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The protected species *Pseudophilotes bavius hungarica* (Diószeghy, 1913): oviposition strategy, new records and conservation (Lepidoptera: Lycaenidae)

Andrei Crişan, Tibor-Csaba Vizauer & László Rákosy

Abstract

Pseudophilotes bavius hungarica (Diószeghy, 1913) is a Transylvanian endemic of conservation interest. We made observations regarding its oviposition strategy, and established its preference for floral stems of *Salvia nutans* L. taller than five centimeters. *Salvia transsilvanica* Schur was also confirmed as a host plant for *P. bavius hungarica.* The distribution map of this subspecies was completed in two localities from the south of the Transylvanian Plain, Râpa Lechinţa and Râpa Dătăşeni. We reported presence of *P. bavius* in Tulcea County for the first time, thus supporting the hypothesis that it colonised Dobrogea via Ukraine alongside *P. bavius bavius* (Eversmann, 1832) and it did not spread from Turkey and Bulgaria alongside *P. bavius egea*. Many conservation threats that may lead to population fragmentation and extinction have been identified.
Keywords: Lepidoptera, Lycaenidae, *Pseudophilotes bavius hungarica*, *Salvia transsilvanica*, oviposition, new records, conservation, Romania.

La especie protegida *Pseudophilotes bavius hungarica* (Diószeghy, 1913): estrategia de oviposición, nuevos registros y conservación (Lepidoptera: Lycaenidae)

Resumen

Pseudophilotes bavius hungarica (Diószeghy, 1913) es un endemismo transilvano de interés para la conservación. Realizamos observaciones sobre su estrategia de oviposición y establecimos su preferencia por tallos florales de *Salvia nutans* L. de más de cinco centímetros de altura. También se confirmó que *Salvia transsilvanica* Schur es una planta nutricia de *P. bavius hungarica*. El mapa de distribución de esta subespecie se completó en dos localidades del sur de la llanura transilvana, Râpa Lechinţa and Râpa Dătăşeni. Informamos de la presencia de *P. bavius* en el condado de Tulcea por primera vez, apoyando así la hipótesis de que colonizó Dobrogea a través de Ucrania junto con *P. bavius bavius* (Eversmann, 1832) y no se propagó desde Turquía y Bulgaria junto con *P. bavius egea*. Se han identificado muchas amenazas para la conservación que pueden conducir a la fragmentación y extinción de la población.
Palabras clave: Lepidoptera, Lycaenidae, *Pseudophilotes bavius hungarica*, *Salvia transsilvanica*, oviposición, nuevos registros, conservación, Rumanía.

Introduction

Pseudophilotes bavius (Eversmann, 1832) is a xerothermophilous species associated with steppe meadows on loamy or calcareous soils where *Salvia nutans* L. is present (Rákosy et al. 2021). Due to

the vulnerability of the species it has been of interest to lepidopterists for over 35 years and a large number of scientific articles have been published examining not only its biology and ecology (Crişan et al. 2011; Dincă et al. 2011a; Jutzeler et al. 1997; Kolev, 2017; König, 1992; Német et al. 2016; Rákosy & Weidlich, 2017), but also its more complex population dynamics (Crişan et al. 2014), and molecular characterisation (Bartoňová et al. 2020; Dincă et al. 2011b).

P. bavius is included in the annexes of the Council Directive 93/43/EEC1 on the conservation of natural habitats and wild fauna and flora (Annex II. and IV.). However, in the European Red List of Butterflies (Van Sway et al. 2010), *P. bavius* is considered only Least Concern. Maes et al. (2019) raise the degree of endangerment / threat to Vulnerable.

