SHILAP Revista de lepidopterología, 53 (209) marzo 2025: 147-153 eISSN: 2340-4078 ISSN: 0300-5267
https://doi.org/10.57065/shilap.1033 https://shilap.org

Description of *Scythris quinquepraedia* Garre & Ortiz, sp. nov. from the Iberian Peninsula (Lepidoptera: Scythrididae)

Manuel Garre & Antonio S. Ortiz

Abstract

A new *Scythris* species (Lepidoptera: Scythrididae), *S. quinquepraedia* Garre & Ortiz, sp. nov., is described from the Iberian Peninsula. Male adult and genitalia are illustrated and the genetic distance, based on DNA barcodes are compared against the closely species of *pascuella-, punctivittella-* and *seliniella*-group from the public BOLD database.
Keywords: Lepidoptera, Scythrididae, *Scythris quinquepraedia*, new species, taxonomy, DNA barcoding, Iberian Peninsula.

Descripción de *Scythris quinquepraedia* **Garre & Ortiz, sp. nov. de la Península Ibérica (Lepidoptera: Scythrididae)**

Resumen

Se describe *Scythris quinquepraedia* Garre & Ortiz, sp. nov. (Lepidoptera: Scythrididae) en la Península Ibérica utilizando la morfología del adulto macho, el andropigio y la distancia genética, basada en códigos de barras de ADN que se compara con las especies cercanas de los grupos *pascuella*, *punctivittella* y *seliniella* disponibles en la base de datos en The Barcode of Life (BOLD).
Palabras clave: Lepidoptera, Scythrididae, *Scythris quinquepraedia*, nueva especie, taxonomía, ADN código de barras, Península Ibérica.

Introducción

The family Scythrididae is a medium-sized family within the Gelechioidea, with approximately 669 species worldwide (Nieukerken et al. 2011) and currently reaching 923 species (Falck 2023). The family have a great diversity in the Mediterranean area with most species belonging to the genus *Scythris* Hübner, [1825] with more than 400 species (Bengtsson, 1997). The checklist of Scythrididae of Spanish mainland comprises 109 species according to Vives Moreno (2014: 102 species) that considers *Scythris flavilaterella* (Fuchs, 1886) and *Scythris lampyrella* (Constant, 1865) as synonyms of *Scythris cuspidella* ([Denis & Schiffermuller], 1775), with later addition of *Enolmis delnoydella* Groenen & Schreurs, 2016 and *Scythris spiniferella* Nupponen & Savenkov, 2019 described based on Spanish material (Groenen & Schreurs, 2016; Nupponen & Savenkov, 2019) and new records for the Iberian Peninsula as *Scythris camelella* Walshingham, 1907 (Richter & Sumpich, 2020), *Scythris larzacensis* Delmas, 2010 (Requena & Pérez De-Gregorio, 2017) and *Scythris sinensis* (Felder & Rogenhofer, 1875) (Corley et al. 2021).

The use of DNA barcoding as an effective tool for species diagnosis in the animal kingdom has been proposed as the best option to overcome the gap between existing taxonomic information and the need for efficient and reliable species identification, particularly for butterflies (Hebert et al. 2003). DNA barcoding provides a fast and inexpensive alternative strategy for identifying described species and discovering new species (Hebert et al. 2003; Savolainen et al. 2005; Mitchell, 2008). Combining molecular methods with morphological species identification can speed up biodiversity inventories and help elucidate the status of suspect species.

The present investigation was prompted by results obtained during an effort to barcode all Macroheteroceran Lepidoptera species in the Iberian Peninsula, which revealed that new *Scythris* specimens were grouped into unique sequence clusters. The species collected from Spain differs from other *Scythris* species in morphological and molecular traits and is described here as a new species, *Scythris quinquepraedia* Garre & Ortiz, sp. nov.

Material and Methods

The images of the adults (Figure 1A) were taken with a Nikon D70 digital camera. Images were z-stacked using the software Zerene. Morphology of the male genital structures (Figure 1B) were studied using a Zeiss Stemi 508 stereomicroscope with a Zeiss Axiocam ICc5 digital camera and were compared with those published by Bengtsson (1997) and, subsequently, he was asked for his personal opinion on its taxonomic status. Specimens were deposited in the Research Collection of Biología Animal in the Department of Zoology and Physical Anthropology of the University of Murcia (Spain).

