for the current conditions were done at the scale of 30 arc s.

Besides modelling the potential distribution of *P. collaris* in current climatic and topographical conditions, we projected the model to four past periods, which were: the Last Glacial Maximum (LGM; ~21 kya), the Last Interglacial (LIG; ~130 kya), the mid-Pleistocene (~787 kya) and the mid-Pliocene (~3.2 Mya). We only used the BioClim variables for the past projections, and Bio2 was excluded because it was not available for all the past periods. Past climatic data were downloaded collectively from the PaleoClim database (Brown *et al.*, 2018), with the following original sources: Karger *et al.* (2017) for the LGM; Otto-Bliesner *et al.* (2006) for the LIG; Brown *et al.* (2018) for the mid-Pleistocene; and Hill (2015) for the mid-Pliocene. The background onto which the models were projected had to be expanded to account for the different continental outlines in the past. For this reason, we converted the background that was used for the current conditions into an envelope, i.e. a rectangle defined by minima and maxima of the *x*- and *y*-coordinates of the input. The past projections were all done at a resolution of 2.5 arc min.

RESULTS

DNA amplification

For the fresh material of *P. collaris* (25 samples) we produced 133 new DNA sequences of the six markers

analysed, resulting in a genetic data matrix with few missing data (89% gene sampling).

For the type material of *C. r. thracius*, we were able to obtain the targeted 148-bp-long *cytb* fragment for nine of the ten specimens. DNA concentrations of these specimens ranged between 0.01 and 3.42 ng/μL, and average number of reads generated for them was 5735 (range 2113–10 302; Table 2). Artefactual variants and contaminations were identified based on a BLAST analysis and discarded manually. One specimen was removed from further analysis (CRT05) because the reads showed a mix of various contaminations (predominantly *Homo*). There were several synonymous substitutions along the 148 bp fragment, indicating that the amino acid content of this gene fragment was similar across all samples.

Phylogenetic analyses

The samples of *P. collaris* form two well-differentiated clades in all analyses. One comprises all samples from Bulgaria (including the types of *C. r. thracius*), western Turkey and most samples from southern Turkey (ML bootstrap = 98/BI pp = 1.00; support values are given in this order hereafter), and we term it here the Balkan–Anatolian clade. The other clade comprises samples from Israel, Jordan and Syria, and we term it the Levantine clade, although this clade has only moderate support in the ML analysis (Fig. 1; support 65/1.00; for original ML and BI trees, see Supporting Information, Figs S1, S2, respectively). Relationships within both clades remain unresolved owing to low branch support. The position of sample DJ8199, from a locality in extreme southern Turkey (locality 19 in Fig. 1), differs in the two analyses. It was reconstructed and supported as sister to the Bulgarian and other Turkish samples in the ML analysis (support 74), but the BI analysis recovers it as sister to the Levantine clade (support 0.94). Both support values are on the edge of interpretability, but the haplotype networks constructed for each marker independently show the sample to be closer to the Levantine clade in its mitochondrial DNA, whereas the nuclear markers are more similar to the Balkan–Anatolian clade (networks in Fig. 1). When only mitochondrial markers are analysed, topologies of the ML and BI trees remain similar to those of the complete dataset, including the varying position of sample DJ8199 (Supporting Information, Figs S3, S4). The haplotype networks clearly differentiate the two clades described above, but the nuclear ones show a certain degree of allele sharing.

The phylogenetic network analysis resulted in similar groups to those recovered in the ML and BI analyses (Fig. 2). The reciprocal monophyly of the Balkan–Anatolian and Levantine clade is strongly supported (bootstrap 99.9). Sample DJ8199, whose phylogenetic position varied in the ML and BI analysis, is clustered at the base of the Levantine clade. No signs of recombination are detected for the three nuclear loci (*P*-values ranging between 0.68 and 1.00).

Genetic *p*-distances between the Balkan–Anatolian and Levantine clades are (±SD): 1.3 ± 0.2% in 12S, 2.2 ± 0.2% in *cytb* and 1.6 ± 0.2% in *coi*. Genetic diversity within the Balkan–Anatolian clade is 0.2% in 12S, 0.6% in *cytb* and 0.3% in *coi*; within the Levantine clade it is 0.3% in 12S, 0.7% in *cytb* and 0.4% in *coi*. Genetic distances of *P. collaris* to its sister species *P. najadum* are 10.6 ± 0.3% in 12S, 11.2 ± 0.3% in *cytb* 10.8 ± 0.2% in *coi*.

Table 2. Type specimens of *Coluber rubriceps thracius* included in the genetic analysis

Voucher	Sample	DNA concentration (ng/μL)	BLAST results	Number of reads after filtering	
				First coverage	Second coverage
NMP-P6V 75259/1	CRT01	0.038	*Platyceps collaris*	2113	3988
NMP-P6V 75259/2	CRT02	1.08	*P. collaris*	3022	6351
NMP-P6V 75257	CRT04	2.61	*P. collaris*	6405	6817
NMP-P6V 75259/5	CRT05	0.038	Contamination	4208	6372
NMP-P6V 75259/4	CRT06	0.128	*P. collaris*	7015	8626
NMP-P6V 75261	CRT11	< 0.01	*P. collaris*	6272	5512
NMP-P6V 75258	CRT12	3.42	*P. collaris*	4546	6947
NMP-P6V 75259/3	CRT13	< 0.01	*P. collaris*	3349	5910
NMP-P6V 75262	CRT14	2.07	*P. collaris*	3018	5019
NMP-P6V 75260	CRT15	< 0.01	*P. collaris*	8910	10 302

The DNA concentration was measured using a Qubit 1.0 fluorometer (Qubit High Sensitivity Kit). Results of the BLAST search showed that one of the specimens was contaminated by human DNA. The columns headed 'Number of reads after filtering' show the total number of sequences obtained from the two independent duplicates.

