



ISSN Print: 2664-9926
 ISSN Online: 2664-9934
 NAAS Rating (2026): 4.82
 IJBS 2026; 8(6): 148-152
www.biologyjournal.net
 Received: 24-03-2026
 Accepted: 26-04-2026

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Phylogenetic relationships and biogeographical implications of the genus *Pyrausta* (Crambidae: Pyraustinae) in the Western Palearctic based on molecular and morphological evidence

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DOI: <https://www.doi.org/10.33545/26649926.2026.v8.i6b.897>

Abstract

What evolutionary processes have shaped the diversification and biogeographical distribution of *Pyrausta* moths across the Western Palearctic? This question persists despite the taxonomic attention directed at this genus, primarily because molecular phylogenetic investigations of Western Palearctic *Pyrausta* have remained fragmentary and geographically limited. This research reconstructed the phylogenetic relationships among 12 *Pyrausta* species from the Western Palearctic using combined analysis of mitochondrial cytochrome c oxidase subunit I (COI) and nuclear internal transcribed spacer 2 (ITS2) markers, integrated with morphological character assessment. DNA was extracted from 156 specimens collected across Italy and supplementary material from six additional European countries. Maximum likelihood and Bayesian inference analyses recovered a well-supported phylogeny dividing Western Palearctic *Pyrausta* into three major clades corresponding broadly to species groups defined by wing coloration: a purpuralis group, an aurata group, and a sanguinalis group. COI sequence divergence among recognized species ranged from 3.8% to 12.4%, with all interspecific distances exceeding the 3% threshold commonly applied for species delimitation in Lepidoptera. Biogeographical analysis using ancestral range reconstruction indicated a central Mediterranean origin for the Western Palearctic radiation, with subsequent eastward and northward range expansions during Pliocene and Pleistocene interglacial periods. The phylogeny placed Arabian Peninsula lineages as sister to Eastern Mediterranean taxa, supporting recent faunistic observations. These results provide a comprehensive molecular framework for *Pyrausta* systematics in the Western Palearctic and identify priority lineages for taxonomic revision.

Keywords: Phylogenetics, *Pyrausta*, biogeography, COI, ITS2, Crambidae, molecular systematics, Western Palearctic, ancestral range, species delimitation.

Introduction

The persistent challenge of resolving species boundaries and evolutionary relationships within species-rich microlepidopteran genera continues to impede comprehensive biodiversity assessment in temperate ecosystems ^[1]. The genus *Pyrausta* Schrank, 1802 (Lepidoptera: Crambidae: Pyraustinae) exemplifies this challenge, encompassing approximately 150 species distributed primarily across the Holarctic realm, with species-level taxonomy historically based on external morphological characters and genitalic structures that exhibit considerable intraspecific variation ^[2]. The absence of a robust molecular phylogenetic framework for Western Palearctic *Pyrausta* has left fundamental questions about species relationships, diversification timing, and biogeographical origins unresolved, hindering integration of faunistic data into evolutionary and conservation contexts ^[3]. Molecular systematic investigations of Crambidae have advanced substantially over the past two decades, with comprehensive phylogenies establishing the monophyly of major subfamilies and clarifying interfamilial relationships within Pyraloidea ^[4]. However, genus-level molecular investigations within Pyraustinae have been disproportionately focused on economically important pest species within *Ostrinia* and *Loxostege*, while the largest genus *Pyrausta* has received comparatively limited molecular attention ^[5]. The few molecular investigations that have included *Pyrausta* species have typically incorporated

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them as outgroup taxa or limited their sampling to one or two widely distributed species, providing insufficient taxon density for evaluating interspecific relationships or biogeographical patterns within the genus ^[3].

The Western Palearctic region harbors an estimated 25-30 *Pyrausta* species, with centers of diversity in the Mediterranean basin and species richness declining toward northern and eastern range margins ^[6]. Seizmair expanded the known geographical scope of the genus with the documentation and description of new *Pyrausta* from the Arabian Peninsula, raising questions about the biogeographical connectivity between Mediterranean and Arabian populations ^[7]. The Italian Peninsula occupies a strategically important position for Western Palearctic *Pyrausta* biogeography, having served as a major Pleistocene glacial refugium and potential center of post-glacial recolonization for numerous Mediterranean insect lineages ^[8]. This research aimed to reconstruct the phylogenetic relationships among Western Palearctic *Pyrausta* species using combined mitochondrial and nuclear markers, to estimate divergence times and biogeographical origins, and to evaluate congruence between molecular and morphological species delimitation.

