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Crossing the Mid-Aegean Trench: vicariant evolution of the Eastern pine processionary moth, *Thaumetopoea wilkinsoni* (Lepidoptera: Notodontidae), in Crete

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Thaumetopoea wilkinsoni Tams, 1924 occurs in the southeast Mediterranean basin and infests pine and cedar stands. Our objective was to decipher the biogeography of the species and, in particular, to explore its evolutionary history on the island of Crete. We collected 135 individuals from 14 sites on Crete, Turkey, Samos and Rhodes. We sequenced one mitochondrial fragment (cytochrome *c* subunit I) and used 12 microsatellite loci for population genetic analyses. All results supported the deep divergence of Cretan populations from the neighbouring areas, with the differentiation between Crete and neighbouring regions being similar to the interspecific divergence between *T. wilkinsoni* and its sibling species, *Thaumetopoea pityocampa* (Denis and Schiffermüller, 1775). Both mitochondrial and microsatellite markers showed a clear longitudinal (east–west) differentiation of populations within Crete. Our results thus suggest that *T. wilkinsoni* crossed the Mid-Aegean Trench, became isolated on Crete and differentiated through vicariance after the end of the Messinian Salinity Crisis, favouring the emergence of an endemic lineage. There, Quaternary climatic oscillations coupled with geographical barriers to gene flow have given rise to the currently observed east–west differentiation of Cretan populations.

ADDITIONAL KEYWORDS: Aegean region – biogeography – genetic structure – phylogeographical patterns.

INTRODUCTION

The Aegean Archipelago, located in the eastern part of the Mediterranean basin, has a long and intense geological history (Angelier *et al.*, 1982; Meulenkamp, 1985; Dermitzakis, 1990). The region experienced recurrent alterations of sea and land configurations and great changes in land mass connectivity over time (Anastasakis & Dermitzakis, 1990). Two of these main geological events were the opening of the Mid-Aegean Trench (MAT), separating the eastern from the western part of the Archipelago (12 Mya), and the 'Messinian Salinity Crisis' (Krijgsman *et al.*, 1999), when a major drop in the Mediterranean sea level (5.9–5.3 Mya) allowed land masses to emerge. This complex geological history and the subsequent dramatic changes in land mass connectivity (for more details, see Poulakakis *et al.*, 2015 and maps and references theirein) greatly affected the phylogenetic and phylogeographical structure of many organisms, resulting in the high levels of endemism and diversification rates currently observed (Blondel *et al.*, 2010). The Aegean region was thus acknowledged to be 'an ideal natural laboratory for evolutionary and biogeography studies' (Sfenthourakis & Triantis, 2017). Poulakakis *et al.* (2015) proposed the categorization of the organisms of this region into four main groups based on

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their timing of arrival and biogeography, namely the 'old colonizers', the 'post-MAT colonizers', the 'new settlers' and the 'human-aided dispersers'. Acknowledging that most studies on the Aegean Archipelago have involved vertebrates, they called for more studies on invertebrates and other microorganisms, which should use both mitochondrial and nuclear markers.

Crete is the largest island in the Aegean Sea, located ~100 km from the Greek coast and > 150 km from Turkey. Many studies have highlighted the strong divergence of the endemic lineages found in Crete, suggesting a long isolation of the island (Parmakelis *et al.*, 2006; Kornilios *et al.*, 2016). Moreover, recent studies have shown that a clear east vs. west differentiation often exists within Crete (Velonà *et al.*, 2010; Thanou *et al.*, 2017), possibly attributable to the complex geological history of Crete. The current configuration of the islands appeared only ~3.0–1.9 Mya, because before then the initial palaeo-islands went through cycles of fusion and separation as a result of tectonic shifts during the Messinian Salinity Crisis (Angelier *et al.*, 1982; Meulenkamp, 1985).

