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RESEARCH ARTICLE

Detection of *Zabrotes subfasciatus* and *Bruchidius glycyrhizae* (Chrysomelidae: Bruchinae) in Romania

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Abstract
Although beans are an important part of the human diet, their long-term storage is still problematic due to pest damage by seed beetles. This study provides data on a documented detection of Mexican bean beetle *Zabrotes subfasciatus* in Romania. A great number of individuals were found inside packages of small white beans (*Phaseolus vulgaris*) imported from Syria and Ethiopia. Based on the investigation of the infested seeds, rearing and biology of the species, it is unlikely that this alien species is established in Romania. We also provide the first known record of *Bruchidius glycyrhizae* in Romania, based on its previously known distribution and the range of its host plants, it is more likely an overlooked native species.

Keywords
Alien species, beans, Coleoptera, distribution, licorice, native, seed beetles, storage pests.

Introduction

Beans are an important part of the human diet in many areas of the world. The production of leguminous plants can be affected in the field by various biotic and abiotic factors. After the harvest, beans can also be affected in storehouses by humidity, fungal infestation or seed beetles. In the case of common beans (*Phaseolus vulgaris* L.), various alien beetle species like *Acanthoscelides obtectus* Say, 1831, *Bruchus rufimanus* Boheman, 1833, *Callosobruchus maculatus* (Fabricius, 1775), *Callosobruchus phaseoli* (Gyllenhal, 1833), *Mimosestes mimosae* (Fabricius, 1781), *Zabrotes subfasciatus* (Boheman, 1833) are known to use the seeds for larval development (Yus-Ramos et al. 2014). In Europe, only *Acanthoscelides obtectus* is known to be established and also cause problems on *Phaseolus vulgaris* in the field and storehouses but the other mentioned species can survive in storehouses and therefore can affect the long-term storage of beans (Yus-Ramos et al. 2014). Although seed beetles can be easily introduced by importing infested seeds, in Romania only two alien species that damage seeds of *Phaseolus vulgaris* were detected so far. *Acanthoscelides obtectus* which develops on common beans (Săpunaru et al. 2006) and *Bruchus rufimanus* which typically develops in broad beans (Stana and Ghizdavu 1990), both species being recorded in the wild and storehouses. But, recent studies on alien seed beetles in Romania showed that many species were already widespread at firsts records (Pintilioaie et al. 2018; Rădac et al. 2021). Thus, many species can be overlooked or misidentified until detection. This may be the case of Mexican bean beetle *Zabrotes subfasciatus*, a frequent pest species in countries with a warmer climate. A similar situation may occur in *Bruchidius glycyrhizae* (Gyllenhal, 1839), a narrow monophagous species which develops in seeds of *Glycyrrhiza* spp. (licorice). Although the species is reported as a pest of licorice in the field and in storehouses (Hagstrum and Subramanyam 2009; Al-e Mansoor and Zarei 2003) there are not so many reports of *Bruchidius glycyrhizae* attacking the cultures of *Glycyrrhiza glabra* L. in Europe. Instead, the native *Glycerrhiza echinata* L. is attacked (Delobel and Delobel 2006).

Bruchidius glycyrhizae and *Zabrotes subfasciatus* were not recorded so far in Romania but were reported from neighboring countries (Jermy and Szentesi 2003; Beenen and Roques 2010; Yus-Ramos et al. 2014; Szentesi et al. 2017). The aim of this study was to investigate the potential presence of *Zabrotes subfasciatus* and *Bruchidius glycyrhizae* in Romania and to provide information regarding the status of the species in Romania, biology and potential introduction pathways.

Material and Methods

For detection of *Zabrotes subfasciatus* samples of imported small white beans (*Phaseolus vulgaris* L.) were collected from three different supermarkets from Timişoara (Romania) in February 2021. Based on the information from the products label, all the beans were packed in Romania and imported from Ethiopia, Syria or

Turkey (Table 1). Each sample consisted of one kilogram of seeds. The samples were unpacked and checked for adult individuals, damaged seeds or seeds with eggs. After the initial examination, the seeds were contained in 10 L plastic containers, kept at room conditions and checked regularly for any emerging adults. In order to track back physical treatments applied to the seeds, for each sample a number of 30 undamaged seeds were selected to check seed germination. The seeds were soaked in water for 1 hour and stored in plastic containers with a moist paper towel at room temperature. After 7 days the germination rate was evaluated. All seedlings with emerging hypocotyls were considered germinated, regardless of their shape, size or presence and size of the primary root.

