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Phylogeographical patterns in *Coenosia attenuata* (Diptera: Muscidae): a widespread predator of insect species associated with greenhouse crops

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The tiger-fly *Coenosia attenuata* is a globally widespread predatory fly which is not only associated with greenhouse crops, but also occurs in open fields. It is a potential control agent against some of the more common pests in these crops. Assessing the genetic structure and gene flow patterns may be important for planning crop protection strategies and for understanding the historical processes that led to the present distribution of genetic lineages within this species. In the present study, the phylogeographical patterns of this species, based on mitochondrial cytochrome oxidase I and nuclear white and elongation factor-1α genes, are described, revealing relatively low genetic diversity and weak genetic structure associated with a recent and sudden population expansion of the species. The geographical distribution of mitochondrial haplotypes indicates the Mediterranean as the most likely region of origin of the species. Some dispersal patterns of the species are also revealed, including at least three independent colonizations of North and South America: one from Middle East to North America with a strong bottleneck event, another from Europe to South America (Chile), with both likely to be a result of unintentional introduction, and a third one of still undetermined origin to South America (Ecuador). © 2014 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, **114**, 308–326.

ADDITIONAL KEYWORDS: Coenosiinae – introductions – mitochondrial DNA – nuclear genes – tiger-fly.

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INTRODUCTION

Studies of the genetic structure and dispersal patterns of species with wide distributions, whose dispersal may have been facilitated by humans, has proved crucial for understanding the causes of spread for many crop pests and diseases (Magiorkinis *et al*., 2009; Faria *et al*., 2012; Karsten *et al*., 2013), as well as new adaptations, such as resistance to pesticides (Alvarez *et al*., 2007). These types of studies are more scarce for natural enemies of pest species (but see Gebiola *et al*., 2014), although they may be important when planning crop protection strategies that involve inoculative, and especially inundative (Eilenberg, Hajek & Lomer, 2001), release of commercially produced biocontrol agents of unknown origin because non-native genotypes can be less adapted to the environment where they are going to be released and they can also originate the loss of genetic variation (Laikre *et al*., 2010). High dispersal does not always imply low genetic structure, as seen, for example, in the migratory locust, *Locusta migratoria* (L.), which, despite its high migratory abilities, shows high genetic differentiation between different geographical regions (Ma *et al*., 2012). On the other hand, even in cases where dispersal events are rare, they may be sufficiently frequent to homogenize the genetic composition of populations. Besides dispersal, other factors may shape the distribution of genetic variation across populations, namely geographical isolation, genetic drift and founder effects, selection, and range expansions and contractions (Avise, 2000; Wilson *et al*., 2009). By describing the population structure and patterns of gene flow, phylogeographical studies are important for understanding the historical processes that have led to the present distribution of genetic lineages within species (Avise, 2000).

The tiger-fly *Coenosia attenuata* Stein is a polyphagous predator (Kühne, 2000; Prieto, Figueiredo & Mexia, 2005) that has been recognized as an effective biological control agent, especially for insect pests of protected crops (Kühne, 2000; Prieto *et al*., 2005; Martins *et al*., 2012; Mateus, 2012; Pohl *et al*., 2012). The adults of *C. attenuata* are the only known predators of the adult stage of several insect pests of those crops, such as whiteflies and leafminers (Prieto *et al*., 2005; Mateus, 2012). They may attack and kill their prey without feeding (Martinez & Cocquempot, 2000). In the first half of the 20th Century, *C. attenuata* was reported as being present in the Palaearctic, Afrotropical, Oriental, and Australian regions (Hennig, 1964). Subsequently, *C. attenuata* has been expanding its distribution range and was detected in the Nearctics and Neotropics (Table 1). *Coenosia attenuata* is considered to be native to the Paleotropical region (Pohl *et al*., 2012) where Hennig (1964) reported a widespread distribution. However, other studies noted a Palearctic or Mediterranean origin (Martinez & Cocquempot, 2000; Téllez & Tapia, 2006; Ugine *et al*., 2010), probably misunderstanding Hennig (1964), who only reported its occurrence in the Palearctic (Table 1) but did not mention this region as the place of origin of the species. Its polyphagy, as well as the high diversity of host plants, the increasing trade of vegetable and ornamental plants (Kühne, Schiller & Dahl, 1997; Kühne, 2000; Salas & Larraín, 2011; Martins *et al*., 2012; Mateus, 2012; Pohl *et al*., 2012), and its tolerance to high temperatures, such as those recorded inside greenhouses during the Mediterranean summer (Gilioli, Baumgärtner & Vacante, 2005), are included among the factors that may be responsible for the successful worldwide dispersal of this predatory species. The patterns of genetic diversity of *C. attenuata* across its distribution range may give indication about the origin and range dynamics of the species.

Mitochondrial (mt)DNA is the most used genetic marker in phylogeographical studies because of its rapid mutation rate, its short coalescent time as a result of haploidy and maternal inheritance of this genome, and its lack of recombination (Avise, 2000; Ballard & Whitlock, 2004). However, the mtDNA history may not always reflect the history of the species because the analysis of a single molecule may be affected by random sampling of coalescent processes, selective sweeps or introgression (Ballard & Whitlock, 2004). Independent data from nuclear DNA is thus valuable to allow a more complete interpretation of phylogeographical patterns.

Assessing the levels of divergence between closely-related species is essential for understanding the patterns of genetic variation within a species (Koutroumpa *et al*., 2013; Pfeiler *et al*., 2013). Because no phylogenetic study to date has included the species *C. attenuata*, we evaluated the position of the haplotypes found in relation to haplotypes from other *Coenosia* species: *Coenosia humilis* Meigen, *Coenosia tigrina* (Fab.), and *Coenosia testacea* (Robineau-Desvoidy), and from two other calyptrate genera, *Delia* and *Anthomyia* (family Anthomyiidae).

In the present study, we analyze the phylogeography of *C. attenuata* at a global scale, based on one mitochondrial gene and two nuclear genes, aiming to test hypothesis about the origin of the species (Palaearctic, and eventually Mediterranean), colonization patterns (either natural or human-mediated), and demographic events (expansions and inferred times).

