Articles

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First record of *Euchloe tagis* (Hübner, 1804) in the province of Tarragona (Catalonia) based on morphology and DNA data (Lepidoptera: Pieridae)

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Abstract. The pierid *Euchloe tagis* (Hübner, 1804) is recorded for the first time in the province of Tarragona, Catalonia, Iberian Peninsula. DNA barcoding was shown to allow the unambiguous identification of all Iberian *Euchloe* species and confirmed the morphology-based identification of the *E. tagis* specimen from Tarragona. DNA barcodes also revealed that two main mitochondrial lineages of *E. tagis* occur in Iberia. These lineages have a parapatric distribution and contact zones in the latitudinal centre of the Iberian Peninsula. It is expected that further research will identify new populations of *E. tagis* in suitable habitats that are present in the province of Tarragona.

Resum. Primer registre de *Euchloe tagis* (Hübner, 1804) a la província de Tarragona (Catalunya) basat en la morfologia i en dades del DNA (Lepidoptera: Pieridae). Es presenta la primera citació del pièrid *Euchloe tagis* (Hübner, 1804) per a la província de Tarragona. Es demostra que el codi de barres genètic (DNA barcoding) permet la identificació de totes les espècies ibèriques del gènere *Euchloe* i mitjançant aquest mètode es confirma la identificació basada en morfologia de l'exemplar d'*E. tagis* de Tarragona. El codi de barres genètic també revela que a la península Ibèrica existeixen dos llinatges mitocondrials d'*E. tagis*. Aquests llinatges tenen una distribució parapàtrica amb zones de contacte al centre latitudinal de la península Ibèrica. S'espera que futures recerques portin al descobriment de noves poblacions d'*E. tagis* a la província de Tarragona, on existeixen hàbitats aparentment adequats per aquesta espècie.

Key words: *Euchloe tagis*, Pieridae, Lepidoptera, distribution, DNA barcoding, habitat, genetic lineages.

Introduction

Euchloe tagis (Hübner, 1804) has a fragmented distribution, occurring locally in North Africa (Morocco and Algeria), the Iberian Peninsula, southern France and northwestern Italy (Tarrier & Delacre 2008; Tshikolovets 2011; García-Barros *et al.* 2013). In Iberia, the species is relatively widespread in the south, and has more isolated popu-

lations in the centre, north and north-east (García-Barros *et al*. 2004, 2013). The north-eastern Iberian range of *E. tagis* includes Catalonia, where the species is local and has been recorded exclusively from the province of Lleida (e.g. Domènech 1982; Pérez De-Gregorio 1989; Viader 1992, 1993; Sarto i Montenys 1993; Taymans 1997) (Fig. 1).