In order to detect *Bruchidius glycyrhizae*, 80 seed pods of *Glycerrhiza echinata* L. were collected on April 2021 from a natural ecosystem in the predelta of the Brațul Sfântu Gheorghe located near the city of Tulcea (45.20888°N, 28.78027°E). The seed pods were stored in plastic containers at optimal conditions of temperature (22°C) and humidity (50%) and checked regularly for any emerging adults.

Voucher specimens of *Bruchidius glycyrhizae* (9 individuals) have been preserved in the entomological collection of the Natural Sciences Museum Galați and of *Zabrotes subfasciatus* (1 individual) in Zoological Museum from Cluj Napoca.

Results

We detected the Mexican bean beetle in 11 out of 12 samples collected during the study (Table 2). Although *Acanthoscelides obtectus* is a common seed pest of beans, we didn't find any adult individuals belonging to this species. Therefore, all the individuals

Table 1. The beans samples collected during the study and their origin.

Sample	Product name	Lot number	Origin
A1	Atifco foods Fasole albă	L2062 1	Syria
A2	Atifco foods Fasole albă	L2062 1	Syria
A3	Atifco foods Fasole albă	L2062 1	Syria
B1	Bitar Fasole albă cu bob mic	2210132	Ethiopia
B2	Bitar Fasole albă cu bob mic	2210132	Ethiopia
B3	Bitar Fasole albă cu bob mic	2210133	Turkey
C1	Campo Largo Fasole albă cu bob mic	L2212 1	Ethiopia
C2	Campo Largo Fasole albă cu bob mic	L2215 1	Ethiopia
C3	Campo Largo Fasole albă cu bob mic	L2215 1	Ethiopia
P1	Proxi Fasole albă cu bob mic	2760181	Ethiopia
P2	Proxi Fasole albă cu bob mic	2760181	Ethiopia
P3	Proxi Fasole albă cu bob mic	2760182	Ethiopia

Table 2. The number of individuals of *Zabrotes subfasciatus* and damaged seeds found in each sample.

Sample	Individuals among the seeds	Seeds with emergence holes			Seeds with eggs	Total affected seeds	Germination rate
		1 hole	2 holes	3 holes			
A1	37	16	2	0	1	19	73%
A2	7	17	1	0	5	23	27%
A3	6	16	3	1	7	27	70%
B1	0	2	0	0	0	2	60%
B2	0	3	0	0	0	3	63%
B3	0	2	0	0	0	2	67%
C1	14	28	16	3	5	52	97%
C2	9	26	6	2	4	38	87%
C3	15	32	15	4	6	57	77%
P1	0	15	4	0	1	20	60%
P2	2	18	9	1	1	29	73%
P3	0	7	1	0	0	8	80%

were considered *Zabrotes subfasciatus* including some damaged adults or larvae. The species was found in samples as eggs, larvae, pupae, adults in seeds and adults among the seeds (Fig. 1). All the specimens found in the samples were dead at the moment of investigation, including pupae and larvae in different stages of development. From the seeds stored in containers, no beetles emerged. In all samples, there were seeds that germinated (Table 2), therefore the imported beans were not previously exposed to control treatments incompatible with seed germination.

In the case of *Bruchidius glycyrhizae* 9 individuals hatched after a month from the collection date. Although the pods of *Glycerrhiza echinata* have 1–2 seeds, the ones from which the bruchids hatched have only a single emergence hole in all cases. In the cases in which the infested pod had 2 seeds, the larva consumed around 90% of only one licorice seed leaving the other seed undamaged.