Table 1. Geographical distribution of *Coenosia attenuata*

Zoogeographical region	Country/region	References
Palearctic		
Mediterranean basin	Algeria	Hennig (1964)
	Cyprus	Pont (1986)
	Egypt	Hennig (1964)
	France	Martinez & Cocquempot (2000)
	Greece	Pont, 2004
	Israel	Kugler (1969), Pont (1986)
	Italy	Colombo & Eördegh (1990)
	Sicily	Present study*
	Libya – Cyrenaica	Hennig (1964)
	Malta	Ebejer & Gatt (1999)
	Morocco	Pont (1986)
	Portugal	Prieto (2002); Prieto *et al*. (2005)
	Spain	Hennig (1964), Rodríguez-Rodríguez & Aguilera (2002), Téllez & Tapia (2006)
	Syria	Hennig (1964)
	Turkey	Pohl, Uygur & Sauerborn (2003), Pohl *et al*. (2012)
Europe (excluding Mediterranean countries)	Germany	Schrameyer (1991), Kühne (2000)
	The Netherlands	Blind (1999)
	Slovakia	Suvák (2008)
Asia	Afghanistan	Hennig (1964)
	China – Xinjiang region	Xue & Tong (2003)
	Iran	Shirazi, Kaviani & Parchami-Araghi (2010)
	Iraq	Pont (1986)
	Tajikistan	Hennig (1964)
Macaronesia	Azores Islands – Terceira	Prieto *et al*. (2005)
	– San Miguel	Borges (2008)
	Canary Islands	Hennig (1964)
	Cape Verde Islands	Hennig (1964)
	Madeira Islands	Pont (1986), Prieto *et al*. (2005)
Ethiopian	South Africa	Hennig (1964)
	Yemen – Sokotra Island	Hennig (1964)
Oriental	China: Xishuangbanna (Yunnan Province)	Xue & Tong (2003) (*C. attenuata* ssp. *brunea*)
	Indonesia: Lombok and Flores Isl.	Hennig (1964)
	Taiwan	Hennig (1964)
Australian	Australia – Sydney	Hennig (1964)
Neartic	USA: New York, Maine, Illinois, Connecticut, California	Hoebeke *et al*. (2003), Sensenbach (2004)
	Canada: Québec, Ontario	Roy & Fréchette (2006)
Neotropical	Chile	Couri & Salas (2010)
	Colombia	Pérez (2006)
	Costa Rica	Hernández (2008)
	Ecuador	Martinez-Sánchez, Marcos-Garcia & Pont (2002)
	Peru	Martinez-Sánchez *et al*. (2002)

*As far as we are aware, this is the first reference of *Coenosia attenuata* in Sicily.

MATERIAL AND METHODS

SAMPLING

A total of 150 specimens of *C. attenuata* from 52 localities of 17 countries were analyzed (Fig. 1, Table 2), covering a large area of the worldwide distribution range of this species (Asia, Australia, Europe, Macaronesian islands, North Africa, and North and South America). Most specimens were captured in greenhouses directly into boxes and plastic

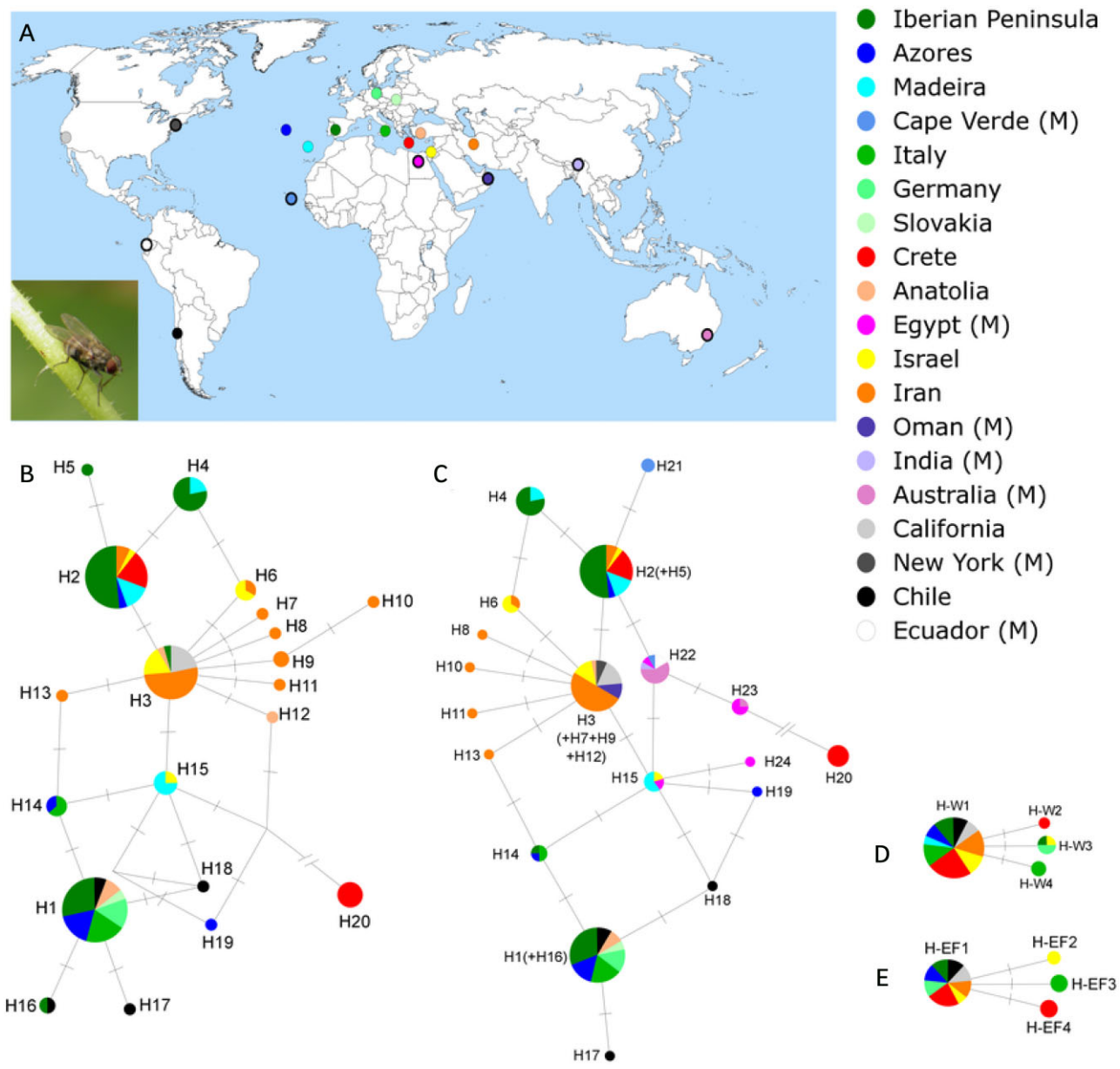

Figure 1. A, sampling locations of *Coenosia attenuata*. Circles with black margins and the the letter M represent the museum sample locations. B, C, D, E, haplotype networks for *Coenosia attenuata* for cytochrome oxidase I (COI) (647 bp) (B), COI-1 + COI-2 (270 pb) (C), white (D) and EF-1α (E). The size of the circular nodes (haplotypes) is proportional to the number of individuals (or haplotypes, in nuclear genes). Tick marks on branches indicate the number of mutational steps between nodes, except in H20, separated by seven mutational steps in (B) and by three mutational steps in (C).

bags or by using sticky traps, and then preserved in absolute ethanol. Seventeen specimens came from the Natural History Museum in London (NHM) collection. They had been collected between 1935 and 2002 in Cape Verde islands, Ecuador, Egypt, India, Oman, and USA, and have been preserved dried. Eight specimens from NHM, collected in Australia and identified as *Coenosia subvittata* (Malloch) (four male specimens) and *Coenosia imitatrix* (Malloch) (four female specimens), were also used. Five specimens of other species were also included to be used as outgroups: three belonging to two other species of the same genus (one specimen of *C. humilis*, and two specimens of *C. tigrina*), and the others belonging to two different genera (one *Anthomyia* sp. and one *Delia* sp.) from another dipteran family, Anthomyiidae.