Tissue of unique sample was processed and sequenced at the Canadian Centre for DNA Barcoding (CCDB, Guelph) to obtain DNA barcodes using the standard high-throughput protocol described by deWaard et al. (2008) which can be accessed at www.dnabarcoding.ca/pa/ge/research/protocols. Voucher data, GPS coordinates, images, sequences, Genbank Accession, and trace files are publicly available through the public data set (dx.doi.org/10.5883/DS-SCYTHRIS) in BOLD.

Figure 1. Adult specimen of *Scythris quinquepraedia* sp. n. **A.** Male holotype AOCLep02710. **B.** Male genitalia AOCLep02710.

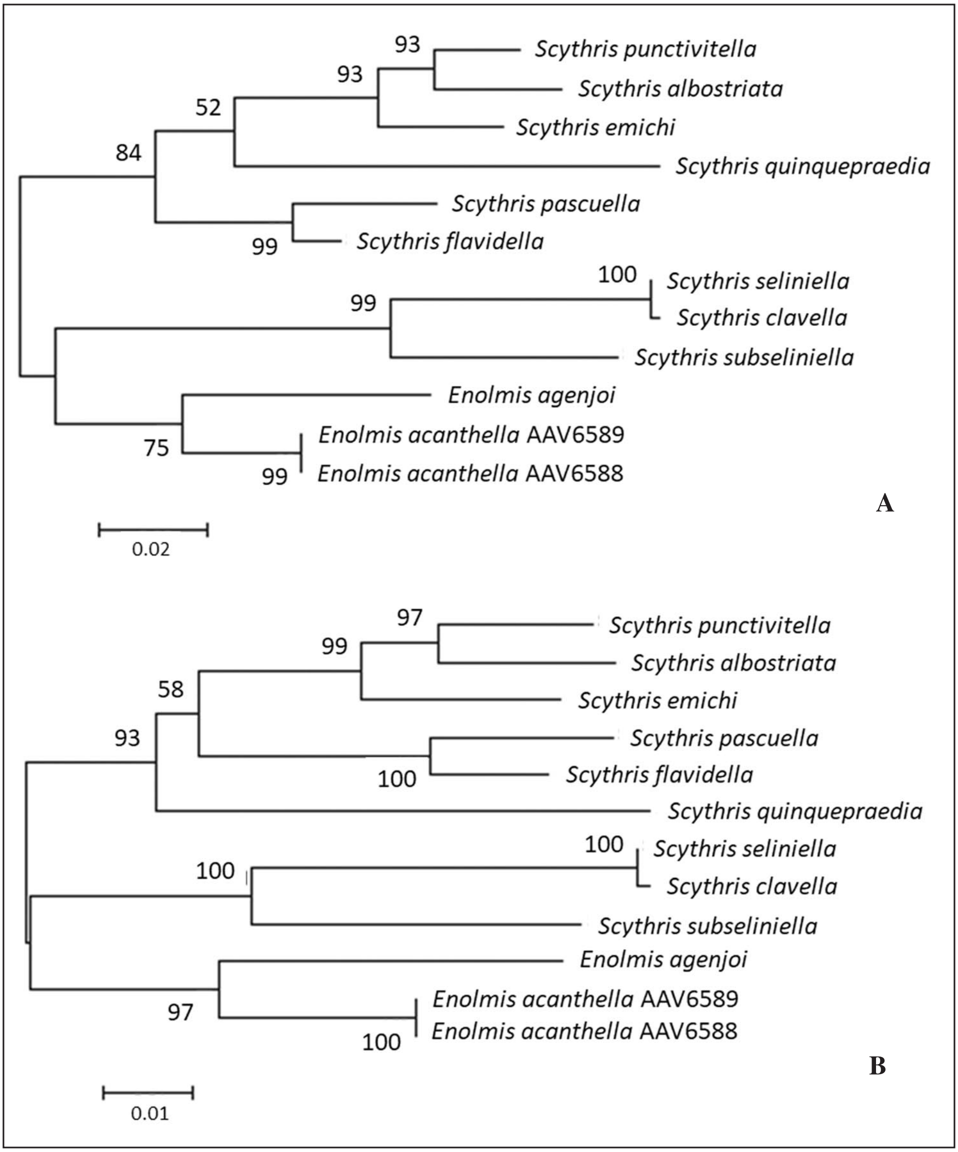

Figure 2. Phylogenetic trees obtained from 46 nucleotide COI sequences. **A.** Maximum Likelihood tree. **B**. Neighbour-Joining tree. The depth of each branch shows divergence within lineages. Bootstrap values are provided at major nodes. The scale bar represents 0.01 genetic difference.

Sequence was compared to a reference library of Lepidoptera barcodes using the identification engine (BOLD-ID). The reference barcode database for Scythrididae used by BOLD-ID is continually validated by specialists to ensure accurate identifications and is particularly well parameterized due to a global campaign to barcode the 618 species of the family (accessed May 20, 2024).

Sequence divergences for the barcode region were calculated using the Kimura 2-parameter (K2P) model (Kimura 1980) and the degrees of interspecific genetic variation were calculated using the analytical tools of BOLD. All the new and public species sequences were downloaded and aligned with the CLUSTAL algorithm of the MEGA6 software (Tamura et al. 2013). Boot-strap values were calculated with 1000 replicates, and initial Neighbor-joining (NJ) and Maximum Likelihood (ML) trees based on distance were constructed with the MEGA6 software. We selected other *Scythris* species as *S. flavidella* Preissecker, 1911 and *S. pascuella* (Zeller, 1855) belonging to *pascuella* species group; *S. albostriata* Hannemann, 1961, *S. punctivittella* (Costa, [1836]) and *S. emichi* (Anker, 1870) belonging to *punctivittella* species group; and *S. clavella* (Zeller, 1855), *S. seliniella* (Zeller, 1839) and *S. subseliniella* (Heinemann, [1876]) belonging to *seliniella* species group for congeneric comparison and, *Enolmis acanthella* (Godart, 1824) and *Enolmis agenjoi* Passerin d'Entrèves, 1988, which are taxonomically related into family Scythrididae (Bengtsson, 1997), as