P. bavius is also protected by Romanian legislation (OUG no. 57/2007, approved with amendments by Law 49/2011), in seven Natura 2000 sites. Conservation efforts are specifically targeted towards the *hungarica* subspecies (a Transylvanian endemic), because changing land use practices in the region mean that most of its natural habitats have disappeared and it is therefore considered endangered in Romania (Rákosy et al. 2021). The abandonment of traditional haymaking causes the accumulation of decayed plant material and litter, which is a major threat to the survival of the butterfly's host plant *Salvia nutans* (Jakab & Kapocsi, 2005; Német et al. 2016) and other valuable steppic plant species (*Astragalus peterfii* Jáv., *Salvia transsilvanica* Schur, *Nepeta ucranica* L. etc.). Through this study we aim to bring new information regarding the oviposition, distribution and host plant of this endemic subspecies of conservation concern. We would also like to draw the attention of the authorities to the need for additional, active measures to protect this taxon.

Study sites

The study sites are in Transylvania, Romania. In Suatu, the study areas were chosen on the north side of the road DN16 Cluj-Napoca – Reghin, on a slope with a southern exposure, clay-sandy soil, partially denuded and steppe vegetation, with a high abundance of *S. nutans.* The coordinates of the centers of the polygons from Suatu are: N 46.798780, E 23.950030, respectively N 46.798705, E 23.950418. In Bărăi, the study areas were designated on a slope with a south-western exposure, steppe vegetation and rich in *S. nutans*. The coordinates of the polygon centers in Bărăi are: N 46.860940, E 23.901262, respectively N 46.861363, E 23.900479. All four study areas have a steep slope of about 25 degrees, and are located at altitudes between 350 and 475 m. The climate of the region is temperate continental with an average annual precipitation of 550-600 mm and a mean annual temperature of 8.5º C (Kun et al. 2007). Suatu plots are located near "Suatu - Cojocna - Crairât" Natura 2000 Site (ROSCI0238) and Bărăi plots are part of "Lacul Ştiucilor - Sic - Puini - Bonţida" Natura 2000 Site (ROSCI0099). Both study areas are part of a mosaic landscape comprising permanent meadows, abandoned farmland and extensively grazed pasture. Due to the abandonment of traditional haymaking and extensive grazing, shrubs are increasing (Crişan et al. 2014).

Materials and methods

The villages of Suatu and Bărăi (Cluj county), have stable populations of *P. bavius hungarica*. In each of these sampling locations, we identified the areas with the highest concentration of the host plant, *S. nutans* and outlined two survey sites of approximately 100 m^2.

On 18-IV-2020, the beginning of *P. bavius hungarica* flight period, we quantified the number of eggs laid on *S. nutans* inflorescences in Suatu (Figure 1). When eggs were found the following data were recorded: 1. the height of the stem, and 2. the number of eggs laid in the inflorescences of the plant. We categorised stem height into set four ranges: 0.1-2.0 cm, 2.1-5.0 cm, 5.1-9.0 cm, >9.0 cm. Four days after the initial assessment, (22-IV-2020), the quantification was repeated on the plots from Suatu, and a comparative count was performed in Bărăi. On this occasion, all the plants were counted, regardless of whether or not they had a developed flower stem.

A measuring tape was used to determine floral stems heights, and all measurements were recorded by the same person to avoid inter-observer bias.