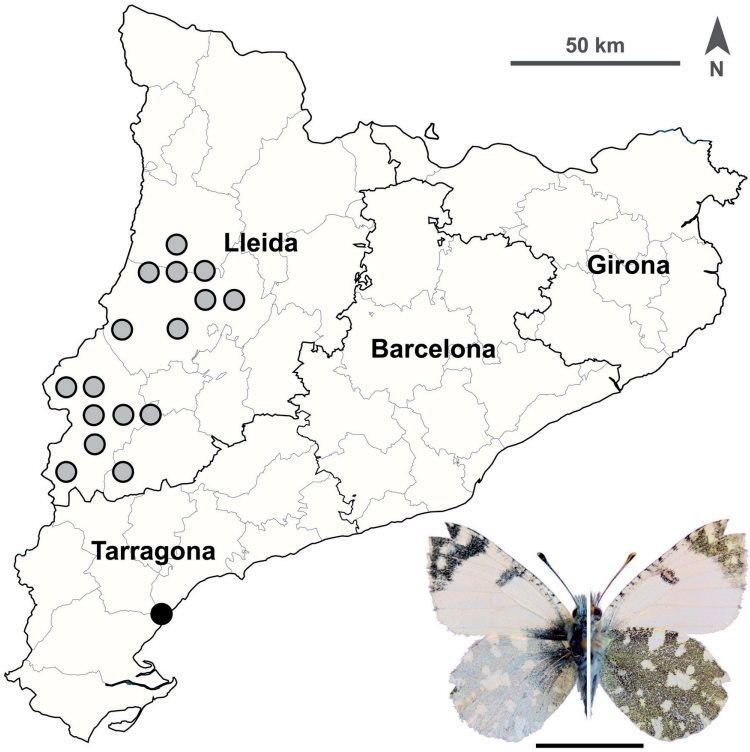

Fig. 1 Distribution of *E. tagis* in Catalonia based on published data. The black dot represents the first record of this species for the province of Tarragona and the southernmost point for Catalonia. The observation site (Calafat, Baix Ebre) is approximately 50 km from the nearest known populations in Lleida and over 170 km from any other Iberian population. The lower right corner illustrates the specimen found north of Calafat on 6.III.2011 (DNA sample ID RVcoll10B498). Scale bar for the specimen is 10 mm.

In this study we report the presence of *E. tagis* in the province of Tarragona, Catalonia. By using DNA barcoding (the use of a short, standardized, DNA fragment to aid species identification) (Hebert *et al.* 2003), we show that (1) all Iberian *Euchloe* species can be reliably differentiated using this technique, (2) confirm the morphology-based identification of *E. tagis* from Tarragona, and (3) provide data on the genetic structure of *E. tagis* in Iberia.

Material and methods

For the DNA analysis, we used all cytochrome *c* oxidase subunit 1 sequences (COI - the standard DNA barcoding markers for animals) of *Euchloe* from a recent study that assembled a comprehensive DNA barcode library of Iberian butterflies (Dincă *et al.* 2015). This dataset has been completed with seven other DNA barcodes of *E. tagis* available from Mutanen *et al.* (2016), resulting in a total of 65 *Euchloe* DNA sequences (Table 1). Most of these represented full-length barcodes (658 bp) with only three sequences being between 621 and 632 bp long. Sequences were aligned using GENEIOUS PRO 6.1.8 created by Biomatters (http://www.geneious.com/) and collapsed to unique haplotypes using TCS 1.21 (Clement *et al.* 2000) (Table 1). Subsequently, a neighbour-joining (NJ) tree was built with MEGA 7 (Kumar *et al.* 2016) using p-distance and 100 bootstrap replicates. *Hesperocharis crocea* Bates, 1866, was used as outgroup.

Results

One specimen of *E. tagis* was captured by the authors in the south-eastern part of Tarragona province (Fig. 1).

Material: 2 km north of Calafat (Baix Ebre, Tarragona), 100 m, lat. 40.946º, lon. 0.836º, 1 specimen, 6.III.2011. DNA sample ID RVcoll10B498.

The specimen of *E. tagis* from Calafat (Fig. 1) displayed curved hindwing costa, morphological feature that distinguishes it from the similar species, *Euchloe crameri* Butler, 1896.

DNA data confirmed the identification of this specimen and also showed that all Iberian *Euchloe* species can be reliably differentiated using DNA barcoding, in accordance with the findings of Dincă *et al.* (2015) (Fig. 2). The single Italian specimen of *E. tagis* was well differentiated from the Iberian specimens (minimum p-distance 1.67 %) and the Iberian specimens formed two well-supported lineages displaying a minimum genetic distance of 0.91 % (Fig. 2). These lineages seem to have a parapatric distribution: one occurs in the southern half of the Iberian Peninsula and the other in the northern half, but they have contact zones in the latitudinal centre of the Iberian Peninsula (e.g. Madrid and Valencia areas) (Fig. 3, table 1). The specimen from Tarragona province belonged to the northern lineage and displayed a unique haplotype (ht 5), that was differentiated by one mutation from haplotype «ht 6» detected in Huesca and La Rioja regions (Fig. 2, Table 1).

Discussion

The site where *E. tagis* has been found in the province of Tarragona (Calafat, Baix Ebre) (Fig. 1, Table 1) is approximately 50 km from the nearest known populations in Lleida and over 170 km from any other Iberian population. It is thus a relatively isolated record and it is very likely that the species has a permanent population in the area.

