

Contribution to the knowledge of *Stygioides italica* Mazzei & Yakovlev, 2016 (Lepidoptera: Cossidae)

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Abstract

With this new report of *Stygioides italica* Mazzei & Yakovlev, 2016 for southern Italy (Calabria: Monte Pollino), we take the opportunity to survey some populations from central and southern Italy from a molecular-genetic point of view and to highlight some morphoanatomical characters that may facilitate the distinction between the recent *S. italica* Mazzei & Yakovlev and *Stygioides colchica* (Herrich-Schäffer, 1851).

Keywords: Lepidoptera, Cossidae, *Stygioides italica*, *Stygioides colchica*, Italy.

Contribución al conocimiento de *Stygioides italica* Mazzei & Yakovlev, 2016 (Lepidoptera: Cossidae)

Resumen

Con este nuevo informe de *Stygioides italica* Mazzei & Yakovlev, 2016 para el sur de Italia (Calabria: Monte Pollino), aprovechamos la oportunidad para sondear algunas poblaciones del centro y sur de Italia desde un punto de vista genético-molecular y destacar algunos caracteres morfoanatómicos que pueden facilitar la distinción entre los recientes *S. italica* Mazzei & Yakovlev y *Stygioides colchica* (Herrich-Schäffer, 1851).

Palabras clave: Lepidoptera, Cossidae, *Stygioides italica*, *Stygioides colchica*, Italia.

Contributo alla conoscenza di *Stygioides italica* Mazzei & Yakovlev, 2016 (Lepidoptera: Cossidae)

Riassunto

Con questa nuova segnalazione di *Stygioides italica* Mazzei & Yakovlev, 2016 per il sud Italia (Calabria: Monte Pollino), si coglie l'occasione per sondare sotto l'aspetto genetico-molecolare alcune popolazioni dell'Italia centrale e meridionale ed evidenziare alcuni caratteri morfo anatomici che possono agevolare la distinzione fra la recente *S. italica* Mazzei & Yakovlev e *Stygioides colchica* (Herrich-Schäffer, 1851).

Parole chiave: Lepidoptera, Cossidae, *Stygioides italica*, *Stygioides colchica*, Italia.

Introduction

The recent description of *Stygioides italica* Mazzei & Yakovlev, 2016 (Lepidoptera: Cossidae) asked for a revision of the scarce records available for Italy concerning *Stygioides colchica* (Herrich-Schäffer, 1851) (= *tricolor* auct. nec Lederer, 1858) (Pinzari & Pinzari, 2020).

First Italian records are very old (Curò, 1890; Ragusa, 1893), followed by other data some of which very recent (Turati, 1919; Dannehl, 1927a,b,c,d; Daniel, 1954-55; de Freina & Witt, 1990; Bertaccini et al. 1997; Parenzan & Porcelli, 2006; Grassi et al. 2007; Cabella & Fiori, 2010; Pinzari & Pinzari, 2023).

The careful examination of a male collected on the 1st of July 2002 in the Abruzzo region, around Campo Felice, L'Aquila, at 1300 m a.s.l., and initially identified as *Stygioides colchica* (Grassi et al. 2007), led to the description of a new species, *Stygioides italica* Mazzei & Yakovlev, 2016. As consequence, all previous Italian records of *Stygioides colchica* need to be revised to ascertain whether both species are present in Italy or not. Our research, supported by molecular analyses, indicate the presence of one species only (*Stygioides italica*). However, further investigation is needed to investigate the presence in Sicily on the Madonie Mountains (Ragusa, 1893) due to data uncertainty and isolation of island populations.

Italian distribution of *Stygioides italica* Mazzei & Yakovlev, 2016 (nec *Stygioides colchica* Herrich-Schäffer, 1851 = *tricolor* auct. nec Lederer, 1858) was largely documented in Mazzei & Yakovlev (2016), Pinzari & Pinzari (2020), here updated by one record in Apulia (Rolli, 2023), several specimens from Latium (Pinzari & Pinzari, 2023), and one more original record.

