

1	TITLE: New large threatened populations of Phengaris nausithous discovered in the SW of Europe					
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15	ABSTRACT					
16	The near threatened Phengaris nausithous is distributed in two large mountainous areas in the northern					
17	part of the Iberian Peninsula, living in small dispersed populations. During July and August of 2012-2015					
18	we systematically searched for the species in the southeastern part of the Cordillera Cantábrica. We found					
19	38 new populations grouped into 7 metapopulations. Two of the latter are the largest known, representing					
20	34% of the species' occurrence range in Spain. The discovery of these new metapopulations does not					
21	improve the conservation status of <i>P. nausithous</i> in the SW of Europe because a land consolidation					
22	project currently underway and an established peat extraction plant could lead to the imminent extinction					
23	of the largest metapopulations. However, there is still time to reverse the present course of change.					
24	Knowledge of these recently discovered populations allows the establishment of management measures to					
25	protect them over the long term.					
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28	KEYWORDS					
29	butterfly, conservation, near threatened, land consolidation, peat extraction					

30 Introduction

31 Butterfly species within the genus Phengaris Doherty, 1891, have complex and highly 32 specialized breeding systems, making them very susceptible to factors adversely affecting their habitat 33 (Kotiaho et al. 2005). The near threatened Phengaris nausithous (Bergsträsser, 1779) (World 34 Conservation Monitoring Centre 1996; van Swaay et al. 2010) lays eggs inside the flowerheads of 35 Sanguisorba officinalis, its exclusive hostplant. When larvae subsequently leave the plant, they are 36 adopted by foraging ants of the genus Myrmica and spend the autumn, winter and spring inside the ants' 37 nest, feeding on ant larvae and pupae (Thomas 1984). In summer, during July and August, P. nausithous 38 individuals fly away as imagoes (García-Barros et al. 2013). Suitable habitat patches include wet and 39 periodically flooded meadows and riverbanks, in which their host plant and host ant are found (Munguira 40 and Martín 1999).

P. nausithous is typically distributed in metapopulations, with small populations occupying
discrete habitat patches (Thomas 1984; Nowicki et al. 2005a; 2007). The average life span is only 2-3
days in the field (Nowicki et al. 2005a). Their mobility is low (commonly within a few hundred metres),
restricted by unsuitable habitat, mainly forests, croplands and urban areas (Hovestadt et al. 2011; Nowicki
et al. 2014). The dispersal rate is very low, but regular disturbance by land use and high density might
lead to increased dispersal (Nowicki et al. 2005a; 2007; Dover and Settele 2009).

47 In Europe, the southernmost populations are located in Spain, where it is listed as vulnerable in 48 the Spanish Catalog of Threatened Species (Real Decreto 139/2011). The species is distributed in two 49 large mountainous areas in the northern part of the Iberian Peninsula (García-Barros et al. 2013). In recent 50 years species surveys have been conducted in many parts of the Spanish distribution range (Munguira et 51 al. 2001; 2011; Manceñido-González and González-Estébanez 2013; Vicente et al. 2013). Previous data 52 for Spain have been detailed in standard 10x10 km or 1x1 km UTM grids (10 000 ha and 100 ha grids, 53 respectively), both at coarser size resolutions than the size of occupied patches (Vicente et al. 2013). 54 Additionally, models on the potential distribution of the species are already available (Romo et al. 2006; 55 2015; Jiménez-Valverde et al. 2008), and according to several studies their current distribution in Spain 56 will be strongly affected by climatic change (Settele et al. 2008; Schweiger et al. 2012; Romo et al. 57 2015).

Here, we present the recent discovery of the largest populations of *P. nausithous* in Spain, andshow that there are imminent threats that hinder the conservation of these important populations.

60 Material and Methods

61 We searched for the species during July and August of 2012-2015 in the north of Palencia and Burgos provinces (south and east of the Cordillera Cantábrica, Fig. 1). We defined habitat patches on the 62 63 basis of land use and vegetation composition: suitable meadows limited by forests, bushes or urban areas. 64 These meadows are normally located at the low parts of mountain valleys. We used Google Earth and 65 Google Street View to systematically identify potential sites that were later visited. Once in the patch, we 66 searched for S. officinalis, which, in the study region, typically grows dispersed or in sparse stands. In 67 each patch with the presence of flowering foodplants, we conducted transect surveys visiting all the 68 identified stands of S. officinalis. We considered the species as absent if it was not detected. The 69 detectability of *P. nausithous* in open habitats is high (Pellet et al. 2012) and it is considered that, in 70 occupied patches, flying individuals are seen within the first five minutes of survey in S. officinalis stands (Jimenez-Valverde et al. 2008). We define a population as an occupied patch of continuous suitable 71 72 habitat. We consider the number of records of *P. nausithous* found in a meadow as a proxy of population 73 size. We define populations located less than 5 km apart as the same metapopulation because this is the 74 maximum recorded displacement of P. nausithous (Nowicki et al. 2005b). We calculated the distance 75 between populations as the Euclidean distance between the centroids of occupied patches. To allow 76 comparison with previous studies made in Spain we also used grids of both 10x10 km and 1x1 km.

