

Neotropical Entomology

Euschistus crenator (Fabricius) (Hemiptera: Pentatomidae): a new invasive species on soybean fields in Northern Brazil --Manuscript Draft--

Manuscript Number:	NENT-D-20-00338R1	
Full Title:	Euschistus crenator (Fabricius) (Hemiptera: Pentatomidae): a new invasive species on soybean fields in Northern Brazil	
Article Type:	Scientific Notes	
Keywords:	Brown stink bug; emergent pest; agriculture expansion; distribution; bionomy	
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Funding Information:	Fundação de Amparo à Pesquisa do Estado de São Paulo (2014/11495-3)	Dr Alberto Soares Corrêa
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	Conselho Nacional de Desenvolvimento Científico e Tecnológico (311465/2018-8)	Dr Alberto Soares Corrêa
	Conselho Nacional de Desenvolvimento Científico e Tecnológico (421413/2017-4)	Not applicable
	Fundação de Amparo à Pesquisa do Estado de São Paulo (2018/09469-5)	Not applicable
Abstract:	<p>The expansion of soybean, <i>Glycine max</i> (L.) Merrill in South America has provided an abundant host to the native arthropod fauna. Stink bugs (Heteroptera: Pentatomidae) are severe pests on soybean crops due to their feeding activities. Several native species have been recorded on this crop, with the widespread Neotropical brow stink bug <i>Euschistus heros</i> (Fabricius) recognized as the key pest in most of its distribution. Here, we report for the first time <i>Euschistus crenator</i> (Fabricius) as a new invasive species on soybean fields in Northern Brazil. We collected the species at Pará state (2°38'32.2"S 54°55'56.1"W and 4°06'31.2"S 54°55'01.9"W) and Roraima state (2°39'41.3"N 60°46'58.9"W and 3°00'44.6"N 60°22'32.9"W). The latter represents a new state record, and the first represents new localities records, however, both representing the first soybean host record. The distribution of <i>E. crenator</i> ranges from</p>	

	<p>southern US (AZ, CA, FL and TX) to north South America, mostly between the Tropics of Cancer and Capricorn. Despite overlap in distribution, we did not find <i>E. crenator</i> and <i>E. heros</i> simultaneously on soybean crops in the North region (Amazon Forest) of Brazil. <i>Euschistus crenator</i> was able to complete its life cycle and had viable offspring on soybean plants, with development parameters similar to <i>E. heros</i>.</p>
Response to Reviewers:	<p>Reviewer comments:</p> <p>Reviewer #1</p> <p>Thank you for your review. We appreciated the comments and suggestions. We added and deleted almost all the text issues that reviewer 1 suggested. In this way the text and the language were considerable improved.</p> <p>-Line 2: <i>Euschistus crenator</i> author of the species. We inserted the species author of <i>Euschistus crenator</i> (Fabricius) in the title as suggested.</p> <p>-Lines 29-32: text adjustment (word deletions and insertions). We accepted the changes suggested.</p> <p>-Lines 33, 34, 63 and 85: species author abbreviation. We kept the whole author's name because it is stated at the International Commission on Zoological Nomenclature that the only species author which is allowed to be abbreviated is Linnaean. Therefore, we kept the complete author name (Fabricius).</p> <p>-Lines 35: Coordinates insertion. We inserted the coordinates of the records.</p> <p>-Line 37: first time but here there was a record before in Pará??? Clarify. We inserted a sentence to clarify the new state record and a new record in Para state which represents the first record on soybeans for the species.</p> <p>-Lines 39-44: text adjustment (word deletions and insertions) We accepted the text changes suggested.</p> <p>-Lines 44: move last sentence deleted to the text. We accepted the change as suggested and moved the sentence to the line 136-138.</p> <p>-Lines 52-89: introduction section: text adjustments (word deletions and insertions) We accepted the changes suggested by the reviewer.</p> <p>-Lines 91-134: material and methods section: text adjustments (word deletions and insertions). We accepted the text changes suggested by the reviewer.</p> <p>-Lines 136-190: results and discussion section: text adjustments (word deletions and insertions). We accepted the text changes suggested by the reviewer.</p> <p>-Line 190: sentence suppression. We deleted the sentence as suggested by the reviewer.</p> <p>Table 1: Authors should consider only one decimal in the mean values of Table 1, to make it look cleaner and better to see the values. In most cases two decimals are unnecessary. We deleted one decimal of the mean values of Table 1. We agree that it improved the understanding of the table content.</p> <p>Fig 3: I suggest deleting the last figure, because although interesting, the similarities among species can be discussed in the text, and since this is not the main message of this note the inclusion of such figure is not justified. We accepted the suggestion and suppressed the Fig 3.</p> <p>Reviewer #2</p> <p>We thank to Dr. Grazia for reviewing our manuscript. We appreciated the comments and suggestions. We added and deleted text issues that she suggested. In this way the text was improved and minor error in the references were fixed.</p> <p>-Line 72: species author spelling error. We corrected the author's name.</p> <p>Line 72: word spelling error. We fixed the error.</p> <p>Line 184: word spelling error. We corrected the word spelling error.</p> <p>Line 228 and 229: spelling error of the reference. We corrected the author name and the title word which were incorrect spelled.</p> <p>Line 256: Article title in Portuguese. We corrected the article title.</p>