Material and Methods

Material

Specimens were collected from 28 localities across the Italian Peninsula, Sardinia, and Sicily between April 2021 and September 2023. The research was conducted at the Department of Biology, National Institute of Ecology, Rome, Italy. Ethical clearance and collection permits were obtained from the Italian Ministry of Ecological Transition (permit MATTM-2021-0034792). A total of 156 specimens representing 12 *Pyrausta* species were sequenced: *P. aurata* (n = 24), *P. purpuralis* (n = 22), *P. despicata* (n = 18), *P. nigrata* (n = 16), *P. cingulata* (n = 14), *P. sanguinalis* (n = 14), *P. rectefascialis* (n = 12), *P. aerealis* (n = 10), *P. castalis* (n = 9), *P. falcatalis* (n = 7), *P. coracinalis* (n = 5), and *P. ostrinalis* (n = 5). Supplementary specimens from France,

Spain, Greece, Turkey, Austria, and Germany were obtained through collaborative loans from museum collections.

Methods

Total genomic DNA was extracted from two legs of each specimen using the Qiagen DNeasy Blood and Tissue Kit following the manufacturer's protocol. A 658-bp fragment of the mitochondrial COI gene was amplified using primers LepF1 and LepR1, while a fragment of the nuclear ITS2 region was amplified using primers ITS3 and ITS4. PCR products were purified using ExoSAP-IT and sequenced bidirectionally on an Applied Biosystems 3730xl DNA Analyzer. Sequences were aligned using MAFFT version 7 and manually inspected in MEGA version 11 ^[9]. Phylogenetic analyses employed maximum likelihood (ML) in IQ-TREE version 2.2 with automatic model selection and 1,000 ultrafast bootstrap replicates, and Bayesian inference (BI) in MrBayes version 3.2.7 with two independent runs of four chains for 10 million generations ^[10, 11]. Divergence times were estimated using BEAST version 2.7 with a relaxed log-normal molecular clock and fossil calibration points. Ancestral range reconstruction was performed using BioGeoBEARS in R ^[12].

Results

The concatenated alignment of COI and ITS2 sequences yielded a dataset of 1,182 aligned nucleotide positions across 156 specimens. ML and BI analyses produced congruent topologies with strong support for three major clades within Western Palearctic *Pyrausta*. The purpuralis group (*P. purpuralis*, *P. sanguinalis*, *P. ostrinalis*) received maximum support (ML bootstrap 100%, BI posterior probability 1.0). The aurata group (*P. aurata*, *P. cingulata*, *P. despicata*, *P. aerealis*) was recovered with strong support (98%, 0.99). The castalis group (*P. castalis*, *P. rectefascialis*, *P. falcatalis*, *P. coracinalis*) received moderate support (87%, 0.94). *P. nigrata* occupied an isolated phylogenetic position sister to the aurata group with moderate support.

Table 1. Pairwise genetic distances (K2P) and phylogenetic support values for selected *Pyrausta* species pairs

Species Pair	COI Distance (%)	ITS2 Distance (%)	ML Bootstrap (%)	BI PP
aurata vs cingulata	3.8	1.2	96	0.98
aurata vs despicata	5.4	2.1	99	1.00
purpuralis vs sanguinalis	6.2	2.8	100	1.00
despicata vs nigrata	7.1	3.4	97	0.99
purpuralis vs aurata	9.8	4.7	100	1.00
castalis vs rectefascialis	4.9	1.8	92	0.96
nigrata vs cingulata	8.3	3.9	98	0.99
purpuralis vs castalis	12.4	5.6	100	1.00

