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SEED PREDATION IN LEGUMINOUS TREES AND SHRUBS: NEW INVASIVESEED BEETLES (COLEOPTERA: CHRYSOMELIDAE: BRUCHINAE) TO THE SERBIAN FAUNA

SUMMARY

Three species of genera *Bruchidius* and one from *Megabruchidius*as n. sp. are established in Republic of Serbia during studies from 2012 to 2014. These seed-beetles larvae develop in seeds within pods of five woody legumes, a widely grown species of ornamental trees and shrubs in Serbia. Several recent reports reveal that this species are well established in France, Hungary, and Bulgaria. The relationships of this new species are investigated using both morphological data and idioecological analyses. Our results indicate that this species is likely related to a mostly Paleotropical group, including members of genera *Bruchidius, Megabruchidius* and *Acanthoscellides*. Hypotheses on the geographic origin of this new species for Serbian entomafauna have beenalso discussed.

Keywords: Bruchinae, invasion, Leguminosae, seed predators, Serbia

INTRODUCTION

Adaptation to host-plant defenses through key innovations is a driving force of evolution in phytophagous insects. Typical hosts of bean beetles are legumes (Fabaceae). Species of the neotropical Bruchine genus *Bruchidius, Megabruchidius* and sister genus *Acanthoscelides* Schilsky are known to be associated with specific host plants. Delobel (2006) suggest that the speciation in

European seed beetles are sequential evolution or sequential radiation that follows plant diversification as response to insect evolution against plant defensive traits. Insect's species exhibited significant conservatism in host utilization, and process was contrasted, also resulted with the reciprocal co-evolution, with continuous mutual adaptation. (Most seed beetles are either monophagous or oligophagous, so their host range is limited to restricted plant taxa (the speciation processes involved in such specialization pattern has produced these specific associations) and all may reflect on radiations linked to particular kinds of host plants. So, an indirect method has been used method to

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address the issue of the existence of seed beetles in large, ornamental and economically important leguminous woody species in Republic of Serbia. The value of the system lies in the accuracy of host affiliations. Indication of (Fabaceae host plant vs. Bruchine seed predator) – *pairs existence,* scientifically examined and geographically established, all in numerous previous research, were a leading for insect detection and their ecology investigation. Bruchine and their legume hosts were observed by extensive field sampling throughout Serbia over three years and by rearing sampled collected beetles in the laboratory. This study should also be of interest to those involved in the numerous biological control programs which either already use or aim to use seed-beetles as auxiliary species to limit the propagation of several invasive legume tree species(Jermy and Szentesi, 2003).Identifying plant traits that promote invasiveness has been a major goal in invasion ecology. Germination plays a central role in the life cycle of plants and therefore could be a key trait in determining species invasiveness. Furthermore, transportation of economic plants can be accompanied by and expand distribution of associated insect herbivores. In particular, internal seed predators that are protected and concealed inside hard host seeds are more likely to be transported successfully than external feeders through commercial trading, as in the bruchine *Acanthoscelides pallidipennis* Motschulskyinfesting the seeds of the North American legume, *Amorpha fruticosa* L. introduced to Eurasia (Szentesi, 1999; Tuda et al., 2001; Gagić et al., 2008). Once introduced, novel enemy-free environments could promote their establishment as in most non-indigenous organisms (Torchin et al., 2003). Confamilial, it could co-occurring with species that share ecological characteristics, the exotic invasive The honey locust- *Gleditsia triacanthos* L., and introduced The Kentucky Coffeetre *Gimnocladus dioicus*(L.) K. Koch (Fabaceae: Caesalpinioideae), and Persian silk tree, pink silk tree - *Albizia jullibrisin (Durazz., 1772. non sensu Baker, 1876*) in Serbia. Also with controlled ornamental shrubs: *Laburnum anagyroides* (Fabaceae:Faboideae: Genisteae) known as Golden chainand *Cersis siliquastrum* L.(Judas tree).All were host plants – subject of pods material sampling (Ferreras et al.2014). Our findings are *Megabruchidius tonkineus* (Pic, 1904) as endophagous - of *G. triacanthos* L., as well of introduced *Gimnocladus dioicus*,. *Bruchidius terrenus*(Sharp) was recognised in August, 2013as seed predator of *A. jullibrisin* and *Bruchidius villosus* (Fabricius) feeding in seed of *L. anagyroides*in May, 2012 are first recorded and completely new to Serbian fauna. *Bruchidius siliquastri* (Delobel) spermatophagous of *C. siliquastrum* was also identified in April, 2013,but although not idoecologicaly studied in detail could also be considered as new a species. This was also a case with *M. tonkineus* because discovering of insect species presence, its biology and diet couldn't be concluded or described as verified, if it is based just on few specimens reared without method, accidentally and absolutely not related to environment. Until now several Individual specimensof *M. tonkineus* and *B. siliquastri* were caught from surrounding greenery in North Serbia (Gavrilovic B and Savic D, 2013), but both