Materials and methods

The field collecting was carried out on the South slope of the Mount Pollino, on a dry rocky prairie (Figures 1-2). Snow melting was incomplete and spring flowering just started. During collecting day there were sunny sky, no wind, and warm temperatures (16-18°C).

The legs of four *Stygioides italica* specimens, 1 ♀, Monte Pollino, Calabria, Italy, 2050 m, 08-VI-2022, sample ID: BC_ZSM_Lep_116421, leg. Bertaccini, coll. Bertaccini; 1 ♀, Vallemare, Lazio, Italy, 1455 m, 2-VI-2022, sample ID: BC_ZSM_Lep_116423, leg. M. Pinzari, coll. Pinzari; 1 ♀, Aranova, Fiumicino, Lazio, Italy, 50 m, 3-VI-2020, sample ID: BC_ZSM_Lep_116422, leg. Mn. & M. Pinzari, coll. Pinzari; 1 ♂, Vallemare, Lazio, Italy, 1455 m, 2-VI-2022, sample ID: BC_ZSM_Lep_117095, leg. M. Pinzari, coll. Bertaccini) were submitted to molecular barcoding analysis to explore intra-specific genetic diversity. The standard protocol of the Canadian Centre for DNA Barcoding (CCDB) was used for sequencing the barcode fragment (658bp) of the mitochondrial cytochrome oxidase gene, subunit 1 (COI 5'), which is accepted as a standard marker for the identification of most animals. LepF1 and LepR1 were the primers used for PCR and sequencing (Hajibabaei et al. 2006). Sequences are deposited in the Barcode of Life DataSystems (BOLD), accessible at www.boldsystems.org in the public dataset DS-STYGIOID (doi: <https://dx.doi.org/10.5883/DS-STYGIOID>).

Comparisons of male genitalia of *S. italica* with its nearest taxa were carried out by using images available in de Freina & Witt (1990) for *S. colchica* and in Saldaitis et al. (2007) for *Stygioides colchica dercetis* (Grum-Grshimailo, 1899).

Results and Discussions

Original record: Mount Pollino, Cosenza, Italy, 2050 m a.s.l., 1 ♀, 8-VI-2022.

The female was found on the South slope of the Mount Pollino, on a dry rocky prairie (Figures 1-2). We observed very scarce butterflies and small geometrids such as several *Cleta filacearia* (Herrich-Schäffer, [1847]) and rare *Lythria cruentaria* (Hufnagel, 1767). At 13:30 a specimen supposedly belonging to the Psychidae family was observed flying frenetically on the ground, jumping from one flower to the next. Its correct identification as a specimen of *Stygioides*, very rare in Italy, was early recognized and here specifically identified as *Stygioides italica* Mazzei & Yakovlev, 2016.

To the best of our knowledge, this is the record at the highest altitude for this species. Previously a female was found on the Montalto, Aspromonte Mountains, at 1700 metres above the sea level (Bertaccini et al. 1997).

Stygioides italica was found in very different habitats despite the paucity of records, being collected from lowland to more than 2000 metres of altitude. Larval foodplants are unknown, but we can suppose it feeds on some Boraginaceae such as *Echium* and *Cynoglossum* like the congeneric *Stygioides colchica* (Korb, 1910).

Stygioides italica seems to be an Italian endemic, whilst *Stygioides colchica* is known from Greece (Peloponnese peninsula), SW Russia, Ukraine (Crimea, Zaporozhskaya Reg.) Turkey, Lebanon, Syria, Israel, Armenia, and Iran (Alipanah et al. 2021).