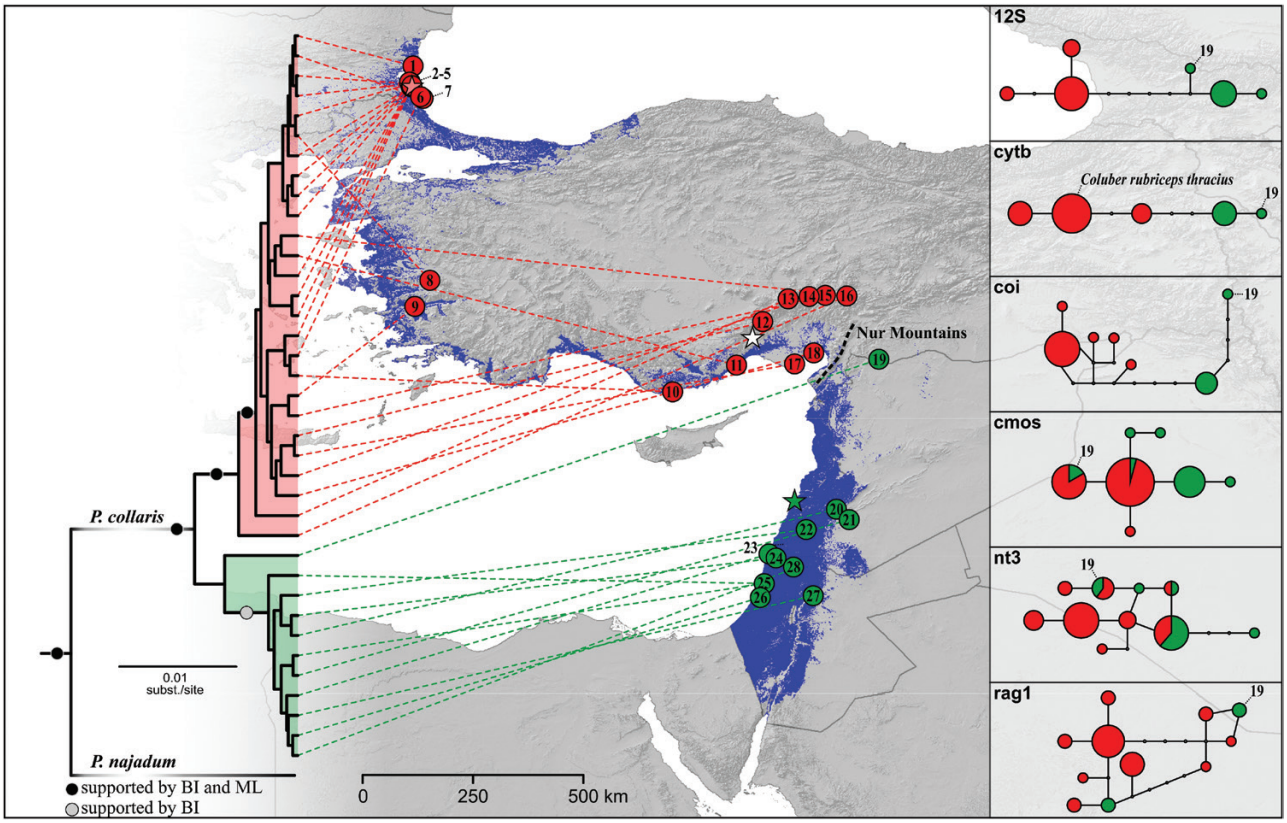

Figure 1. Phylogenetic tree resulting from the Bayesian inference (BI) analysis of three mitochondrial and three nuclear genes concatenated. Nodes were considered supported when Bayesian posterior probability was ≥ 0.95 and maximum likelihood (ML) bootstrap values ≥ 70. Lengths of branches connecting the split between *Platyceps collaris* and *Platyceps najadum* and the crown nodes of those species are not proportional to the rest of the tree and the scale, which is indicated by their partial transparency. The two clades, the Balkan–Anatolian and the Levantine, are highlighted in the tree with the red and green shading, respectively. Four species of *Telescopus* used to root the tree are not shown. Each tree tip is connected by a dashed line with the locality of its sample, which is marked by a number (for details, see Table 1). Type localities are marked with stars: *P. collaris collaris* in green, *P. collaris rubriceps* in white and *Coluber rubriceps thracius* in pink. The potential current distribution of *P. collaris* based on the species distribution model with the maximum training sensitivity plus specificity threshold applied is shown in blue. Haplotype networks reconstructed for the six markers are on the right. Circles are colour coded according to the clade assignment, and their size is proportional to the number of individuals. Lines represent mutational steps. The network for the *cytb* gene was constructed using the 148-bp-long fragment that was available for the types of *Coluber rubriceps thracius*. The position of sample DJ8199 from locality 19, whose phylogenetic placement differed in the maximum likelihood and Bayesian inference analyses, is marked with the locality number in each network.

Species distribution modelling

Species distribution models of all replicates performed well, with AUC values ranging between 0.8779 and 0.8905 (mean = 0.8864). The standard deviations of the models are low (0.0385–0.0457; mean = 0.0429), indicating model stability regardless of the input data. Models based on real data performed significantly better than null models (AUC range = 0.649–0.762; mean = 0.705). The main environmental predictors explaining the distribution of *P. collaris* are Bio4 (47.1% contribution averaged over the ten random thinning replicates, range 45.9–49.3%), Bio2 (mean contribution 22.4%, range 20.5–23.6%) and Bio17 (mean contribution 10.6%, range 10.0–10.9%; for the contribution of all variables and their response curves, see Supporting Information, Fig. S5). The predicted distribution of suitable habitat spans from south-eastern Bulgaria and north-western Turkey in a narrow band continuously along the Turkish Mediterranean coast all the way to the coast of Syria and further across Lebanon to Israel and Jordan. The suitable habitat is rarely found > 200 km inland from the Mediterranean coast, and especially in southern Turkey it is formed by a considerably narrow coastal zone (Figs 1, 3).

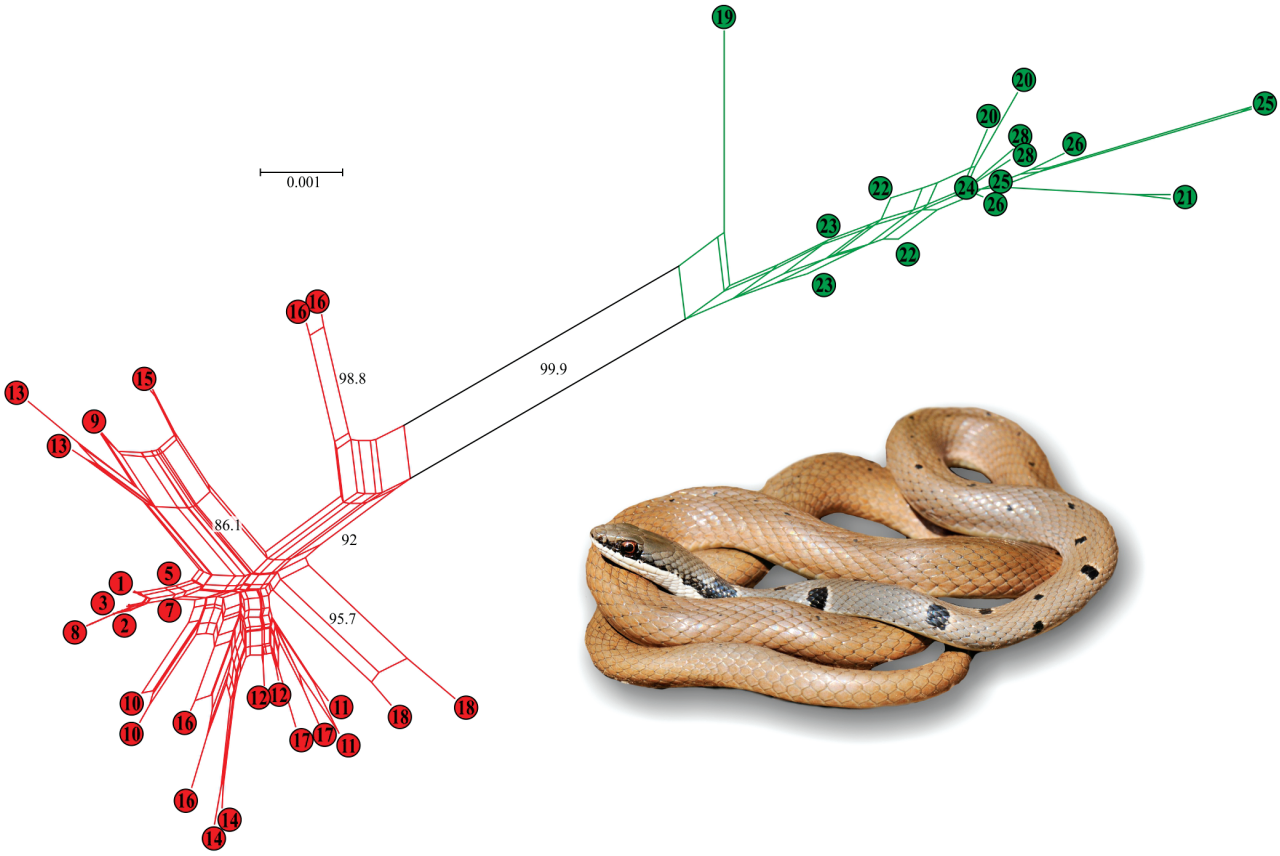

Figure 2. Phylogenetic network from SPLITSTREE, showing the reticulate relationships within the two clades of *Platyceps collaris*, the Balkan–Anatolian clade (red) and the Levantine clade (green). Numbers in circles are locality numbers shown in Figure 1 and detailed in Table 1. Bootstrap support values for major nodes ≥ 70 are indicated. The specimen depicted is from Ropotamo, Bulgaria.

Projections of the suitable conditions into the past show that certain parts of the range of *P. collaris* have been stable since the mid-Pliocene. The overall extent of suitable habitat was smallest in the mid-Pleistocene, when it was restricted to the Levant and western Anatolia, with a thin coastal strip that might have been a corridor connecting these two refugia. During the climatic optimum of the LIG, this coastal strip was much broader and most probably allowed population connectivity. During the LGM, western Anatolia had the most suitable conditions, whereas the southern Levant was mostly unsuitable. Since the LGM, the western and southern Anatolian part of the suitable area contracted noticeably, whereas it expanded considerably into the southern Levant (Fig. 3).