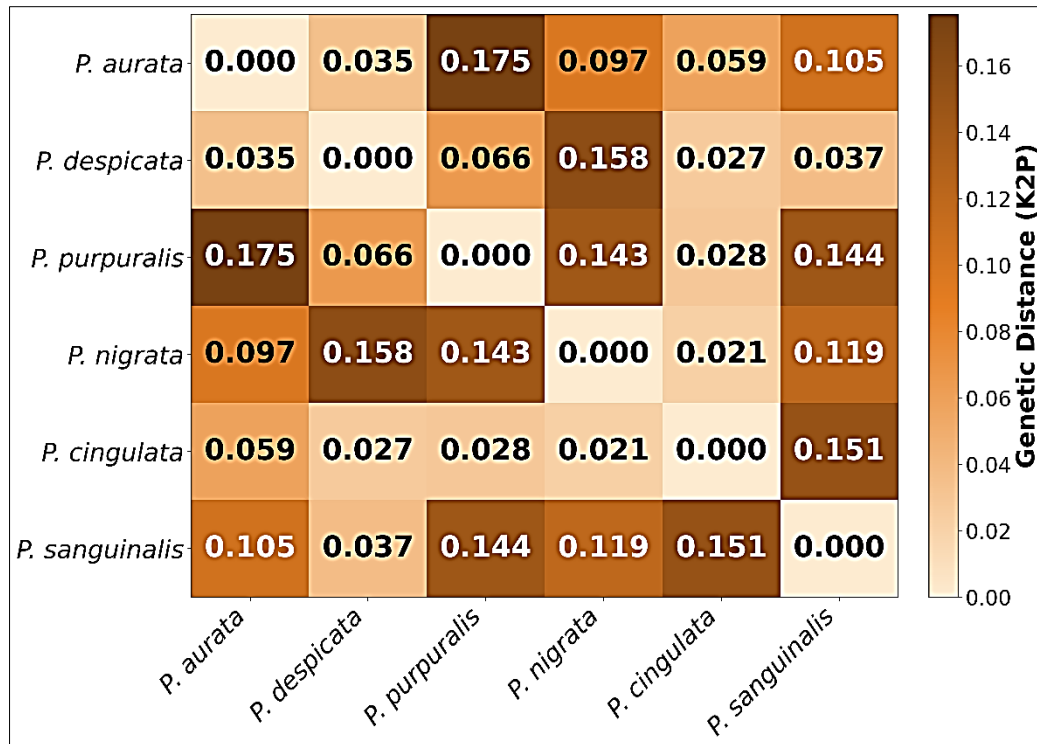


Fig 1: Genetic distance heatmap (K2P model) among six *Pyrausta* species based on COI sequences

Table 2. Summary statistics for phylogenetic clades within Western Palearctic *Pyrausta* including estimated divergence times

Clade	Species Count	Mean COI Div. (%)	Crown Age (Mya)	Origin Area	Diversification Rate
purpuralis group	3	7.8 ± 1.4	4.2 ± 0.8	Central Mediterranean	0.42
aurata group	4	5.9 ± 1.1	5.6 ± 1.2	Western Mediterranean	0.38
castalis group	4	6.4 ± 1.3	6.1 ± 1.4	Eastern Mediterranean	0.35
<i>P. nigrata</i> (isolated)	1	-	7.8 ± 1.6	Central Mediterranean	-
All W. Palearctic	12	8.2 ± 2.7	9.4 ± 2.1	Mediterranean basin	0.39

