

DNA barcoding relationships and habitat description of *Psorosa ferrugatella* Turati, 1924 in Spain (Lepidoptera: Pyralidae, Phycitinae)

Manuel Garre, John Girdley, Rosa M. Rubio, Juan J. Guerrero & Antonio S. Ortiz

Abstract

First DNA barcode sequences of *Psorosa ferrugatella* Turati, 1924 from the Iberian Peninsula is published and compared with other European *Psorosa* and closely related species. Three different habitats where *P. ferrugatella* inhabits are described.

Keywords: Lepidoptera, Pyralidae, Phycitinae, *Psorosa ferrugatella*, taxonomy, occurrence, cytochrome oxidase, mitochondrial DNA, habitat, Murcia, Spain.

ADN Código de barras genético y descripción de los hábitats de *Psorosa ferrugatella* Turati, 1924 en España (Lepidoptera: Pyralidae, Phycitinae)

Resumen

Se publica las primeras secuencias del código de barras genético (barcode) de *Psorosa ferrugatella* Turati, 1924 de la península ibérica comparándolo con otras especies europeas de *Psorosa* y especies sistemáticamente relacionadas. Se describen tres hábitats diferentes donde la especie ha sido observada.

Palabras clave: Lepidoptera, Pyralidae, Phycitinae, *Psorosa ferrugatella*, taxonomía, presencia, citocromo oxidasa, ADN mitocondrial, hábitat, Murcia, España.

Introduction

The genus *Psorosa* Zeller, 1846 belongs to the tribe Phycitini of the subfamily Phycitinae in the family Pyralidae. The world fauna of this genus includes 24 species (Slamka, 2019). Seven of them were reported for Europe (Leraut, 2014; Slamka, 2019). *Psorosa* species are distributed mostly in southern areas of the European region except *P. nucleolella* (Möschler, 1866) known from Central Europe. In the Iberian Peninsula, the genus was represented by two species: *P. dahliella* (Treitschke, 1832) and *P. mediterranella* Amsel, 1953 until Corley (2019) added one specimen of *Psorosa ferrugatella* (Turati, 1924) from Portugal to the first record of a specimen captured in Granada on 14-VI-1914 and deposited in the Museum of Natural History in Vienna (Slamka, 2019), thus confirming its presence in the Iberian Peninsula. Recently, Girdley et al. (2020) recorded a preliminary data of *P. dahliella* and *P. ferrugatella* flying sympatrically in Murcia (Southeastern Iberian Peninsula) which were later updated in Garre et al. (2022). Even more recently, Ranki et al. (2022) have found *P. ferrugatella* in the Trebujena Marshes (Cádiz).

The biology of almost all species of the genus is poorly known with preimaginal stages and

hostplants unknown and inhabiting in open arid habitats and various types of steppes. Different *Psorosa* species can be very similar in appearance, so that genitalia examination is often necessary for determination.

In this article, we provide the first DNA barcoding of *Psorosa ferrugatella* collected in the wetlands of the Humedal del Ajauque and Rambla Salada (Murcia, Spain) and these mtDNA sequence (COI) were used to assess genetic divergence with *Psorosa* species and other systematically related species from Europe. Additionally, the habitats where *Psorosa* species live are characterised.