Here, we present a phylogeographical study of the Eastern pine processionary moth, *Thaumetopoea wilkinsoni* Tams, 1924, in the southeast of the Mediterranean basin, focusing on the island of Crete. It is the sister species of the winter pine processionary moth, *Thaumetopoea pityocampa* (Denis and Schiffermüller, 1775), which occurs in the Western Mediterranean region and westernmost Turkey (Kerdelhué *et al.*, 2009). The distribution of this species complex in the Aegean Archipelago is rather entangled, with *T. pityocampa* occurring on the Greek mainland, in the Thrace region in Turkey and on the Northern Aegean islands, and *T. wilkinsoni* in Turkey, the Near East, Cyprus and Crete (Kerdelhué *et al.*, 2009; Korsch *et al.*, 2015). Recently, these species were proved to hybridize, both in laboratory conditions and in the field in a narrow contact zone in north-western Turkey (İpekdal *et al.*, 2015; Petrucco-Toffolo *et al.*, 2018). Preliminary molecular data available from Crete, based on a limited sample size and a single mitochondrial marker, suggested that Cretan populations were monophyletic and formed a sister clade to Cypriot and Turkish populations (Kerdelhué *et al.*, 2009), which should now be confirmed using nuclear markers. Our objectives were to characterize the differentiation of Crete compared with nearby continental and other island populations and to test the congruence between differently inherited markers. In addition, we aimed to identify possible within-island diversification patterns and develop inferences of past demographic events.

MATERIAL AND METHODS

Sampling

Larvae of *T. wilkinsoni* were collected from 14 sites [seven in Crete, one in Samos, one in Rhodes and five in Western Turkey (Fig. 1A; Supporting Information, Table S1)]. Each individual was taken from a different tree, transferred to 70% ethanol and stored at –20 °C.

Mitochondrial DNA analyses

DNA was extracted using either the Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich) or the PureLink Genomic DNA Mini kit (Invitrogen). Polymerase chain reaction (PCR) amplifications of a part of the mitochondrial cytochrome *c* subunit I (*COI*) gene were performed using C1-J-2183 (Jerry) 5′-CAACATTTATTTTGATTTTTTGG-3′ and TL2-N-3014 (Pat) 5′-TCCAATGCACTAATCTGCCATATTA-3′ (Simon *et al.*, 1994), and PCR products were purified with the PureLink PCR Purification Kit (Invitrogen). Sequencing took place at CeMIA SA (Larissa, Greece) using an ABI 3730XL for Cretan individuals, whereas it was performed by the MWG Eurofins Genomics company for the other samples.

The sequences obtained were checked with CHROMAS LITE (Australia Technelysium Pty Ltd) and aligned using CLUSTAL_X (Thompson *et al.*, 1997). No double peaks, insertion, deletion or stop codons were observed. To compare the genetic distances obtained in the present study with already known interclades or interspecific distances within the complex, we added to the final alignment seven haplotypes for *T. wilkinsoni* (five from Eastern Turkey and two from Cyprus; İpekdal *et al.*, 2015) and three haplotypes of *T. pityocampa* from Greece, France and Portugal (accession numbers KP676150, GU385906 and GU385933).

The number of haplotypes, haplotype diversity (H_d) and nucleotide diversity (π) were computed for each region and sampling site using DNASP 5.0 (Librado & Rozas, 2009). We constructed a haplotype network using the TCS method (Clement, Posada & Crandall, 2000) in the software POPART (Leigh & Bryant, 2015). Finally, pairwise genetic distances (Kimura 2-parameter, K2P) between haplotypes were estimated using MEGA v6 (Tamura *et al.*, 2013).

Microsatellite analyses

Twelve microsatellite loci were used to genotype all the 89 individuals from Crete, namely Ppit34, Ppit20, Ppit37, Ppit09, Ppit39, Ppit16, Ppit35, Ppit38, Ppit48 (Sauné, Abella & Kerdelhué, 2015), Thpit10 (Molecular Ecology Resources Primer Development Consortium *et al.*, 2012), Thpit03 and Thpit04 (Rousselet, Magnoux & Kerdelhué, 2004). Only ten of those were successfully used for Turkey, Samos and Rhodes, because Ppit38 and Thpit10 could not be amplified. Fluorescent PCR products were run on an ABI 3730 sequencer, and allele calling was done using GENEMAPPER 4.0 (Applied Biosystems, Carlsbad, CA, USA).