DISCUSSION

In this study, we applied a multidisciplinary approach to disentangle the phylogeographical structure, genetic diversity and distribution dynamics of a racer snake, *Platyceps collaris*. We used a multilocus genetic dataset generated by Sanger sequencing in combination with a newly developed method for amplifying and Illumina sequencing short DNA barcodes for museum specimens, which often suffer from DNA fragmentation and degradation. The main advantage of this approach is that it allows separation of individual reads of each sequence and exclusion of those that are obviously contaminated, typically by human DNA (Galan *et al.*, 2012), which also happened with one of our samples. The biggest potential of this method is, in our view, that it allows an alignment-based placement of important specimens (e.g. types, extinct populations) within the phylogenetic context to resolve outstanding taxonomic or conservation questions.

INTRASPECIFIC GENETIC DIVERSIFICATION

Platyceps collaris is endemic to the Eastern Mediterranean, a region that experienced intense

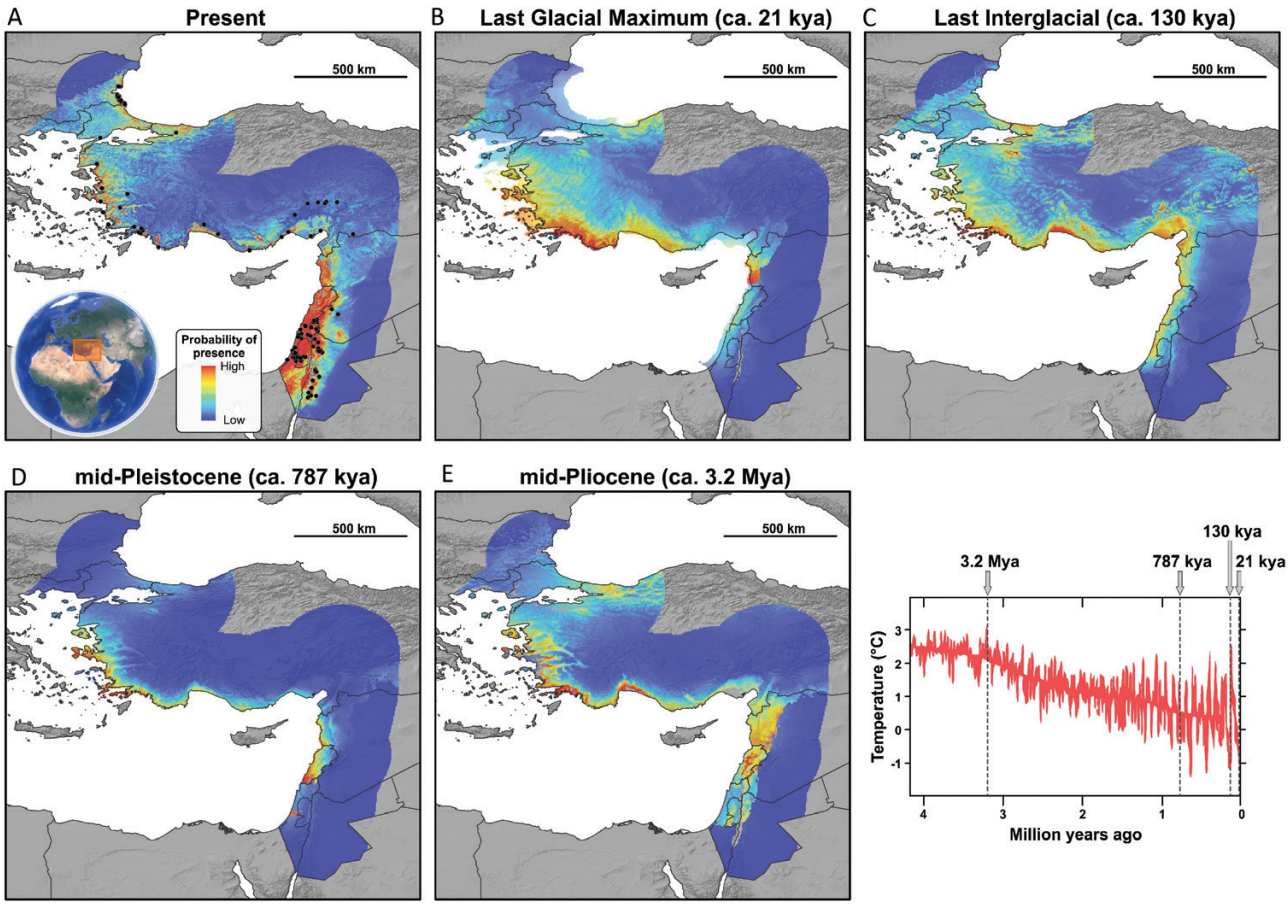

Figure 3. Species distribution models for *Platyceps collaris* in current conditions (A) and in conditions during the Last Glacial Maximum (B), the Last Interglacial (C), mid-Pleistocene (D) and mid-Pliocene (E). Black points in A indicate the records that were used for the species distribution modelling. The bottom right panel shows reconstructions of global temperature in the last 4 Myr relative to the peak Holocene temperature (Hansen & Sato, 2012), with grey arrows and dashed lines highlighting the temperature in the time periods used in the present study.

phylogeographical research on squamates in the past decade that has often resulted in uncovering hidden genetic diversity or even cryptic species (Moravec *et al.*, 2011; Bellati *et al.*, 2015; Jandzik *et al.*, 2018; Kotsakiozi *et al.*, 2018; Jablonski & Sadek, 2019; Šmíd *et al.*, 2019). The results of all phylogenetic analyses conducted in the present study show a clear genetic differentiation of *P. collaris* into two geographically well-delineated clades. One, the Balkan–Anatolian clade, occupies the north-western part of the species range. It spans from the Black Sea coast in south-eastern Bulgaria along the Mediterranean coast of Turkey to the Antakya region at the Turkish–Syrian border. The second, the Levantine clade, covers the southern part of the species range from extreme southern Turkey through Syria to Israel and Jordan. The two clades are clearly differentiated in their mitochondrial DNA, but they share some alleles in the *cmos* and *nt3* nuclear loci. As the networks show, these shared nuclear alleles are either ancestral or shared across multiple samples from both clades, which indicates retention of ancestral polymorphism rather than them being a result of hybridization. The boundary between the two clades corresponds well with the geographical position of the Nur Mountain range in Hatay Province of south-central Turkey (Fig. 1), which runs parallel to the Gulf of İskenderun and separates the Anatolian part of the Eastern Mediterranean from the Levant. These mountains are part of the so-called Anatolian Diagonal, a natural and probably the most important biogeographical barrier in the region, which has repeatedly been proved effective in separating closely related taxa (Davis, 1971; Tamar *et al.*, 2015; Kornilios, 2017; Jandzik *et al.*, 2018; Jablonski *et al.*, 2019; Jablonski & Sadek, 2019).

Genetic diversity within the Balkan–Anatolian and Levantine clades is of a comparable magnitude. All mitochondrial markers show the presence of several

substitutions within both clades, and the nuclear loci also show a considerable degree of variance, despite their generally slower substitution rates compared with the mitochondrial genes. Relationships within these two clades could not be resolved, which was most probably because the phylogenetic relationships at this shallow evolutionary depth do not necessarily form a bifurcating tree but rather a reticulate pattern, which becomes obvious when they are visualized using the phylogenetic network (Fig. 2).

Distribution and historical range dynamics

The distribution of *P. collaris* is well documented in the literature thanks to the unique appearance of the snake, although misidentifications with the sister species, *P. najadum*, can happen (e.g. Berger-Dell'Mour, 1986). Although the range of the species spans a relatively large territory, from south-eastern Bulgaria to southern Israel and Jordan, the species distribution modelling analysis shows that the predicted suitable habitat is found only in a narrow stretch of land along the Mediterranean coast (Fig. 1). The potential distribution reaches further inland in the Levant, even beyond the Dead Sea Rift in Jordan, but there it is restricted to the Mediterranean ecozone (Disi, 1996; Disi *et al.*, 2001). The character of the environmental variables that contribute most to the potential distribution of the species suggests that *P. collaris* prefers areas of low climatic seasonality, without fluctuating temperatures (Supporting Information, Fig. S5). For example, the probability of presence of the species decreases significantly with increasing elevation. This means that in south-western Turkey, where the mountains come close to the sea, the suitable habitat is present only along the immediate vicinity of the coast.

