Neotrop Entomol (2013) 42:288–292
DOI 10.1007/s13744-013-0122-8

SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Description of the Immatures of *Scaptocoris carvalhoi* Becker (Hemiptera: Cydnidae)

LM Vivan[1], C Nardi[2], J Grazia[3], JMS Bento[4]

[1]Fundação MT, Rondonópolis, MT, Brasil
[2]Univ Estadual do Centro-Oeste, Guarapuava, PR, Brasil
[3]Univ Federal do Rio Grande do Sul, Porto Alegre, RS, Brasil
[4]Univ de São Paulo, ESALQ/USP, Piracicaba, SP, Brasil

Keywords

Burrower bugs, descriptive morphology, nymphs, morphology, soil pests

Correspondence
C Nardi, Univ Estadual do Centro-Oeste, CEP 85040-080, Guarapuava, PR, Brasil; cnardi@unicentro.br

Edited by Roberto A Zucchi – ESALQ/USP

Received 27 February 2012 and accepted 7 February 2013
Published online 21 March 2013

Abstract

Nymphs and adults of the burrower bug *Scaptocoris carvalhoi* Becker feed on vegetal sap of their host plants through the roots, and little is known on the morphology and biology of its immature stage. Therefore, we aimed to characterize the immatures of *S. carvalhoi* by describing the egg and the morphology of each instar. Eggs of *S. carvalhoi* have a smooth chorion surface without visible micropylar processes. The presence of five instars was confirmed by the coefficient of determination (R^2>0.95) and by the growth constant (*K* between 1.2 and 1.6). Nymphs have an elliptical body and fossorial scythe-like forelegs. The tarsi are absent as in adults, and the prototarsal insertion region becomes visible only in the fourth instar. Nymphs from first to fourth instar of *S. carvalhoi* showed the presence of 1+1 trichobothria in urosternites III to VII, close to the anterior margin and inside the spiracles; besides these trichobothria, fifth instars presented 1+1 pre-trichobothria in urosternites III to V located posteriorly, almost in the row of spiracles close to the posterior margin of the urosternites. This is the first detailed morphological record of immatures belonging to *Scaptocoris*.

Introduction

The main species of *Scaptocoris* Froeschner occurs from the Nearctic region to the south of the Neotropical region. In these regions, this genus is represented by seven species, three of which occur in cultivated plants, *Scaptocoris buckupi* Becker, *Scaptocoris carvalhoi* Becker, and *Scaptocoris castanea* Perty (Froeschner 1960, Becker 1996, Grazia *et al* 2004).

Scaptocoris carvalhoi is one of the most important soil pest species in Brazil and is associated with different plants, mainly grasses. As with other Scaptocorini species, it is characterized by its underground mode of life and by the expanded tibiae adapted for digging (Froeschner 1960, Becker 1967). After nomenclatural and taxonomic arrangements made by Grazia *et al* (2004), *S. carvalhoi* was defined as senior synonym to *Atarsocoris brachiariae* Becker, whose adults have reduced tarsi or tarsal insertion in fore and mid-tibiae, and notably elongated distal tubercles in corbiculum (Becker 1967, Grazia *et al* 2004). Recently, Nardi *et al* (2008) reported that this species presents wing dimorphism, with migrant macropterous and non-migrant brachypterous forms.

Studies on burrower bugs are rare and the lack of information, mainly on their biology and behavior, limits the research and development of management techniques for this insect. Up to now, the main studies on *S. carvalhoi* were related to the adult stage, but knowledge on immatures is also important for the characterization of their biology and establishment of laboratory rearing. The morphological study of immatures may also be useful to differentiate *Scaptocoris* species, widening the possibilities for their recognition. Thus, we describe in here the morphological characteristics of immatures of *S. carvalhoi* in order to provide information to support the development of future basic and applied research.

Material and Methods

Insects

Specimens of *S. carvalhoi* were collected from excavated soil in a soybean field (*Glycine max*) from October 2009 to February 2010 and in a *Brachiaria* field (*Brachiaria* sp.) from February to September 2010, in Primavera do Leste, MT, Brazil (14°48′59.5″S, 54°11′04.8″W). The specimens collected were identified according to the diagnostic traits of *S. carvalhoi*, such as the absence of tarsi, characteristics of the head, and shape of the clypeus (Becker 1967, Grazia *et al* 2004).

The eggs used for morphometry were obtained from females of *S. carvalhoi* collected in the field and maintained in laboratory conditions. Parts of these eggs (*N*=10) were collected after oviposition in the soil and parts (*N*=260) were collected directly from the reproductive tract of adult females following dessication. Females were dissected by a lateral incision in their abdomen and all eggs in the common oviduct were collected. This procedure was adopted due to the great difficulty in finding eggs in the field and the low rate of oviposition under laboratory conditions. In order to guarantee a reliable description, eggs obtained from the reproductive tract were previously compared to those from the field and no apparent differences were identified.

The morphological analysis of nymphs was done using 70% ethanol-preserved specimens of different sizes randomly collected in the field (*N*=478). To ensure the presence of first instars in the sample, eggs laid by females in laboratory were maintained under controlled conditions (23±2°C; 60±10% RH; 14 L:10 D) until eclosion (*N*=10). First instars were analyzed immediately after hatching, while the remaining instars obtained in the field were later processed.