records are formally insufficient for species existence proof or establishing, methodologically and scientifically. *Bruchidius* species are hosts to many experimentally reared specimens of parasitoids (Hymenoptera: Braconidae; Pteromalidae; Eulophidae; Eupelmidae) and the presence of hyperparasitism is also detected.

MATERIAL AND METHODS

Just pods of *G. dioicus* were collected in 2013 at the University of Belgrade, Forestry Faculty Arboretum. During 2012-2014 seed pods of *C. siliquastrumG. triacanthos,* L. *anagyroides* and *A. jullibrisin* were collected at 13 localities.. Pods were held in the laboratory in plastic boxes and transparent bags until emergence of adult beetles and parasitoids. We studied the seed beetles *Bruchidius terrenus* (Sharp) and *B. siliquastri* Delobel, which infest the seeds of *A. julibrissin* and *C. siliquastrum*, respectively, and their parasitoids. Each sampled population of pods and/or seeds was placed in a bag connected to a clear bottle or tube (Fursov, 2004) and kept under semi-natural room conditions -. In the laboratory, bruchids and the primary parasitoids were mass-reared in climate controll rooms under conditions close to those of their area of origin: 12:12 L:D , 3-23° C and ≤80% RH (depends of host plant ongoing phenology or experimental needs- proof of weevil monophagous or else feeding preferences)or experimental needs as it is in the regions where collections were made. Emerging bruchine beetles and their parasitoids captured in bottles were collected daily for a month, and later on weekly until all adults emerged. Bruchine adults were identified by external morphological traits. The insect specimens and part of plant specimens were deposited at the Institute of Forestry - Belgrade and Faculty of Forestry - Belgrade. We The published work were extensively reviewedfor bruchine beetles associated with *studied host plants* and their geographical distribution to study host ranges of the beetles. Percentages of total pre- dispersal and post-dispersal (in reinfested material), makes these insects serious host plant suppression candidates (Table 1). Their bionomic were monitored by continuous collection ofinfested seeds, itsdissection and observed also in correlation with host specificity ranged from monophagy (at least ecological monophagy) to possible oligophagy. On the basis of presence/absence data the null hypothesis was tested assuming that plant taxa and seed consuming bruchid species form congruent phyletic relations at the species level (Tuda et al., 2009).

RESULTS AND DISCUSSION

Bruchine beetle specimens emerged from the seeds of legume host plants (Table 1, Fig. 1, 2, 3, 4, 5) and mean level of infested seed explored for the localities in Serbia are present in Tabels 1 and 2. Data about insect stocks cultures bionomy and life cycle development collect by seed dissection (100 seed per locality) are also presented.

Table 1. Host plant seed infestation cause's and results in % with total number of reared insects

Host plant(a)	*Albizia julibrissin*						
	01.08. – 01. 10. 2012; 2013; -*main generation appeareance*	TSE	TIS	EH	A	PA	PP
	01.04. - 31.06. 2013; 2014.- *owerwintreder specimens generation*	(1)	(2)	(3)	(4)	(5)	(6)
Seed beetle	*Bruchidius terrenus*	300	265	246	207	39	
	%		*88%*	*82%*	*69%*	*13%*	*15%*
Host plant(b)	*Cercis siliquastrum*						
	1.04.2014 - 31.05.2014.	TSE	TIS	EH	A	PA	PP
		(1)	(2)	(3)	(4)	(5)	(6)
Seed beetle	*Bruchidius siliquastri*	300	202	146	135	11	
	%		*67%*	*49%*	*45%*	*4%*	*5%*
	LOC1	100	46	46	42	4	
			46%	*46%*	*42%*	*4%*	*9%*
	LOC2	100	75	49	45	4	
			75%	*49%*	*45%*	*4%*	*5%*
	LOC3	100	81	51	48	3	
			81%	*51%*	*48%*	*3%*	*4%*
Host plant(c)	*Laburnum anagyroides*						
	01.08. – 01. 10. 2012; 2013; *main generation appeareance*	TSE	TIS	EH	A	PA	PP
	01.04. - 31.06. 2013; 2014. *owerwintreder specimens generation*	(1)	(2)	(3)	(4)	(5)	(6)
Seed beetle	*Bruchidius villosus*	300	69	55	43	12	
	%		*23%*	*18%*	*14%*	*4%*	*17%*
	LOC1	100	34	34	29	5	
			34%	*34%*	*29%*	*5%*	*15%*
	LOC2	100	24	15	11	4	
			24%	*15%*	*11%*	*4%*	*17%*
	LOC3	100	11	6	3	3	
			11%	*6%*	*3%*	*3%*	*27%*