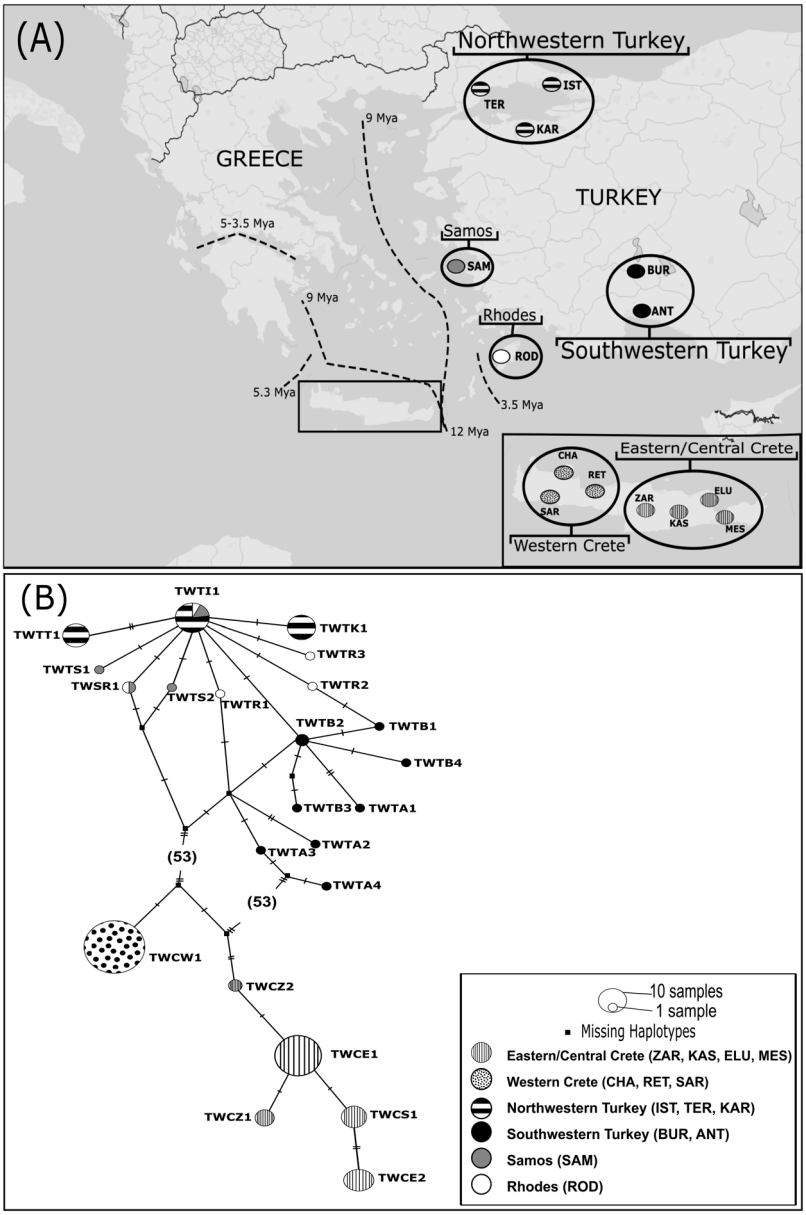

Figure 1. A, map of the Aegean region showing the sampling sites (presented with their sample codes, see Table S1 for further details), the main palaeogeographical barriers (black dashed lines) and their respective ages (redrawn after Kornilios *et al.*, 2016) B, maximum parsimony network showing mutational relationships among haplotypes of the *COI* gene. The mutations are shown as lines in the branches or numbers in parentheses.