Discussion

The status of the recorded species in Romania

According to Yus-Ramos et al. (2014) *Zabrotes subfasciatus* requires an optimal temperature of 32°C and 70% humidity but other studies show that the species can reproduce at suboptimal values like 27°C and 70% humidity (Dendy and Credland 1991) or even 25°C and 57% relative humidity (Zekarias and Haile 2018). All individuals found in the samples were dead at the moment of the investigation and although adult longevity of *Z. subfasciatus* is relatively short (about 11 days) (Tigist

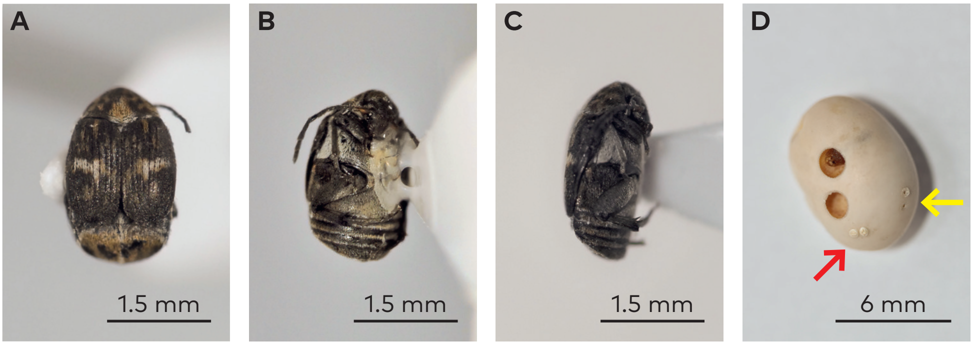

Figure 1. *Zabrotes subfasciatus*: **A–C** adults; **D** infested seed displaying emerging holes, eggs (red arrow) and larval holes (yellow arrow).

2020), the fact that all stages were affected is more likely due to pest control treatments. There was at least one heat treatment, as we identified seeds with possible burn marks on the seed coat. This hypothesis is also supported by the fact that germination in *Phaseolus vulgaris* is not affected by a 90 minutes exposure to a temperature of 60°C (Miceli and Miceli 2012). Moreover, sunning and heat treatment are used to control the Mexican bean beetle (Chinwada and Giga 1996) as most stored-product pests die from prolonged exposure at temperatures higher than 35°C and death occurs within minutes at around 60°C (Fields 1992).

We were unable to check the storehouses or the facilities where the products were packed, so there are two possibilities regarding its establishment: the imported individuals were already dead by the time they reached Romania (the species did not establish) or infested beans were imported with alive individuals and killed prior to packing process (the species survives in storehouses from Romania). However, based on fact that the species *Zabrotes subfasciatus* was not found in the wild in Europe, its biology and the fact that all intercepted individuals were dead, make us consider that the species should be treated as unestablished in Romania until further evidence.

In the case of *Bruchidius glycyrhizae* (Fig. 2), although it is rarely reported, it is a widespread species being recorded from Azerbaijan, Armenia, Georgia, Hungary, Russia (South European Territory), Iran, Iraq, Kazakhstan, Mongolia, Turkmenistan and Uzbekistan (Anton 2010). It is considered a native species in the listed countries. In Germany, *B. glycyrhizae* was introduced (Anton 2010). In Europe, it is associated with *Glycyrrhiza echinata* L. (Jermy and Szentesi 2003; Delobel and Delobel 2006; Szentesi et al. 2017; this study) and in Asia with *Glycyrrhiza glabra* L. and *Glycyrrhiza uralensis* Fisch. ex DC. (Anton 1998; Zarnegar 2014). *G. echinata* is a native species in Romania, it is found sporadically along the banks of rivers, ditches or irrigation canals (Ciocârlan 2009), therefore *Bruchidius glycyrhizae* should be treated as a native species till further evidence. However, we cannot totally exclude a past introduction in Europe from infested seeds of *Glycyrrhiza glabra* as apparently healthy seeds (but infested) could easily have gone unnoticed. Interceptions of infested seeds of *G.*