outgroups to root the trees. In order to assess the COI divergences between the taxa, we included all sites with the pairwise deletion option. All trees presented similar topology with some differences in the position of *S. quinquepraedia* and, therefore, both trees (ML and NJ) are presented here (Figures 2A, B). Because one gene is far too little for reasonable phylogenetic analysis (Gatesy et al. 2007), the trees presented here do not reliably illustrate evolutionary relationships among the sequenced taxa.

Results and discussion

Scythris quinquepraedia Garre & Ortiz, sp. nov.

Barcode Index Number. BOLD:AFB2144

Type material: Holotype, Spain, 1 ♂, Murcia, Alquerías [coordinates 38.007, -1.033], 32 m altitude, 28-VIII-2020, leg. M. Garre, Genprep and BOLD sample ID: AOCLep02710.

Diagnosis: *S. quinquepraedia* is easly confused with other dark, small Scythrididae by the external appearance. All characters indicate that belongs to the *S. pascuella* species group, which initially comprised 13 species (Bengtsson, 1997) and actually includes 36 species (Savela, 2024). Externally this group is characterized by unicolorous and dark fuscous forewing, sometimes with greenish lustre and glossy but exceptions can be seen in specimens from North Africa and in the east part of the Mediterranean area. Uncus prominent, bifurcate; gnathos curved, pointed and somewhat hooked. Aedeagus medium-sized, slightly curved, pointed. Valvae longish, bent, with rounded or tapering ends. Sternum 8 subtriangular with a posterior furcation. In female genitalia for species of this group no common feature has been observed (Bengtsson, 1997). Examination of the male genitalia is essential for safe determination of *S. quinquepraedia.*

Description (Figure 1A): Wingspan 9.4 mm. Head and haustellum covered with golden, appressed, slender, parallel-sided scales oriented longitudinally. Labial palp slightly upturned, right, terminal segment brownish pointed and segments 2-3 yellowish white mottled. Antenna blackish brown, approximately the same length as the forewing. Vertex, neck tuft, collar, tegula and thorax dark brown, mottled dorsally with golden scales, especially around the neck and thorax, and ventrally with silver glossy scales. Forewing dark brown mottled with bright white scales. Whitish fringe in the antemedian fascia degradated through submedian interfascia to the base. Hindwing width about 1/2 of the forewing, dark grey. Abdomen greyish mottled with golden scales.