Table 1 Specimens used for the DNA analysis. The haplotype numbers correspond to those in Fig. 2. The specimen from Tarragona province bears sample ID RVcoll10B498.

Sample ID	COI haplotype	E. tagis Iberian lineage	GenBank accession number	Taxon	Region	Exact Site	Country
RVcoll08M415	ha 1		HQ004465	*E. ausonia*	Dobrogea	Constanta, 1 Km NE of Gura Dobrogei reserve	Romania
RVcoll08M435	ha 1		HQ004468	*E. ausonia*	Dobrogea	Constanta, 1 Km NE of Gura Dobrogei reserve	Romania
RVcoll08M416	ha 1		HQ004466	*E. ausonia*	Dobrogea	Constanta, 1 Km NE of Gura Dobrogei reserve	Romania
RVcoll08M434	ha 1		HQ004467	*E. ausonia*	Dobrogea	Constanta, 1 Km NE of Gura Dobrogei reserve	Romania
RVcoll07D013	ha 1		HQ004464	*E. ausonia*	Dobrogea	Constanta, Canaraua Fetei (Baneasa)	Romania
RVcoll08M455	ha 1		HQ004469	*E. ausonia*	Dobrogea	Constanta, Esechioi forest	Romania
RVcoll06K685	hba 3		GU676775	*E. bazae*	Andalusia	Granada, Hermanillas, Depresión de Baza	Spain
RVcoll06K683	hba 3		GU676773	*E. bazae*	Andalusia	Granada, Hermanillas, Depresión de Baza	Spain
RVcoll06K686	hba 3		GU676776	*E. bazae*	Andalusia	Granada, Hermanillas, Depresión de Baza	Spain
RVcoll06K684	hba 4		GU676774	*E. bazae*	Andalusia	Granada, Hermanillas, Depresión de Baza	Spain
RVcoll08J313	hba 1		KP870796	*E. bazae*	Aragón	Huesca, Barranco de Valcuerna, Candasnos	Spain
RVcoll08J300	hba 2		GU676545	*E. bazae*	Aragón	Huesca, Barranco de Valcuerna, Candasnos	Spain
RVcoll10A205	hba 2		KP870305	*E. bazae*	Aragón	Zaragoza, SE of Caspe	Spain
RVcoll10A201	hba 2		KP870994	*E. bazae*	Aragón	Zaragoza, SE of Caspe	Spain
RVcoll10A200	hba 2		KP870846	*E. bazae*	Aragón	Zaragoza, SE of Caspe	Spain
RVcoll10A203	hba 2		KP871161	*E. bazae*	Aragón	Zaragoza, SE of Caspe	Spain
RVcoll10A204	hba 2		KP870967	*E. bazae*	Aragón	Zaragoza, SE of Caspe	Spain
RVcoll09V797	hba 2		KP871081	*E. bazae*	Aragón	Zaragoza, SE of Caspe	Spain
RVcoll12L593	hb 5		KP870709	*E. belemia*	Algarve	Nave, Monchique	Portugal
RVcoll11D959	hb 3		KP870468	*E. belemia*	Andalusia	Cádiz, Arroyo de Prior, Algeciras	Spain
RVcoll11D960	hb 5		KP870272	*E. belemia*	Andalusia	Cádiz, Arroyo de Prior, Algeciras	Spain
130209KL19	hb 1		JN276232	*E. belemia*	Andalusia	Cádiz, Bolonia,Tarifa	Spain
RVcoll11D925	hb 5		KP870546	*E. belemia*	Andalusia	Málaga, Colmenar	Spain
RVcoll11D924	hb 3		KP870588	*E. belemia*	Andalusia	Málaga, Colmenar	Spain
RVcoll11D641	hb 4		KP871073	*E. belemia*	Andalusia	Málaga, Colmenar	Spain
RVcoll08J871	hb 5		GU675919	*E. belemia*	Castilla y León	León, Villamarco	Spain
RVcoll08J876	hb 5		GU675920	*E. belemia*	Castilla y León	León, Villamoratiel de las Matas	Spain
RVcoll08H305	hb 5		GU676698	*E. belemia*	Com. de Madrid	Aranjuez, Aranjuez	Spain
RVcoll08R038	hb 2		GU676722	*E. belemia*	Com. Valenciana	Alicante, Arenal de Almorxo	Spain