The Levantine part of the species range corresponds well to the distribution of the Eastern Mediterranean conifer–broadleaf forest ecoregion (Dinerstein *et al.*, 2017), which covers the eastern Mediterranean coastal zone (Supporting Information, Fig. S6). The ecoregion forms a narrow coastal strip in southern Turkey, and it reaches eastwards along the entire length of the Turkish–Syrian border all the way to the isolated *P. collaris* populations. However, these populations lie well away from the suitable habitat of the species. It might be that the environmental variables used in the modelling process did not capture all dimensions of the biotic requirements of the species and thus failed to identify these localities to have a suitable habitat. It is nonetheless interesting that there have not been more observations of *P. collaris* in the broadleaf forest ecoregion along the Turkish–Syrian border. These eastern localities might represent microclimatic pockets where the species survives in isolation from the Mediterranean populations, a hypothesis that could be addressed easily if the specimens were sequenced and placed in a phylogenetic framework. More enigmatic in this respect is the record from central Anatolia, which is > 200 km away from other records of the species (İğci *et al.*, 2015). Given that a voucher of the specimen is catalogued in a collection [Zoology Museum of Adıyaman University, Turkey (ZMADYU) 2013/57], we recommend collecting a tissue sample and genotyping it in order to assess its phylogenetic affinities.

The models of suitable habitat distributions in the past show that the climatic conditions of the Eastern Mediterranean have been stable since the Late Tertiary and suitable for the occurrence of *P. collaris*. Despite some oscillations, western and southern Anatolia and the northern Levant have remained suitable for the species for the past 3 Myr (Fig. 3). This contrasts with south-eastern Bulgaria and the southern Levant, which lacked suitable habitats in the LGM and mid-Pleistocene. Western Anatolia, one of the current hotspots for the species distribution, had a larger extent of suitable habitats in the LGM and LIG, whereas further in the past its suitability corresponded more or less to that of the current conditions. Together with the northern Levant, these areas might have provided refugia where populations of *P. collaris* survived climatically challenging times, such as the LGM, and from where they subsequently colonized the rest of the current range. Here, it is of importance to note that the historical oscillations of suitable habitats did not necessarily have to lead to population expansions and contractions for reasons associated with factors such as dispersal limitations, competitive exclusion and predation (Peterson, 2011). However, the genetic evidence that confirmed the presence of the two distinct clades provides credibility to the presence of the two refugia. It is therefore likely that the Anatolia refugium served as a source for the colonization of the south-eastern Balkans after the LGM, whereas the south of the Levant was repopulated from the north, from the Syrian coastal region, as has been suggested for the genus *Lacerta* Linnaeus, 1758 (Ahmadzadeh *et al.*, 2013).

Subspecific taxonomy of *Platyceps collaris*

The genetic differentiation between the two *P. collaris* clades is, to some degree, mirrored in the morphological traits. Specimens from Bulgaria (including the types of *Coluber rubriceps thracius*) and south-western Turkey have been shown to have a lower number of ventral (185–203) and subcaudal scales (79–105) compared with specimens from the Levant (ventrals, 189–220; subcaudals, 89–128; Rehák, 1985, 1986; Rehák & Obst, 1993; Schätti *et al.*, 2001). However, the morphological

variability seems to be more of a cline, with specimens from southern Turkey being intermediate in these traits. Unfortunately, all authors who have collected morphological data for *P. collaris* compared populations and did not provide values for individual specimens. For example, Baran (1976) gave only trait means and variations for specimens from across the species range, Rehák (1986) compared populations from Bulgaria and Israel, and Rehák & Obst (1993) compared populations from Bulgaria with those from Turkey, Israel and Jordan. The most detailed comparison was provided by Schätti *et al.* (2001), who compared the following five geographical groups: Bulgaria, western Anatolia, southern Anatolia, Lebanon and north-western Syria. However, as our results show, southern Anatolia is occupied by both clades, and the groupings by Schätti *et al.* (2001) might have meant that both were represented in this category, thus obfuscating the potential difference between the clades. Therefore, future morphological comparison of ideally genotyped specimens from southern Anatolia will be necessary to clarify this issue.

Based on the genetic results obtained in the present study, we are able to draw some conclusions regarding the subspecific taxonomy of *P. collaris*. Considering the phylogeographical structure found within the species, the parapatry of the two clades and their complete segregation of mitochondrial haplotypes, the two clades can be considered distinct subspecies. Given that the type locality of *P. collaris* in Beirut falls within the range of the Levantine clade, this clade retains the nominotypical name *Platyceps collaris collaris* (Müller, 1878).

In the case of the Balkan–Anatolian clade, the name *Zamenis dahlii rubriceps* Venzmer, 1919 has priority over the name *Coluber rubriceps thracius* Rehák, 1985. By sequencing the holotype and eight paratypes of *C. r. thracius*, we show that they are genetically identical with all other specimens of *P. collaris* from Bulgaria included in the analysis and also with two specimens from western and southern Turkey. Although we could not analyse the type material of *Zamenis dahlii rubriceps* directly in the present study, we had samples collected not far away from its type locality that have proved to represent the Balkan–Anatolian clade. In fact, the type locality is surrounded by samples of that clade, and we believe that it is reasonable to assume that, if genotyped, it would cluster with the Balkan–Anatolian clade. Therefore, following the principle of priority, we suggest that this clade should bear the name *Platyceps collaris rubriceps* (Venzmer, 1919) comb. nov., and the name *Coluber rubriceps thracius* Rehák, 1985 should be its junior synonym.

Genetic methods have enabled a boom in species descriptions that is unprecedented since the times of Linnaeus. Despite this, subspecific taxonomy shows the opposite trend in squamates (Torstrom *et al.*, 2014). However, recognition of subspecies can serve as a useful formalization that reflects character differentiation (genetic, morphological and geographical) within species and should be adhered to (Kindler & Fritz, 2018), especially when there is congruence between more lines of evidence. In our case, these congruent lines are the genetic differentiation between the Levantine clade (*P. c. collaris*) and the Balkan–Anatolian clade (*P. c. rubriceps*), which is probably a result of different Quaternary refugia used by the clades. However, morphological differentiation between the clades needs to be verified.

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SUPPORTING INFORMATION

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

Figure S1. Phylogenetic tree of *Platyceps collaris* resulting from the maximum likelihood analysis of all genes concatenated. Bootstrap support values ≥ 60 are shown above branches. Type specimens of *Coluber rubriceps thracius* are in red.

Figure S2. Phylogenetic tree of *Platyceps collaris* resulting from the Bayesian inference analysis of all genes concatenated. Posterior probability values ≥ 0.90 are shown above branches. Type specimens of *Coluber rubriceps thracius* are in red.

Figure S3. Phylogenetic tree of *Platyceps collaris* resulting from the maximum likelihood analysis of the three mitochondrial genes concatenated. Bootstrap support values ≥ 60 are shown above branches. Type specimens of *Coluber rubriceps thracius* are in red.

Figure S4. Phylogenetic tree of *Platyceps collaris* resulting from the Bayesian inference analysis of the three mitochondrial genes concatenated. Posterior probability values ≥ 0.90 are shown above branches. Type specimens of *Coluber rubriceps thracius* are in red.

Figure S5. Response curves of the environmental variables used to develop the distribution model, showing how each variable affected the prediction and its relative contribution to the model (averaged over ten runs). Each plot shows the probability of presence of the species (*y*-axis) as a function of the environmental predictor value (*x*-axis). Red lines show mean responses of ten replicate runs; standard deviations are in blue. The key in the lower right refers to the categorical land cover variable in the lower left.

Figure S6. Geographical distribution of the Eastern Mediterranean conifer–broadleaf forest ecoregion (Dinerstein *et al.*, 2017).