glabra were reported in the past in Iran with *Bruchidius glycyrhizae* (Al-e Mansoor and Zarei 2003) and with *Bruchidius halodendri* (Gebler, 1825) in seeds from Greece and USRSS (United States Department of Agriculture 1934; Wadhi and Verma 1970). However, there are many taxonomic misunderstandings around the name of *Bruchidius glycyrhizae.* Also, old records of both *B. glycyrhizae* and *B. halodendri* can represent misidentifications as *B. halodendri* in Europe typically feeds on seeds of *Caragana halodendron* (Pall.) and not with *G. glabra* (Delobel and Delobel 2006). While in Hungary *B. glycyrhizae* is reported from *G. echinata* and not from *G. glabra* meanwhile the same seed beetle attacks *G. glabra* in the eastern part of its distribution range (Anton 1998; Al-e Mansoor and Zarei 2003; Delobel and Delobel 2006; Zarnegar 2014). Furthermore, Lukjanovitch and Ter-Minassian (1957) proposed two subspecies in *Bruchidius glycyrhizae*, *B. g. glycyrrhizae* recorded in the southern part of the former Soviet Union (European region and Caucasus) in seeds of *Glycyrrhiza echinata*, *G. hirsuta* L. (currently a synonym of *G. glabra*) and *B. g. obscuripennis* recorded in Eastern Kazakhstan in seeds of *Glycyrrhiza uralensis*. Although Anton (2010) treats *obscuripennis* merely as a synonym of *Bruchidius glycyrhizae* it cannot be excluded that there are several host races within this species. Therefore, a review of the records from *Bruchidius halodendri* group in all its range is needed in order to better understand the host species and the native area of distribution of different populations in the realm of this species.

Authorship and spelling of *Bruchidius glycyrhizae*

The species was described in Schönherr (1839) and although the volume includes the work of three entomologists (Gyllenhal, Boheman and Fåhraeus), the authorship of each description can be easily traced back by the abbreviations mentioned at the end of the description. In the case of *Bruchidius glycyrhizae,* the description has the "Ghl" abbreviation at the end, because the description was made by Leonard Gyllenhal. He described the species as *Bruchus glycyrhizae* mentioning "Steven *in*

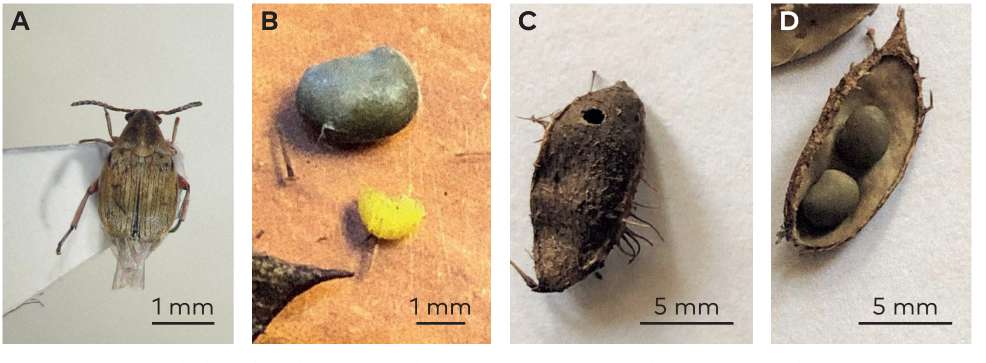

Figure 2. *Bruchidius glycyrhizae*: **A** adult; **B** larva; **C** emergence hole; **D** seeds of the host plant (*Glycerrhiza echinata*).

litteris", meaning that the name was previously proposed by Steven. Gyllenhal also mentioned the origin of the species and the host plant stating "*Patria*: Sibiria, in Glycyrhyzae glandulis.", this record made it obvious that the name of the species was related to the name of the host plant. However, the spelling of the correct name of the plant genus is *Glycyrrhiza* (see Linnaeus 1753) and it is noticeable that the name of the plant and the specific epithet of the seed beetle and the spelling of the genus name of the plant by Gyllenhal are not the same. The spelling of "*glycyrhizae*" is the same also in the index of species of the volume in which it was described (Schönherr 1839). In the corrigenda of the same volume (Schönherr 1840), there are no records of errors in the name of *B. glycyrhizae*. Although it may seem that "*glycyrhizae*" was misspelled, there is no explicit mention that the species was named after the host plant and if it was an error, it was not mentioned in the corrigenda. Furthermore, ten years later the same spelling is maintained in Jekel (1849). However, subsequent papers are mentioning the taxon with various misspellings (Table 3).