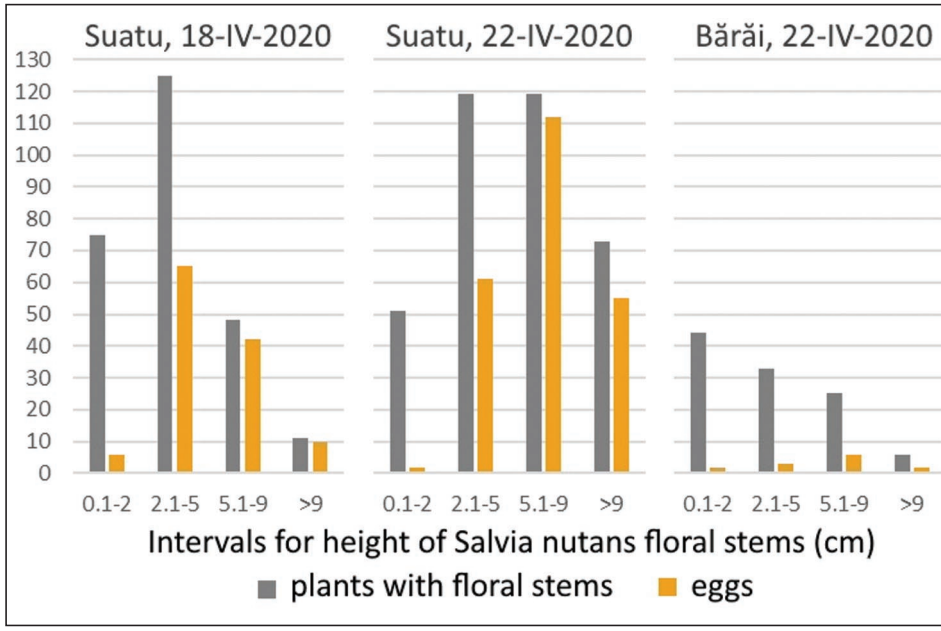

Figure 1. Histograms representing the number of *S. nutans* plants stratified into four categories according to their floral stems height (0-2 cm; 2.1-5.0 cm; 5.1-9.0 cm; >9.0 cm), and the number of *P. bavius hungarica* eggs found on plants in each height range. Data were recorded in study plots of about 100m^2 from Suatu and Bărăi.

On 25-IV-2019, a transect was used to try and identify new populations of *P. bavius hungarica* in the "Râpa Lechinţa" region of Mureş Valley, Natura 2000 Site ROSCI0210 (N 46.463368, E 24.243547). Mapping/inventoring of *P. bavius hungarica* was also conducted in the "Râpa de la Dătăşeni" (N 46.487270, E 24.150318), and ROSCI0040 Coasta Lunii (N 46.530037, E 23.939893) areas of Mureş and Arieş Valleys on 23-IV-2020, 5-V-2021, 10-V-2021, 8-VI-2021, and 11-V-2022.

Results

A total of 1387 *S. nutans* plants were examined (Table 1). In Suatu, over 900 plants were checked for the presence/absence of *P. bavius hungarica* eggs. On 18-IV-2020, 69 and 54 eggs were recorded in the first plot (S1), second plot (S2), respectively. After just four days (on 22-IV-2020), the number of eggs almost doubled, reaching 127 in S1, and 103 in S2. In Bărăi (22-IV-2020) only six eggs were found in the first plot (B1), and seven in the second (B2).

When the egg counts were conducted, we occassional observed ants belonging to the species *Lasius paralienus* Seifert, 1992 and *Camponotus aethiops* (Latreille, 1798) on the flower buds of the stems, but never aphids.

As part of our effort to identify new *P. bavius hungarica* populations in Transylvania from April 2019 to April 2020, we discovered two small populations in the "Râpa Lechinţa" (four adult females) and "Râpa de la Dătăşeni" (four adult females and two adult males) regions of the Mureş Valley.

Since *S. nutans* is very rare in the southern Transylvanian Plain (near the Mureş Valley; Prodan, 1931), the identification of another plant that could be exploited by female *P. bavius hungarica* for oviposition was attempted. After examining over 200 plants (most of them without flowers), we found a hatched egg on a leaf of *S. transsilvanica*.

Table 1. Number of *Salvia nutans* plants, the number and height of the floral stems and the number of *Pseudophilotes bavius hungarica* eggs identified in the study areas of Suatu and Bărăi.