RVcoll 12L551	hc 2		KP870237	*E. crameri*	Algarve	Porches, Lagoa	Portugal
RVcoll08H616	hc 4		GU676607	*E. crameri*	Andalusia	Cádiz, El Gastor	Spain
RVcoll08L015	hc 2		GU676462	*E. crameri*	Andalusia	Granada, Ascenso al Veleta, Guejar Sierra	Spain
RVcoll06A038	hc 2		GU676836	*E. crameri*	Andalusia	Granada, Barranco de los Lagartos, Cadiar	Spain
RVcoll 12L543	hc 2		KP870638	*E. crameri*	Andalusia	Huelva, Campos de cultivo, Cartaya	Spain
RVcoll08H442	hc 2		GU676658	*E. crameri*	Cantabria	Puerto de Pozazal	Spain
RVcoll08J870	hc 3		GU675859	*E. crameri*	Castilla y León	León, Villamarco	Spain
RVcoll06G471	hc 1		GU676802	*E. crameri*	Catalonia	Barcelona, Osona, El Brull	Spain
RVcoll06H840	hc 2		GU676783	*E. crameri*	Catalonia	Barcelona, Vallès Occidental, Campus UAB	Spain
RVcoll08H407	hc 5		GU676670	*E. crameri*	Com. de Madrid	Cantoblanco	Spain
150308PP31	hc 2		GU675840	*E. crameri*	Com. Valenciana	Valencia, Alpuente, Alto del Viso	Spain
RVcoll08H987	hc 6		GU676925	*E. crameri*	Extremadura	Plasencia, Cáceres	Spain
RVcoll08M981	hs 1		GU675955	*E. simplonia*		Pas d`Envalira	Andorra
RVcoll08M982	hs 1		GU675956	*E. simplonia*		Pas d`Envalira	Andorra
RVcoll09T144	hs 2		HM901723	*E. simplonia*	Castilla y León	León, Laguna de las Verdes, Cabrillanes	Spain
RVcoll09T075	hs 1		JF847983	*E. simplonia*	Catalonia	Lleida, Vall d`Aran, Val d`Hurno,	Spain
BC Back 0029	ht 1		KX070996	*E. tagis*	Tuscany	Livorno, San Vincenzo	Italy
BC Back 0027	ht 14	Southern	KX072011	*E. tagis*	Andalusia	Cádiz, Gibraltar	Spain
BC Back 0025	ht 8	Southern	KX071489	*E. tagis*	Andalusia	Granada, Baza	Spain
BC Back 0032	ht 13	Southern	KX071394	*E. tagis*	Andalusia	Granada, Rio Genil, Moraleda de Zafayona	Spain
BC Back 0026	ht 10	Southern	KX071531	*E. tagis*	Andalusia	Huelva, Aracena	Spain
BC Back 0024	ht 12	Southern	KX071848	*E. tagis*	Andalusia	Huelva, Aracena	Spain
RVcoll08J317	ht 3	Northern	GU676537	*E. tagis*	Aragón	Huesca, Candasnos, Barranco de Valcuerna	Spain
RVcoll08J316	ht 6	Northern	HM901242	E. tagis	Aragón	Huesca, Candasnos, Barranco de Valcuerna	Spain
RVcoll08J315	ht 6	Northern	HM901241	E. tagis	Aragón	Huesca, Candasnos, Barranco de Valcuerna	Spain
BC Back 0030	ht 6	Northern	KX071608	E. tagis	Aragón	Huesca, Costean	Spain
RVcoll08H541	ht 3	Northern	GU676625	E. tagis	Castilla-La Mancha	Pastrana, La Alcarria, Guadalajara	Spain
RVcoll08H542	ht 2	Northern	HM901216	E. tagis	Castilla-La Mancha	Valdeconcha, La Alcarria, Guadalajara	Spain
RVcoll10B498	ht 5	Northern	JN276245	E. tagis	Catalonia	Tarragona, Baix Ebre, 2 km north of Calafat	Spain
RVcoll08H306	ht 13	Southern	GU676690	E. tagis	Com. de Madrid	Aranjuez, Aranjuez	Spain
RVcoll08H429	ht 9	Southern	HM901211	E. tagis	Com. de Madrid	Campo Real	Spain
RVcoll08H427	ht 3	Northern	GU676662	E. tagis	Com. de Madrid	Campo Real	Spain
RVcoll08J003	ht 4	Northern	HM901226	E. tagis	Com. Valenciana	Valencia, Ayora (Palomera)	Spain
RVcoll08J008	ht 11	Southern	GU676934	E. tagis	Com. Valenciana	Valencia, Cortes de Pallas (Sierra Martes)	Spain
RVcoll 12L046	ht 7	Northern	KP870370	E. tagis	La Rioja	Cellorigo	Spain
RVcoll 12L047	ht 6	Northern	KP870997	E. tagis	La Rioja	Cellorigo	Spain