Male genitalia (Figure 1B): Unique and very easy to identify when it is compared with the other species in the family Scythrididae. Uncus with a roundish and minutely warted plate, slightly incurved posteriorly. Gnathos broad at base, then tapering, straight and pointed. Socii rounded at tip, long and setose. Valvae symmetrical, club-shaped, strongly bent in posterior half; at tip furnished with a dense row of short bristles. Tergum 8 as a squarish and warty plate. Sternum 8 subtriangular, posterior extension very long with bifurcated apex; prongs short and thick. Aedeagus long, evenly curved and tapering to the tip.

Distribution and abundance: Probably, an endemism from southeast of the Iberian Peninsula (known only from one locality in the Murcia Region). Scarce and local in agricultural landscape of Huerta de Murcia.

Biology: Flight period end-August, most likely in a single generation in the Huerta of Alquerías. This characterized by an ancient canal network feeds an agricultural landscape watered by the Segura River through an ancient canal network. The primary crops are citrus groves with natural vegetation in the margins of paths, borders of crops, abandoned fields and banks of irrigation canals. With the exceptions of some hygrophilous plants as *Phragmites australis* (Cav.) Trin. ex Steud., *Arundo donax* L. and *Apium nodiflorum* (L.) Lag., nitrophilous and ruderal species predominate (Figure 3). Early stages unknown.

Genetic remarks: BIN BOLD:AFB2144 (n=1; Table 1; sequence length 658 bp). Based on COI divergence, the new species is separated from the other *Scythris* species compared in our study by genetic distances of 11.4% (mean divergence; n= 43) (Figure 2). This value is high compared to 10.2% mean divergence among all the studied species (Table 2, Figure 2) and belongs to a phylogenetically *S. pascuella* isolated lineage well supported by morphology and genetic data.

Table 1. Interspecific mean K2P (Kimura 2-Parameter) divergences (mean pairwise distances in %) based on the analysis of COI fragments (>500 bp) among *Scythris quinquepraedia* and other *Scythris* species (SPU: *S. punctivittella*; SEM: *S. emichi*; SFL: *S. flavidella*; SPA: *S. pascuella*; SQU: *S. quinquepraedia*; SCL: *S. clavella*; SSE: *S. seliniella;* SSU: *S. subseliniella*).

	SPU	SEM	SFL	SPA	SQU	SCL	SSE	SSU
Scythris albostriata	3.9	5.2	8.8	8.8	10.2	13.0	12.7	11.1
Scythris punctivittella		4.9	8.7	8.8	10.8	13.0	12.7	11.6
Scythris emichi			8.2	8.8	8.8	12.9	12.6	12.2
Scythris flavidella				4.0	9.9	13.1	12.8	12.5
Scythris pascuella					10.9	14.3	14.0	13.0
Scythris quinquepraedia						14.0	13.7	12.8
Scythris clavella							0.3	8.1
Scythris seliniella								7.8

Derivation of name: Lat. *quinque* = five and *praedia* = farm, refers to the original name of the locality of the first studied specimen, in Spanish Cinco Alquerías, actually the locality of Alquerías, a district belonging to the municipality of Murcia (Spain).

Molecular analysis: DNA barcoding data results obtained in our study indicate that *Scythris quinquepraedia* sp. n. is rather isolated from other congeneric species studied and, even, from *Enolmis* species (Figure 2). Interspecific distances of the new species compared to the other *Scythris* species spanned from 8.8% to *S. emichi* and 14.0% to *S. clavella* and differs from the rest of the *Scythris* species with mean values of 10.2% (Table 2). Differences in barcode sequences were higher than 2% and, according to Hausmann et al. (2011), in different groups of invertebrate taxa, a sequence divergence in the barcode region higher than 2% are typical of interspecific variation and recognized as distinct MOTUs, while lower values often correspond to intraspecific differences.