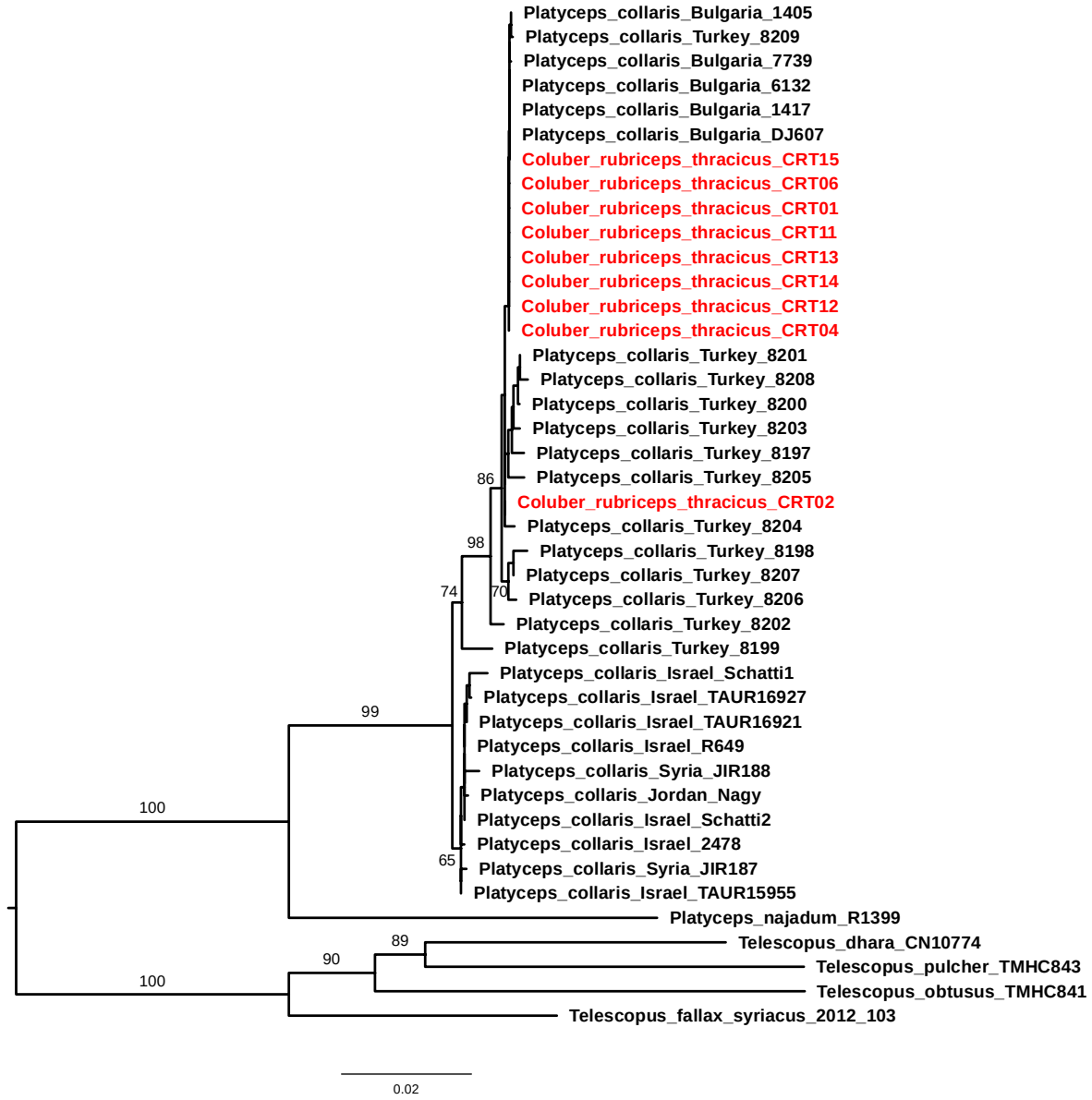
Table S1. Locality details of records used for the species distribution modelling and their original sources. Coordinates from Bulgaria have been truncated to two decimal places for conservation purposes.

Supplementary Figures and Table for

Quaternary range dynamics and taxonomy of the Mediterranean collared dwarf racer, *Platyceps collaris* (Squamata: Colubridae)

by

Šmíd *et al*.



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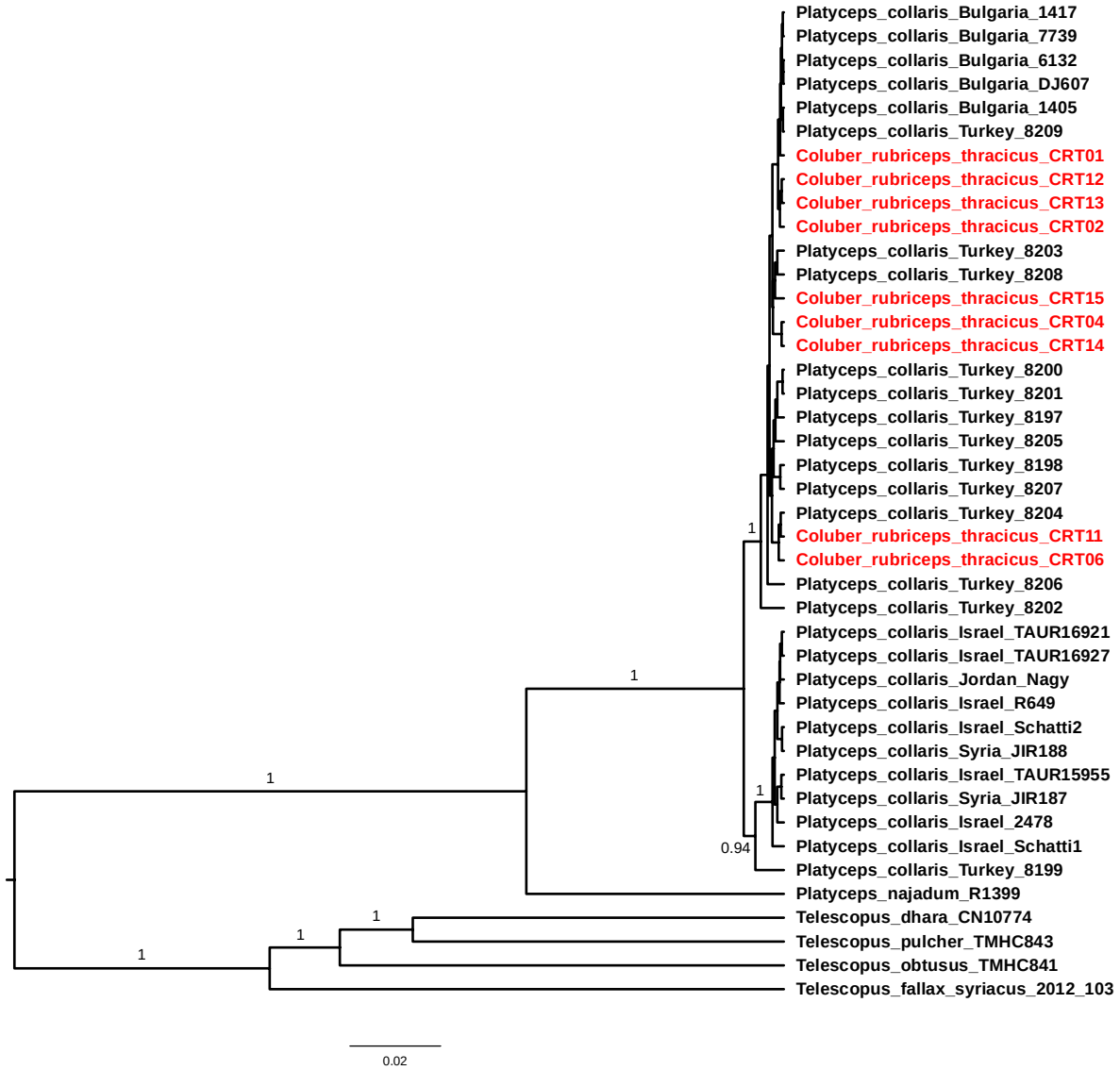