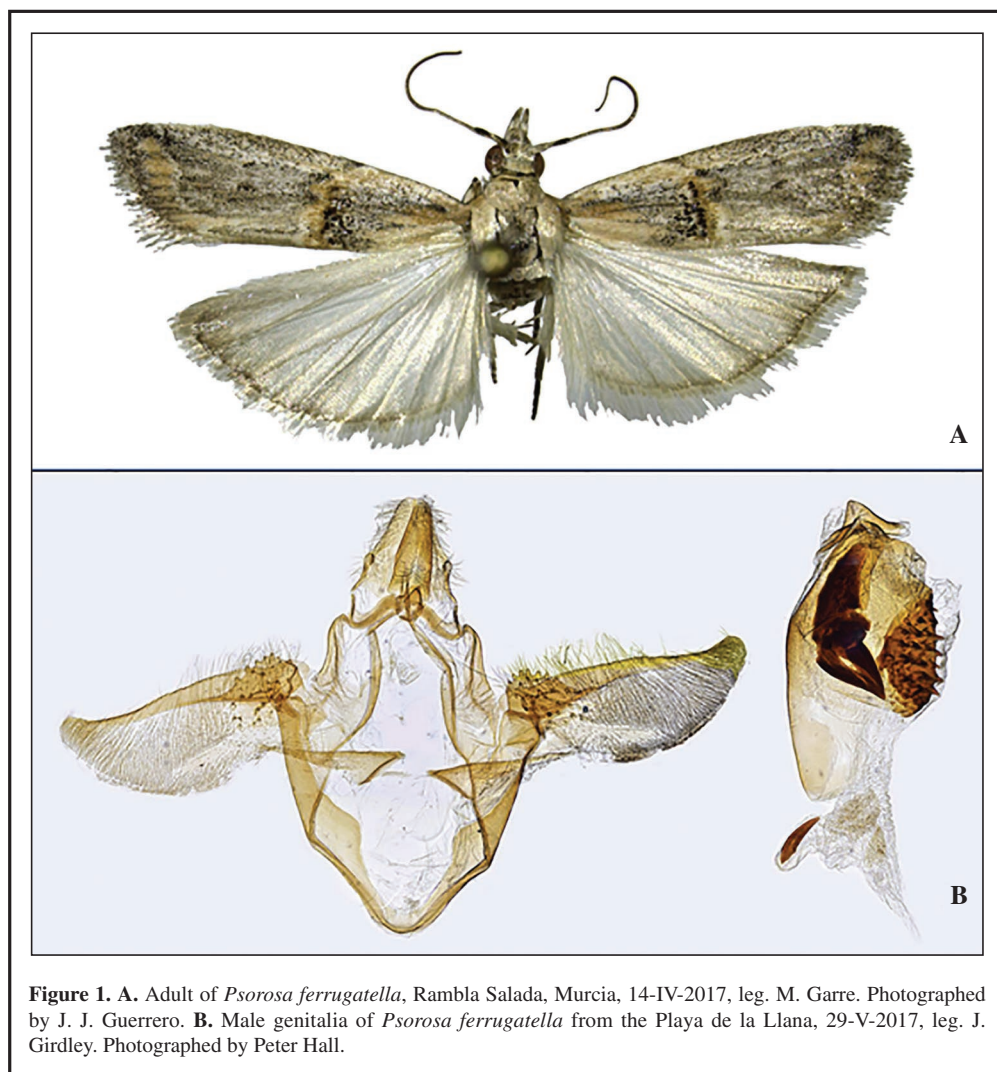


Figure 1. A. Adult of *Psorosa ferrugatella*, Rambla Salada, Murcia, 14-IV-2017, leg. M. Garre. Photographed by J. J. Guerrero. B. Male genitalia of *Psorosa ferrugatella* from the Playa de la Llana, 29-V-2017, leg. J. Girdley. Photographed by Peter Hall.

Materials and methods

Specimens examined: SPAIN. MURCIA, Fortuna, Humedal del Ajauque y Rambla Salada, 30SXH62, 150 m, (38.176; -1.101), 17-IV-2017, 1 ♂; 9-VI-2017, 2 ♂♂, 1 ♀; 28-VI-2017, 1 ♂, 1 ♀;

29-IV-2018, 1 ♂; 18-V-2018, 2 ♂♂; 12-VI-2018, 1 ♀; 3-IV-2019, 1 ♂; 1-V-2019, 1 ♂; 9-V-2021, 2 ♂♂, 1 ♀, M. Garre leg.; San Pedro del Pinatar, Playa de La Llana, 30SXG98, 0 m, (37.807; -0.756), 29-V-2017, 1 ♂, J. Girdley leg.; Murcia, Huerta de Alquerías, 30SXH70, 30 m, (38.006; -1.032), 21-IV-2021, 1 ♂, M. Garre leg.