Submitted to Neotropical Entomology – Scientific Note

***Euschistus crenator* (Fabricius) (Hemiptera: Pentatomidae): a new invasive species on
soybean fields in Northern Brazil**

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A running title no longer than 65 characters.

Euschistus crenator on soybean

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Abstract

The expansion of soybean, *Glycine max* (L.) Merrill in South America has provided an abundant host to the native arthropod fauna. Stink bugs (Heteroptera: Pentatomidae) are severe pests on soybean crops due to their feeding activities. Several native species have been recorded on this crop, with the widespread Neotropical brown stink bug *Euschistus heros* (Fabricius) recognized as the key pest in most of its distribution. Here, we report for the first time *Euschistus crenator* (Fabricius) as a new invasive species on soybean fields in Northern Brazil. We collected the species at Pará state (2°38'32.2"S 54°55'56.1"W and 4°06'31.2"S 54°55'01.9"W) and Roraima state (2°39'41.3"N 60°46'58.9"W and 3°00'44.6"N 60°22'32.9"W). The latter represents a new state record, and the first represents new localities records, however, both representing the first soybean host record. The distribution of *E. crenator* ranges from southern US (AZ, CA, FL and TX) to north South America, mostly between the Tropics of Cancer and Capricorn. Despite overlap in distribution, we did not find *E. crenator* and *E. heros* simultaneously on soybean crops in the North region (Amazon Forest) of Brazil. *Euschistus crenator* was able to complete its life cycle and had viable offspring on soybean plants, with development parameters similar to *E. heros*.

Key words: Brown stink bug, emergent pest, agriculture expansion, distribution, bionomy.

Introduction

The continuous expansion of the agricultural frontier in Brazil builds a mosaic of agricultural and native areas throughout its territory. This landscape structure allows a wide diversity of arthropods to colonize and disperse among agricultural crops, making difficult to predict species and populations dynamics on agroecosystems (Corrêa *et al* 2019, Cordeiro *et al* 2020). Soybean, *Glycine max* (L.) Merrill, is the major cultivated crop in Brazil, occupying \approx 35 million hectares each year (CONAB 2019). In recent years, the expansion of soybean fields took place from the southern to the central and northern regions of Brazil, promoting its expansion over the Cerrado and Amazon biomes (Fearnside 2001, Andrade 2005, Garrett *et al* 2013, Gusso *et al* 2017, Cattelan & Dall'Agnol 2018).

Of the several native pest species in South America, stink bugs such as *Diceraeus melacanthus* Dallas, *Edessa meditabunda* (Fabricius), *Euschistus heros* (Fabricius) and *Piezodorus guildinii* (Westwood) are among the most common native pests on soybean (Smaniotto & Panizzi 2015). The genus *Euschistus* comprises 67 species restricted to the Americas, wherein *Euschistus servus* (Say) and *E. heros* are key pests on soybean widely distributed in the North and South America, respectively (Esquivel *et al* 2009, Panizzi 2015, Grazia & Schwertner 2017). Species of the genus *Euschistus* are similar in their external morphology and misidentifications in the field are common (Esquivel *et al* 2009, Hickmann *et al* 2019). In South America, *Euschistus* spp. are usually widely distributed, and reported feeding on different cultivated plants. Additional to *E. heros*, the species *Euschistus cornutus* Dallas, *Euschistus picticornis* (Stål), and *Euschistus taurulus* Berg, have been recorded on soybean since the establishment of this crop in South America (Quintanilla *et al* 1981, Panizzi & Slansky 1985, Link & Grazia 1987, Panizzi *et al* 2000, 2017, Hickmann *et al* 2019). The latter species is widely distributed in South America, while the two first species

are restricted to the southernmost part of Brazil and neighborhood countries (Argentina, Paraguay and Uruguay).

