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Doctoral School of Integrative Biology

Distribution, ecology and conservation of three protected butterfly species in Romania PhD thesis

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Outline of the thesis

This doctoral thesis contributes to the conservation of butterfly (Lepidoptera: Rhopalocera) biodiversity in the context of implementing the Habitats Directive and the European Green Deal, with a focus on species of conservation interest. It presents new findings on the distribution, ecology, biology, and population structure of three butterfly species of community interest protected in Romania. This knowledge is essential for assessing the conservation status of these species at both national and biogeographical region levels and supports the foundation of conservation measures.

The general objectives of this thesis are:

- 1. Compilation of data on protected butterfly species in Romania listed in various legal acts issued by state institutions after the political regime change in 1989.
- 2. Analysis of the implementation status of the Habitats Directive in Romania with a view to conserving protected butterfly species of community interest
- 3. To clarify the historical and current distribution, the biology and ecology and review conservation efforts in Romania for three protected butterfly species: *Colias myrmidone*, *Pseudophilotes bavius hungarica*, and *Euphydryas aurinia*.
- 4. To assess the ecological requirements of *Colias myrmidone* based on its distribution data in Romania and to describe the socio-economic conditions in the area of three Natura 2000 sites designated in 2016 in Cluj and Harghita counties for the conservation of this species.
- 5. To characterize the structure of a newly discovered *Colias myrmidone* population in a hay meadow habitat fragment and to provide recommendations for the conservation of the populations and colonies identified between 2021 and 2024 in the Ciuc Basin, Harghita County.

Chapter 1 sets the context for the main theme of the thesis: the global paradigm of biodiversity decline (with a focus on the butterflies), as demonstrated over the past half-century, Romania's socio-economic and political orientation in the post-communist period, and the status and dynamics of the number and composition of protected butterfly species listed in legal acts issued by Romanian state institutions. Law no. 13/1993 on Romania's accession to the Bern Convention marks the beginning of legal acts that include protected butterfly species. For over three decades, a series of legal acts issued by the Romanian Government and the Ministry of Environment has placed under strict protection a total of 57 butterfly species, representing 27% of the country's fauna. In 2025, 46 species of butterflies are protected at the national level in Romania.

Romania's accession to European structures required the implementation of EU biodiversity conservation directives. Butterfly species are afforded protection at the European Union level through the Habitats Directive. Starting in 2007, the Natura 2000 ecological network was established in Romania. The Sites of Community Importance (SCIs), designated in part for the conservation of butterfly species populations, have been established in three waves: in 2007, 2011, and 2016. A total of 442 SCIs has been designated, 93 of which aim to conserve populations of the 12 butterfly species of community interest. The conservation status of these populations is largely unknown, with an average data deficiency of 68.11%.

The thesis further presents data, information, and results concerning the updated regional or national distribution of three protected butterfly species (*Colias myrmidone*, *Pseudophilotes bavius*, and *Euphydryas aurinia*), their ecological requirements, as well as proposals and discussions regarding conservation measures and the socio-economic context of these measures.

Chapter 2 focuses on the ecological requirements and socio-economic conditions in the areas of the three Natura 2000 sites designated in 2016 for the conservation of the protected species *Colias myrmidone* in Romania. We used a dataset of species distribution collected in the field during 2011, 2014, and 2015, along with data on distribution and ecological parameters from the territory of the three newly designated Natura 2000 sites, collected during August–September 2016 and 2017. A total of 114 distribution points were identified, and data were collected from 127 grid cells of 100x100 meters within the newly designated Natura 2000 sites. Information was gathered regarding land use, the extent of woody vegetation cover, and the abundance of the host plant. In the autumn and winter of 2016, face-to-face interviews were conducted with land users

within these Natura 2000 sites (n = 36), and an additional 12 narrative interviews were analyzed concerning the communication between authorities and land users. The results show that average altitude, mean annual precipitation, and mean annual temperature were significant negative predictors for the presence of adult C. myrmidone individuals. The abundance of adults was positively predicted by the abundance of the host plant *Chamaecytisus* sp. The extent of coniferous woody vegetation cover had a weak effect on population abundance. Land parcels within the Natura 2000 areas were used for grazing (n = 30), haymaking (n = 20), agriculture (n = 4), and timber production (n = 3). More than half (54%) of the land parcels in the study area were smaller than 10 hectares, and 10% were larger than 50 hectares. All farmers applied for and received financial subsidies for grazing (n = 22) and haymaking (n = 15), in many cases for both. The local population appreciated the conservation efforts for the butterfly but expressed concerns about potential restrictions that could limit their activities and economic benefits. Future changes in land use may threaten C. myrmidone populations in Romania. The maintenance of C. myrmidone populations depends on coordination between policy, the acceptance of local communities and farmers, and adequate financial support to sustain traditional, ecologically feasible land use, rather than harmful developments such as land use intensification, abandonment, and afforestation.