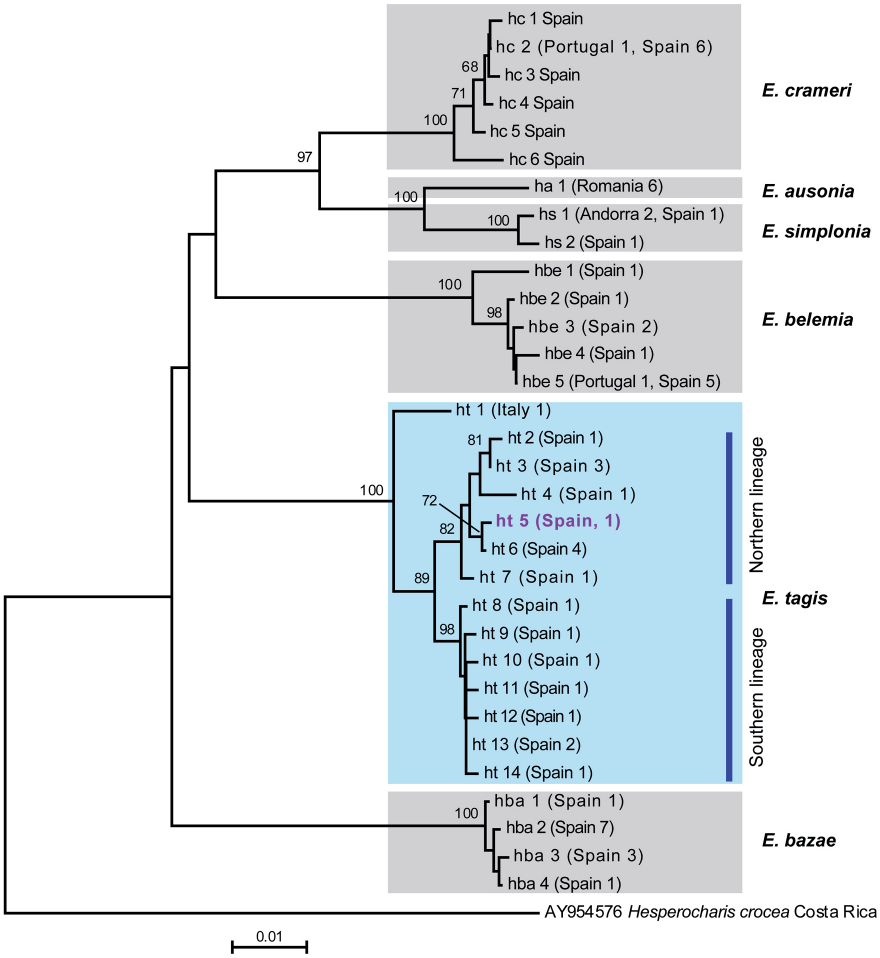

Fig. 2 Neighbour-joining tree based on unique COI haplotypes of *Euchloe*. Bootstrap supports (> 60) are shown next to recovered nodes. The two Iberian lineages of *E. tagis* are indicated with blue vertical bars and the specimen from Tarragona province, representing a unique haplotype (ht 5) is indicated in bold violet.