Table 2. Sampling localities, collection date, and number of individuals (*N*) of *Coenosia attenuata* analyzed

Code	Locality	Country	Collection date	*N* COI	COI haplotypes†	N W	White accession numbers	N EF	EF accession numbers
AGU	Sto André, Aguçadoura (Póvoa do Varzim) – greenhouses	Portugal (mainland)	16 November 2010	2	H4				
AMO	Amorim (Vila do Conde) – greenhouses	Portugal (mainland)	16 November 2010	3	H2				
ALG	Estói (Faro) – greenhouses	Portugal (mainland)	3 March 2011	2	H2	1	KJ652092; KJ652093	1	KJ652114
CAMP	Campelos (Torres Vedras) – greenhouses	Portugal (mainland)	22 August 2013	1	H2				
CAT	Silveira (Torres Vedras) – greenhouses	Portugal (mainland)	3 May 2010; 20 September 2010	5	H1(1),H2(2),H4(2)				
HORTI	Cilha Queimada (Alcochete) – greenhouses	Portugal (mainland)	4 July 2005	1	H1				
LIS	Tapada da Ajuda, Alcântara (Lisboa) – vineyard; eucalyptus	Portugal (mainland)	June to September 2012; August 2013	2	H2,H3				
ODE	Fataca (Odemira) – open high tunels	Portugal (mainland)	1 July 2011	2	H1				
RAM	Ramalhal (Torres Vedras) – open high tunels	Portugal (mainland)	10 July 2013	1	H16				
SALV	Foros de Salvaterra (Salvaterra Magos) – processing tomato	Portugal (mainland)	21 August 2013	1	H1				
SANT	Santo Antão (Batalha) – greenhouses	Portugal (mainland)	26 July 2011	1	H1				
TAV	Tavira (Tavira) – citrus groves	Portugal (mainland)	15 November 2012	2	H2				
ZAMB	Carvalhal, Zambujeira do Mar (Odemira) – greenhouses	Portugal (mainland)	31 May 2013	1	H2				
ALM	Murcia (Murcia) & Almeria (Andalusia) – greenhouses	Spain (mainland)	21–22 June 2011	7	H2(3),H4(3),H5(1)	1	KJ652094		
GIRMOR	Girona (Catalonia) – greenhouses	Spain (mainland)	August 2012	4	H1(3),H2(1)				
ANG	Posto Santo, Angra Heroísmo (Terceira Island) – greenhouses	Portugal, Azores	September 2012 to July 2013	1	H19				
CA-VP	Calheta (São Miguel Island) – greenhouses	Portugal, Azores	14 January 2012	2	H1(1),H14(1)				
RP-JL	Calheta (São Miguel Island) – greenhouses	Portugal, Azores	14 January 2012	4	H1(3),H2(1)	1	KJ652112	1	KJ652115
SR-JB	S. Roque (São Miguel Island) – greenhouses	Portugal, Azores	14 January 2012	2	H1				
MAD	Ponta do Sol & Calheta (Madeira Island) – greenhouses	Portugal, Madeira	19 April 2012; 3 May 2012	9	H2(4),H4(2),H15(3)	1	KJ652095		
CAL	Lombardy – greenhouses	Italy (mainland)	October to November 2010	5	H1(3),H14(2)	1	KJ652096	1	KJ652116; KJ652117
SIC	Acate & Marina di Ragusa, Ragusa – greenhouses	Italy, Sicily	12 October 2012	4	H1	1	KJ652097		
KL	Kleinmachnow – greenhouses	Germany	17 September 2010	5	H1	1	KJ652098	1	KJ652118
ESLOV	Košice – greenhouse	Slovakia	8 January 2013	2	H1				
CRE1	Arvi – greenhouses	Greece, Crete	28 May 2012	4	H2(2),H20(2)	3	KJ652099–KJ652102	2	KJ652119; KJ652120
CRE2	Ierapreta – greenhouses	Greece, Crete	29 May 2012	2	H20				
CRE3	Stomio – greenhouses	Greece, Crete	5 June 2012	5	H2(4),H20(1)	1	KJ652103	1	CRE3_5EF‡
ANT	Antalya – greenhouses	Turkey	15 October 2010	5	H1(3),H3(1),H12(1)				
ISRA	Nahal Ye'elim – open field	Israel	23 April 2012	2	H3(1),H6(1)	1	KJ652104; KJ652105	1	KJ652121; KJ652122
ISRB	Nahal Qedem – open field	Israel	April 2012	1	H6				
ISRC	En Boqeq – open field	Israel	21 April 2012	2	H2(1),H3(1)				
ISRD	Hazeva Reservoir – open field	Israel	22 April 2012	3	H3	1	KJ652106		
IRA_A	Varamin-Research Station (Tehran) – greenhouses	Iran	31 August 2010	2	H3(1),H8(1)	1	KJ652107	1	KJ652123
IRA_B	Takhteh Chenar, Pakdasht (Tehran) – greenhouses	Iran	16 December 2010	2	H3(1),H7(1)				

IRA_C	Sharifabad, Pakdasht (Tehran) – greenhouses	Iran	1 July 2012	2	H2(1), H3(1)			
IRA_D	Karimabad, Pakdasht (Tehran) – greenhouses	Iran	1 July 2012	2	H3(1), H13(1)			
IRA_E	Ghaleh Khajeh, Javadabad, Varamin (Tehran) – greenhouses	Iran	14 June 2012	2	H3(1),H9(1)			
IRA_F	Mohammadabad Arabha, Pishva, Varamin (Tehran) – greenhouses	Iran	14 June 2012	2	H3			
IRA_G	Tiran (Isfahan) – greenhouses	Iran	1 June 2012	1	H6			
IRA_H	Falavarjan (Isfahan) – greenhouses	Iran	30 May 2012	1	H3			
IRA_I	Dehaghan (Isfahan) – greenhouses	Iran	31 May 2012	1	H3			
IRA_J	Shahreza (Isfahan) – greenhouses	Iran	1 October 2010	1	H11			
IRA_K	Shahreza (Isfahan) – greenhouses	Iran	31 May 2012	1	H2			
IRA_M	Tehran-BCR Dept. Campus (Tehran) – green area	Iran	3 July 2012	5	H3(3),H9(1),H10(1)	1	KJ652108		
CALIF	Davis (California) – greenhouses	USA	2 August 2012	5	H3	1	KJ652109	1	KJ652124
CHI	Coquimbo – greenhouses	Chile	February to March 2011	5	H16(1),H17(1),H18(1)	1	KJ652110	1	KJ652125
Museum samples									
AUS*	Newport (New South Wales)	Australia	November 1972 to January 1973	8	H22(7),H23(1)‡				
CV	Rib.Lagoa & P.Vaz (Maio Isl.); Rib. Brava (S.Nicolau Isl.)-open field	Cape Verde	3 February 1954	3	H21(2),H22‡				
EGI	Siwa	Egypt	April to May 1935	6	H15(1),H22(1), H23(3),H24(1) 3				
EQU	Atuntaqui (Imbabura) – greenhouses	Ecuador	3 June 1999	2	H22‡				
IND	Tinsukia (Assam)	India	26 March 1944	1	H22‡				
NY	East Syracuse (New York) – greenhouses	USA	28 August 2002	2	H3				
OMA	Fossil Valley (= Jebel Huwayya) (Buraimi) – water trap	Oman	23 March 2000	3	H3				
Other species									
C. humilis									
CHU_EstG1	Silveira (Torres Vedras) – greenhouses	Portugal (mainland)	28 February 2013	1	CHU_EstG1	1	KJ652111		
C. tigrina									
CTI_ANGPR1	Ribeirinha, Angra Heroísmo (Terceira Island) – greenhouses	Portugal, Azores	November to December 2012	1	CTI_ANGPR1				
CTI_FA1	Fataca (Odemira) – open high tunels	Portugal (mainland)	5 June 2013	1	CTI_FA1				
Anthomyia sp.									
ANT_IRAL1	Karaj-Hashtgerd (Alborz) – greenhouses	Iran	24 May 2012	1	ANT_IRAL1	1	KJ652113	1	KJ652126
Delia sp.									
DEL_ANGPS1	Angra Heroísmo (Terceira Island) – greenhouses	Portugal, Azores	1 July 2013	1	DEL_ANGPS1				

GenBank accession numbers for the three genes are also indicated.