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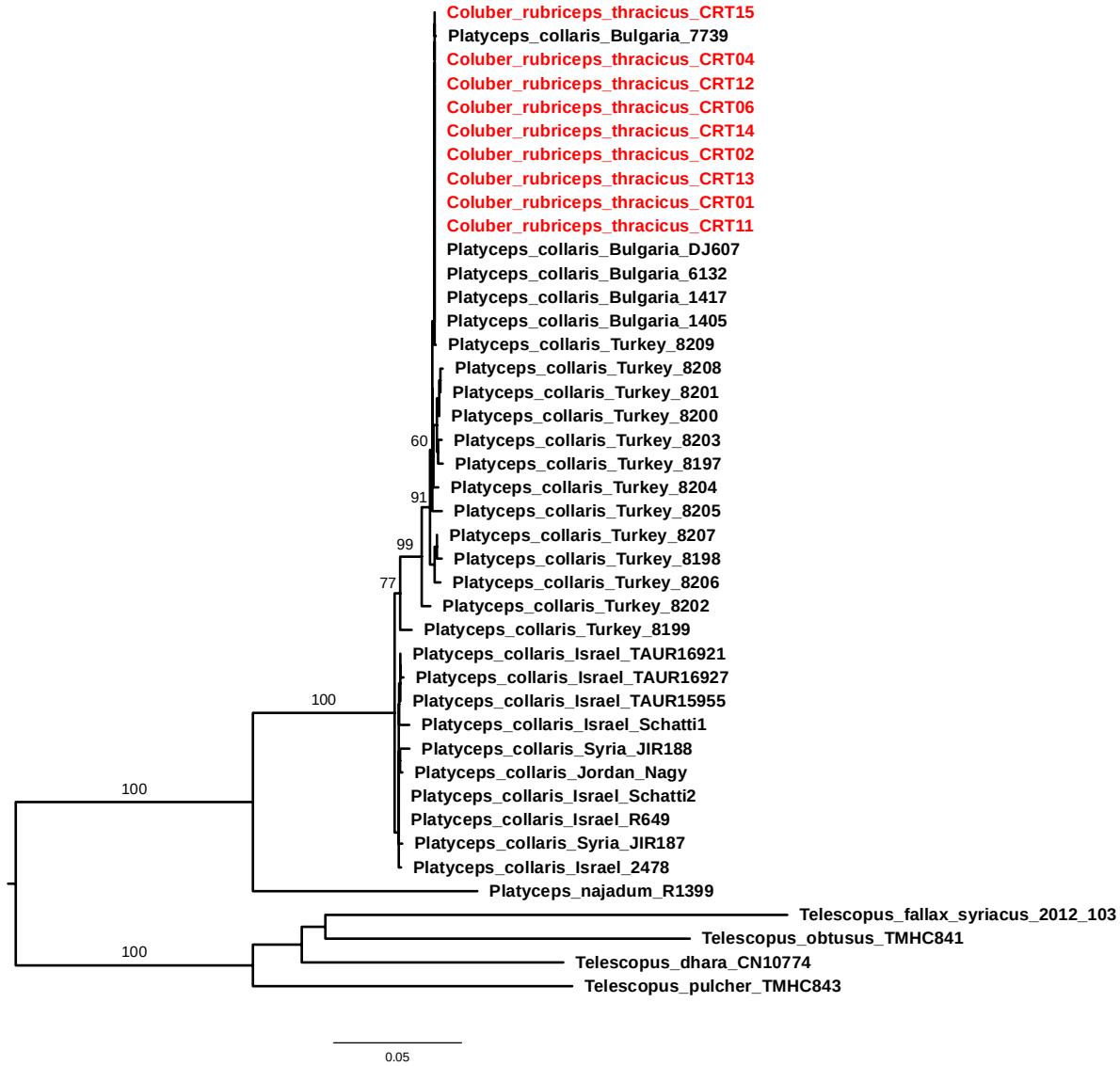

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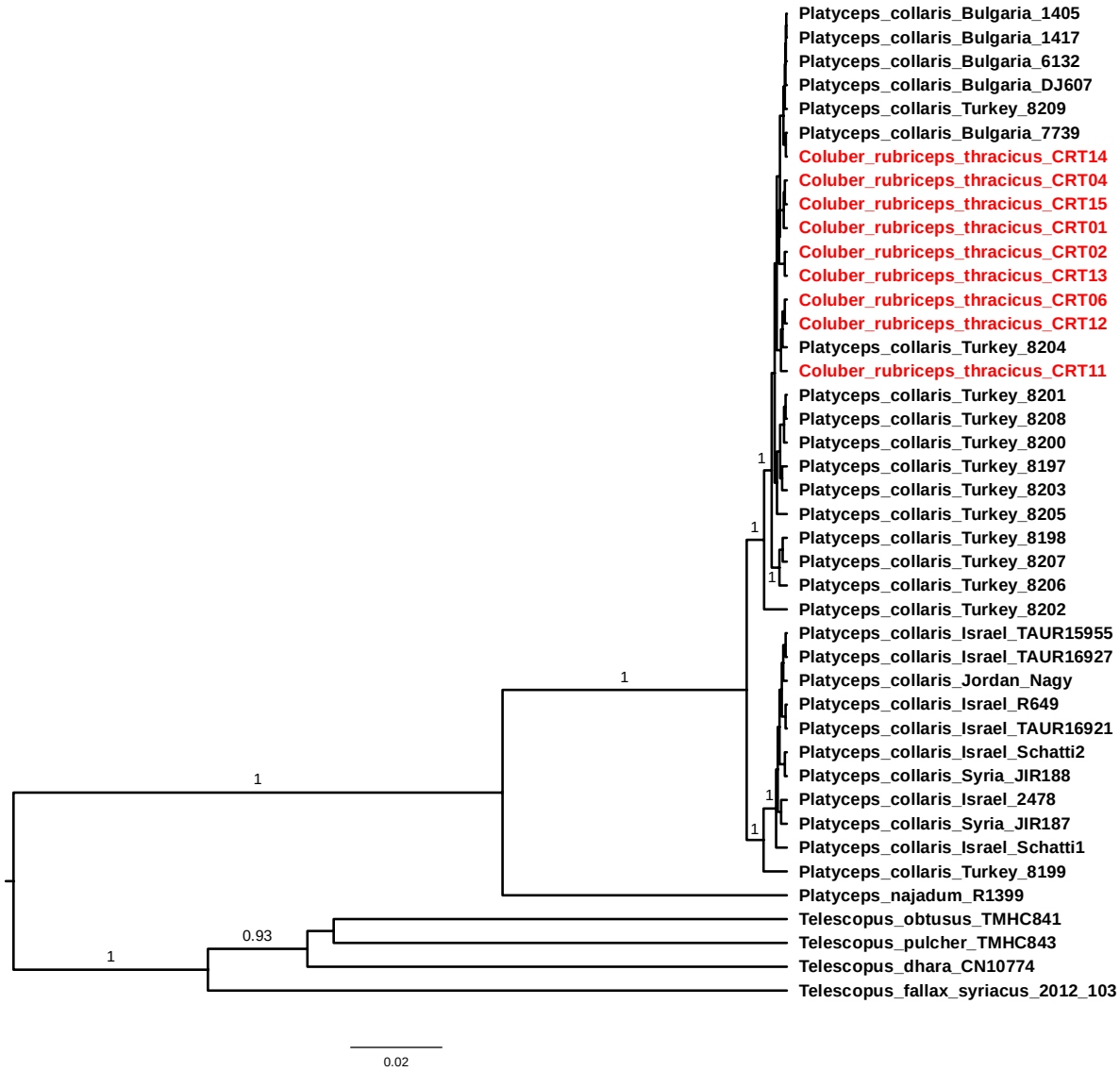