Chapter 3 provides an in-depth analysis of the population structure of *Colias myrmidone* discovered in 2021 in the northern part of the Ciuc Basin (Hargita County), specifically on Garadoş Hill near the village of Sândominic. Between 2021 and 2024, habitat surveys demonstrated that this population occupies at least 110 hectares on a terraced slope composed of dry grassland over dolomitic substrate, primarily used as hay meadow. The area includes a mosaic of actively managed and abandoned land parcels with minimal woody vegetation cover. In addition to the core population on Garadoş Hill, seven to eight additional local populations and colonies were identified within a 7–8 km radius, situated across the neighboring communes of Tomeşti, Cârţa, and Mihăileni, probably forming a metapopulation in the northern part of the Upper Ciuc Basin. Population structure was investigated using the mark–recapture method during the second-generation flight period, from August 3 to September 14, 2023. Fieldwork was conducted over 22 days by a team of seven observers. A total of 105 individuals (47 females and 58 males) were marked in the central study area on Garadoş Hill. Recapture data indicated a significant difference between sexes: 20 males (34.5%) and only three females (6.4%) were recaptured at least once.

The estimated size of the superpopulation was approximately 400 individuals, with peak population sizes recorded on August 11 and 13, 2023. The mean dispersal distance for marked and recaptured individuals was 792 meters. Outside the core study area, additional individuals were marked in habitat fragments located 2–3 km away to the west, northeast, and east, with 7, 3, and 1 individual recorded in each direction, respectively. No dispersal events between these fragments and the central population were observed during the study period. Populations rediscovered and newly documented between 2020 and 2024 in the communes of Racu and Mihăileni fall within the boundaries of the existing Natura 2000 site ROSCI0323 (Ciuc Mountains). However, the remaining colonies and populations identified during this period are not currently included within any protected areas. In December 2024, a formal proposal was submitted to the Romanian Ministry of Environment requesting the designation of a new Natura 2000 site—the Harghita-Hăşmaş-Ciuc Mountains Corridor. The proposed site would cover an area of 19,080 hectares and aims to include all known populations, colonies, and potential habitat fragments identified in the region.

Chapter 4 contributes to the understanding of the distribution, ecology, and biology of the butterfly Pseudophilotes bavius (Lepidoptera: Lycaenidae). The taxon P. bavius hungarica (Diószeghy, 1913) is an endemic subspecies characteristic of the steppe slopes of the Transylvanian Plain and is a protected species of Community interest. Since its original description, the taxon has been considered monophagous, with larvae feeding exclusively on the inflorescences of Salvia nutans. During field investigations in 2021–2022, new populations were identified along the Mure\$ River Valley, within the Natura 2000 site ROSCI0210 Râpa Lechinţa, and at Râpa DătăSeni (Mures County). In these habitat fragments, located at the southeastern edge of the subspecies' known range, larval feeding on Salvia transsilvanica Schur was confirmed for the first time in natural conditions. An oviposition strategy study conducted on the populations at Suatu (Cluj County) demonstrated a marked preference for flowering stems of Salvia nutans taller than five centimeters. Additionally, the presence of P. bavius was reported for the first time in Tulcea County. This new record suggests that the species may have colonized the Dobrogea region from Ukraine (P. bavius bavius Eversmann, 1832), rather than from the Balkans (Turkey and Bulgaria), where the subspecies P. bavius egea is present, as previously assumed. Several conservation threats have been identified that could lead to the fragmentation and eventual

extinction of the reported populations, highlighting the need for urgent conservation measures.