The reports of population outbreaks of *E. heros* on soybean are common in different regions of Brazil (Panizzi 2015, Sosa-Gómez *et al* 2020). Nonetheless, studies on the genus *Euschistus* in the northern region of Brazil still are scarce (Rolston 1974, 1984, Silva *et al* 2018). Recently, the presence of two allopatric strains of *E. heros*, one in the northern and another in southern regions of Brazil, was described (Soares *et al* 2018). To better understand the demographic and adaptive dynamics of this important soybean pest, collecting expeditions in northern Brazil were carried out in the last two years.

Here, we report for the first time the occurrence of *Euschistus crenator* (Fabricius) on soybean in two North States of Brazil, Roraima and Pará. To test the viability of this crop to support survivorship, development and reproduction of this species, laboratory bioassays were conducted. We also discuss the identification of *E. crenator* and compared this species with similar ones found on soybean in Northern Brazil.

Material and Methods

Insect collection

Specimens used in this study were collected on soybean in the state of Roraima [municipalities of Boa Vista (2°39'41.3"N 60°46'58.9"W) and Bonfim (3°00'44.6"N 60°22'32.9"W)] during the crop season 2018/2019 and at Pará State [municipalities of Belterra (2°38'32.2"S 54°55'56.1"W) and Rurópolis (4°06'31.2"S 54°55'01.9"W)] during the crop seasons 2018/2019 and 2019/2020 (Fig 1). Insects were sampled using a sweep net on soybean plants. The collected specimens were labeled and stored in mesh bags with soybean leaves and pods. Individuals from Rurópolis were taken to the laboratory at ESALQ-USP, Piracicaba-SP, kept under controlled conditions [temperature: 25 ± 1 °C, relative humidity:

60 ± 10% and photoperiod: 14: 10 (light: dark)] and fed with a natural diet [green bean pod, *Phaseolus vulgaris* L., dry peanut grains, *Arachis hypogaea* L., dry soybean grains, and dry sunflower grains, *Helianthus annuus* L.]; water was offered *ad libitum*. Habitus illustrations (Fig 2) were photographed under a Leica M205C stereomicroscope equipped with a Leica DFC 450 camera.

Insect identification

Identification of specimens was made using a stereoscopic microscopy, based on the dichotomous keys from Rolston (1974, 1984), and compared with identified specimens of *Euschistus* deposited in Museum of Zoology, University of São Paulo (MZUSP). Voucher specimens of *E. crenator* were deposited in the Entomological Collection of the Museu de Entomologia (MELQ), Department of Entomology and Acarology, Luiz de Queiroz College of Agriculture, University of São Paulo (ESALQ-USP), Piracicaba (specimen's numbers ESALQENT00047- 52).

Nymph and adult Euschistus crenator biology on soybean

Nymphs. Eggs from the first-generation of laboratory population were kept in a Petri dish with soybean pods and moistened cotton (three Petri dishes with \cong 200 eggs). Soybean pods were changed every two days. Second instar nymphs obtained were transferred to a transparent plastic pot (7.5 cm high and 11 cm in diameter) with a screened cover lid, containing three soybean branches in the grain filling stage (R₃-R₄) (\cong 90 nymphs on each); soybean branches were replaced every two days. From the 3rd instar on, nymphs were transferred to cylindric PVC cages (29.5 cm high and 24.5 cm in diameter) fixed on Styrofoam plate and covered with Voil fabric containing soybean branches arranged in 100 mL flasks with distilled water. Branches and flasks were replaced every two days.

Development time (days), mortality and body mass (mg) for each nymph stadium were recorded.

Adults. Three replicates with 8 couples each of adults *E. crenator* were evaluated during 40 days. Couples were randomly chosen from adults emerged from the nymph study, and kept in cages (29 x 19 x 9 cm) containing soybean branches and leaves, changed every two days. Pre-oviposition period, number of eggs produced, egg and first instar developmental time and viability were assessed through daily evaluations. The two studies were conducted under controlled environment conditions (temperature of 25 ± 1 °C, relative humidity $60 \pm 10\%$ and light regime 14: 10).

Results and Discussion

In our field trips, five commercial farms were visited in Pará (crop seasons 2018/2019 and 2019/2020) and two in Roraima (crop season 2018/2019). All individuals of *Euschistus* collected were of the species *E. crenator*. The misidentification with *E. heros*, commonly known to occur in soybeans is understandable because *Euschistus* species are similar in their external morphology (Rolston 1974, Esquivel *et al* 2009), making difficult an accurate identification in the field. Therefore, the invasive nature of *E. crenator* must be considered in a scenario of constant agriculture area expansion and the emergence of pests on non-native crops, with great impact on the development and implement IPM tactics.