DNA barcoding analyses recovered a full sequence of 658bp for the Pollino and one of the Vallemare specimens and a shorter sequence of 627bp for the second specimen from Vallemare and of 345bp for the Aranova specimen as follows:

Sample ID: BC_ZSM_Lep_116421; sequence ID: GWOUK940-22; Pollino (658bp)

AACATTATATTTTTATTTTTGGTATTTGATCTGGATTAGTAGGAACCTCTCTTAGTCTTTTT
AATTCGAGCTGAATTAGGTAATCCTGGATCTTTAATTGGTAATGATCAAATTTATAATA
CTATTGTTACAGCTCATGCTTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGG
AGGTTTTGGTAATTGATTAGTACCATTAATGTTAGGAGCCCCTGATATAGCTTTCCCAC
GAATAAATAATATAAGTTTTGATTACTCCCCCCTCTTTAACCCTTTAAATTTCTAGAA
GAATCGTTGAAAATGGTGCTGGAACAGGATGAACAGTTTATCCACCCTTATCTTCTAAT
ATCGCCCATAGAGGAAGTTCAGTTGATTAGCTATTTTTCCCTTCATTTAGCTGGTATT
TCCTCAATTTTAGGAGCTATTAATTTTTATTACCACTATTATTAATATACGACCCTATAAT
ATATCATTTGACCAAAATACCTCTTTTTGTCTGAGCAGTTGGCATTACCGCTTTATTATTA
CTTCTTTCTCTTCTGTATTAGCAGGAGCTATTAATATATTATTAATGATCGAAATTTA
AATACTTCATTTTTTGACCCAGCAGGAGGTGGAGATCCAATTTTATATCAACATTTATTT

Sample ID: BC_ZSM_Lep_116423; sequence ID: GWOUK942-22; Vallemare (658bp)

AACATTATATTTTTATTTTTGGAATTTGATCTGGATTAGTAGGAACCTCTCTTAGTCTTTTT
AATTCGAGCTGAATTAGGTAATCCTGGATCTTTAATTGGTAATGATCAAATTTATAATA
CTATTGTTACAGCTCATGCTTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGG
AGGCTTTGGTAATTGATTAGTACCATTAATATTAGGAGCCCCTGATATAGCTTTCCCAC
GAATAAATAATATAAGTTTTGATTACTCCCCCCTCTTTAACCCTTTAAATTTCTAGAA
GAATCGTTGAAAATGGTGCTGGAACAGGATGAACAGTTTATCCACCCTTATCTTCTAAT
ATCGCCCATAGAGGAAGTTCAGTTGACTTAGCTATTTTTCCCTTCATTTAGCTGGTATT
TCCTCAATTTTAGGAGCTATTAATTTTTATTACCACTATTATTAATATACGACCCTATAAT
ATATCATTTGACCAAAATACCTCTTTTTGTCTGAGCAGTTGGCATTACCGCTTTATTATTA
CTTCTTTCTCTTCTGTATTAGCAGGAGCTATTAATATATTATTAATGATCGAAATTTA
AATACTTCATTTTTTGACCCAGCAGGAGGTGGAGATCCAATTTTATACCAACATTTATT T

Sample ID: BC_ZSM_Lep_117095; sequence ID: GWOUL189-23; Vallemare (627bp)

AACATTATATTTTTATTTTTGGAATTTGATCTGGATTAGTAGGAACCTCTCTTAGTCTTTTT
AATTCGAGCTGAATTAGGTAATCCTGGATCTTTAATTGGTAATGATCAAATTTATAATA
CTATTGTTACAGCTCATGCTTTTTATTATAATTTTTTTTATAGTTATACCTATTATAATTGG
AGGCTTTGGTAATTGATTAGTACCATTAATATTAGGAGCCCCTGATATAGCTTTCCCAC
GAATAAATAATATAAGTTTTGATTACTCCCCCCTCTTTAACCCTTTAAATTTCTAGAA
GAATCGTTGAAAATGGTGCTGGAACAGGATGAACAGTTTATCCACCCTTATCTTCTAAT
ATCGCCCATAGAGGAAGTTCAGTTGACTTAGCTATTTTTCCCTTCATTTAGCTGGTATT
TCCTCAATTTTAGGAGCTATTAATTTTTATTACCACTATTATTAATATACGACCCTATAAT
ATATCATTTGACCAAAATACCTCTTTTTGTCTGAGCAGTTGGCATTACCGCTTTATTATTA
CTTCTTTCTCTTCTGTATTAGCAGGAGCTATTAATATATTATTAATGATCGAAATTTA
AATACTTCATTTTTTGACCCAGCAGGAGG