Morphological study

All specimens were examined externally to evaluate possible differences in their colouration and wing shape. Furthermore, they were dissected using standard procedures (Hausmann, 2001) with minor modifications. Male adult image (Figure 1A) was taken with a Nikon D70 digital camera and were z-stacked using Zerene software. Morphology of genital structures (Figure 1B) were studied using a Zeiss Stemi 508 stereomicroscope with a Zeiss Axiocam ICc5 digital camera. All specimens are deposited in the Research Collection of Animal Biology (RCBA-UMU) in the Department of Zoology and Physical Anthropology of the Universidad de Murcia (Spain).

Molecular procedures

Psorosa specimens used for mitochondrial gene cytochrome oxidase subunit 1 (COI) sequencing are reported in Table I. For DNA extraction, two or three legs were removed from the specimens to sequence the 658 base-pair long barcode segment of the mitochondrial COI gene (cytochrome c oxidase 1, 5' terminus). The tissue samples were submitted to the Canadian Centre for DNA Barcoding (CCDB, Biodiversity Institute of Ontario, University of Guelph) to obtain DNA barcodes using the high-throughput protocol described in deWaard et al. (2008) which can be accessed at www.dnabarcoding.ca/pa/ge/research/protocols. The DNA extracts are currently stored at the CCDB, and the sequences are deposited in GenBank according to the iBOL data release policy (Table I). Sequences were compared with a reference library of Lepidoptera barcodes using the identification engine (BOLD-ID). The reference barcode database for Pyralidae used by BOLD-ID is continually validated by specialists to ensure accurate identifications and is particularly well parameterised due to a global campaign to barcode more than 2,563 species of the family (Ratnasingham & Hebert, 2007).

Table I. List of DNA barcoded (COI 5') specimens of *Psorosa ferrugatella* (Turati, 1924) used for comparison and tree construction.

	Sample ID	Process ID	BIN	GenBank nr
<i>P. ferrugatella</i>	AOC Lep 01310	IBLAO1318-20	AEF6784	OP270249
<i>P. ferrugatella</i>	AOC Lep 01530	IBLAO1538-20	AEF6784	OP270251
<i>P. ferrugatella</i>	AOC Lep 01531	IBLAO1539-20	AEF6784	OP270250

Voucher data, images, sequences, and trace files are publicly available on the Barcode of Life Data System (BOLD) (Ratnasingham & Hebert, 2007). Sequence divergences for the barcode region were calculated using the Kimura 2-parameter (K2P) model and the degrees of interspecific genetic variation were calculated using the analytical tools of BOLD. All the new and related species sequences were downloaded and aligned with the CLUSTAL algorithm of the MEGA6 program (Tamura et al. 2013). In order to assess the COI divergences between *P. ferrugatella* and the other systematically related species from Europe, we included all sites with the pairwise deletion option (Table I). Our sequences of *P. ferrugatella* (BOLD:AEF6784; n=3 seqs) and the public ones of *P. nucleolella* (BOLD:AAU2037; n=1) and *P. dahliaella* (BOLD:ACA9753; n=1) and, closely related species according to (Slamka, 2019): *Altophia combustella* (Herrich-Schäffer, 1852) (BOLD:ADK9057; n=2), *Catastia kistrandella* Opheim, 1963 (BOLD:AAI7381; n=3), *Catastia marginata* ([Denis & Schiffermüller], 1775) (BOLD:AAE9528; n=18), *Rhodophaea formosa* (Haworth, 1811) (BOLD:AAC8900; n=30), and *Selagia spadicella* (Hübner, 1796) (BOLD:AAE1543; n=24) were obtained from BOLD. Neighbour-Joining (NJ) and

Maximum Likelihood (ML) trees were calculated to visualise similarity among selected species. All trees presented the same topology and were practically identical, therefore, only the ML tree is presented here (Figure 2). Due to the fact that one gene is too few for reasonable phylogenetic analysis (Gatesy et al. 2007), the trees presented here do not reliably illustrate evolutionary relationships among the sequenced taxa. For the parameter values considered (e.g., sensitivity to codon bias and unequal rates of evolution) the statistical inconsistency of Maximum parsimony (MP) method may occur and was not performed in this study.