77 Results

78 We found several new locations where the species is present. In Palencia Province we obtained 79 568 individual records, distributed in 34 populations, grouped into five metapopulations (Figure 1; Table 80 1). The largest metapopulation is located in the upper basin of the Pisuerga River. This metapopulation 81 has the lowest mean distance between populations (Table 1). In this province, the new records occupy 40 82 cells of the 1x1 km grid and 9 cells of the 10x10 km grid, in 4 of which the species had not been 83 previously recorded. In Burgos Province, we obtained 53 individual records, distributed in four 84 populations, grouped into two metapopulations. Nine of these records belong to the three populations of 85 the Tozo-Loras metapopulation which shows the greatest mean inter-population distance (Table 1). In 86 two of these populations we found only one individual, whereas the third population occupies three grid 87 cells of 1x1 km. The remaining 44 records belong to the Nava River basin where we found a single large 88 population occupying 10 grid cells of 1×1 km. In all populations, most records are confined to vegetation 89 adjacent to streams and waterlogged peatlands.

90 Discussion

91 Our study provides an update to the distribution of *P.nausithous* in the southwest of Europe. This 92 information indicates that the species is distributed in dispersed and small populations (Vicente et al. 93 2013). It is not easy to compare the new data with previously known sites because no common resolution 94 and terminology were employed. Munguira et al. (2011) used a 1x1 km grid as reference, recording the 95 study species in 41 grid cells. In the most recent review, Vicente et al. (2013) used both locality and 96 colony alike, mentioning 15 new small and isolated populations. Only two were larger than 5 ha, while 8 97 were smaller than 1 ha (without specifying how area was calculated). Assuming that each population 98 represents a single grid cell at a 1x1 km resolution, the previously known distribution of *P. nausithous* in 99 Spain would cover 56 grid cells. The 55 new cells described in the present study nearly double the 100 previous estimations.

Previous data also indicated a high level of isolation: the 56 known 1x1 km grid cells were
distributed in 36 10x10 km grid cells (Munguira et al. 2011; Vicente et al. 2013). In our study,
aggregation is higher in both the Pisuerga metapopulation, with 28 1x1 km grid cells occupying four
10x10 km grid cells, and the Nava basin, with 10 1x1 km grid cells present in a single 10x10 grid cell.
Measured at the 1x1 km resolution the two metapopulations represent 34% of the area occupied by the
species in Spain.

107 Unfortunately, we do not think that the discovery of these new populations represents an 108 improvement in the conservation status of the species in Spain. There are serious threats affecting them 109 that could lead to their imminent extinction. The Pisuerga, Carrión and Otero de Guardo metapopulations 110 are completely included in Fuentes Carrionas and Fuente Cobre Natural Park, which is part of the Natura 111 2000 network. In this protected area a land consolidation plan has been approved for the whole area. If 112 implemented, all the pastures and croplands will be rearranged at the landscape scale in order to increase 113 their average size, including the removal of hedgerows, the levelling-off of the new patches of land, the 114 eventual drainage of pastures and river banks, and the canalization of streams (by straightening them and 115 lowering their beds), effectively destroying the habitat of *P. nausithous*. The project has already been 116 implemented in San Salvador de Cantamuda municipality, where, in spite of its potential, the species is 117 absent. The remaining localities are waiting for funds to be allocated, mainly by the European Regional 118 Development Fund, to undertake the land consolidation.

119	Likewise, the habitat of the Nava basin population is being destroyed by the extraction of peat					
120	(permit granted in the 1990s) across a large area of the habitat patch occupied by the population. To date,					
121	about 40 ha of habitat have been directly destroyed, and the surrounding habitat belt is being drained.					
122	These threats make the largest populations recently discovered at serious risk of extinction in the					
123	near future. However, we still have time to reverse the present course of change. This work should allow					
124	the rethinking of land consolidation projects as well as the inclusion of measures allowing for the					
125	conservation and management of populations of P. nausithous. The most important action is to avoid the					
126	drainage of pastures and surrounding areas, or other works that could adversely affect the conservation of					
127	P.nausithous populations (for example, the dumping of waste products resulting from work projects,					
128	repairs and new construction of service roads near populations, etc.). If this is not possible, a key action is					
129	to halt the funding of land consolidation by the European Union within the Natural Park of Fuentes					
130	Carrionas.					
131	In the population of the Nava basin, it is necessary to check whether the peat extraction grant					
132	complies with the Habitats Directive as well as other Spanish and European legislation.					
133	Knowledge of these recently discovered populations, allows the establishment of management					
134	measures to protect them over the long term.					
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Metapopulation		Province	Number of populations	Inter-population distance (m)	
		Flovince		Mean± SD	Range (m)
А	Guardo	Palencia	2	1149	-
В	Otero de Guardo	Palencia	1	-	-
С	Carrión	Palencia	5	1626±202	1407-1821
D	Pisuerga	Palencia	25	837±338	360-1415
E	Quintanaluengos	Palencia	1	-	-
F	Tozo-Loras	Burgos	3	3607±986	3038-4745
G	Nava basin	Burgos	1	-	-

Table 1 Number of populations in each metapopulation and mean nearest distance between the
 nearest neighbouring populations. The range represents the shortest and the longest nearest distances in
 each metapopulation.



Fig. 1 a) Distribution of *P. nausithous* in Spain (standard 10x10 km UTM). Empty bold cells correspond
to published localities where the species is present. Gray bold cells correspond to new locations found in
this study (note that we also detected the species in cells where it was already recorded). b) Study area
(shaded). Black dots correspond to the location of populations. Different metapopulations are encircled
and identified with letters: A. Guardo, B. Otero de Guardo, C. Carrión, D. Pisuerga, E. Quintanaluengos,

- 222 F. Tozo-Loras and G. Nava basin.