Sample ID: BC_ZSM_Lep_116422; sequence ID: GWOUK941-22; Aranova (345bp)

CCTCCCCCTCTTTAACCCCTTTTAATTTCTAGAAGAATCGTTGAAAATGGTGCCGGAAC
 AGGATGAACAGTCTATCCACCTTTATCTTCTAATATCGCCCATAGAGGAAGTTCAGTTG
 ACTTAGCTATTTTTCCCTTCATTTAGCTGGTATTTCCCTCAATTTTAGGAGCTATTAATTT
 TATTACCACTATTATTAATATACGACCCCTATAATATATCATTGACCAAATACCTCTTTT
 TGTCTGAGCAGTTGGCATCACCGCTTTATTATTACTTCTTTCTCTTCCTGTATTAGCAGG
 AGCTATTACTATATTATTAAGTATCGAAATTTAAATACTTCATTT

Specimens submitted to DNA barcoding analysis were found in very different habitats ranging from 50 to 2050 m of altitude. The completely uniform morphology of adults corresponds to a genetic difference (BOLD Barcode Gap Analysis) comprised between the 0.97% of the Pollino-Vallemare pair and the 1.76% of the Vallemare-Aranova pair. The two sequences from Vallemare specimens were identical. The short length of the sequence recovered for the Aranova specimen (345bp) suggest caution in the interpretation of data.

Comparisons of male genitalia with available iconography of nearest taxa showed a clear affinity of *S. italica* with *S. colchica dercetis* that should be better evaluated when molecular data will be available also for *S. colchica colchica* and *S. colchica dercetis*.

Lastly, in addition to the adult habitus of *Stygioides italica* (Figures 4-6), some important distinctive characters, such as the antennae (Figures 3a-c), the scales that cover the upper surface of the female forewings (Figures 7-9) and the male genitalia of nearest species (Figures 10-12), were shown.

Conclusions

Stygioides italica Mazzei & Yakovlev, was recorded after its description for few localities of Central and South Italy, but comparisons with the congeneric *S. colchica* are lacking. In this paper we provided original distribution data, the first molecular data for *S. italica*, and contributed to the knowledge of some distinctive characters such as antennae, scales covering forewings of females, and male genitalia of nearest species. The availability of full DNA barcode sequence for all taxa can strongly contribute in the future to investigate the interspecific relationships within the genus *Stygioides*.

Acknowledgements

We want to thank Paul Hebert, Evgeny Zakharov, and Sujeevan Ratnasingham (Centre for Biodiversity Genomics (CBG), University of Guelph, Canada), Josef J. de Freina (München, Germany), and Aidias Saldaitis (Institute of Ecology of Vilnius University, Lithuania) for their collaboration.