This is suggested by the suitable habitat (dry rocky slopes with maquis) (Fig. 4), as well as by the genetic data. The specimen of *E. tagis* from Calafat is genetically similar to the nearest populations analysed (Huesca region), but it represents a distinct haplotype (Fig. 2).

The species can usually be reliably distinguished from *E. crameri* based on external morphology, especially taking into account the shape of the hindwing costa, but also the smaller size and more numerous small white mottled spots on the underside hindwing. If

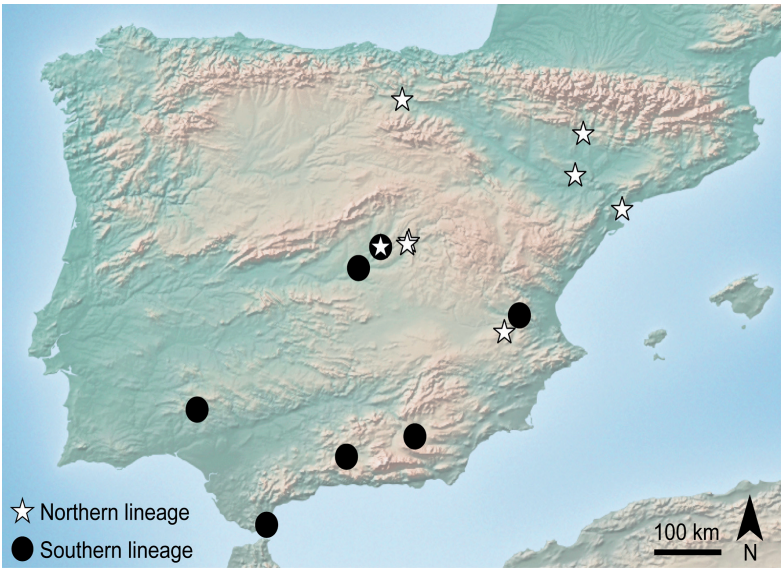

Fig. 3 Geographic distribution of Iberian COI lineages of *E. tagis*. The two lineages appear to have contact in the latitudinal centre of the Iberian Peninsula.

morphological features are not clear (e.g. very worn specimens), or if difficult to identify preimaginal stages are involved, DNA data allows for reliable identification (Fig. 2).

Interestingly *E. tagis* revealed spatial genetic structure represented by the well diverged Italian specimen (Fig. 2) and by the two parapatric lineages detected in Iberia (Figs 2, 3). Further sampling and directed studies will be needed to better understand the mechanisms behind the formation and maintenance of these lineages, as well as their evolutionary and conservation significance. It is however clear that the populations from Catalonia, including the one from Tarragona, belong to the northern Iberian lineage of *E. tagis*. It would be interesting to analyse additional material from southern France and north-western Italy and determine whether the northern Iberian lineage and the Italian one have any potential contact zone and where that is located.

It is likely that further research will reveal more populations of *E. tagis* south-west of Tarragona city because potential habitats as the one near Calafat (Fig. 4) extend in a larger area along Serra de la Talaia and Serra de Tivissa, or more south in Serra de Cardó and Serra de Montsià. Directed research has, for example, allowed the identification of several unknown populations in Portugal (Marabuto 2008).

According to the Climatic Risk Atlas of European butterflies (Settele *et al.* 2008), the present distribution of *E. tagis* can be well explained by climatic variables. A large part of Catalonia is climatically suitable for the species and, although the species is predicted to reduce its range in Iberia, its presence continues to be predicted in Catalonia

especially for the coastal parts, under different climate change scenarios (Settele *et al*. 2008).

Euchloe tagis is not listed as a taxon of conservation concern in the red list of European butterflies (van Swaay *et al.* 2010). However, the species is relatively local and rare in northern Iberia. We hope that the new record from Tarragona will stimulate further research that will lead to the discovery of other populations of this species in Catalonia.

Fig. 4 Habitat of *Euchloe tagis*, north of Calafat (Baix Ebre, Tarragona), 6.III.2011 [Photo: V. Dincă].

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