Host plant(d)	*Gleditchia triacanthos*						
	1.4.2014 - 31.05.2014.	TSE	TIS	EH	A	PA	PP
		(1)	(2)	(3)	(4)	(5)	(6)
Seed beetle	*Megabruchidius tonkineus*	300	140	135	15	0	0
	%		*47%*	*45%*	*5%*	*0%*	*0%*
	LOC1	100	0	0	0	0	0
			0%	*0%*	*0%*	*0%*	*0%*
	LOC2	100	60	55	5	0	0
			60%	*55%*	*5%*	*0%*	*0%*
	LOC3	100	75	75	5	0	0
			75%	*75%*	*5%*	*0%*	*0%*
Host plant(e)	*Gimnocladus dioicus*						
	LOC1	100	5	5	5	0	0
			5%	*5%*	*5%*	*0%*	*0%*

Table notes and legend:

a. Emergence of the seed beetle *Bruchidius terrenus* from seeds of *Albizia julibrissin* with emergence of beetle parasitoids (eupelmid and braconid) from *B. terrenus*.
b. Emergence of the seed beetle *Bruchidius siliquastri* from seeds of *Cercis siliquastrum* with emergence of parasitoids (pteromalid, eupelmid, eulophid) from *B. siliquastri.*
c. Emergence of the seed beetle *Bruchidius villosus* from seeds of *Laburnum anagyroides* with emergence of its parasitoids (pteromalid and braconid)
d. Emergence of the seed beetle *Megaruchidius tonkineus* from seeds of *G. triacanthos*
e. Emergence of the seed beetle *Megaruchidius tonkineus* from seeds of *G. dioicus*

(1)TSE: Total seeds examined; (2) TIS: Total infested seeds and percentage of infested seeds; (3) EH: Number of seeds with emergence holes; (4) A: Number of adults, (5) PA: Number of parasitoids (pteromalid, eupelmid, eulophid), (6) PP: (5) PA: Number of parasitoids divided with (2) TIS: Total infested seeds (percentage)

Life cycle was found to be synchronized with host phenology in each location. It is important to point that this seed predators have emerged also from an introduced *plants* (Fabaceae) in Serbia. The Old World genus Bruchidius Schilsky, 1905 (Coleoptera: Chrysomelidae: Bruchinae) comprises about 300 species of seed beetles (Kingsolver, 2004). Bruchine chrysomelids are economically important pests of agricultural and stored products. Larval host plants of most *Bruchidius* species are legumes (Fabaceae), as well as species of Apiaceae and Asteraceae (Borowiec, 1987). *A. julibrissin* and *Cercis siliquastrum* L. (Fabales: Fabaceae) are introduced in Serbia and planted as ornamental trees. *Megabruchidius tonkineus* was described from „Tonkin" by PIC (1904) as *Laria tonkinea*. Four years later Borowiec (1984) established *Megabruchidius*. In that paper *Megabruchidius tonkineus* was moved into this genus. The European presence of *Megabruchidius tonkineus* was first recorded

by Wendt (1980) from Germany (albeit with host record based on mistaken speculation.

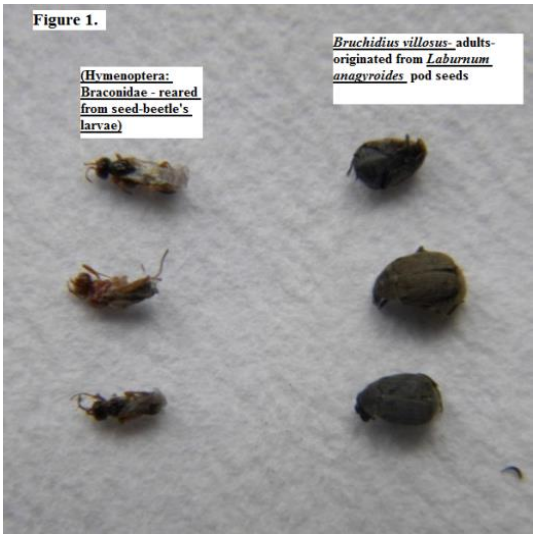

Figure 1. *B. villosus* adults andreared specimens of its parasitoids (original)