Parcel	**S1**		**S2**		**B1**	**B2**
Data	18-IV-2020	22-IV-2020	18-IV-2020	22-IV-2020	22-IV-2020	22-IV-2020
Total number of examined *Salvia nutans* plants	429		538		146	274
The number of *S. nutans* plants with inflorescences	123	173	136	189	109	203
Number of *S. nutans* plants with at least one egg of *Pseudophilotes bavius hungarica*	37	58	34	56	4	4
The average height of the floral stems of *S. nutans* (cm) with at least one egg *P. bavius hungarica*	3.8	5.6	4.2	7.5	3.9	4.4
Number of identified *P. bavius hungarica* eggs	69	127	54	103	6	7
The maximum number of *P. bavius hungarica* eggs identified on a inflorescence	9	10	4	5	2	3

On 5-V-2021, 10 individuals of *P. bavius hungarica* (8 males, 2 females) were observed at Dătăşeni. The observed male-to-female ratio indicated that the flight had only recently begun, presumably because of the unusually cool spring temperatures recorded in 2021. On 10-V-2021, 11 individuals of *P. bavius hungarica* (6 males, 5 females) were observed at Dătăşeni, and >200 plants (most of them without flowers) were examined. Overall, 30 eggs of *P. bavius hungarica* deposited on *S. transsilvanica* plants, 28 of which were deposited on inflorescences (with a maximum number of 9 eggs per inflorescence). The remaining two eggs were deposited on the leaves of the plants that have not yet developed floral buds. Inside an inflorescence were observed 2 larvae in the first instar.

In Râpa Dătăşeni on 8-VI-2021, we recorded the presence of six *P. bavius hungarica* larvae; each of which was found on a different *S. transsilvanica* plant. During the same survey effort we examined 100 *S. nutans* plants in the Râpa Lechinţa and did not find a single *P. bavius hungarica* larva. Similarly, no larvae were found on any of the >200 *S. nemorosa* plants that were examined at Râpa Dătăşeni and Râpa Lechinţa.

On 11-V-2022, at Coasta Lunii we observed the largest number of *P. bavius hungarica* eggs laid on *S. transsilvanica.*

Our field observations confirmed the following plants to be nectar sources for the adults: *Cytisus nigricans* (L.), *Euphorbia cyparissias* L.*, E. seguieriana* Neck.*, Fragaria vesca* L., *F. viridis* Duchesne*, Ornithogalum umbellatum* L., *Salvia nutans, S. transsilvanica, Thymus marschallianus* Willd., *T. vulgaris* L.*, Veronica prostrata* L., *Vinca minor* L.

Discussion

It is already known that the phenology of the host plant as well as the flight period of the butterfly differs from one year to another due to the weathering peculiarities of each year (Crişan et al. 2011; König, 1992). Although the flight period does not exceed 15-17 days, the start of the flight has been observed to vary between 2nd April and 18th April and the exact timing is dictated by the

prevailing weather conditions in any given year (Crişan et al. 2011; Crişan et al. 2014; pers. comm. L. Rákosy).

Within 4 days of the first count in Suatu, the number of stems in *S. nutans* plants increased by approximately 40%, and the number of eggs laid increased by almost 100% in both S1 and S2. It is interesting to note that most eggs were laid on flower stalks in the height category 5.1-9.0 cm, both in S1 and S2. Even when the number of plants with stems larger than 9.0 cm increased, the highest concentration of eggs was in the category 5.1-9.0 cm. This highlights, the preference of females to lay their eggs on flower stalks with heights of 5-9 cm or higher.

The *P. bavius hungarica* population of Bărăi is much smaller than the population of Suatu, however, the abundance of the host plant is comparable in both areas. In Bărăi the vegetation is thicker meaning there is a lack of exposed topsoil that which can slow the plant's development, and cause an associated phenological delay in *P. bavius hungarica* (Jakab & Kapocsi, 2005) (Figure 2). Additionally, the high density vegetation in Bărăi hinders the growth of ant populations (specifically *L. paralienus* and *C. aethiops*) which are involved in a commensal myrmecophilous relationship with *P. bavius hungarica*, whereby they provide protection to larvae and facilitate increased survival rates (Német et al. 2016).