Chapter 5 expands current knowledge on the distribution of the Community interest species Euphydryas aurinia (Lepidoptera: Nymphalidae) in Romania. The findings demonstrate that this species is also distributed in the regions of Oltenia and Muntenia, with these populations likely connected to those in Bulgaria. A distribution map of the species is presented, based on both published literature and new records from Romania and Bulgaria. In Transylvania, the majority of new observations recorded between 2019 and 2021 originate from Harghita County, particularly within the Natura 2000 site ROSCI0323 Ciuc Mountains. Based on distribution data, the species is widespread in wet hay meadows along river and stream valleys in the Ciuc Basin and Mountains, with populations extending up to spring zones. For the first time, it has been demonstrated that in Romania, E. aurinia occurs above 1000 meters in elevation, as evidenced by certain populations in the Suhardu Mic and Ciuc Mountains. In Romania, E. aurinia is closely associated with wet meadows and hayfields, where its larvae develop on the leaves of Succisa pratensis. An earlyseason observation of the species near the Natura 2000 site ROSCI0137 Pădurea Bejan (Hunedoara County) on April 24, 2019, in an atypical habitat suggests that this population may represent a dry grassland ecotype—an ecological form known from various regions across Europe. This chapter also highlights several threats to E. aurinia populations in Romania, including habitat isolation and fragmentation, changes in land use, drainage, afforestation, and excessive use of herbicides, pesticides, and chemical fertilizers. Furthermore, it emphasizes the need for continuous monitoring of known populations and the continued mapping of as-yet undocumented populations.

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General conclusions

- (1) This doctoral thesis presents the first synthetic analysis of the status and dynamics of the number and composition of protected butterfly species (Lepidoptera: Rhopalocera) listed in legal acts issued by Romanian state institutions during the post-communist period. Of the 206 butterfly species recorded on the current territory of Romania, 57 species have been granted protected status over the past 35 years. As of now, 46 species are nationally protected, including some endemic to Romania.
- (2) The Natura 2000 ecological network includes 93 Sites of Community Importance (SCIs) in Romania (representing 21.04% of all SCIs), designated for the conservation of 12 butterfly species of community interest. The conservation status of these butterfly populations remains largely unknown, with a data deficiency rate of 68.11%.
- (3) Our team contributed to clarifying both the historical and current distribution of *Colias myrmidone*, a species considered critically endangered within the European Union. These results influenced the designation and dynamics of SCIs selected for the conservation of this species in Romania. The species has disappeared from most EU Member States, currently being known from only one metapopulation in Poland and two to three metapopulations in Romania.
- (4) Based on the distribution of *Colias myrmidone* populations and habitats within three SCIs designated in 2016 for its conservation in Cluj and Harghita counties. We demonstrated that adult abundance is positively predicted by the abundance of its host plant, *Chamaecytisus triflorus*.
- (5) We identified significant positive relationships between *C. myrmidone* abundance and certain physical-geographic habitat features (such as slope and aspect), as well as specific habitat types and land-use categories, including transitional woodland-shrub habitats, pastures, and natural grasslands. Mean altitude, mean annual precipitation, and mean annual temperature were significant negative predictors for the presence of adult *C. myrmidone* individuals.
- (6) Between 2021 and 2024, we identified a previously unknown metapopulation of *Colias myrmidone* in the Upper Ciuc Basin area, Harghita County. The metapopulation consists of 7–8 populations, subpopulations, and colonies. The central population, located in the largest continuous habitat area used as hay meadow, was estimated in 2023 (second

- generation) to include 410 individuals across approximately 110 hectares. The total area encompassing all *C. myrmidone* populations in this region was proposed as a new Natura 2000 site, titled Harghita-Hăşmaş-Ciuc Mountains Corridor, and submitted for approval in 2024.
- (7) The butterfly species *Pseudophilotes bavius*, protected at the EU level, is characteristic of steppe slopes in Romania, where the larval host plant *Salvia nutans* predominates. We demonstrated that the range of the endemic subspecies *P. b. hungarica*, specific to the Transylvanian Plain, extends southeast along steppe slopes in the middle course of the Mureş River. In these areas, we observed for the first time that larvae also feed on the inflorescences of *Salvia transsilvanica*.
- (8) Euphydryas aurinia populations are more widely distributed in Romania than previously known during the last decade. While mostly reported from Transylvania, we also identified new populations outside the Carpathian Arc, in the regions of Muntenia and Oltenia. In the Eastern Carpathians, the species occurs at elevations exceeding 1000 m. In the intramontane depressions of the central Eastern Carpathians (Giurgeului and Ciucului), we identified several populations that likely form metapopulations within the upper catchment basins of the Olt, Mureş, and Trotuş rivers.