Figure 2. *M. tonkineus* - adults with damaged seeds and pods of *G. triacanthos*

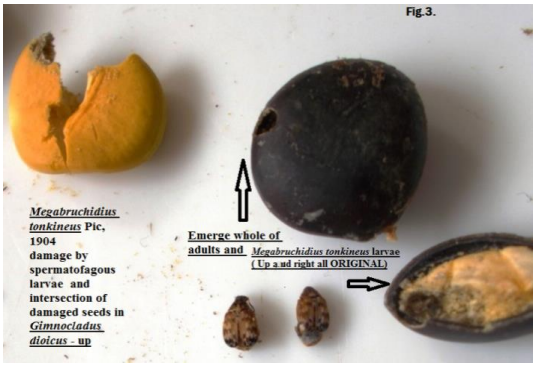

Figure 3. *M. tonkineus*- adults with damaged seeds and pods of *G. diocus*

Figure 4. *C.siliquastrum* seeds with beetle emerge holes and *B. siliquasty* liveadult

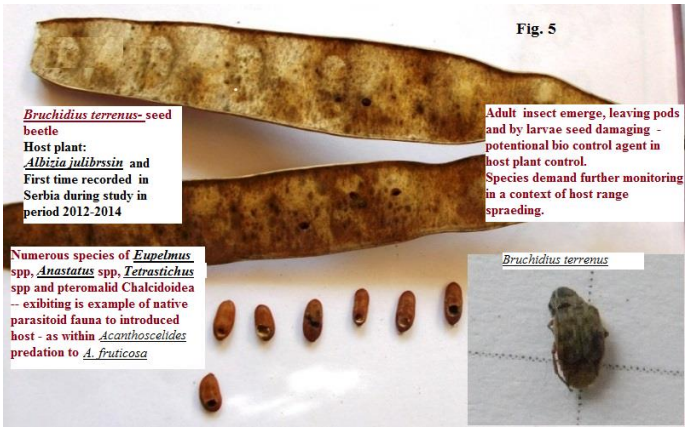

Figure 5. *A.jullibrissin*- damaged seeds and pods with *B. terrenus* adult

Earlier studies have shown two bruchid species that develop in the seeds of woody plants in Hungary: (Fabricius, 1792) in *Laburnum anagyroides* (Szentesi 2006, Szentesi and Wink 1991) and our specimens were identified as *Megabruchidius tonkineus* (Jermy et al. 2002, Jermy and Szentesi 2002, György, Z. 2007). We confirmed that *Megabruchidius tonkineus* that was reared on *G. triacanthos* was able to utilize *Gimnocladus dioicus* as well.

Many leguminous plants are used as ornamentals, green manure, land cover, food crop and for erosion control. Human transportation of seeds of these economic legumes can expand the distribution not only of plants (e.g *Gimnocladus dioicus,G. triacanthos, and A. jullibrisin*) but also their internal seed predators such as*Megaruchidius* and *Bruchidius*species (Figure 1-5). A recent example is the North American *Acanthoscelides pallidipennis* infesting *Amorpha fruticosa* seeds introduced to East Asia and Europe (Szentesi 1999; Tuda et al. 2001, Gagic Serdar et al. 2013). Endophages tend to have narrower diet breadth than ectophages (Lewinsohn, 1991; Gaston et al., 1992; Frenzel and Brandl, 1998; Okamoto et al. 2008 of the host range of ectophagous insects) and therefore are probably more suitable as biological control agents.