*Identified previously as *C. subvittata* (males) and *C. imitatrix* (females).

†The GenBank accession numbers for these haplotypes are: KJ585390 to KJ585409 (H1 to H20); KJ585410 (CHU_EstG1); KJ585411 (CTI_ANGPR1); KJ585412 (CTI_FA1); KJ585413; (DEL_ANGPS1); KJ585414 (ANT_IRAL1).

‡Some sequences (H21 to H24 for COI-1 + COI-2 and CRE3_5EF for elongation factor) were not submitted to GenBank as a result of small size (< 200 bp) and are shown in the Supporting information (Fig. S4).

DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING

The head and thorax of the recently collected specimens were used for DNA extraction with EZNA® Tissue DNA Isolation kit (Omega Bio-Tek) in accordance with the manufacturer's instructions. Initially, the tissues were macerated inside each tube with the tip of a pipette, and then left digesting overnight. For the dried museum samples, single middle or front legs were used for DNA extraction with DNeasy Blood & Tissue Kit (Qiagen), and the elution was carried out in a reduced volume of 50 µL of buffer AE that was reloaded in the spin-column once for higher recovery of DNA.

A fragment of approximately 800 bp of cytochrome oxidase I (COI) mitochondrial gene was amplified by the polymease chain reaction (PCR) and for all the non-museum specimens (125 *C. attenuata* and five specimens from four outgroups species) (Table 2) using primers C1-J-2195 (5′-TTGATTTTTT GGTCATCCAGAAGT-3′) and TL2-N-3014 (5′-TCCAA TGCACTAATCTGCCATATTA-3′) (Simon *et al*., 1994). DNA of the 25 dried museum samples was degraded and new primers were designed for amplification of two smaller fragments of COI (COI-1 and COI-2), one with 180 bp, using primers C1-J-2195 and OXIcat183R (5′-CCTACAGTAAATATATGATGAG-3′), and the other with 200 bp, using primers OXIcat583F (5′-CTTTTTAGGATTAGCAGGAATACC-3′) and TL2-N-3014. These were designed based on the COI sequences obtained for the field collected samples and included most of the variable positions, mostly located near the 5′ end and the 3′ end of the COI fragment.

A fragment of exon 3 of the white nuclear gene (approximately 600 bp) was sequenced for 19 individuals (Table 2) using primers WEC-F21 (5′-GTTTGTGGCGTAGCCTATCC-3′) and WEC-R12 (5′-AATGTCACTCTACCYTCGGC-3′) (Ready *et al*., 2009) and a fragment of the elongation factor-1α (EF-1α) nuclear gene (approximately 700 bp) was amplified for 12 individuals using primers EF0 (5′-TCCGGATGGCAYGGCGAGAAYATG-3′) and EF2 (5′-ATGTGAGCAGTGTGGCAATCCAA-3′) (Villablanca, Roderick & Palumbi, 1998).

PCR conditions for COI comprised a 12.5-µL reaction containing 1 × Colorless GoTaq® Flexi Buffer, 0.1 mM dNTPs, 2 mM $MgCl_2$, 1 µM of each primer, 0.02 U GoTaq® DNA Polimerase (Promega), and approximately 30 ng of DNA, amplified by an initial denaturation step at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 45 s and extension at 72 °C for 1 min, with a final extension period at 72 °C for 7 min. For the two smaller fragments, COI-1 and COI-2, PCR conditions comprised a 12.5-µL reaction containing 1 × Colorless GoTaq® Flexi Buffer, 0.1 mM dNTPs, 1.8 mM $MgCl_2$, 0.6 µM of each primer, 0.04 U GoTaq® DNA Polimerase (Promega), and approximately 30 ng of DNA, amplified by an initial denaturation step at 94 °C for 1 min, followed by 45 cycles of denaturation at 94 °C for 30 s, annealing at 57 °C for 1 min and extension at 72 °C for 45 s, with a final extension period at 72 °C for 7 min. For the White gene and EF-1α nuclear genes, PCR conditions comprised a 10-µL reaction containing 1 × Colorless GoTaq® Flexi Buffer, 0.25 mM dNTPs, 2 mM $MgCl_2$, 0.5 µM of each primer, 0.05 U GoTaq® DNA Polimerase (Promega), and approximately 30 ng of DNA, amplified by an initial denaturation step at 94 °C for 1 min, followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 45 s and extension at 72 °C for 45 s, with a final extension period at 72 °C for 5 min. For the nuclear genes, several nonspecific fragments were amplified in the PCR; thus, the desired fragment of the expected size was isolated from the agarose gel for each individual and then reused as template in a new PCR reaction with the same conditions as described above.

As a result of the risk of contamination between samples when using museum material, precautions were taken to avoid contamination in every step of the protocol, namely different PCR reagent stocks and replicated PCR reactions.

PCR products were purified with Sureclean (Bioline) in accordance with the manufacturer's instructions. Forward and reverse sequences were sequenced by external companies, either Stabvida (http://www.stabvida.com), Macrogen (http://dna.macrogen.com) or Beckman Coulter (https://www.beckmancoulter.com).

SEQUENCE EDITING AND PHYLOGENETIC ANALYSIS

Forward and reverse sequences were aligned and edited in SEQUENCHER, version 4.0.5 (Gene Codes Corporation) and the consensus sequences were aligned in the sequence alignment editor BIOEDIT, version 7.0.9 (Hall, 1999). Two sequences from another study (Kutty *et al*., 2008) available from GenBank were also included in the analysis, identified as *C. tigrina* (accession number FJ025606.2) and *C. testacea* (accession number FJ025605.1). Input file conversions were conducted in CONCATENATOR, version 1.1.0 (Pina-Martins & Paulo, 2008) and PGDSPIDER, version 2.0.3.0 (Lischer & Excoffier, 2012). MEGA, version 5.2 (Tamura *et al*., 2011) was used to obtain the translation to amino acids using the invertebrate genetic code and to calculate pairwise uncorrected genetic distances. The phylogenetic relationships based on mitochondrial COI and the two nuclear genes were inferred by

Bayesian inference using MrBayes, version 3.2.2 (Ronquist *et al*., 2012). The posterior probabilities of the phylogenetic trees were estimated by a Metropolis-coupled, Markov chain Monte Carlo sampling algorithm. Stationarity of the likelihood scores of the trees with the generation time was checked using TRACER, version 1.5 (Rambaut & Drummond, 2007); a total of 2×10^6 generations were sampled every 1500 generations with a 'burn-in' of 1000. Maximum likelihood analysis was performed using PAUP, version 4.0.d99 (Swofford, 2002), with a heuristic search performed using 100 replicates, and branch support was obtained by performing 1000 replicates of nonparametric bootstrap. For each dataset, the best fit model of sequence evolution was estimated under the Akaike information criterion using JMODELTEST, version 2 (Posada & Crandall, 1998) and MRMODELTEST, version 2 (Nylander, 2004).