Figure 2. The difference between the vegetation structure from Suatu, set on fire in autumn / winter 2020 (a) compared to the one from Bărăi, unharmed, not set on fire (b).

The vegetation structure in Suatu differs from Bărăi which could account for the observed differences in butterfly population dynamics. In Suatu, there are many patches of bare soil which were created by the burning of dry vegetation in the autumn of 2019. It seems that the burning of dry vegetation at this time of year did not adversely affect *P. bavius hungarica*, and it favoured the expansion of *S. nutans* population. Ants are present in this area but visit flower buds or inflorescences after the appearance of *P. bavius hungarica* larvae and the installation of aphids.

Captive *P. bavius hungarica* larvae readily accept *Salvia nemorosa* (König, 1992) and *Salvia pratensis* (Jutzeler et al. 1997) in addition to *S. nutans*. However, despite the fact that Leraut (2016) and Tshikolovets (2011) list *S. transsilvanica* among *P. bavius* host plants this is the first time the *S. transsilvanica* has been confirmed as a host plant for the *P. bavius hungarica* subspecies in the wild. The discovery of two small populations of *P. bavius hungarica* in areas without *S. nutans*, coupled with the observation of active ovipositioning and larval feeding, confirm *S. transsilvanica* as a host plant, and extends this butterfly species' oligophagous range (Figures 3-4). Confirmation of the new host-

plant makes it possible to conceive of the existence of populations of *P. bavius hungarica* in areas without *S. nutans*, provided *S. transsilvanica* is present. Considering the many flowers that adults visit for feeding, *P. bavius hungarica* is also a successful pollinator. Similarly to other lycaenid species, *P. bavius hungarica* show no egg avoidance behavior (Árnyas et al. 2009; Dinesh & Venkatesha, 2013; Jansson, 2013; Van Dyck & Regniers, 2010) (Figure 3). The maximum number of eggs observed at Coasta Lunii on a single plant (*S. transsilvanica*) was 34.

Figure 3. Eggs of *P. bavius hungarica* laid on the inflorescence of *Salvia nutans* (a), respectively on the flower bud of *S. transsilvanica* (b, c).

Protection and conservation

Pseudophilotes bavius hungarica is a protected endemic Lepidoptera subspecies threatened by changing agricultural practices leading to overgrazing and abandonment of traditional hay making

followed by afforestation (e.g. Crişan et al. 2011, 2014). Both of these threats reduce the quality and size of *P. bavius hungarica* habitats. Populations are generally well protected in areas that have a steep gradient comprised of sage, which prevents them from being converted to arable land. *P. bavius hungarica* is also common in landscapes with steep slopes and clay-sandy soils because regular landslides create large areas of bare soil that are immediately colonised by *Salvia* spp. (including *S. nutans*). In recent years it has been observed that *S. nutans* in these optimal habitats being replaced by invading *S. nemorosa,* a ruderal relative of *S. nutans* (Bădărău & Maloş, 2019) that is not able to support *P. bavius hungarica* populations.

Figure 4. Larvae of *P. bavius hungarica* feeding on the inflorescence of *Salvia nutans* (a), and on the flower of *S. transsilvanica* (b).

In Transylvania, most steppe habitat has already been converted into arable land, intensive pasture, vineyards, orchards, or pine and acacia plantations, all of which lead to high levels of habitat fragmentation and subsequent isolation of existing populations of *P. bavius hungarica*. When this habitat loss is considered alongside its low-mobility (30 m per day; Crişan et al. 2014), *P. bavius hungarica* must be considered especially vulnerable to rapid, landscape scale land-use change.

Until now, conservation efforts have focussed solely on managing host plant populations. However, given that the larvae are facultatively myrmecophilic, conservation managers must also consider the need to preserve areas with large patches of bare soil and low vegetation density that sustain the ant populations (e.g. *Camponotus* spp.) which aid *P. bavius hungarica* larval survival (Német et al. 2016).