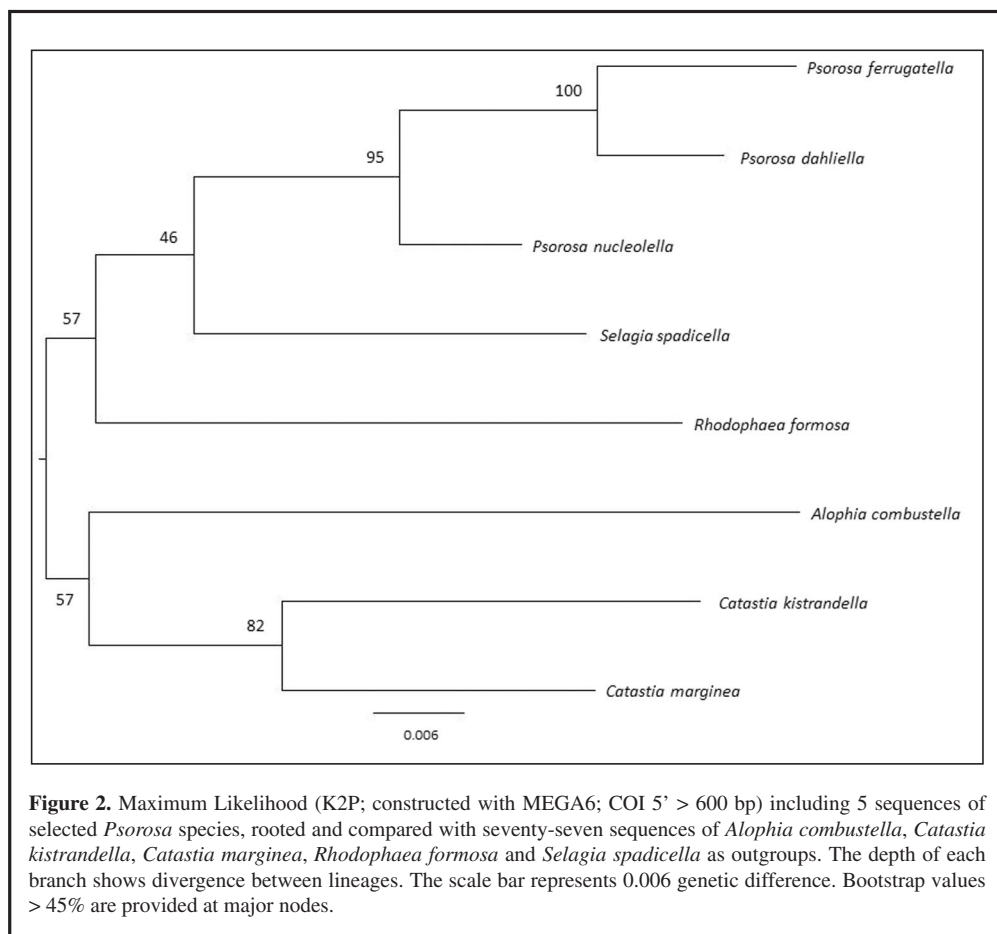


Figure 2. Maximum Likelihood (K2P; constructed with MEGA6; COI 5' > 600 bp) including 5 sequences of selected *Psorosa* species, rooted and compared with seventy-seven sequences of *Alophia combustella*, *Catastia kistrandella*, *Catastia marginea*, *Rhodophaea formosa* and *Selagia spadicella* as outgroups. The depth of each branch shows divergence between lineages. The scale bar represents 0.006 genetic difference. Bootstrap values > 45% are provided at major nodes.