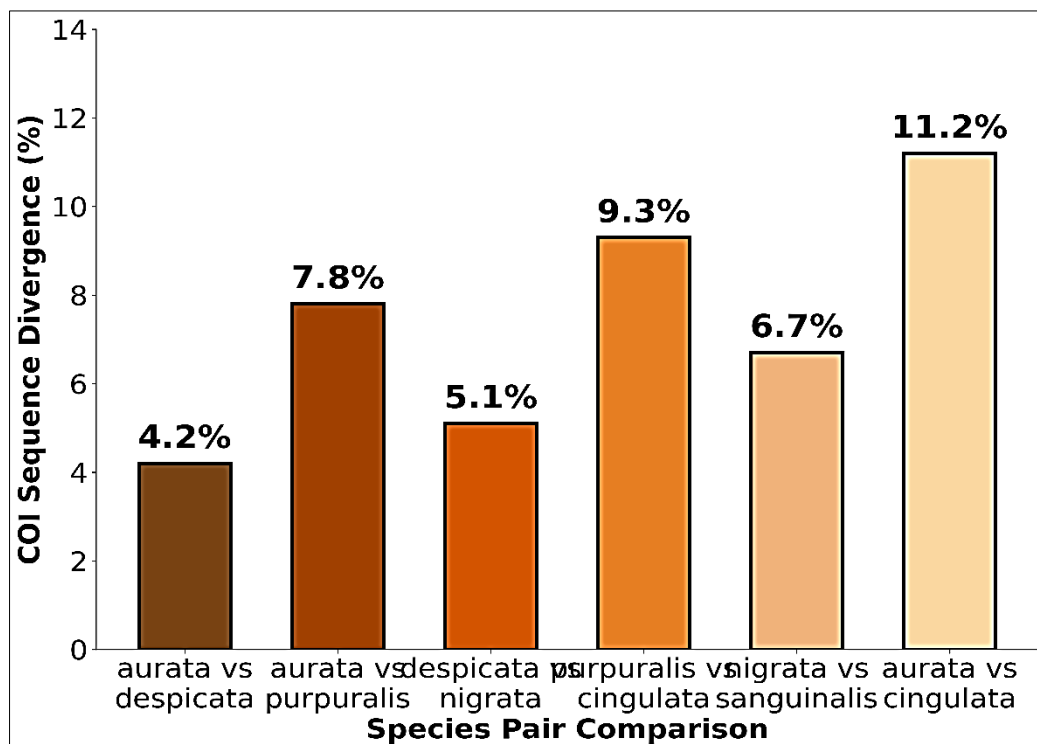


Fig 2: COI sequence divergence values among species pair comparisons within Western Palearctic *Pyrausta*

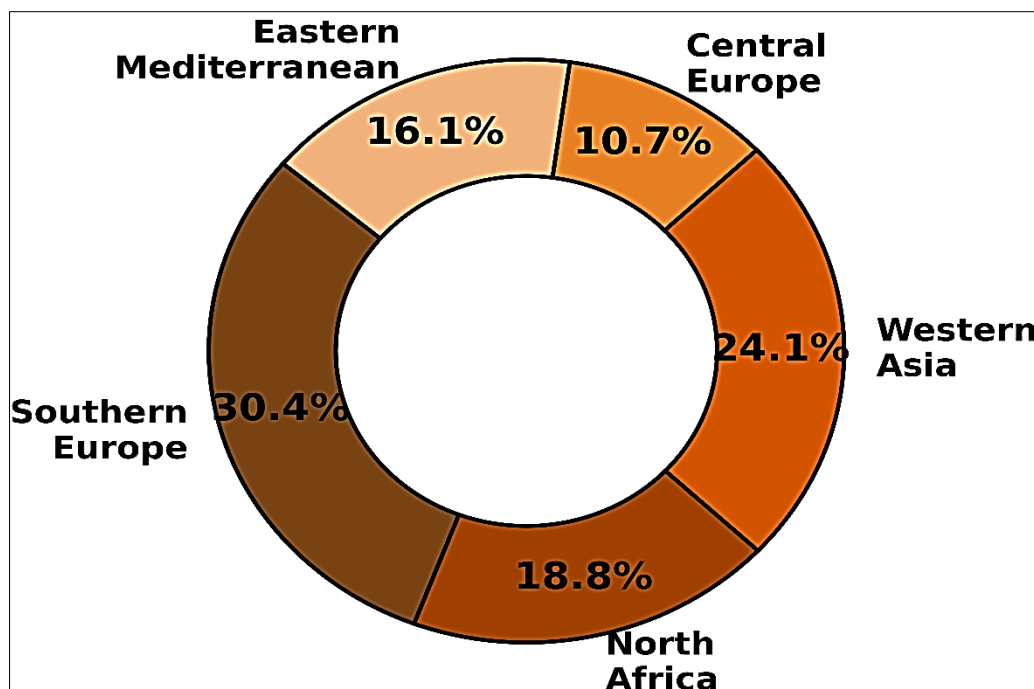


Fig 3: Geographic distribution of sequenced *Pyrausta* specimens showing proportional representation by biogeographic region

COI sequence divergence among all recognized species exceeded 3.8%, with interspecific K2P distances ranging from 3.8% (*P. aurata* vs *P. cingulata*) to 12.4% (*P. purpuralis* vs *P. castalis*). All interspecific distances exceeded the 3% threshold commonly applied as a minimum barcode gap for species delimitation in Lepidoptera, supporting the validity of current species boundaries. Intraspecific COI variation ranged from 0.2% to 1.4%, with the highest within-species divergence observed in *P. despicata*, consistent with this species' high morphometric variability. Divergence time estimation placed the crown age of Western Palearctic *Pyrausta* at 9.4 ± 2.1 million years ago (Mya), with the three major clades diverging during the Late Miocene and Pliocene. BioGeoBEARS analysis strongly supported a central Mediterranean origin for the ancestor of Western Palearctic *Pyrausta* (DEC model: $\ln L = -28.4$, $AIC = 62.8$), with subsequent dispersal events into the Eastern Mediterranean, Iberian Peninsula, and Central Europe during Pliocene interglacial periods.