Contrary to expectation, it has been found that the burning of dry vegetation in late autumn or very early spring does not negatively affect the pupae of *P. bavius hungarica*; at least in areas where the uneven microtopography does not allow fire to completely destroy the habitat. In fact, fire may have a beneficial impact on *P. bavius hungarica* by reducing the dry plant mass and creating patches of bare soil that support *Lasius* spp. and *Camponotus* spp. ant colonies (Német et al. 2016).

The poor management of the Suatu I scientific reserve, which was home to the largest known population of *P. bavius hungarica* until 1990-93, led to a rapid and sharp decline in the population. The main cause of the population decline in this 4.28 ha area is the loss of traditional farming practices. Abandonment of mowing and grazing has caused an undesirable thick vegetal layer, with damaging

impacts not only for *P. bavius hungarica* but also for the many rare plant species protected in the reserve (Enyedi et al. 2008).

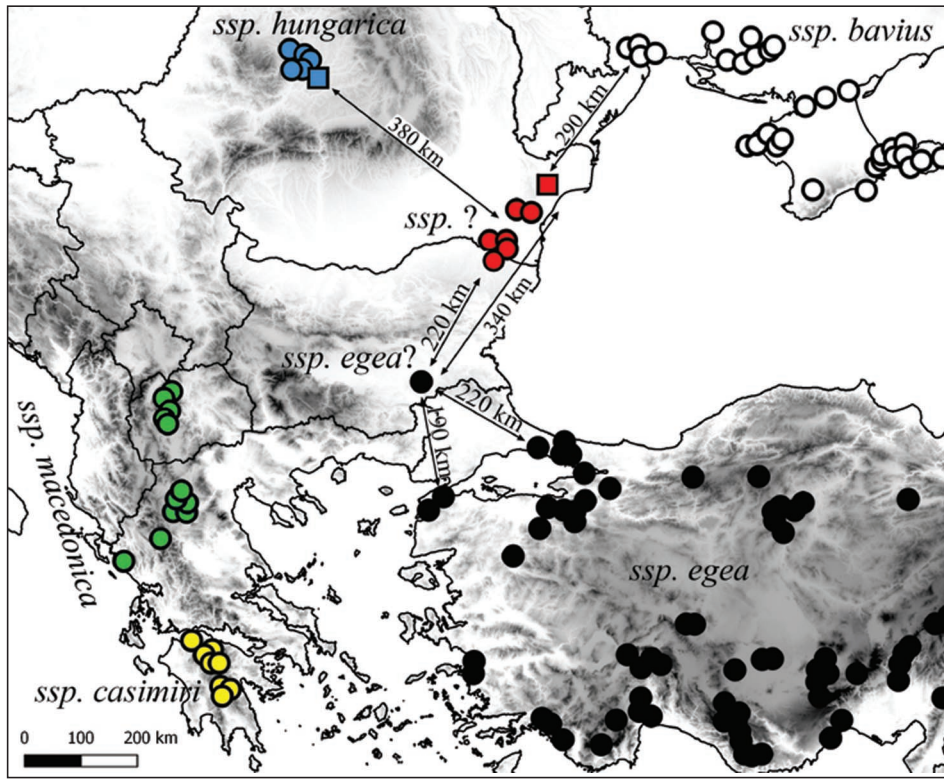

Figure 5. Western distribution of *Pseudophilotes bavius*. Blue square - new records from Transylvania (Râpa Dătăşeni and Râpa Lechinţa), Red square - a new record from Tulcea county (northern Dobrogea, Babadag area). General distribution after Rákosy & Widlich (2017).