Results and discussion

The *P. ferrugatella* specimens showed morphological traits typical of European individuals according to diagnosis in Slamka (2019). Previously *P. dahliaella* specimens referred in Girdley et al. (2020) were re-identified as *P. ferrugatella* and sequenced to match correct identification. Integrating the evidence from COI mitochondrial DNA sequences and adult morphology, we conclude that the *P. ferrugatella* specimens collected in the wetlands of the protected landscape of Humedal del Ajaque and Rambla Salada (Murcia) are genetically different to those co-generic species previously sequenced

from Europe based on mitochondrial data. Molecular data indicates significant divergence between *P. ferrugatella* with 2.1% mean distances to *P. dahliella*, 2.9% to *P. nucleolella* (Table II, Figure 2).

Divergence between *Psorosa* and the other closely related species varies between 4.2% and 8.1% (mean 6.6%; Table II) where the highest interspecific values were found between *P. ferrugatella* and *P. dahliella* with *Alophia combustella* (8.1% and 7.9%, respectively), whereas the lowest one was found between *P. nucleolella* and *Selagia spadicella* (4.2%). Differences among the other genera varies with the highest interspecific values between *Rhodophaea formosa* and *Alophia combustella* (8.2%) and the lowest one between *Catastia marginea* and *Rhodophaea formosa* with *Selagia spadicella* (5.9% and 6.1%, respectively) (Table II, Figure 2). The total number of nucleotide substitutions between species is 106 variable sites. In this sense, it seems that *Psorosa* is more closely related to *Selagia* (mean divergence: 5.3%) and with *Catastia* (mean: 6.3%) and *Rhodophaea* (mean: 6.5%).

Habitat description and biology

P. ferrugatella is a species previously known from across North Africa, with records from Morocco, Algeria, Tunisia, and Libya. The Portuguese specimen was recorded in the Algarve (Southwest of the Iberian Peninsula) at the salt marsh in the estuary of the Guadiana River, while the new specimens were recorded in a salt ravine in the protected landscape Humedal del Ajaque y Rambla Salada, in the La Llana beach in the Regional Park of Salinas y Arenales de San Pedro del Pinatar and in the Huerta de Alquerías in the plain of Murcia (Huerta de Murcia) (In the Southeast of the Iberian Peninsula).

Table II. Interspecific mean K2P (Kimura 2-Parameter) divergences (mean pairwise distances) based on the analysis of COI fragments (>600 bp).

	<i>Catastia kistrandella</i>	<i>Catastia marginea</i>	<i>Psorosa ferugatella</i>	<i>Psorosa dahliella</i>	<i>Psorosa nucleolella</i>	<i>Selagia spadicella</i>	<i>Rhodophaea formosa</i>
<i>Alophia combustella</i>	7.4	7.0	8.1	7.9	7.4	7.6	8.2
<i>Catastia kistrandella</i>		4.7	6.5	6.5	5.6	6.7	7.1
<i>Catastia marginea</i>			7.0	6.7	5.6	5.9	7.1
<i>Psorosa ferugatella</i>				2.1	3.3	5.8	7.1
<i>Psorosa dahliella</i>					2.9	5.9	6.5
<i>Psorosa nucleolella</i>						4.2	6.1
<i>Selagia spadicella</i>							6.1

The landscapes of the riverside plain of the Guadalquivir River, in the furthest point south of the Iberian Peninsula where *P. ferrugatella* was recorded by Ranki et al. (2022), are characterised by the irregular regime of water inputs, which can go from flooding the marshland to turning it into a desert dryland. These circumstances, and the marine influence due to its proximity to the river mouth, produce soils with varied salinity content that characterise the vegetation. This habitat is characterised by a series of hyperhalophilic Mediterranean-Ibero-Atlantic thermomediterranean edaphohydrophilic vegetation (EH20) which forms estuaries, salt marshes and marshes with a mixture of salty and fresh waters which is represented by the communities *Spartinetum maritimae*, *Puccinellio-Sarcocornietum perennis*, *Halimiono-Sarcocornietum alpini*, *Cistancho-Arthrocnemetum macrostachyi*, *Polygono-Limonastretum monopetalii* and, on the edge of the estuaries, the halonitrophilic community,

Cistancho-Suaedetum verae. Sometimes, a plantation of *Polygono-Tamaricetum africanae* may appear. The land use bordering the habitat are mainly agricultural fields (Figure 3A) (CMAOT, 2015).