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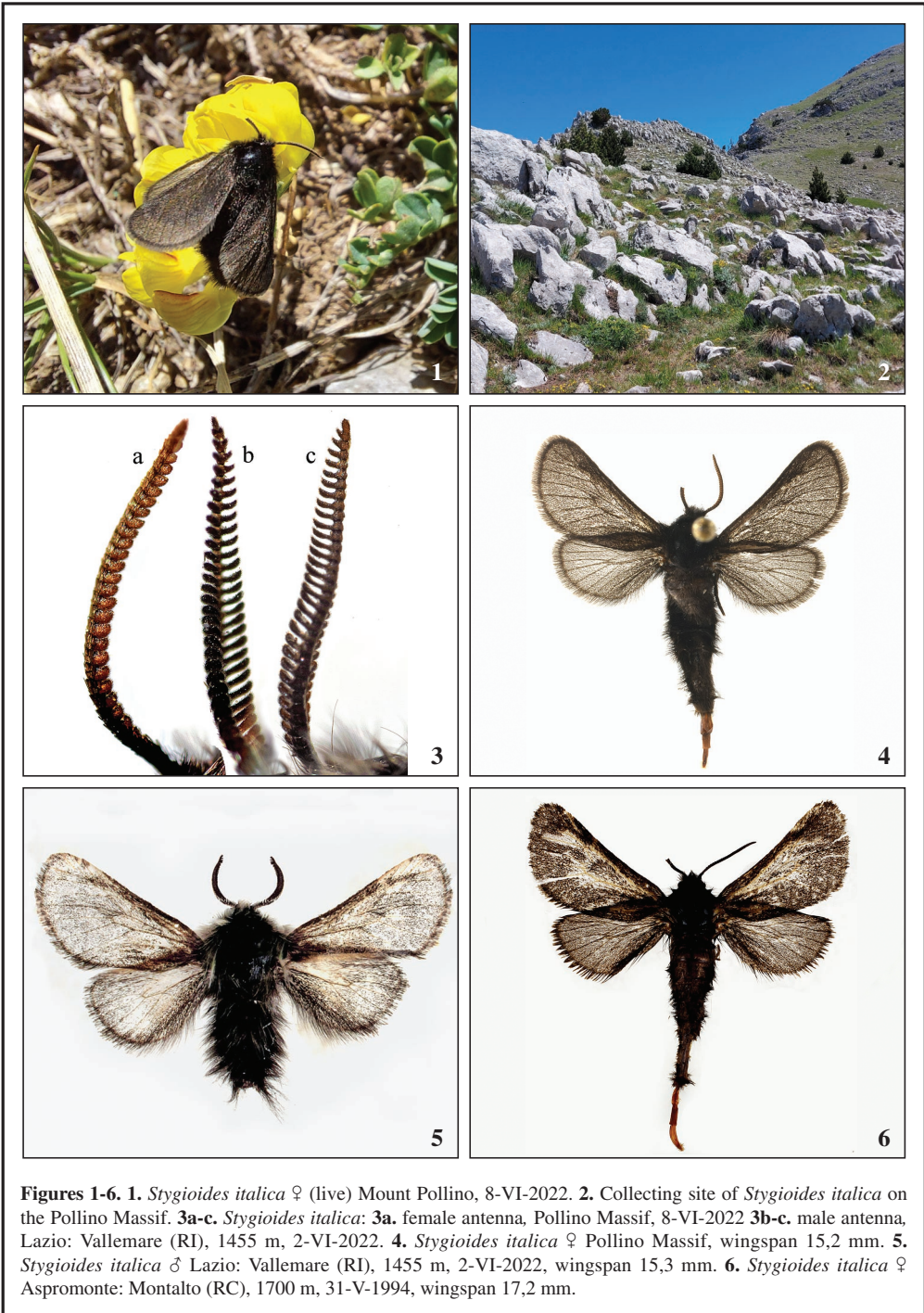
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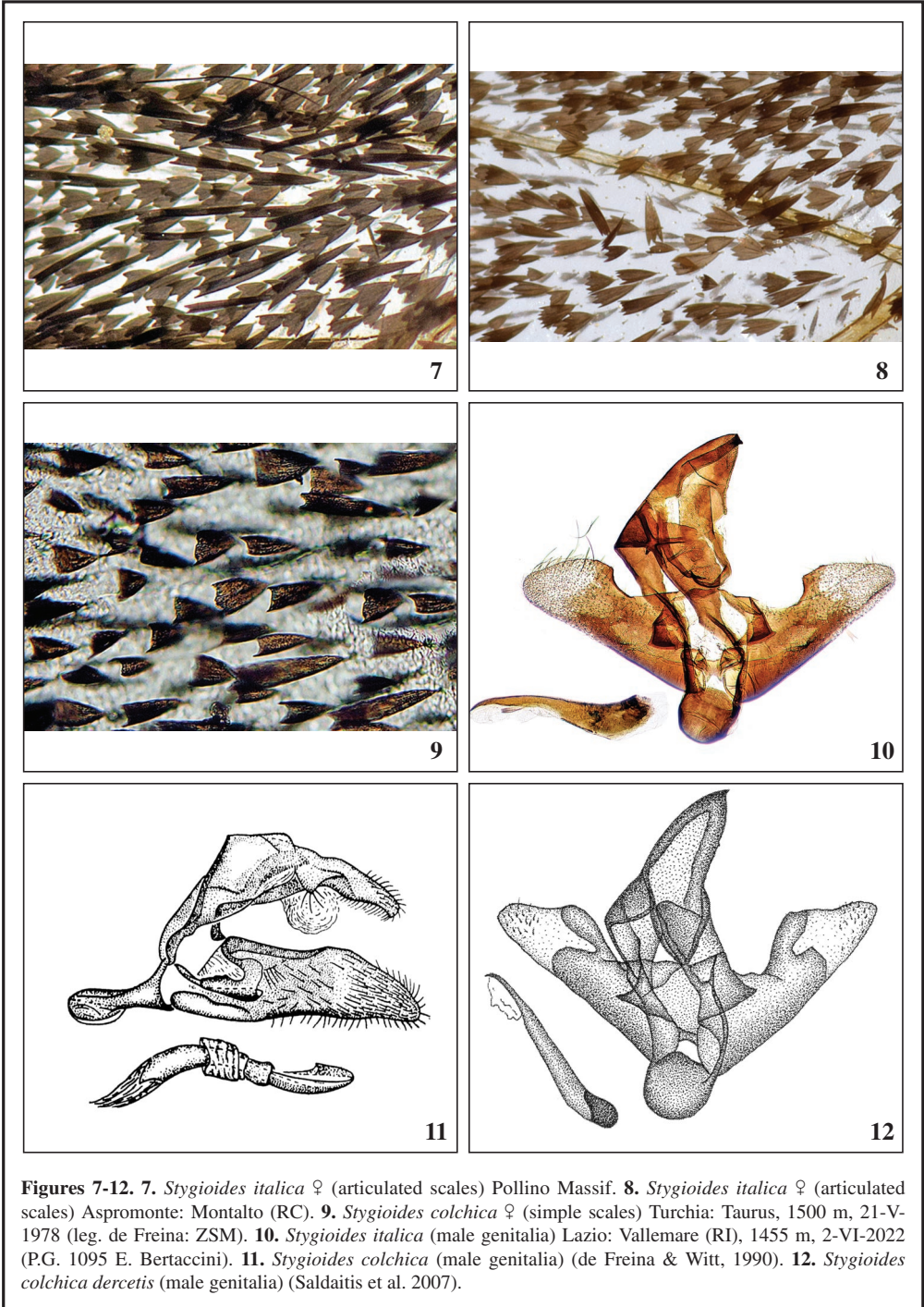
(Recibido para publicación / *Received for publication* 4-XII-2023)

(Revisado y aceptado / *Revised and accepted* 10-I-2024)

(Publicado / *Published* 30-VI-2024)

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Figures 7-12. 7. *Stygioides italica* ♀ (articulated scales) Pollino Massif. 8. *Stygioides italica* ♀ (articulated scales) Aspromonte: Montalto (RC). 9. *Stygioides colchica* ♀ (simple scales) Turchia: Taurus, 1500 m, 21-V-1978 (leg. de Freina: ZSM). 10. *Stygioides italica* (male genitalia) Lazio: Vallemare (RI), 1455 m, 2-VI-2022 (P.G. 1095 E. Bertaccini). 11. *Stygioides colchica* (male genitalia) (de Freina & Witt, 1990). 12. *Stygioides colchica dercetis* (male genitalia) (Saldaitis et al. 2007).



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Shilap Revista de Lepidopterología

vol. 52, no. 206, p. 227 - 234, 2024

Sociedad Hispano-Luso-Americana de Lepidopterología,

ISSN: 0300-5267

ISSN-E: 2340-4078

DOI: <https://doi.org/10.57065/shilap.898>