Although Table 3 does not represent a complete list of papers, we can observe in the last years a tendency to correct the specific epithet of the seed beetle in order to match the spelling of the plant genus. According to the International Code of Zoological Nomenclature (ICZN) an incorrect original spelling must be corrected if "in the original publication itself, without recourse to any external source of information, clear evidence of an inadvertent error, such as a lapsus calami or a copyist's or printer's error" and that the corrections made in the corrigendum of the volume are "accepted as clear evidence of an inadvertent error". Therefore, in view of the above arguments, we consider that the emendation criteria of ICZN were not met and the original spelling from the description made by Gyllenhal should be maintained. Besides the misspellings from the published papers, there are also other cases on websites or online databases. A particularly interesting case is the listing of

Table 3. Misspellings of *Bruchidius glycyrhizae* in various papers along the time.

The auteurs of the paper	The spelling used in the paper
Allard (1868)	*glycirrhizae* Sch.
Baudi (1868)	*glycyrrhizae* Gyll.
Schilsky 1905	On *Bruchidius halodendri* (Gebler, 1825) as: var. *glyzyrrhizae* quoting *Bruchus glyzyrrhizae* Fahr. Schönh.
Pic (1913)	On *Bruchidius halodendri* (Gebler, 1825) as: var. *glycyrrhizae* Fåhr. in Schönh.
Lukjanovitch and Ter-Minassian (1957)	*Bruchidius glycirrhizae* (Fåhr.)
Karapetjan (1985)	*Bruchidius glycirrhizae* (Fåhrreus)
Borowiec (1987)	*Bruchidius glyzyrrhizae* (Fahr.)
Delobel and Delobel (2006)	*Bruchidius glycyrrhizae* (Fahr.)
Ghahari and Borowiec (2017)	*Bruchidius glycyrrhizae* (Gyllenhal, 1839)
Szentesi et al. (2017)	*Bruchidius glycyrhizae* (Fåhraeus, 1839)

Bruchidius glycyrrhizae (Fabricius, 1839) on the Fauna Europaea database (Audisio et al. 2015) where, besides the frequent misspelling of the species name, the authorship is attributed to Fabricius. This is most likely a confusion of the abbreviation Fahr. with Fabr. maid most likely when transcribing from old papers, as Fabricius died in 1808, and therefore he could not have described the species in 1839.

Introduction pathways of *Zabrotes subfasciatus* in Romania

The imported small white beans on the market came mainly from non-European countries. We found adult individuals of *Zabrotes subfasciatus* in samples from Ethiopia and Syria. In the sample from Turkey, we found only 2 damaged seeds. Therefore, we cannot confirm Turkey as a potential source of seeds infested with the Mexican bean beetle. Moreover, a small number of damaged seeds or individuals can remain in the packing machine from a previous lot. Such a case may be also in sample A1 (see Table 1) where we found more individuals among the seeds than seeds with opened emergence holes. This clearly shows that individuals from other seeds can end up in other packages and therefore further studies should include multiple packages from a lot in order to confirm the presence of seed beetle species in a particular lot. Based on the fact that all individuals were found dead, it is a chance that the specimens found in the packages were imported dead, after a pest control treatment in the country from where the beans were imported. Furthermore, seeds with burn marks suggest at least one heat treatment which can be sunning or artificial heating as both are efficient in controlling the Mexican bean beetle (Fields 1992; Chinwada and Giga 1996) but other physical, chemical or biological methods can be applied (Mikami et al. 2010; Zekarias and Haile 2018; Iturralde-García et al. 2020) and many of them are hard to traceback just from investigating the seeds.

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