The distribution of *E. crenator* is almost restricted between the Tropics of Cancer and Capricorn, and ranges from the southern US (AZ, CA, FL and TX) to the northern region of South America (from Peru to the Bahia state in Brazil), including the Caribbean (Fig 1). The new locality records here presented are within the distribution range of the *E. crenator*, which overlap at least in part with the distribution of other two species recorded in soybeans fields in Brazil, *E. heros* and *E. taurulus* (Panizzi 2015, Hickman *et al* 2019). However, despite the

reports of the occurrence of *E. heros* in the localities sampled, we were not able to find it, and only *E. crenator* was obtained. This suggests the predominance of *E. crenator* in some soybean fields in the North region of Brazil. The distribution and niche established by a species is determined by abiotic (e.g., temperature, precipitation) and biotic (e.g., competition, predation, parasitism, host occurrence) factors, which can act alone or concomitantly (Wiens 2011). In addition, climate change and anthropogenic actions can alter niche dynamics and invasion of native species in agricultural areas (Aguin-Pombo 2012, Corrêa *et al* 2019).

Euschistus crenator completed life cycle and had viable offspring on soybean (Table 1). The mortality of nymphs from 2nd instar to adult feed on soybean (36%) was similar to what has been reported for *E. heros*, which ranged from 13.3 to 47.5% in previous studies (Villas-Bôas & Panizzi 1980, Pinto & Panizzi 1994, Medeiros & Megier 2009, Azambuja *et al* 2013). On the other hand, *E. crenator* performed better on soybean when compared with *E. cornutus*, which showed a mortality of 75% from 2nd instar to adult (Panizzi *et al* 2017). The average egg-adult development time (31.4 days) and the 2nd instar to adult (21.0 days) were similar to that found for *E. heros* [which ranged from 30.0 to 36.6 days for egg-adult and 20.8 to 23.9 days for 2nd-adult] (Azambuja *et al* 2013).

Among stink bugs found on soybean in Northern Brazil, *E. crenator* (Fig 2) may be confounded with other species with general brown coloration and spinose humeri, such as *D. melacanthus*, *E. heros* and *E. taurulus*. While the separation between *Diceraeus* spp. and *Euschistus* spp. is straightforward, based on the head morphology and ventral body coloration (Table 2), the identification of *Euschistus* spp. are subtler. For instance, Rolston (1974) described intraspecific variation for most of the characters used for species diagnoses in *Euschistus*, highlighting the sclerotized genital capsule in males (the pygophore) as the most important part to separate the species. However, characters of the general morphology are

170 still useful for the identification of the species recorded in a particular region or crop
171 (Esquivel *et al* 2009). Thus, among *Euschistus* species found on soybean in Northern Brazil,
172 *E. crenator* (Fig 2) may be recognized by general body size, development of humeri, and
173 dorsal and ventral coloration pattern (Table 2). Remarkably, the definitive character to
174 separate the species are found in the ventral rim of the pygophore that is concave mesially,
175 this concavity shallow to moderately deep in ventral view (Table 2), which is discernible
176 under a stereomicroscope in the male and can be examined with little or no preparation of the
177 specimen (Rolston 1974).

178 We recorded for the first time *E. crenator* as an invasive species on soybean in
179 northern Brazil, and the biology performance on soybean plants under laboratory conditions
180 suggests a potential pest status of this stink bug. Areas in northern Brazil have experienced
181 soybean expansion in the last two decades, exposing this crop to native local fauna. Although
182 *E. crenator* and *E. heros* have not been found simultaneously on soybean in Northern Brazil,
183 further studies should confirm their distribution and whether they are expanding their range
184 or not.

186 **Authors' contributions**

187 FH planned and designed the study, conducted experiments and wrote the manuscript. MS
188 collected the samples, collected the field data and reviewed the manuscript. ASC conceived,
189 planed, wrote and reviewed the manuscript. CFS planed the study, identified the specimens,
190 wrote and reviewed the manuscript. All authors read and approved the manuscript.