Table 2. Host plant and seed beetles native range, localities and date

	Host plant	**Seed beetle**	Sampling localities and sampling date
	Gleditchia triacanthos	*Megaruchidius tonkineus*	1.Belgrade, 25 May Museum – January, 2013
			2.Belgrade, Banjica forest- January, 2013
Native range	Central North America	Vietnam	3.Ruma- January, 2014
	Gimnocladus dioicus	*Megaruchidius tonkineus*	1.Belgrade University, Faculty of forestry, Arboretum– January, 2013
Native range	Midwest of North America	Vietnam	
	Cercis siliquastrum	*Bruchidiussiliquastri*	1.Belgrade, Tasmajdan- April, 2013
			2.Novi Sad, Telep - April, 2014
Native range	*Southern Europe and Western Asia*	*Asia*	3.Ruma, Deteline - April, 2014
	Albizia julibrissin	*Bruchidius terrenus*	1.Ruma, Deteline – August, 2013;
Native range	Southwestern and Eastern Asia	Eastern Asia	2.Ruma, Kudoš - August, 2014
	Laburnum anagyroides	*Bruchidius villosus*	1. Belgrade, Bežanijska kosa- April, 2014
			2. Belgrade, Cukarica – May, 2012; August, 2013
Native range	Mountains of Southern Europe from France to the Balkan Peninsula	Native European range	3. Novi Sad, City Fair – April, 2014,

Bruchine beetles have been suggested (Southgate, 1979) and used as control agents of weeds (Julien, 1992; van Klinken 2005; review in Tuda 2007). Furthermore, the present study indicates the possibility that established species could play double role of a pre- and post-dispersal seed predator. On the other hand, there is a concern about the efficacy of recorded beetles as control agents: unexpected host expansion. Potential new host plants may not be indigenous but introduced ones as in the case of the bruchine control agent of *G. triacanthos*, a newly established invasive plant in Serbia. Future studies are needed to clarify the ecology of host utilization and parasitoid accumulation process of effective biocontrol of invading legumes that has become a pantropic species (Table 3).

Table 3. Parasitoid complex as potential biological threatening agents for seed pests

Insect	**Biology and host preference of pod pests**
***Eupelmus* spp.** and ***Anastatus* spp.**(Hymenoptera: Chalcidoidea: Eupelmidae)	ectoparasitoids of ***B. siliquatry***, and ***B. terrenus*** larvae
Pteromalidwasps (Hymenoptera: Chalcidoidea: Pteromalidae)	ectoparasitoids of ***B. siliquatry*** and ***B. villosus*** larvae
***Tetrastichus* spp.** (Hymenoptera: Chalcidoidea: Eulophidae)	encompass (here) ***B. siliquatry*** and ***B. villosus*** parasitoids of the first and second order, so it is needed to proceed the research in order to determine their status -hyperparasitism
Braconidwasps (Hymenoptera: Braconidae)	reared specimens as***B. terrenus*** and ***B. villosus*** larvae parasitoids. Investigation needs to be continue in a goal of getting more specimens, data, status confirmation and species determination

CONCLUSIONS

Bruchine chrysomelids are economically important pests of agricultural and stored products. Larval host plants of most Bruchid species are legumes (Fabaceae). *G. triacanthos, G. triacanthos*, *A. julibrissin* and *C.siliquastrum*were introduced in Serbia and planted as ornamental trees. We studied the seed beetles *B. terrenus* and *B. siliquastri* which infest the seeds of *A. julibrissin* and *C. siliquastrum* as well their parasitoids.Our findings include *Megabruchidius tonkineus* as endophagous - of *G. triacanthos* L., and also of introduced *Gimnocladus dioicus*. *Bruchidius villosus* feeding in seed of *Laburnum anagyroides*, and *Bruchidius terrenus*are first recorded and completely new to Serbian fauna.

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ŠTETOČINE SEMENA LEGUMINOZNIH STABLAŠICA I ŽBUNJA: NOVE INVAZIVNE VRSTE ŽIŽAKA (COLEOPTERA: CHRYSOMELIDAE: BRUCHINAE) ZA FAUNU SRBIJE

SAŽETAK

Tri vrste roda *Bruchidius*i jedna *Megabruchidius* evidentirane su kao nove za faunu Republike Srbije u istraživanju u periodu od 2012. do 2014. godine. Ove spermatofage razvijaju se u semenu pet vrsta drvenastih mahunarki, koje su često gajene ukrasne stablašice i žbunje. Dosadašnji nalazi ovih vrsta, potvrđeni su u Francuskoj a zatim i u susednim, Mađarskoj i Bugarskoj. Novi taksoni za Srbiju su kao takvi determinisani pomoću morfoloških karakteristika, ali su ispitavane i njihove idioekološke preference. Dobijeni rezultati ukazuju na pripadnost Paleotropskoj grupi, čiji članovi su rodovi *Bruchidius*, *Megabruchidius* i *Acanthoscelides*. Izvorno geografsko poreklo ovih semenojeda (žižaka), ali i njihovih biljaka-domaćina takođe su razmatrane kao predmet rada.

Ključne reči: Bruchinae, invazivnost, Leguminoze, semenojedi, Srbija