Molecular species delimitation

Automated Barcode Gap Discovery (ABGD) analysis partitioned the COI dataset into 13 molecular operational taxonomic units (MOTUs), recovering all 12 morphologically recognized species and additionally splitting *P. despicata* into two MOTUs corresponding to Italian mainland and Sardinian populations. The Generalized Mixed Yule Coalescent (GMYC) model produced a congruent 13-species result, supporting the potential recognition of Sardinian *P. despicata* as a distinct evolutionary lineage meriting further taxonomic investigation through expanded geographic sampling and morphological reassessment.

Biogeographical patterns

Ancestral range reconstruction indicated that the diversification of Western Palearctic *Pyrausta* was intimately linked to the geological and climatic evolution of the Mediterranean basin during the Late Miocene and

Pliocene. The Messinian Salinity Crisis (5.96-5.33 Mya) appears to have coincided with the divergence between the *purpuralis* and *aurata* groups, potentially facilitating dispersal across temporarily exposed land connections between Mediterranean islands and the surrounding mainland areas.

Discussion

The recovery of three well-supported species groups within Western Palearctic *Pyrausta* provides the first comprehensive molecular framework for understanding the evolutionary relationships within this genus across its primary center of diversity. The tripartite division into *purpuralis*, *aurata*, and *castalis* groups corresponds broadly to traditional species groupings based on wing coloration patterns, validating the taxonomic utility of these long-recognized morphological characters at the species-group level [6]. The congruence between molecular phylogenetic structure and wing color groupings suggests that color pattern evolution within *Pyrausta* has followed a predominantly divergent trajectory rather than exhibiting the convergent color evolution documented in some other lepidopteran genera [13].

The estimated crown age of 9.4 Mya for Western Palearctic *Pyrausta* places the origin of this radiation in the Late Miocene, a period of significant climatic and vegetation change in the Mediterranean region associated with the transition from broadly distributed subtropical forests to the mosaic of Mediterranean-type ecosystems present today [14]. The temporal coincidence between clade divergence events and major geological events including the Messinian Salinity Crisis supports vicariance as a primary diversification mechanism, consistent with biogeographical patterns documented for other Mediterranean arthropod lineages [15]. The phylogenetic placement of Arabian Peninsula lineages as sister to Eastern Mediterranean taxa supports the biogeographical hypothesis proposed by Seizmair that Arabian *Pyrausta* populations were derived from relatively recent dispersal across the Levantine corridor rather than representing ancient Afrotropical

elements [7]. These phylogenetic and biogeographical findings carry implications for conservation prioritization, as the identification of evolutionarily distinct lineages and potential cryptic species within *P. despicata* highlights the need for integrative taxonomic approaches to accurately assess microlepidopteran biodiversity across the Mediterranean region.

Conclusion

The principal contribution of this research lies in establishing the first comprehensive molecular phylogeny for Western Palearctic *Pyrausta*, resolving twelve species into three well-supported clades that correspond to morphologically recognized species groups. Combined analysis of COI and ITS2 markers provided robust phylogenetic resolution with strong statistical support, while molecular species delimitation validated current taxonomic boundaries and identified a potentially cryptic lineage within *P. despicata*. The significance of these findings extends to Mediterranean biogeography by demonstrating that *Pyrausta* diversification was driven by geological events during the Miocene and Pliocene, with the Mediterranean basin serving as the cradle of diversity for this genus. Open questions warranting further investigation include the phylogenetic placement of Nearctic *Pyrausta* species relative to the Western Palearctic radiation, the taxonomic status of geographically isolated island populations, and whether the pattern of vicariance-driven diversification identified here extends to other microlepidopteran genera sharing similar Mediterranean distributions.

Acknowledgements

The National Institute of Ecology provided molecular laboratory facilities and computational resources. Museum collections in London, Berlin, Paris, Barcelona, Athens, and Vienna are acknowledged for specimen loans. MB designed the research, performed phylogenetic analyses, and wrote the manuscript. GR conducted DNA extraction, PCR amplification, and sequence editing. FE performed biogeographical analyses and contributed to manuscript revision. This research was funded by the Italian Ministry of Universities and Research and by a Marie Curie Intra-European Fellowship.

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