The Humedal del Ajauque y Rambla Salada is an area characterised by semi-desert landscapes around seasonal or sporadic water courses at 150 m above sea-level with halophytic habitats in a semi-arid, thermo-Mediterranean bioclimatic environment, colonised by highly specialised fauna and flora adapted to the extreme conditions imposed by soil moisture and salinity which is represented by the plant association *Frankenio corymbosae-Arthrocnemetum macrostachyi* including the main following plants: *Arthrocnemum macrostachyum* (Moric.) Moris, *Sarcocornia fruticosa* (L.) A. J. Scott, *Frankenia corymbosa* Desf., *Suaeda vera* Forsk. ex J. F. Gmel., *Halimione portulacoides* (L.) Aellen, *Tamarix boveana* Bunge, *Lygeum spartum* L., *Limonium caesium* (Girad) Kuntze, *L. cossonianum* Kuntze, *Inula crithmoides* L. and *Phragmites australis* (Cav.) Trin. ex Steud (Figure 3B).

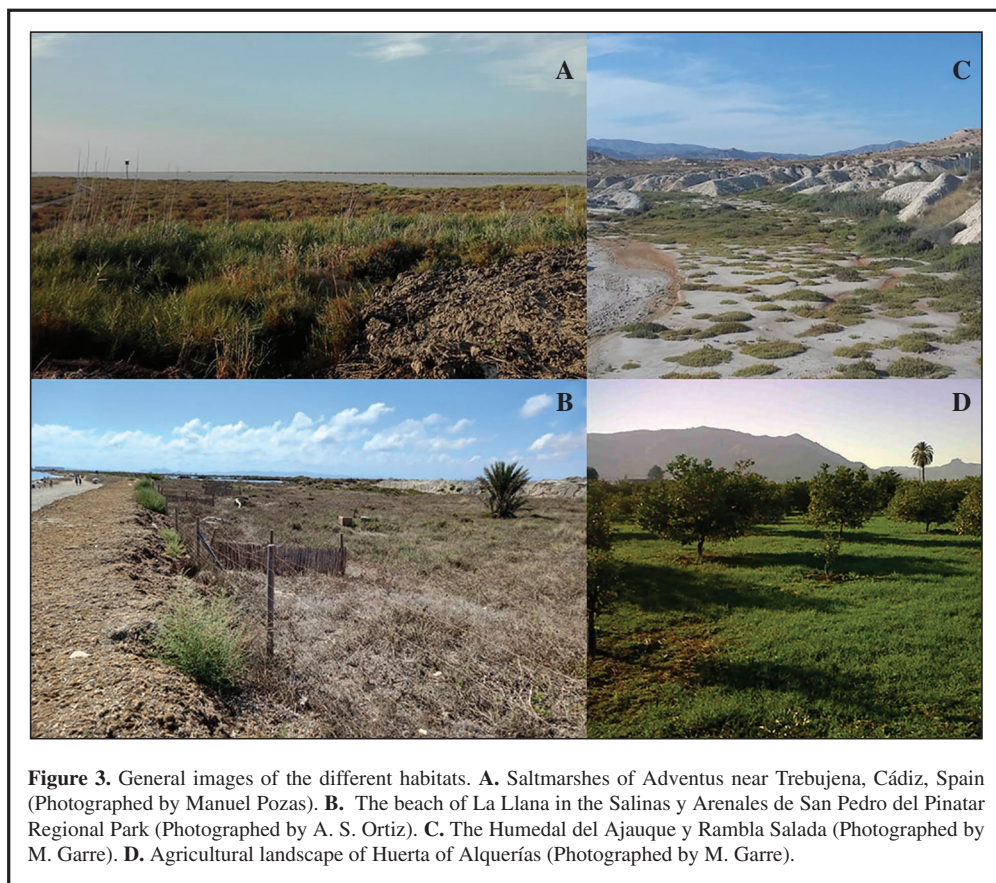


Figure 3. General images of the different habitats. **A.** Saltmarshes of Adventus near Trebujena, Cádiz, Spain (Photographed by Manuel Pozas). **B.** The beach of La Llana in the Salinas y Arenales de San Pedro del Pinatar Regional Park (Photographed by A. S. Ortiz). **C.** The Humedal del Ajauque y Rambla Salada (Photographed by M. Garre). **D.** Agricultural landscape of Huerta of Alquerías (Photographed by M. Garre).