Genetic diversity and population differentiation

Haplotype and nucleotide diversities were calculated in DNASP, version 5.10.01 (Librado & Rozas, 2009) for all genes. Mitochondrial data was used to test the level of population structure among regions by performing an analysis of molecular variance with 10 000 permutations in ARLEQUIN, version 3.5 (Excoffier & Lischer, 2010). To test for a correlation between genetic and geographical distances, a Mantel test with 10 000 permutations was performed in ARLEQUIN. Geographical distances were measured as great circle distances between the geographical coordinates of the sampling location (or of the centroid, if there were different sampling points) using GPS VISUALIZER (http://www.gpsvisualizer.com/calculators). Median-joining haplotype networks for the mitochondrial and nuclear haplotypes were obtained using NETWORK, version 4.6.1.0 (http://www.fluxus-engineering.com; Bandelt, Forster & Röhl, 1999).

Demographic analysis

Neutrality tests of Tajima's *D* (Tajima, 1989) and Fu's F_S statistics (Fu, 1997) were calculated and tested for deviations from neutrality using 10 000 coalescent simulations in ARLEQUIN. Mismatch distributions of pairwise sequence differences were also performed in ARLEQUIN with 1000 permutation replicates.

Estimated expansion values were obtained using ARLEQUIN and graphics of frequency distribution using DNASP, version 5 (Librado & Rozas, 2009). To test the observed mismatch distribution goodness-of-fit to the sudden expansion model and to obtain confidence intervals around the estimated mode of mismatch distribution, 1000 permutation replicates were used (Schneider & Excoffier, 1999). Statistically significant differences between observed and expected distributions were evaluated with the sum of the square deviations (SSD) and Harpending's raggedness index (Harpending, 1994).

RESULTS

Genetic variability and diversity

A fragment of 647 bp at the 3′ end of cytochrome oxidase I (COI) mitochondrial gene was obtained, after trimming, for 125 *C. attenuata* specimens (the museum ones were not included here) and five specimens from other four species. Within *C. attenuata*, 19 characters were variable of which 14 were parsimony informative and 20 haplotypes were found (Table 3). The G + C content was on average 27.6% and differed with codon position (39.5% in the first position; 38.0% in the second; and 5.4% in the third). No evidence of nuclear copies was found because there were no stop codons within the sequence and the base composition was similar with no indels among all sequences. A low number of transversions and of nonsynonymous substitutions were found (see Supporting information, Table S1).

The two smaller fragments amplified in the degraded museum samples, one at the 5′ end and the other at the 3′ end of the above COI fragment, were concatenated (COI-1 + COI-2) and sequences of 270 bp were obtained for the 25 museum specimens. These were aligned with the same fragments of the remaining samples, resulting in a matrix of 150 individuals of *C. attenuata*. The smaller fragment size resulted in the merging of some haplotypes in the original COI fragment (Table 4). On the other hand, the addition of the museum samples to the analysis revealed four new haplotypes (H21, H22, H23, and H24). When excluding the third codon positions, only one variable character remained: one transition in CHI5 (Chile).

A fragment of 533 bp of the white nuclear gene was obtained for 17 *C. attenuata* and one *C. humilis*. This aligned with the white gene of *Drosophila melanogaster* (FBgn0003996, http://flybase.org/) with 27% average nucleotide divergence (*p*-distance) from *C. attenuata*. Within *C. attenuata*, three characters were variable of which one was parsimony informative, which was also the only nonsynonymous substitution, resulting in an amino acid change from valine to methionine. Four haplotype types were found (Table 5). Three individuals were heterozygous in one nucleotide position (ISR1, Israel; CRE1_2, Crete; ALG2, Iberian Peninsula).

Table 3. Number of individuals of *Coenosia attenuata* from each geographical region and each mitochondrial haplotype in the COI fragment using all the non-museum samples

	COI (647 bp)																			
	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20	Total
Iberian Peninsula	10	15	1	7	1											1					35
Azores	6	1												1					1		9
Madeira		4		2											3						9
Italy	7													2							9
Germany	5																				5
Slovakia	2																				2
Crete		6																		5	11
Anatolia	3		1									1									5
Israel		1	4			2									1						8
Iran		2	12			1	1	1	2	1	1		1								22
California			5																		5
Chile	2															1	1	1			5
Total	35	29	23	9	1	3	1	1	2	1	1	1	1	3	4	2	1	1	1	5	125

The total number of individuals per population and haplotype type are also indicated.

A fragment of 300 bp of EF-1α nuclear gene was obtained, after trimming, for 11 *C. attenuata* and one *Anthomyia* sp. Elongation factor has paralogous copies in several insect species, including Diptera (Djernaes & Damgaard, 2006). The fragment considered here aligned with *D. melanogaster* elongation factor 1α48D (FBgn0000556, http://flybase.org/) with 17% average nucleotide divergence (*p*-distance) from *C. attenuata*. Within *C. attenuata*, six characters were variable of which one was parsimony informative. One nonparsimony informative site had a nonsynonymous substitution, in sample CRE3_5 (Crete), resulting in aminoacid change from arginine to histidine. Some samples had unreadable sequence in the start of the sequence and were included as *N* in the initial matrix. After trimming, to exclude these *N*, only 140 bp remained, which included three variable sites. Four haplotype types were found (Table 5). Two individuals were heterozygous in one nucleotide position (ISR1, Israel; CAL1, Italy).

GenBank accession numbers for all genes are: KJ585390 to KJ585414 for COI; KJ652092 to KJ652113 for White; and KJ652114 to KJ652126 for EF (Table 2).

Phylogenetic and population structure analysis

Mean uncorrected sequence divergence between the four *Coenosia* species considered here ranged from 6.8% (between *C. attenuata* and *C. humilis*) to 11.3% (between *C. attenuata* and *C. tigrina*). Between *Coenosia* species and the other dipteran species, divergence was higher than 12% (see Supporting information, Table S2). Within *C. attenuata*, the haplotypes diverged from 0.2% to 0.9%, except the H20 haplotype that diverged from the others by 1.2% to 1.7%. These divergences are visible in the phylogenetic tree (Fig. 2) in which *C. attenuata* haplotypes form a well-supported monophyletic group with low genetic differentiation within it, having only one more divergent haplotype, found in Crete (H20). The nuclear genes showed no distinct lineages within *C. attenuata* (see Supporting information, Fig. S1).