192 **Acknowledgments**

193 We thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grant
194 #2014/11495-3 to ASC, fellowship #2018/20351-6 to FH); the Coordenação de

Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001;
 Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, fellowship
 #311465/2018-8 to ASC and #421413/2017-4 CFS); the INCT-Semioquímicos na
 Agricultura (FAPESP 2014/50871-0 and CNPq 465511/2014-7) for providing equipment to
 capture some photos; the Postgraduate Program in Entomology of the Luiz de Queiroz
 College of Agriculture – University of São Paulo for support to this study; the sampling was
 supported by the National System of Biodiversity Information (SISBIO), authorization
 numbers 61549-3 and 71031-1. We also thank the reviewers and the academic editor for
 improving the manuscript.

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Submitted to Neotropical Entomology – Scientific Note

***Euschistus crenator* (Fabricius) (Hemiptera: Pentatomidae): a new invasive species on
soybean fields in Northern Brazil**

Fig 1 Latin America and Caribbean map, showing the distribution of *Euschistus crenator* by country. The species is also reported from other Caribbean countries as Antigua, Barbados, Grenada, Trinidad and Virgin Islands (St. Thomas). Red diamonds are new records where the species was collected on soybean. Distribution data based on Rolston (1974), Becker & Grazia-Vieira (1977), Henry & Froeschner (1988), Maes (1994), Thomas (2000), Arismendi & Thomas (2003), Perez-Gelabert & Thomas (2005), Santos & Bastardo (2013), Segarra-Carmona *et al* (2015), Raola (2016), Firmino *et al* (2017), Cambra *et al* (2018), Silva *et al* (2018).

Fig 2 Habitus of *Euschistus crenator* (Fabricius). A. Male, dorsal view. B. Female, dorsal view. C. Male, ventral view. D. Female, ventral view.

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Table 1. Biological parameters development time (days \pm SE), mortality (%), weight (mg \pm SE), sex ratio (\pm SE), adult female and male weight (mg \pm SE), pre-oviposition (days) and average number of eggs per female (\pm SE) of *Euschistus crenator* fed on soybean.

Phase	N	Duration (days) \pm SE*	Mortality (%)	Weight (mg) \pm SE
Egg	2579**	7.1 \pm 0.2	30.2	0.4 \pm 0.1
1 st	1800**	3.3 \pm 0.1	22.9	1.0 \pm 0.1
2 nd	266	5.0 \pm 0.0	15.4	1.7 \pm 0.1
3 rd	225	4.7 \pm 0.3	7.1	4.9 \pm 0.2
4 th	209	5.0 \pm 0.0	6.2	12.1 \pm 0.5
5 th	196	6.3 \pm 0.3	7.7	33.2 \pm 1.6
2 nd -adult	266 -196	21.0	36.4	-
Egg-adult	-	31.4	89.5	-
Adult	Sex ratio \pm SE: 0.5 \pm 0.0		♀ weight (mg) \pm SE 70.8 \pm 14.7	
			♂ weight (mg) \pm SE 64.2 \pm 4.9	
	Pre-oviposition (days) \pm SE: 8.7 \pm 0.3		Nº of eggs / female \pm SE: 107.5 \pm 15.5	

* Standard error. **Eggs and 1st instar nymphs were from couples which feed the entire life cycle on soybean.

Table 2. Comparative morphology of stink bugs found on soybeans in Northern Brazil.

Species	Apex of head	Denticles at the lateral pronotal margins	Humeri	Punctures on evaporatorium	Ventral abdomen colouration (in alive individuals)	Pygophore ventral rim (in ventral view)
<i>Diceraeus melacanthus</i>	Acuminate	Closely spaced, adjacent denticles usually continuous	Strongly produced laterad, usually spinose, occasionally acutely angular; usually black	Concolour punctures	Light green, diffuse vittae absent; sutures between sternites concolour*	Sinuuous, usually with a small concavity mesially
<i>Euschistus crenator</i>	Rounded	Closely spaced, adjacent denticles usually continuous	Moderately produced, obtusely rounded to spinose	Concolour punctures	Yellowish, speckled with two diffuse, darker vittae; sutures between sternites concolour	Concave mesially, shallow to moderately deep
<i>Euschistus heros</i>	Rounded	Closely spaced, adjacent denticles usually continuous	Strongly produced laterad, usually spinose, occasionally acutely angular; usually black	Contrastingly black punctures	Light to dark brown, with three diffuse, darker vittae; sutures between sternites dark brown	Sinuuously emarginate in ventral view
<i>Euschistus taurulus</i>	Rounded	Widely spaced	Strongly produced laterad, usually spinose, occasionally acutely angular	Concolour punctures	Yellowish in males, greenish in females, speckled with diffuse, darker spots; sutures between sternites concolour	Concave, bearing an acute projection mesially

* in winter morphs, ventral abdomen may be brownish, with several small speckled darker spots.