The beach of La Llana in the Salinas y Arenales de San Pedro del Pinatar Regional Park is integrated in a sand dune system composed of mobile sands (strandline, embryo, and mobile dunes) and consolidated sands (semi-fixed and fixed dunes) and alternating with these dune slacks are formed. This ridge of dunes delimits a salt marsh. The vegetation that colonises sand dunes is adapted to limiting factors such as sand burial, salt spray and xeric conditions, so few plant species can survive in these environments. The specimen was caught on the semi-fixed dunes in the domain of the plant association *Loto cretici-Crucianelletum maritimae*. Chamaephytes such as *Teucrium dunense* Sennen,

Crucianella maritima L., *Helichrysum stoechas* subsp. *caespitosum* (C. Presl.) DC. and *Ononis natrix* subsp. *ramosissima* (Desf.) Batt. in Batt. & Trab. are the most characteristic species (Figure 3C).

The Huerta of Alquerías is an agricultural territory irrigated by the Segura River through an ancient network of canals. Citrus groves are the main crops and natural vegetation is restricted to the margins of paths, borders of crops, abandoned fields and banks of irrigation canals. Nitrophilous and ruderal species are predominant, besides some hygrophilous plants such as *Tamarix canariensis* Willd., *Cynanchum acutum* L., *Phragmites australis* (Cav.) Trin. ex Steud., *Arundo donax* L. and *Apium nodiflorum* (L.) Lag (Figure 3D).

The characteristics of these habitats suggest that *Psorosa ferrugatella* inhabits habitats that are not conditioned by the prevailing macroclimate, but to edaphic conditions, particularly soil moisture, salinity gradient and soil structure, that determine the selection of several plant communities of a non-climax character.

In relation to the biology of the species, Slamka (2019) states that it flies from March to October in several generations, while in our territory sightings took place from early April to late June. Host plant and early stages are also unknown.

We emphasise again the combination of traditional morphological analysis and ecological traits with the additional dataset of DNA sequences for those taxonomic groups whose identification is particularly difficult due to the small size, handling and that it is mainly based on differences in the genitalia.

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Manuel Garre
Gran Vía Escultor Salzillo, 7
E-30004 Murcia
ESPAÑA / SPAIN
E-mail: manuel.garre@fripozo.com
<https://orcid.org/0000-0002-5846-8621>

John Girdley
Atlántico, 114-2ºB
Hacienda Riquelme Golf Resort
E-30590 Sucina (Murcia)
ESPAÑA / SPAIN
E-mail: john@birdtours.co.uk
<https://orcid.org/0000-0001-7976-7439>

Rosa M. Rubio
Departamento de Zoología y Antropología Física
Área de Biología Animal
Facultad de Veterinaria
Universidad de Murcia
Campus de Espinardo
Apartado 4021
E-30071 Murcia
ESPAÑA / SPAIN
E-mail: rmrubio@um.es
<https://orcid.org/0000-0002-0109-7874>

Juan J. Guerrero
Departamento de Zoología y Antropología Física
Área de Biología Animal
Facultad de Veterinaria
Universidad de Murcia
Campus de Espinardo
Apartado 4021
E-30071 Murcia
ESPAÑA / SPAIN
E-mail: juanjogf@um.es
<https://orcid.org/0000-0002-9645-5266>

y / and

*Antonio S. Ortiz
Departamento de Zoología y Antropología Física
Área de Biología Animal
Facultad de Veterinaria
Universidad de Murcia
Campus de Espinardo
Apartado 4021
E-30071 Murcia
ESPAÑA / SPAIN
E-mail: aortiz@um.es
<https://orcid.org/0000-0002-3877-6096>

*Autor para la correspondencia / *Corresponding autor*

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