The haplotype networks for COI, COI-1 + COI-2, White and EF (Fig. 1) showed low diversity and high similarity between haplotypes, with the exception of one mitochondrial haplotype from Crete that was more divergent (H20), as reported above. However, the nuclear divergence did not correspond to this mitochondrial divergence because the individuals with H20 mitochondrial haplotype had nuclear sequences identical to the remaining *C. attenuata*. The networks also showed the lack of geographical differentiation. The nuclear genes had a more common haplotype, present around the world, and

Table 4. Number of individuals of *Coenosia attenuata* from each geographical region and each mitochondrial haplotype in the COI-1 + COI-2 concatenated fragment for all the samples

	COI-1+COI-2 (270 bp)																				
	H1 (+H16)	H2 (+H5)	H3 (+H7+ H9+H12)	H4	H6	H8	H10	H11	H13	H14	H15	H17	H18	H19	H20	H21	H22	H23	H24	Total
Iberian Peninsula	11	16		7						1										35
Azores	6	1								1				1						9
Madeira		4		2							3									9
Cape Verde																2	1			3
Italy	7									2										9
Germany	5																			5
Slovakia	2																			2
Crete		6													5					11
Anatolia	3		2																	5
Egypt											1						1	3	1	6
Israel		1	4		2						1									8
Iran		2	15		1	1	1	1	1											22
Oman			3																	3
India																	1			1
Australia																	7	1		8
California			5																	5
New York			2																	2
Ecuador																	2			2
Chile	3											1	1							5
Total	37	30	31	9	3	1	1	1	1	4	5	1	1	1	5	2	12	4	1	150

The total number of individuals per population and haplotype type are also indicated.

Table 5. Number of nuclear haplotypes in each geographical region and each haplotype type in the fragments white and EF-1α

	White					Elongation factor				
	H-W1	H-W2	H-W3	H-W4	Total	H-EF1	H-EF2	H-EF3	H-EF4	Total
Iberian Peninsula	3		1		4	2				2
Azores	2				2	2				2
Madeira	2				2					0
Italy	2			2	4			2		2
Germany			2		2	2				2
Slovakia					0					0
Crete	7	1			8	4			2	6
Anatolia					0					0
Israel	3		1		4	1	1			2
Iran	4				4	2				2
California	2				2	2				2
Chile	2				2	2				2
Total	27	1	4	2	34	17	1	2	2	22

The total number of haplotypes per population and haplotype type are also indicated.

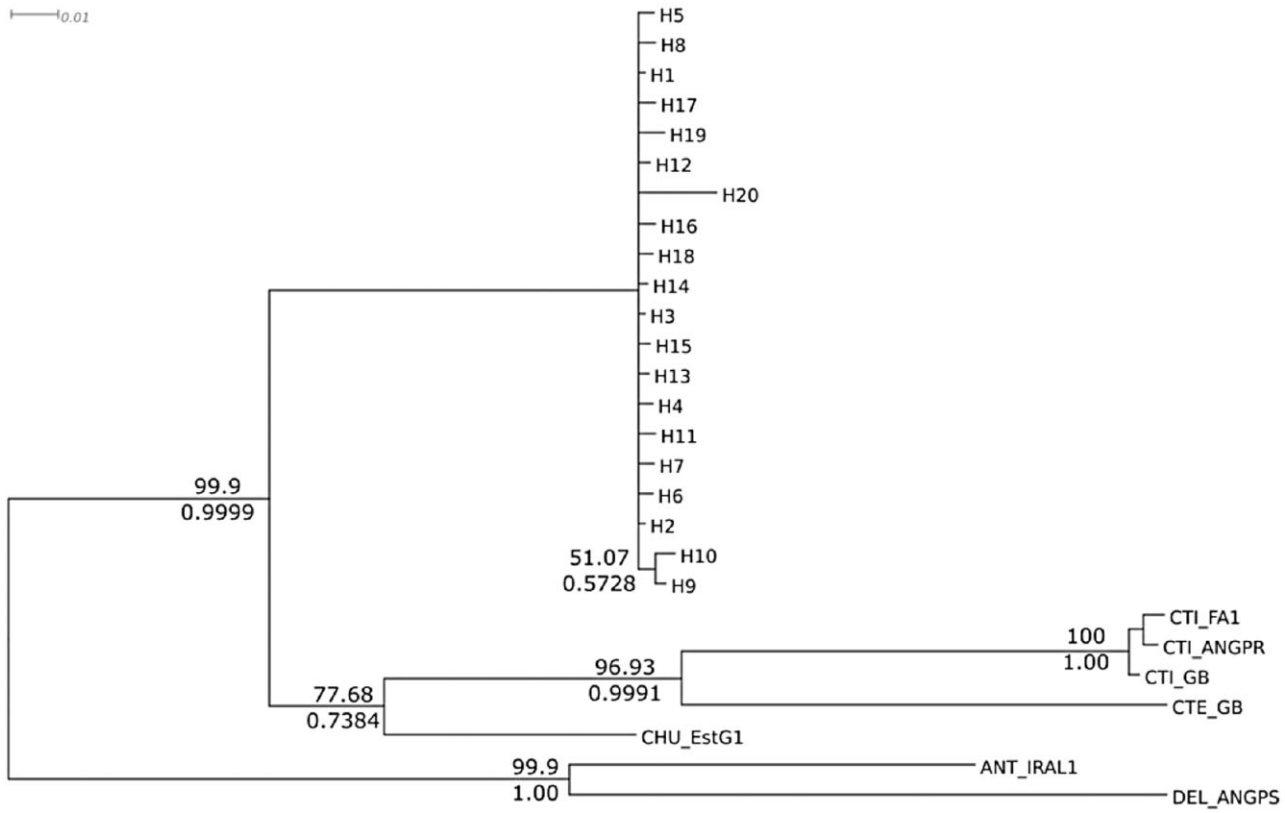

Figure 2. Bayesian tree of the haplotypes found in cytochrome oxidase I (COI) (647 bp) in *Coenosia attenuata* (H1 to H20) and the other species CHU, *Coenosia humilis*; CTI, *Coenosia tigrina*; CTE, *Coenosia testacea*; ANT, *Anthomyia* sp.; DEL, *Delia* sp.; GB, sequences from GenBank. Values above branches are the maximum likelihood bootstrap values (> 50%) and values below branches are Bayesian posterior probabilities.

Table 6. Number of haplotypes (N_H), haplotype diversity (h) and nucleotide diversity (π) of COI fragment (647 bp) and of COI-1 + COI-2 (270 bp) in *Coenosia attenuata* for each geographical grouping considered

	COI (647 bp)				COI-1 + COI-2 (270 bp)			
	N	N_H	h	π	N	N_H	h	π
Europe	51	7	0.690	0.0036	51	5	0.660	0.0085
Mediterranean	68	11	0.793	0.0049	74	11	0.806	0.0099
Mediterranean without H20	63	10	0.763	0.0034	69	10	0.781	0.0083
West Mediterranean	44	7	0.723	0.0037	44	5	0.685	0.0085
Iberian Peninsula	35	6	0.703	0.0033	35	4	0.661	0.0076
East Mediterranean	24	7	0.837	0.0063	30	9	0.876	0.0107
East Mediterranean without H20	19	6	0.795	0.0026	25	8	0.853	0.0071
Macaronesia	18	6	0.810	0.0037	21	8	0.857	0.0089
West Asia	35	12	0.755	0.0021	38	9	0.595	0.0037
South America	5	4	0.900	0.0019	7	4	0.810	0.0068
Australia	–	–	–	–	8	2	0.250	0.0009
North America	–	–	–	–	7	1	0.000	0.0000
Paleotropical	–	–	–	–	5	3	0.700	0.0052
Egypt	–	–	–	–	6	4	0.800	0.0055