DATA ANALYSES

Marker validation and genetic diversity indices

Sampling sites with fewer than ten individuals were used only in individual-based analyses. We first tested linkage disequilibrium and Hardy–Weinberg equilibrium (HWE) with ARLEQUIN 3.5 (Excoffier *et al.*, 2010) with a significance level of 5%, 1000 permutation steps and 100 000 steps in the Markov chain for HWE and applying sequential Bonferroni corrections. Allelic richness was calculated with FSTAT 2.9.3 (Goudet, 2002), and observed–expected heterozygosities were calculated using GENETIX v4.05 (Belkhir *et al.*, 2004). Null allele frequencies were estimated for each locus using an expectation maximization algorithm performed in the FreeNA package (Chapuis & Estoup, 2007).

Population genetic structure

Principal component analysis (PCA) was first performed to explore the population genetic structure

of all individuals, using the *adegenet* 1.4–2 package (Jombart, 2008) implemented in R (R Core Team, 2014). Second, we applied STRUCTURE 2.3.3 (Pritchard, Stephens & Donnelly, 2000) to detect genetically homogeneous clusters (see Supporting Information, Appendix S1 for details). Third, pairwise fixation index (F_{ST}) values were inferred using FreeNA, with or without application of the null alleles (ENA) correction (Chapuis & Estoup, 2007). The 95% confidence intervals (CIs) were obtained by bootstrapping 1000 times over loci.

Demographic inference

Demographic inferences were conducted on the Cretan dataset only, with the software MIGRAINE (http://kimura.univ-montp2.fr/~rousset/Migraine.htm), which uses the class of importance sampling algorithms developed by De Iorio & Griffiths (2004a, b) and De Iorio *et al.* (2005) to infer model parameters under a maximum likelihood framework. MIGRAINE was initially used considering an isolated population with a single past variation in population size and a generalized stepwise mutation model for microsatellite markers (Leblois *et al.*, 2014), and then with a time-constant model of two populations exchanging migrants. All details are provided in the Supporting Information (Appendix S1).

RESULTS

Mitochondrial analyses

A total of 23 mitochondrial DNA (mtDNA) *COI* haplotypes (720 bp) were found among the 135 *T. wilkinsoni* individuals (Supporting Information, Table S1). Haplotype diversity was higher in Turkey, Samos and Rhodes than in Crete. The number of haplotypes, haplotype diversity and nucleotide diversity are given for each sampling site in the Supporting Information (Table S1). All sequences were deposited in GenBank, with accession numbers MH092867–MH092889.

The haplotype network (Fig. 1B) showed two highly divergent haplotype groups (separated by 60–68 mutation steps), one corresponding to Crete and the other to Turkey, Samos and Rhodes. The intergroup K2P distances ranged from 8.9 to 10.2%. Two subgroups of haplotypes were identified within Crete, corresponding to eastern/central vs. western Crete, separated by five to nine mutations (0.6–1.1% K2P distances). The distances between the haplotypes found for *T. wilkinsoni* (from either Turkey or Cyprus) and European *T. pityocampa* ranged between 0.090 and 0.099, i.e. they were similar to distances obtained between Crete and Turkey. All pairwise distances are provided in the Supporting Information (Appendix S2).

Microsatellite analyses

Marker validation and genetic diversity

Null allele frequencies were > 10% in only three cases [Ppit09 and Ppit39 in Chania (CHA) and Ppit38 in Messeleroi (MES)]. After correction for multiple comparisons, all sampling sites were at HWE for all loci. Allelic richness and observed heterozygosities per population are given in the Supporting Information (Table S1). For details on sampling localities names, see Table S1.

Population genetic structure

In the PCA performed on the whole dataset, the first axis (PC1) explained 10.4% of the variance and separated individuals from Turkey, Samos and Rhodes from individuals from Crete, whereas the second axis (PC2; 5.6%) separated eastern/central from western Crete (Fig. 2).

STRUCTURE was applied on the whole dataset. Both Evanno's delta$K$ and the curve of LnProb($X|K$) suggested that the main patterns were found for $K = 2$ and $K = 3$ (Supporting Information, Fig. S1A). In the first case, one cluster corresponded to Crete and the other to all individuals from Samos, Rhodes and Turkey; for $K = 3$, the samples from Crete were split into two clusters, namely eastern/central vs. western regions (Supporting Information, Fig. S2A). In all cases, each individual was clearly assigned to a single cluster. Within Crete, the optimal number of clusters was $K = 2$, corresponding to eastern/central Crete vs. western Crete (Supporting Information, Fig. S2B). Within eastern/central and within western Crete, results strongly suggested the occurrence of a single cluster (Supporting Information, Fig. S2C, D).