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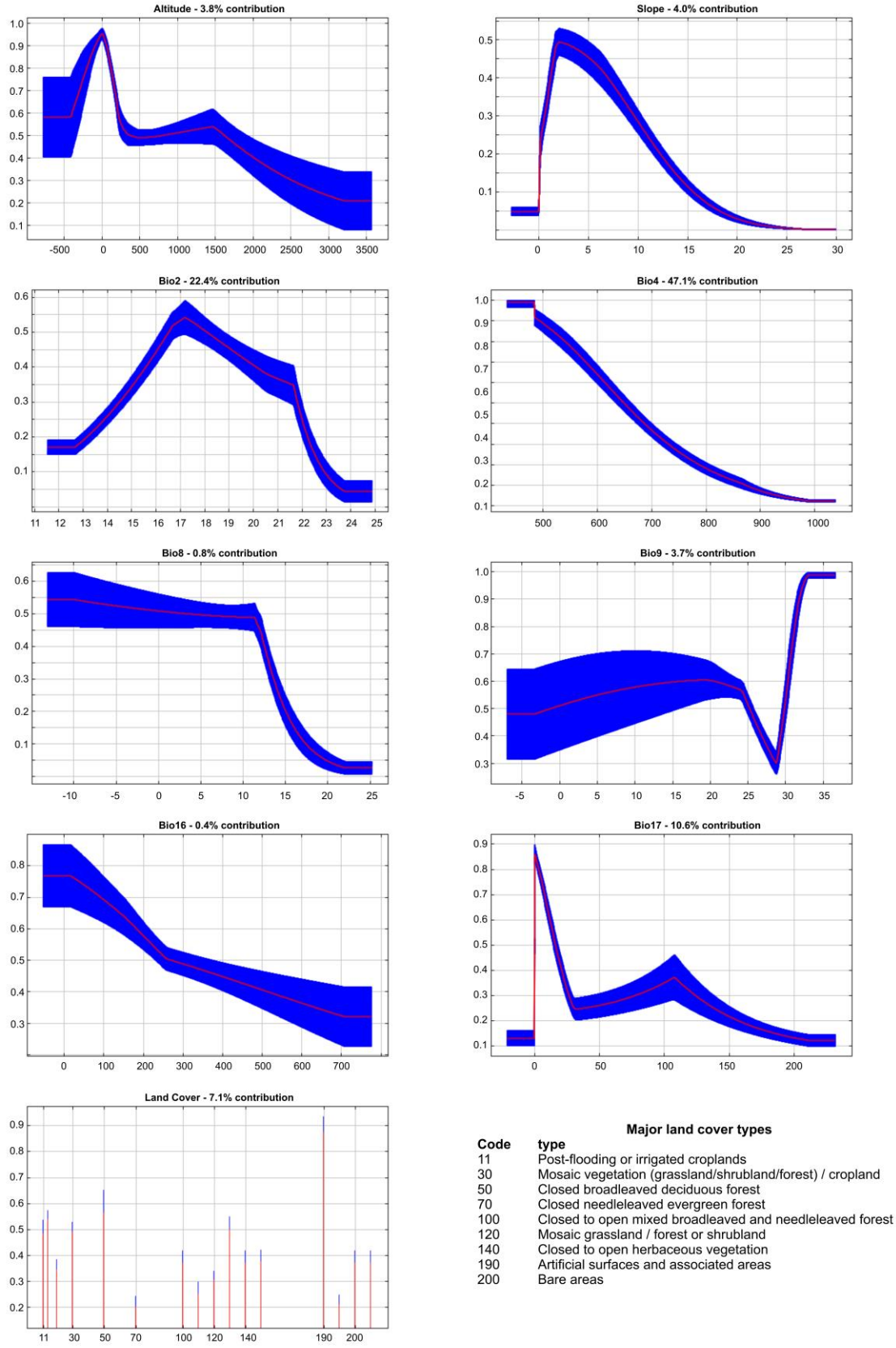

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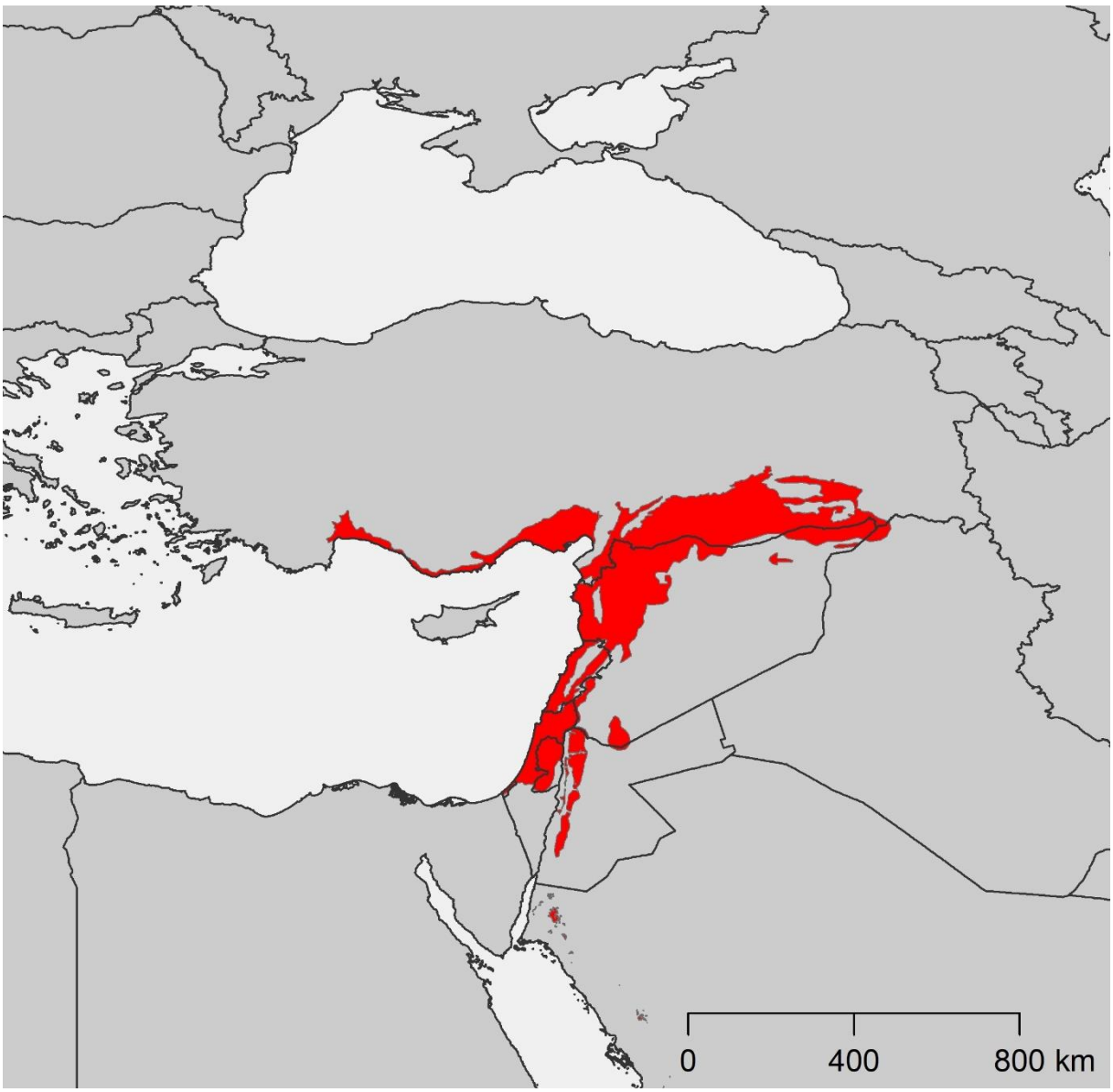

Figure S6. Geographic distribution of the Eastern Mediterranean conifer-broadleaf forest ecoregion (Dinerstein *et al*., 2017).

Table S1. Locality details of records used for the SDM and their original sources. Coordinates from Bulgaria have been truncated to two decimal places for conservation purposes.