N, number of individuals. Europe: Iberian Peninsula, Italy, Germany, Slovakia; Mediterranean: Iberian Peninsula, Italy, Anatolia, Crete, Israel (and Egypt for COI-1 + COI-2); *West Med*: Iberian Peninsula, Italy; East Med: Anatolia, Crete, Israel (and Egypt for COI-1 + COI-2); Middle East: Anatolia, Israel, Iran (and Oman for COI-1 + COI-2); Macaronesia: Azores, Madeira; Palaeotropical: Cape Verde, Oman, India; North America: California (and New York for COI-1 + COI-2); South America: Chile (and Ecuador for COI-1 + COI-2). Calculations that include Crete were also performed without the haplotype H20.

three other low frequency haplotypes differing in a single mutation from this one found in scattered populations. Some patterns are visible in the mitochondrial COI and COI-1 + COI-2 networks: Iberian Peninsula, Azores, Madeira, and Anatolia are represented in diverged haplotypes across the network; Crete has two highly divergent haplotypes; Central Europe has only one haplotype, one of the most abundant ones; Middle East has one more abundant haplotype and several derived and exclusive ones, in a star pattern characteristic of recent expansion events; South Asia and Australia have one haplotype (H22) also present in Cape Verde, North Africa (Egypt), and South America (Ecuador); in South America, Chile has one of the most abundant haplotypes present in Europe and some derived and exclusive ones, and Ecuador has a widespread haplotype as referred above; North America has only one haplotype, the most abundant one in the Middle East. We investigated whether there was any geographical structure in the distribution of haplotypes within the Iberian Peninsula, the region with higher number of samples and populations in the present study. No pattern was found (see Supporting information, Fig. S2).

The haplotypes found in the NHM specimens from Australia were the same as those found in the other specimens of *C. attenuata*, although they had been identified as either *C. subvittata* or *C. imitatrix*.

The haplotype diversity of COI in all *C. attenuata* was 0.839 and the nucleotide diversity was 0.00389. These diversities were also calculated for different geographical groups (Table 6) and revealed a higher haplotype and nucleotide diversity in the East Mediterranean ($h = 0.837$ and $\pi = 0.0063$ for COI; $h = 0.876$ and $\pi = 0.0107$, for COI-1 + COI-2, which included the NHM samples from Egypt), partly as a result of the H20 divergent haplotype. Macaronesia also showed high haplotype and nucleotide diversities ($h = 0.810$ and $\pi = 0.0037$, respectively, for COI; $h = 0.857$ and $\pi = 0.089$, respectively for COI-1 and COI-2, which included the NHM samples from Cape Verde). South America, despite the low number of samples (five from Chile and two from Ecuador), showed a high haplotype diversity, with four different haplotypes found in Chile for COI, with few mutational steps between them ($h = 0.900$, $\pi = 0.0019$), and three haplotypes in Chile and one in Ecuador for COI-1 + COI-2 ($h = 0.810$, $\pi = 0.0068$). Egypt alone (six samples) showed relatively high diversity as well ($h = 0.800$, $\pi = 0.0055$).

Several groupings of populations, chosen based on the visualization of the COI haplotype network, were tested by analysis of molecular variance, and the

Table 7. Parameters from the mismatch distribution for COI (647 bp) in *Coenosia attenuata*, excluding haplotype H20

Demographic expansion	
τ	3.414 (0.713–5.897)
θ_0	0.000 (0.000–0.779)
θ_1	5.005 (3.243–99 999)
SSD	0.012
P_{SSD}	*0.390*
Raggedness	0.033
P_{rag}	*0.675*
Spatial expansion	
τ	1.876 (0.680–4.542)
θ	1.034 (0.0007–2.813)
M	6.441 (1.925–99 999)
SSD	0.015
P_{SSD}	*0.302*
Raggedness	0.034
P_{rag}	*0.754*
Neutrality tests	
Tajima's *D* test	−0.397
P (sim_*D* < obs*D*)	*0.394*
Fu's F_S test	−27.125
P (sim_F_S ≤ obs_F_S)	*0.000*

Numbers in parentheses are the upper and lower bound of the 95% confidence interval (1000 bootstrap replicates). θ_0 and θ_1, pre-expansion and post-expansion populations size; τ, time in number of generations elapsed since the sudden/demographic expansion and spatial expansion episodes; SSD, sum of squared deviations; Raggedness, raggedness index.

percentage of variation observed among groups was always lower than that within populations or that among populations within groups (see Supporting information, Table S3). The correlation between pairwise F_{ST} and geographical distances was low and nonsignificant (Mantel test, $r = 0.020$, $P = 0.411$ for COI and $r = 0.091$, $P = 0.143$ for COI-1 + COI-2).

Demographic analysis

The demographic history of *C. attenuata* was analyzed excluding the highly divergent haplotype H20. The distribution of pairwise nucleotide differences (mismatch distribution) showed a slightly bimodal curve (see Supporting information, Fig. S3). The SSD and raggedness index did not differ significantly from the expected under sudden and spatial population expansion models (Table 7). Significant negative deviations from neutrality were detected with Fu's F_S statistic, which corroborate the hypothesis of past population expansion events.

DISCUSSION

The phylogeographical analysis based on mtDNA and nuclear DNA across the wide distribution range of *C. attenuata* revealed relatively low genetic diversity consistent with a very recent demographic and spatial expansion. For the 150 specimens analyzed, we only found 24 COI haplotypes with a few mutational steps between them. Low mitochondrial diversity may be a result of natural selection acting on mitochondria (Ballard & Whitlock, 2004; Bazin, Glémin & Galtier, 2006; Balloux *et al.*, 2009). Maternally-inherited symbionts, such as *Wolbachia*, are well-known agents of selection in mitochondria (Hurst & Jiggins, 2005). The fact that the number of males in natural populations of *C. attenuata* is much lower than the number of females (male : female ratio of 1 : 4; Mateus, 2012) supports that possibility, considering that the observed sex ratio may be an outcome of male-killing bacteria such as *Wolbachia* (Weeks, Reynolds & Hoffmann, 2002; Martin *et al.*, 2012). This hypothesis deserves to be tested. However, the nuclear DNA in this species shows also very low variability, both in white and EF-1α genes. This suggests a recent divergence within *C. attenuata*. The demographic analysis supports the hypothesis of a recent worldwide population expansion. However, the occurrence of one more divergent mitochondrial haplotype (H20) may indicate that the species started diverging earlier, although only a restricted subset of the gene pool was able to disperse widely. Intermediate lineages may have existed and gone extinct or were not yet sampled. Sorting of mitochondrial lineages may be faster than assumed by the current divergence patterns, as shown in the moth *Hyles euphorbiae* (L.), in which the study of historic DNA from museum specimens revealed a recent demographic change, possibly associated with anthropogenic habitat loss and fragmentation, as well as with recent climate warming that favoured the spreading of one of the potentially better adapted lineages (Mende & Hundsdoerfer, 2013).