The matrices of pairwise F_{ST} obtained with and without applying the ENA correction for the presence of null alleles are given in Table 1. These indices were significant for all pairs involving Istanbul (IST) and any site in Crete (all values > 0.44), and between western and eastern/central populations in Crete.

Demographic inferences in Crete

MIGRAINE analyses under the model with past variation in population size showed clear and significant signals of strong past population contraction for the two western sites (CHA and Rethimno [RET], $N_{ratio} < 0.1$), whereas no significant past change in population size was inferred for the eastern populations (Elounda [ELU], MES and Kastamonitsa [KAS]; N_{ratio} not significantly different from one). For all samples, relatively high *pGSM* values, varying from 0.48 to 0.68, were inferred (see details in Supporting Information, Table S2).

Concerning the past contraction detected in the western sites, current scaled population sizes (θ)

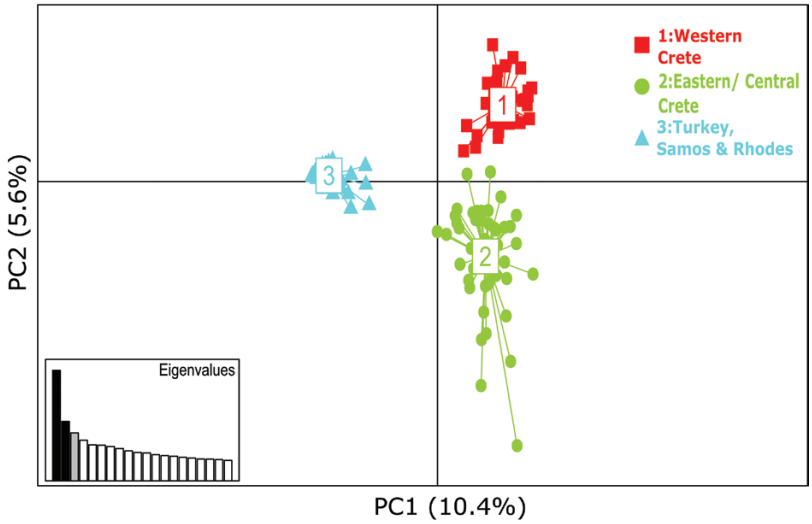

Figure 2. Graph of the first two axes from a principal component analysis of microsatellite genotypes of the whole dataset.

Table 1. Pairwise F_{ST} values without ENA (below the diagonal) and with ENA correction (above the diagonal)

Population	CHA	RET	KAS	MES	ELU	IST
CHA	–	**0.052317**	**0.185829**	**0.111495**	**0.152271**	**0.470925**
RET	**0.052194**	–	**0.197179**	**0.166140**	**0.202501**	**0.553309**
KAS	**0.185048**	**0.201870**	–	**0.100888**	**0.084347**	**0.533546**
MES	**0.111075**	**0.167298**	**0.096478**	–	**0.073887**	**0.456261**
ELU	**0.150487**	**0.207288**	**0.086299**	**0.071895**	–	**0.462611**
IST	**0.467571**	**0.553627**	**0.532703**	**0.453962**	**0.463111**	–

Estimates for which the 95% confidence intervals after 1000 bootstraps did not include zero are reported in bold. CHA, Chania; ELU, Elounda; ENA, excluding null alleles; IST, Istanbul; KAS, Kastamonitsa; MES, Messeleroi; RET, Rethimno.

were first inferred as being close to one in both cases with very good accuracy, which corresponds to a current effective population size, N, of 500 [95% CI, 300–900] individuals for a mutation rate of 5×10^{-4}. Second, the scaled timing ($D = T/4N$) of the contraction was inferred with much less precision, ~6 [95% CI, 2–15] and 12 [95% CI, 2–23] depending on the chosen sample. This corresponded to contractions beginning ~3000 [95% CI, 600–14 000] and 6000 [95% CI, 600–20 000] years ago for RET and CHA, respectively. Third, very large ancestral scaled population sizes were inferred, but with very low precision, as the corresponding 95% CIs were very large and corresponded to ancestral population sizes varying from a few thousands to millions of individuals (Supporting Information, Table S2).