Unfortunately, local authorities appear to be entirely disinterested in the protection of the local endemics *P. bavius hungarica* and *Astragalus peterfii* Javorka despite the fact they provide the area with a unique feature on a European, if not global, scale. After 1960 and as a result of land-use changes within the reserve, including abandonment of mowing and grazing alongside the formation of bush barriers, the two endemic taxa settled on abandoned terraces located east and west of the reservation. The population of *P. bavius hungarica* has developed so well in these areas that the population in 2010 was estimated to be over 1000 individuals (Crişan et al. 2014). In 2016, the small 2 ha area in which the *P. bavius hungarica* population was concentrated began to be intensely grazed (overgrazed) by a flock of sheep. In the spring of 2017, all the *S. nutans* plants were grazed at the same time of year at which the larvae of *P. bavius hungarica* are found in the inflorescences. So far the situation is unchanged, the same flock of sheep consumes all *S. nutans* plants in early May, so the population has been eradicated. Since this increase in grazing intensity, no *P. bavius hungarica* have been observed in the area meaning that the largest population of an endemic taxon has been destroyed because of irrational decision-making, indifference and ignorance. Our efforts to highlight the plight of *P. bavius hungarica*,

including the media coverage of the situation, have not been successful, and immediate material interests are, unfortunately, still prioritised over biodiversity preservation.

The protection of *P. bavius hungarica* in the Mureş Valley is an important objective for the ROSCI0210 Râpa Lechinţa site because it is Transylvanian endemic at its most southeastern distributional limit (Figure 5). It is therefore imperative that this taxon is included on lists of protected taxa. Populations identified outside the site should be included in the site by extending its boundaries.

The species *P. bavius* was first reported from Dobrogea near the Romanian-Bulgarian border based on individuals collected in 1988. These populations were assumed to belong to the subspecies *P. bavius egea* (Herrich-Schäffer, 1852) (Rákosy & Székely, 1996; Székely, 1994). Later, the species was reported from several sites in Dobrogea, including in the central area of the region (Dincă at al. 2011, Székely 2012, 2013), but all these reports were from Constanţa county (southern Dobrogea). The idea of the presence of the *P. bavius egea* subspecies in Romania was later maintained (Rákosy et al. 2003; Rákosy, 2013; Székely, 2008; Székely, 2016). On 13-V-2021, the presence of the species *P. bavius* was also reported in Tulcea County (northern Dobrogea), in the Babadag region (pers. obs. L. Rákosy) (Figure 5). The taxonomic classification of *P. bavius* populations in Dobrogea was discussed by Rákosy & Weidlich (2017), with the hypothesis being formulated that the populations of Dobrogea probably belong to the nominative species *P. bavius bavius*, which came here from the Crimean Peninsula and southeastern Ukraine (*P. bavius bavius*), and not from Turkey (*P. bavius egea*). The discovery of this species in Tulcea County also makes the hypothesis of colonisation from Ukraine with the nominotypic subspecies much more plausible. The latest publications (Rákosy & Goia 2021; Rákosy et al. 2021) no longer include *P. bavius egea* on the list of taxa that can be found in Romania.

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*Andrei Crişan
Cluj-Napoca
Aleea Gârbău, 5, ap. 15
RO-400534 Jud. Cluj
RUMANÍA / *ROMANIA*
E-mail: andrei.crel@gmail.com
https://orcid.org/0000-0003-3501-785X

László Rákosy
Babeş-Bolyai University
Faculty of Biology and Geology
Department of Taxonomy
Str. Clinicilor, 5-7
RO-3400 Cluj-Napoca
RUMANÍA / *ROMANIA*
E-mail: laszlo.rakosy@ubbcluj.ro
https://orcid.org/0000-0002-7793-6996

Tibor-Csaba Vizauer
Dej, Vânătorilor, 4
RO-405200, Jud. Cluj
RUMANÍA / *ROMANIA*
E-mail: vizauercsaba@gmail.com
https://orcid.org/0000-0002-6416-2581

y / *and*

Babeş-Bolyai University
Faculty of Biology and Geology
Department of Taxonomy
Str. Clinicilor, 5-7
RO-3400 Cluj-Napoca
RUMANÍA / *ROMANIA*

*Autor para la correspondencia / *Corresponding author*

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