A phylogenetic hypothesis with Neighbour-Joining (NJ) and Maximum Likelihood (ML) trees of COI barcode region were generated using MEGA software and all the species studied could be unequivocally assigned to one of the clades (Figure 2). The monophyly of the genera *Scythris* was recovered by all those methods and the molecular results enable to preliminarily division into four Scythrididae species groups, *punctivittella-*, *seliniella-* and *pascuella*-group, and the *Enolmis* species, as outgroups to root the tree (Figure 2). Nevertheless, *Scythris quinquepraedia* differs from each *punctivittella-* and *pascuella*-group by 10.1% (Mean K2P distance) and the species is placed in a different position depending on ML or NJ trees construction (Table 2, Figure 2). We are trying to capture new specimens for comparisons and confirm all the differences found in male genitalia and barcode.

Acknowledgements

We are very grateful Dr. Bengt Å. Bengtsson for your help and comments for the right species identification. Also, to the staff at the Canadian Centre for DNA Barcoding for sequence analysis. Dr. Paul D. N. Hebert and many other colleagues of the Barcode of Life project (Biodiversity Institute of Ontario, Guelph, Canada) contributed to the success of this study. Special thanks to our colleagues John Girdley, Rosa María Rubio, Dr. José Luis Yela and Dr. Juan José Guerrero for their comments and technical support.

Environmental Authorities in "Región de Murcia" gave permission for collection and access to field sites. We also thank to various anonymous referees for their comments. We are very grateful for this collegial and kind support. This study has been partially supported by the project Fauna Ibérica XII - Lepidoptera: Noctuoidea I (PGC2018-095851-B-C63) of the Spanish Ministry of Research.

References

Bengtsson, B. Å. (1997). Scythrididae. *Microlepidoptera of Europe* (Vol. 2). Apollo Books. https://doi.org/10.1163/9789004631359

Corley, M. F. V., Cardoso, H., Nunes, J., Valadares, A., & Valkenburg, T. (2022). New and interesting Portuguese Lepidoptera records from 2021 (Insecta: Lepidoptera). *SHILAP Revista de lepidopterología*, *50*(200), 617-631. https://doi.org/10.57065/shilap.270

deWaard, J. R., Ivanova, N. V., Hajibabaei, M., & Hebert, P. D. N. (2008). Assembling DNA barcodes: Analytical Protocols. In C. C. Martin (Ed.). *Methods in Molecular Biology: Environmental Genomics* (pp. 275-293). Humana Press Inc. https://doi.org/10.1007/978-1-59745-548-0_15 PMid:18642605

Falk, P. (2023). New data on the genus *Scythris* Hübner, [1825] from the Canary Islands (Spain), with description of six new species (Lepidoptera: Scythrididae). *SHILAP Revista de lepidopterología, 51*(204), 653-680. https://doi.org/10.57065/shilap.788

Gatesy, J., DeSalle, R., & Wahlberg, N. (2007). How many genes should a systematist sample? Conflicting insights from a phylogenomic matrix characterized by replicated incongruence. *Systematic Biology, 56*, 355-363. http://dx.doi.org/10.1080/10635150701294733. PMid:17464890

Groenen, F., & Schreurs, A. (2016). *Enolmis delnoydella* Groenen & Schreurs, sp. n., a new species from Spain (Lepidoptera: Scythrididae). *SHILAP Revista de lepidopterología*, *44*(173), 45-47. https://doi.org/10.57065/shilap.656

Hausmann, A., Haszprunar, G., & Hebert, P. D. N. (2011). DNA barcoding the Geometrid fauna of Bavaria (Lepidoptera): successes, surprises, and questions. *PloS ONE*, *6*(2), e17134. https://doi:10.1371/journal.pone.0017134. PMid:21423340 PMCid:PMC304064

Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, *270*, 313-322. https://doi.org/10.1098/rspb.2002.2218 PMid:12614582 PMCid:PMC1691236

Kimura, M. (1980). A simple model for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution, 16*, 111-120. https://doi:10.1007/BF01731581. PMid:7463489

Mitchell, A. (2008). DNA barcoding demystified. *Australian Journal of Entomology*, *47*, 169-173. http://doi.org/10.1111/j.1440-6055.2008. 00645.x

Nieukerken, E. J. van, Kaila, L., Kitching, I. J., Kristensen, N. P., Lees, D. C., Minet, J., Mitter, Mutanen, M., Reiger, J. C., Simonsen, T. J., Wahlberg, N., Yen, S.-H., Zahiri, R., Adamski, D., Baixeras, J., Bartsch, D., Bengtsson, B. Å. Brown, J. W., Bucheli, S. R., Davis, D. R., De Prins, J., De Prins, W., Epstein, M. E., Gentili-Poole, P., Gielis, C., Hättenschwiler, P., Hausmann, A., Holloway, J. D., Kallies, A., Karsholt, O., Kawahara, A., Koster, S. J. C., Kozlov, M., Lafontaine, J. D., Lamas, G., Landry, J.-F., Lee, S., Nuss, M., Park, K. T., Penz, C., Rota, J., Schmidt, B. C., Schintlemeister, A., Sohn, J. C., Solis, M. A., Tarmann, G. M., Warren, A. D., Weller, S., Yakovlev, R. V., Zolothuhin, V. V., & Zwick, A. (2011). Order Lepidoptera Linnaeus, 1758. In Z.-Q. Zhang (Ed.). Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness. *Zootaxa*, *3148*, 212-221. https://doi.org/10.11646/zootaxa.3148.1.41

Nupponen, K., & Savenkov, N. (2019). Descriptions of two new species of the family Scythrididae from Europe

(Lepidoptera: Scythrididae). *SHILAP Revista de lepidopterología*, *47*(187), 469-474. https://doi.org/10.57065/shilap.518

Requena, E., & Pérez de-Gregorio, J. J. (2017). Contribució al coneixement del gènere *Scythris* Hübner, [1825], a Catalunya (Lepidoptera; Scythrididae). *Butlletí de la Societat Catalana de Lepidopterologia, 107*, 99-106.

Richter, R., & Sumpich, J. (2020). *Scythris camelella* Walshingham, 1907 recorded in Spain as new species for Europe (Lepidoptera Scythrididae). *SHILAP Revista de lepidopterología, 48*(189), 115-117. https://doi.org/10.57065/shilap.402

Savela, M. (2024). *Lepidoptera and some other life forms*. http://www.nic.funet.fi/pub/sci/bio/life/insecta/lepidoptera/

Savolainen, V., Cowan, R. S., Vogler, A. P., Roderick, G. K., & Lane, R. (2005). Towards writing the encyclopaedia of life: an introduction to DNA barcoding. *Philosophical Transactions of the Royal Society B*, *360*, 1805-1811. https://doi.org/10.1098/rstb.2005.1730 PMid:16214739 PMCid:PMC1609222

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*, *30*, 2725-2729. https://doi.org/10.1093/molbev/mst197 PMid:24132122 PMCid:PMC3840312

Vives Moreno, A. (2014). *Catálogo sistemático y sinonímico de los Lepidoptera de la Península Ibérica, de Ceuta, de Melilla y de las islas Azores, Baleares, Canarias, Madeira y Salvaje*s *(Insecta: Lepidoptera)*. Improitalia.

Manuel Garre
Departamento de Zoología y Antropología Física
Facultad de Veterinaria
Universidad de Murcia
Campus de Espinardo
E-30071 Murcia
ESPAÑA / *SPAIN*
E-mail: mgarre@um.es
https://orcid.org/0000-0002-5846-8621

y / *and*

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

*Autor para la correspondencia / *Corresponding author*

(Recibido para publicación / *Received for publication* 22-III-2024)
(Revisado y aceptado / *Revised and accepted* 19-V-2024)
(Publicado / *Published* 30-III-2025)