For the eastern sites of KAS, ELU and MES, where stable population sizes were inferred, MIGRAINE estimated the θ parameter with good precision (values of 1.3 [0.85–2.0], 2.1 [1.5–2.9] and 2.7 [1.8–3.8] for KAS, ELU and MES, respectively). These correspond to population sizes between 650 and 1350 [425–1900] individuals.

We also ran MIGRAINE under the two-population model with migration, using each pair of sites and then using a pool of western vs. a pool of the eastern samples, and obtaining good estimates for the latter pooled analysis (Table 2). First, relative population size Q_1 was inferred to be 0.17 [0.09–0.28; significantly < 0.5], suggesting that the western group had a three to ten times smaller size than the eastern one. Second, backward migration rates M_1 and M_2 were inferred to be symmetric (~2–2.5 effective migrants per generation in both directions), a relatively small value compared with the estimated total effective population size (3800 [3100–4600] individuals, i.e. a θ value of 7.6 [6.2–9.2]). Similar patterns of asymmetric population size between eastern and western Crete and moderate gene flow between regions are found in most pairwise analyses of west vs. east sampling sites (i.e. RET vs. KAS, RET vs. ELU, CHA vs. KAS and CHA vs. ELU). In all pairwise comparisons of sites within region (e.g.

Table 2. MIGRAINE outputs for the model of two populations exchanging migrants expressed by M_1, M_2 (the scaled migration rate), $2N_{mu}$ (the scaled total population size) and Q_1 (the relative size of populations)

Population pair	Parameter			
	M_1	M_2	$2N_{mu}$	Q_1
Between west and east				
RET vs. KAS	1.3 [0.4–2.5]	1.7 [3.2×10^{-9}–3.7]	6.5 [4.5–9.0]	0.2 [0.03–0.4]
RET vs. ELU	1.2 [0.6–2.2]	1.3 [0.4–2.8]	6.6 [5.2–8.5]	0.2 [0.07–32]
RET vs. MES	1.5 [0.8–2.8]	2.5 [0.2–10]	9.0 [6.5–13]	0.07 [0.012–0.2]
CHA vs. KAS	0.8 [0.3–1.8]	0.8 [0.3–2.1]	5.4 [4.2–7.1]	0.4 [0.2–0.6]
CHA vs. ELU	1.3 [0.5–2.5]	1.4 [0.6–2.9]	6.6 [5.3–8.3]	0.2 [0.1–0.4]
CHA vs. MES	3.2 [1.6–5.4]	7.4 [NA–140]	10 [7.3–15]	0.02 [NA–0.1]
West vs. east (pooled analysis)	2.1 [1.2–3.4]	2.3 [0.9–4.5]	7.6 [6.1–9.2]	0.2 [0.09–0.3]

The 95% confidence intervals are presented in square brackets. CHA, Chania; ELU, Elounda; IST, Istanbul; KAS, Kastamonitsa; MES, Messeleroi; NA, not available; RET, Rethimno.

within eastern or within western Crete), MIGRAINE did not allow good parameter estimations (Supporting Information, Table S3). Nevertheless, our analyses globally showed larger migration rates within eastern and within western Crete than between regions.