No well-defined patterns of geographical structure were found, which may be the result of very recent worldwide colonization or to recurrent high levels of gene flow, as described for other widespread species that lack phylogeographical structure (Vandewoestijne *et al.*, 2004; van Gremberghe *et al.*, 2011; Karsten *et al.*, 2013). The dispersal ability of *C. attenuata* is not known. Because *C. attenuata* is usually found associated with protected crops, often reaching high numbers within greenhouses, it is very likely that dispersal mediated by humans through international trade has facilitated the expansion of its distribution range. The larvae of *C. attenuata* develop in the soil, preying on the larvae of fungus gnats

(Diptera: Sciaridae) or on other soil organisms and are able to survive for long periods (up to 34 days) with low numbers of prey before dying of starvation (Ugine *et al*., 2010). Thus, human-mediated movement of potted plants from one place to another may easily transport its immature stages. The accidental introduction of *C. attenuata* through commercial transport of plant material in potting media was suggested by Kühne *et al*. (1997) and Hoebeke *et al*. (2003). Indeed, the degree of international trade has been shown to be the best predictor of the number of invasive alien species in a country (Westphal *et al*., 2008). Adults of *C. attenuata* may also be carried with plants or plant parts. The hypothesis of long-range dispersal, either by active flight or passive transportation by wind, cannot be ruled out. For example, Diptera have been reported as being generally the second most abundant order of insects in aerial netting studies carried out in the UK (Chapman *et al*., 2004). There was no pattern of isolation-by-distance, at least in the long range, and the analysis of molecular variance showed a lack of genetic structure among geographical regions, which suggests ongoing global gene flow. In the present study, a few samples were collected in wild areas and in nonprotected crops, far away from greenhouses (at least, all sampled populations from Israel, Oman, and Cape Verde, one population from Iran, and a few from Portugal) (Table 2). Despite that, these samples presented the same haplotypes as those found in the respective regions. This does not give us an indication about the type of dispersal (either natural or human-mediated) but does indicate that the gene pool is essentially the same, with no restriction of dispersal both from and to the greenhouses. The colonization of greenhouses by individuals of *C. attenuata* coming from the surrounding environment and their ability to establish there by reproducing and completing development within greenhouses' soil have been documented (Kühne *et al*., 1997).

Our data support the hypothesis that the most likely origin of *C. attenuata* is the Mediterranean Basin, where most of the major haplotypes were found, and haplotype diversity and nucleotide were higher. The Mediterranean region has previously been suggested as the possible origin for this species (Martinez & Cocquempot, 2000). However, the contribution of the Macaronesian islands to the diversification of the species deserves further study. The fact that we have found a haplotype most divergent from all other (H20) only in the eastern Mediterranean (Crete) may indicate that the source has been in this area, where several haplotype groups may have existed, although only one of them has expanded to other regions, as seen before. Given our unsuccessful attempts to obtain samples from East Asia or the African continent (except from Egypt), it is likely that other haplotypes will be found in *C. attenuata* populations from these areas and thus we cannot exclude completely a Paleotropical origin, as suggested by Hennig (1964). The Middle East is an area of very recent diversification, as seen from the star-like pattern of the haplotype network found in Iran.

The colonization of some regions by *C. attenuata* has likely involved founder events given the low diversity of these areas, namely Central Europe and North America. In the case of Central Europe, where only one COI haplotype was found, common to the one present in southern Europe and Azores, the most likely origin of its colonization is southern Europe, a refuge area for many species during the Quaternary glaciations (Hewitt, 1999, 2004; Gibbard *et al*., 2010). In the case of North America, also only one COI haplotype was found, common to one present in Western Asia, the most likely region of origin of the colonization, probably through human accidental introductions. In the case of South America, at least two colonizations have likely happened: one from Europe, and in high numbers that allowed the maintenance of the high genetic diversity found in Chile, and another of undetermined origin, given the wide distribution of the haplotype found in Ecuador. These patterns of colonization of South and North America by *C. attenuata* apparently differ from those that have been suggested for other dipteran species of Mediterranean origin. For example, genetic data revealed that *Drosophila subobscura* Collin has very recently colonized the New World (three decades ago), most likely from the western Mediterranean into South America, and then from there to North America (Pascual *et al*., 2007).

The specimens from Australia analyzed in the present study had originally been identified as *C. subvittata* and *C. imitatrix* using morphological characters. However, their mtDNA haplotypes (COI-1 + COI-2) were identical to those from other specimens identified as *C. attenuata*, suggesting that they were either misidentified or, most likely, that these taxa are conspecific with *C. attenuata*. However, because this was based on a small fragment of COI, we cannot completely exclude the possibility that these are distinct species with a very low divergence from *C. attenuata*.

Museum samples are a valuable source for assessing genetic diversity in specimens from geographical locations that may be difficult to sample, as well as for assessing historical changes in genetic diversity (Goldstein & DeSalle, 2003; Hartley *et al*., 2006). However, they pose several technical challenges as a result of DNA degradation, including amplification of only short fragments (usually less than 200 bp), a risk of contamination, and damage to the DNA in the form

of single nucleotide misincorporations (Wandeler, Hoeck & Keller, 2007). In our case, the amplification success of the museum samples was high: from the total of 28 specimens, 25 amplified both COI-1 and COI-2 fragments. The three specimens for which PCR amplification failed for the two fragments (one specimen from India) or for one of the fragments (two specimens from Egypt) were part of the oldest specimens, collected in the 1930s and 1940s. When analyzing sequence data from museum specimens, we have to be aware that single base errors in PCR products are more likely than in recent specimens (Sefc, Payne & Sorenson, 2007). In the present study, four haplotype sequences were found in the museum samples that were not present in the recent samples (H21 to H24). Although we cannot exclude the possibility of base misincorporations in these sequences, it is unlikely that haplotype H22 would have the same errors in all of the 12 specimens where it was found and which included relatively recent samples from Ecuador (collected in 1999). These four haplotypes differ from those found in recent samples by only one or two nucleotide substitutions and, even if these small differences were all artefacts (which is unlikely), they would not change the overall pattern of genetic diversity discussed in the present study.

This is the first study to characterize the worldwide genetic variation of *C. attenuata*, testing some hypotheses about the origin of the species, patterns of dispersal and demographic processes, including human-mediated spread, and not excluding the action of selective agents (such as symbionts) in the current distribution of its genetic variation. Further sampling and more variable molecular markers will provide a more comprehensive perspective on this species and will allow testing some of the hypotheses raised in the present study.

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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:

Fig. S1. Maximum likelihood trees based on nuclear genes white and elongation factor-1α.

Fig. S2. Distribution of cytochrome oxidase I (COI) haplotypes in the Iberian Peninsula.

Fig. S3. Mismatch distribution of mitochondrial DNA cytochrome oxidase I (COI) haplotypes. The expected frequency is based on a population growth-decline model, determined using DNASP and is represented by a continuous line. The observed frequency is represented by a dotted line. Parameter values for the mismatch distribution are given in Table 6.

Fig. S4. Sequences of haplotypes H21 to H24 [concatenated COI-1(in bold) + COI-2] and CRE3_5EF (elongation factor).

Table S1. Nucleotide substitutions found in the 647-bp fragment of cytochrome oxidase I (COI) for each haplotype of *Coenosia attenuata*.

Table S2. Pairwise p-distances between haplotypes of cytochrome oxidase I (COI) fragment for *Coenosia attenuata* (H1 to H20) and for the remaining species. CHU, *Coenosia humilis*; CTI, *Coenosia tigrina*; CTE, *Coenosia testacea*; ANT, *Anthomyia* sp.; DEL, *Delia* sp.; GB, sequences from GenBank.

Table S3. Analyses of molecular variance of cytochrome oxidase I (COI) haplotypes considering different groupings of populations of *Coenosia attenuata*. Europe: Iberian Peninsula, Italy, Germany, Slovakia; East Med: Anatolia, Crete, Israel; Middle East: Anatolia, Israel, Iran; Macaronesia: Azores, Madeira; North America: California; South America: Chile.