Country	Locality	Latitude	Longitude	Source
Bulgaria	Rezovo	42	27.99	smartbirds.org
Bulgaria	Sinemorec	42.03	27.95	This study
Bulgaria		42.04	27.99	This study
Bulgaria	Sinemorets	42.05	27.98	smartbirds.org
Bulgaria	Sinemorets	42.06	27.92	This study
Bulgaria	Ahtopol	42.06	27.95	smartbirds.org
Bulgaria	Brodilovo	42.11	27.83	smartbirds.org
Bulgaria	Tsarevo	42.17	27.83	smartbirds.org
Bulgaria		42.18	27.75	This study
Bulgaria	Velika	42.19	27.75	smartbirds.org
Bulgaria		42.19	27.8	This study
Bulgaria	Primorsko	42.28	27.74	smartbirds.org
Bulgaria		42.28	27.75	This study
Bulgaria	Primorsko	42.29	27.74	iNaturalist
Bulgaria		42.3	27.69	This study
Bulgaria	Ropotamo	42.3	27.72	Balcanica.info
Bulgaria	Primorsko	42.3	27.74	smartbirds.org
Bulgaria		42.31	27.72	This study
Bulgaria	Primorsko	42.31	27.77	smartbirds.org
Bulgaria	Arkutino	42.33	27.72	Rehák (1985)
Bulgaria	Sozopol	42.37	27.69	smartbirds.org
Bulgaria	Sozopol	42.39	27.68	This study
Bulgaria	Sozopol	42.39	27.69	This study
Bulgaria	Sveti vlas	42.71	27.75	This study
Bulgaria	Elenite	42.71	27.81	Panner (2009)
Bulgaria	Sveti Vlas	42.72	27.75	smartbirds.org
Bulgaria	Sveti Vlas	42.72	27.76	This study
Bulgaria		42.73	27.71	This study
Israel		31.61406	34.92282	GBIF
Israel		31.67055	34.57677	GBIF
Israel		31.7424	35.10922	GBIF
Israel		31.74498	35.00052	GBIF
Israel		31.75104	34.87807	GBIF
Israel		31.7621	34.98781	GBIF
Israel		31.76345	34.80309	GBIF
Israel	Jerusalem	31.79367	35.21999	GBIF
Israel	Mt. Scopus	31.79447	35.2439	Schatti et al. (2001)
Israel		31.79454	34.97507	GBIF
Israel		31.91965	34.86479	GBIF
Israel	Southern Coastal Plain, Nezer Sereni	31.922	34.822	This study
Israel		31.93234	34.89118	GBIF
Israel	Miqwe Yisrael	32.0292	34.7814	Schatti et al. (2001)
Israel		32.15777	34.88299	GBIF
Israel	Haifa	32.5241	35.1407	GBIF
Israel		32.58816	35.00289	GBIF
Israel		32.61228	35.5153	GBIF
Israel		32.62401	35.51427	GBIF
Israel		32.66846	35.03896	GBIF
Israel	Mt. Tabor	32.683	35.4	Schatti et al. (2001)
Israel	Yizre'el (Jezreel) Valley, Qiryat Tiv'on	32.72	35.14	This study
Israel		32.83671	35.8023	iNaturalist
Israel	HaZafon	32.89259	35.09821	GBIF
Israel		32.90455	35.74612	GBIF
Israel		32.90545	35.74613	GBIF
Israel		32.91079	35.7622	GBIF
Israel		32.91084	35.74937	GBIF
Israel		32.91266	35.74617	GBIF
Israel		32.93816	35.79532	iNaturalist
Israel		32.97109	35.54115	GBIF
Israel		33.03372	35.30589	GBIF
Israel	HaZafon	33.17679	35.63402	GBIF
Israel	Golan	33.18039	35.77118	GBIF
Israel	HaZafon	33.241	35.65568	GBIF
Israel		33.24576	35.64843	GBIF
Israel		33.24844	35.65381	GBIF
Israel		33.24897	35.74609	GBIF
Israel		33.28952	35.75171	GBIF
Israel		33.29223	35.75173	GBIF
Israel	Golan	33.29272	35.75448	GBIF
Israel		33.29328	35.75557	iNaturalist
Israel	Hermon	33.29599	35.76239	This study
Israel		33.30293	35.77864	GBIF
Israel		33.30927	35.77116	GBIF
Jordan	Dilāghah	30.13333	35.4	Amr & Disi (2011)
Jordan	Ma'an	30.2	35.73333	Disi et al. (2001)
Jordan	Ayl	30.21667	35.53333	Amr & Disi (2011)
Jordan	Petra	30.3333	35.4333	Disi et al. (2001)
Jordan	Ash Shawbak	30.52	35.53833	Amr & Disi (2011)

Country	Locality	Latitude	Longitude	Source
Jordan	Ash Shawbak	30.5333	35.5667	Disi et al. (2001)
Jordan	Aṭ Ṭafīla	30.83333	35.6	Amr & Disi (2011)
Jordan	Al Mazār	30.97722	35.85889	Amr & Disi (2011)
Jordan	Al Karak	31.1667	35.75	Disi et al. (2001)
Jordan	Al Karak	31.18472	35.70472	Amr & Disi (2011)
Jordan	Rākīn	31.22389	35.70667	Amr & Disi (2011)
Jordan	Batīr	31.26417	35.70472	Amr & Disi (2011)
Jordan	ʻAmmān	31.96667	35.98333	Amr & Disi (2011)
Jordan	Al Jubayhah	32.01667	35.86667	Disi et al. (2001)
Jordan	Şuwayliḥ	32.025	35.83806	Amr & Disi (2011)
Jordan	Yājūz	32.03333	35.91667	Amr & Disi (2011)
Jordan	Salīhī	32.12278	35.83111	Amr & Disi (2011)
Jordan	Dayr ʻAllā	32.19778	35.62111	Amr & Disi (2011)
Jordan	Ibbin	32.36667	35.81667	Disi et al. (2001)
Jordan	Dayr Abu Sa'id	32.5	35.68333	Disi et al. (2001)
Syria	E of Utaibeh, Lake shore	33.50606	36.6274	This study
Syria	2 km N of Seydnaya	33.7173	36.3717	This study
Turkey	Bozyazı-Anamur/Mersin	36.09666	33.04152	Aydın Adnan Menderes University
Turkey	Yılan adası, Kalkan - Antalya	36.21502	29.35472	Dokuz Eylül University
Turkey	Yilan Adasi, Kalkan	36.21517	29.35585	Schatti et al. (2001)
Turkey	Erdemli/Mersin	36.63865	34.34009	Aydın Adnan Menderes University
Turkey	Kaldırım Village-Yumurtalık/Adana	36.67375	35.52499	Aydın Adnan Menderes University
Turkey	Waldgebiet bei Aydolun	36.752	31.774	Kucharzewski (2016)
Turkey	Küplüce Village-Kilis	36.75719	37.23555	Aydın Adnan Menderes University
Turkey	İztuzu Kuzeyi - Dalyan - Muğla	36.78493	28.62834	Dokuz Eylül University
Turkey	Kızılburun Mevkii - Köyceğiz - Muğla	36.80226	28.52348	Dokuz Eylül University
Turkey	Çandır-Köyceğiz	36.82751	28.60819	Dokuz Eylül University
Turkey	Ekincik- Köyceğiz	36.83261	28.55059	Dokuz Eylül University
Turkey	Toroslar, Mersin	36.83932	34.61363	Dokuz Eylül University
Turkey	Ceyhan (Botaş)/Adana	36.89531	35.91594	Aydın Adnan Menderes University
Turkey	Kavak arası Köyceğiz	36.89906	28.72029	Dokuz Eylül University
Turkey	Toparlar Kuzeyi - Köyceğiz - Muğla	36.98732	28.6671	Dokuz Eylül University
Turkey	Gökova - Muğla	37.05563	28.36512	Dokuz Eylül University
Turkey	Beşkonak - Antalya	37.1403	31.19879	Dokuz Eylül University
Turkey	Ömerli Village-Pozantı/Adana	37.53545	34.87516	Aydın Adnan Menderes University
Turkey	Karina und Umgebung	37.632	27.11756	GBIF
Turkey	Işıklı Village/Aydın	37.832	27.7987	Aydın Adnan Menderes University
Turkey	Suna Plateau-Yahyalı/Kayseri	37.99278	35.39736	Aydın Adnan Menderes University
Turkey	Obruk şelalesi - Saimbeyli - Adana	38.001	36.09318	Dokuz Eylül University
Turkey	Gürümze Village-Feke/Adana	38.04202	35.81172	Aydın Adnan Menderes University
Turkey	Temürağa Village-Göksun/Kahramanmaraş	38.0458	36.58581	Aydın Adnan Menderes University
Turkey	10 km North of Saimbeyli/Adana	38.06065	36.14885	Aydın Adnan Menderes University
Turkey	Gümüldür, İzmir	38.0775	27.01762	Dokuz Eylül University
Turkey	Bozdağ-Ödemiş/İzmir	38.36708	28.10343	Aydın Adnan Menderes University
Turkey	Menemen, İzmir	38.60313	27.07201	Dokuz Eylül University
Turkey	Tahtakuşlar - Kaşdağ	39.59337	26.85549	Dokuz Eylül University
Turkey	Yeniköy - Tekirdağ	40.64698	26.99938	Dokuz Eylül University
Turkey	Orhaniye village, Kocaeli	40.85767	30.0669	Afsar et al. (2013)
West Bank		31.62698	35.12515	GBIF
West Bank		31.78608	35.3019	GBIF

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