DISCUSSION

A HIGHLY DIVERGENT ENDEMIC LINEAGE IN CRETE

The winter pine processionary moth is a Mediterranean species complex including *T. pityocampa* in the west and *T. wilkinsoni* in the east (Turkey, Syria, Lebanon, Israel and Cyprus; Simonato *et al.*, 2007). Kerdelhué *et al.* (2009), using only mitochondrial sequences, showed that Cretan individuals appeared as the sister group of *T. wilkinsoni* samples. Here, we used an extensive sampling over Crete, analysed a longer mtDNA fragment together with 12 microsatellite loci developed for *T. pityocampa* (note that only ten loci could be used for individuals outside Crete). Our results confirmed that the populations occurring in Crete are highly divergent whatever the markers used, with the *COI* distances being as high as the *T. pityocampa*–*T. wilkinsoni* divergence, and the pairwise F_{ST} values reaching extreme values of ~0.5. Moreover, two of the microsatellite loci used in Crete failed to amplify the *T. wilkinsoni* samples from Turkey and the studied islands. Given the strength of the estimated divergence, we suggest that the Cretan lineage could be considered a distinct, allopatric species. However, a detailed morphometric study and a thorough phylogenetic analysis including every clade and lineage identified within the *T. wilkinsoni*–*T. pityocampa* complex are needed before any reconsideration of their taxonomic status.

The microsatellite markers that we used showed a very low allelic diversity in Turkey; therefore, we failed to develop demographic inferences including all samples and to estimate the age of the divergence based on the nuclear data. Interestingly, however, Kerdelhué *et al.* (2009) estimated the age of the Cretan subclade to 5.3 Myr. This estimate is fully consistent with the genetic distance (~10%) we obtained here using a 720 bp *COI* fragment and the arthropod mitochondrial divergence rate of 2% per Myr (Brower, 1994; but see Papadopoulou, Anastasiou & Vogler, 2010). The split of Cretan vs. Turkish subclades in *T. wilkinsoni* coincides with the Messinian Salinity Crisis that profoundly modified the land connectivity in the region. Movements between the continents and Crete were favoured when sea level was at its lowest level and land bridges occurred, either between Crete and Peloponnese (Kyriazi *et al.*, 2013; Sagonas *et al.*, 2014) or between Crete and Turkey via the Karpathos–Rhodes bridge (see maps in the study by Parmakelis *et al.*, 2006), as suggested for the trapdoor spiders (Kornilios *et al.*, 2016). Our results suggest that *T. wilkinsoni* is one of the rare examples of migration crossing the MAT after its formation, as they suggest a colonization of Crete from Turkey (Poulakakis *et al.*, 2015). Subsequently, Crete became isolated after the refilling of the Mediterranean Sea, and it has remained isolated since then. Consequently, Cretan populations were trapped and have remained isolated for the last 5.3 Myr. We can thus confidently hypothesize that the endemic lineage diverged through vicariance owing to long-term geographical isolation after the end of the Mediterranean refilling. It is likely that the Cretan lineage could also occur in some close islands, such as Gavdos, and further sampling will be needed to decipher the presence–absence pattern of species and lineages in other parts of the Aegean Archipelago. In particular, the populations of Karpathos, which has

been isolated from Rhodes for the last 3.5 Myr, would need to be analysed further. A large-scale study of *T. wilkinsoni* and *T. pityocampa* in the Aegean region is needed now.

Phylogeography and recent demographic history in Crete

In each lineage studied (*T. wilkinsoni* and the Cretan subclade), local diversification corresponded to recent events. The haplotypes found in Turkey and the islands of Samos and Rhodes differed by a few mutations at most, whereas the maximum distance found between haplotypes identified in Crete was 1.2%, suggesting that diversification events occurred during the Pleistocene. During this period, the glacial–interglacial cycles led to oscillating climate conditions and sea level fluctuations (Kornilios *et al.*, 2016). Consequently, the islands located close to the continent could be connected to the shore during glacial maxima and subsequently disconnected, or even submerged, during interglacials. Consistently, we expect to find local diversity in the southernmost regions that could have acted as glacial refugia, whereas northern territories were recolonized only when climate was suitable (interglacials; Hewitt, 2000).

The patterns we found in Turkey and the neighbouring islands of Samos and Rhodes were fully consistent with these expectations, with very high diversity on both islands and in southern Turkey, and a reduced number of haplotypes in northwestern Turkey (see Simonato *et al.*, 2007; İpekdal *et al.*, 2015). However, the situation in Crete was somewhat different, because this region remained fully isolated from all other places throughout the Quaternary climatic oscillations. The populations trapped on such islands had to endure the recurrent climate changes locally, possibly by shifts in elevation along mountain slopes or by surviving in suitable microhabitats. One would expect that the populations went through strong bottlenecks and that genetic drift and local adaptations would be the main evolutionary drivers. We found signs of local geographical diversification, with an east vs. west differentiation identified using both mtDNA and nuclear markers. Several studies pointed to relatively old east–west divergence within Crete, possibly attributable to Pliocene tectonic events (Velonà *et al.*, 2010; Kornilios *et al.*, 2016) or to the colonization of Crete by already differentiated lineages (Thanou *et al.*, 2017). For *T. wilkinsoni*, however, the east–west differentiation was much more recent and could be attributable to separate refugial areas in the island and/or a role of the barrier to dispersal played by the mountain ranges (e.g. mountain Ida) in central Crete.

The demographic inference clearly suggested distinct histories within each region of Crete. The eastern group revealed a stable population size over time, whereas the western group experienced a strong bottleneck event some thousands of years ago, which could correspond to low survival during the Last Glacial Maximum. Consistently, the current population size was estimated to be up to three times lower than in the east. Interestingly, several studies highlighted marked climatic differences between eastern and western parts of the island (Thanou *et al.*, 2017), with a warmer and dryer climate in the east. The gradual integration of satellite islets into the main body of Crete further promoted genetic isolation, enhancing genetic differentiation (Dermitzakis & De Vos, 1987). Our results thus suggest that climatic and topographic conditions in the eastern part of Crete offered more favourable environmental conditions and allowed the species to survive locally without profound demographic changes, whereas conditions were not optimal in western Crete.

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AUTHOR CONTRIBUTIONS

D.P., F.A.A., C.K. and D.N.A. designed the study; D.P., D.N.A. and K.I. carried out the sampling; D.P. and L.S. generated the molecular data; D.P. and R.L. conducted the analyses; D.P., R.L., C.K. and D.N.A. wrote the manuscript, which was revised and approved by all authors.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Appendix S1. Parameters for STRUCTURE and MIGRAINE implementations.
Appendix S2. Pairwise distance matrix between the haplotypes and the outgroups of the mitochondrial DNA fragment based on a Kimura-2 parameter model.
Table S1. Sampling localities, number of analysed individuals, indices of population genetic diversity of *COI*, allelic richness and observed heterozygosity from microsatellite data.
Table S2. MIGRAINE outputs of past demographic analysis expressed by the geometric distribution of mutation sizes (*pGSM*), the current scaled population size (θ), scaled time of occurrence of past variation (D), ancestral scaled population size (θ_{anc}) and N_{ratio} (N/N_{anc}). The 95% confidence intervals are given in square brackets.
Table S3. MIGRAINE outputs under the model of two populations exchanging migrants expressed by M_1, M_2 (the scaled migration rate), $2N_{mu}$ (the scaled total population size) and Q_1 (the relative size of populations). The 95% confidence intervals are presented in square brackets.
Figure S1. Curves of Evanno's delta$K$ (left) and LnProb[Data$|K$] (right) as a function of K inferred from STRUCTURE simulations. A, whole dataset, i.e. 135 individuals and ten loci, with K varying from one to 16. B, Crete dataset, i.e. 89 individuals and 12 loci, with K varying from one to ten. C, eastern/central Crete (47 individuals). D, western Crete (42 individuals).
Figure S2. Graphical representations of nuclear genetic clusters for samples from the whole dataset and from the dataset restricted to Cretan localities, inferred from STRUCTURE simulations. A, assignment of 135 individuals from the whole dataset for $K = 2$, $K = 3$ and $K = 4$. B, assignment of 89 Cretan individuals for $K = 2$. C, assignment of 47 individuals of eastern/central Crete for $K = 2$ and 42 individuals of western Crete populations for $K = 2$ and $K = 3$, respectively.