Morphology and morphometry

Nymphs of *S. carvalhoi* (*N*=478) were separated by morphotype according to the characteristics described for Cydnidae (García & Bellotti 1980, Sites & McPherson 1982, Stehr 1991) and by other Pentatomoidea (Grazia & Frey-da-Silva 2001, Schwertner *et al* 2002) based on the degree of development of the pteroteca.

Eggs and nymphs were measured with a wide angle micrometric eyepiece (WF 10×/23 mm) with linear and cross graduation (0.1/14 mm) for stereomicroscope (Motic, SMZ 168). Egg length and egg width were recorded, and the presence of micropylar processes was registered. For the nymphs, we assessed the body length, head length, pronotum length and width, scutellum length, abdomen length and width, and head width. All measurements of length were done along the median longitudinal line, while the width measurements were taken along the widest transversal line over the body. Pigmentation and shape features of immatures were analyzed under a stereomicroscope and described according to the terminology of Becker (1967).

Illustrations were made from specimens preserved in alcohol 70% using a camera lucida mirror type connected to a stereo microscope. Voucher specimens were deposited at the "Coleção Entomológica do Departamento de Zoologia da Universidade Federal do Rio Grande do Sul."

Statistical analysis

Morphometric data of the nymphs of *S. carvalhoi* were analyzed by the determination index (R^2) and growth constant (K) (Dyar 1890) using of Microsoft Office Excel 2007®. These parameters were used to confirm the hypothesis of the existence of five instars according to Parra & Haddad (1989).

Results

The initial morphological evaluation of nymphs of *S. carvalhoi* based on the development of the pteroteca enabled the identification of five morphological groups which supported the hypothesis of the existence of five instars. For all parameters assessed, the morphometric analysis resulted in a determination index (R^2) greater than 0.95. Furthermore, the growth constant (K) remained between 1.2 and 1.6 (Table 1).

From the separation of nymphs in five instars, it became possible to describe *S. carvalhoi* immatures according to their shape and color:

Egg. Elliptical; height, 1.6±0.21 mm; width, 0.9±0.12 mm; color uniform, whitish-yellow; chorion surface smooth, aero-micropylar processes absent

First instar (Fig 1a and Table 1). Body elliptical, color uniform whitish-yellow. Head elliptical, flat at apex, lateral margins slightly darkened; dorsal surface with low transversal grooves reaching distal third; ocelli absent; tylus longer than juga, wide at apex; rostrum four-segmented covered by sparse setae, longer on dorsal surface of segment II; antennae four-segmented, segments I to III almost cylindrical, segment IV fusiform longer than segments II and III combined, segment III the shortest. Proportion of antennal segments: I≈II>III<IV. Thorax mostly whitish-yellow, pronotum longer and darker than meso- and metanotum; tibia of

Table 1 Morphometrical data (X±SD) (in millimeter) and statistical parameters (K = growth constant, p = significance value, R^2 = determination index) of immatures of *Scaptocoris carvalhoi* (n=480).

Body part	Measurements (mean±SD) (mm), instar					K	p	R^2
	First	Second	Third	Fourth	Fifth			
AL	1.07±0.08	1.26±0.20	1.79±0.41	2.32±0.40	2.47±0.23	1.23	0.003	0.99
BL	2.28±0.10	2.52±0.24	3.05±0.32	4.28±0.35	5.22±0.23	1.23	0.004	0.99
HL	0.46±0.05	0.46±0.13	0.66±0.08	0.80±0.15	1.02±0.13	1.23	0.007	0.98
SL	0.21±0.02	0.31±0.04	0.53±0.10	0.82±0.15	1.26±0.12	1.57	0.02	0.96
PL	0.42±0.03	0.44±0.08	0.63±0.10	1.00±0.11	1.11±0.15	1.29	0.005	0.99
AW	1.29±0.10	1.64±0.18	2.00±0.25	2.75±0.35	3.29±0.31	1.26	0.003	0.99
HW	0.46±0.02	0.47±0.07	0.58±0.07	0.75±0.07	1.00±0.07	1.22	0.005	0.99
PW	0.99±0.07	1.24±0.18	1.58±0.18	2.08±0.23	2.66±0.18	1.28	0.005	0.99

AL abdomen length, *BL* body length, *HL* head length, *SL* scutellum length, *PL* pronotum length, *AW* abdomen width, *HW* head width, *PW* pronotum width.

anterior and median legs not showing the area of tarsal insertion; legs slightly darker than body, with long and sparse light brown setae, denser at tibiae; anterior legs fossorial, tibiae scythe-like, whitish-yellow, apex darkened, covered with sparse setae, denser at ventral surface; median legs with flat tibiae, fine setae covering ventral surface of femur, longer and tougher setae on dorsal and ventral surfaces of tibiae; posterior legs short and thickened, femur rough and strongly convex laterally, tibiae cylindrical, expanding toward apex, corbiculum in "U" distal surface truncate, marginal tubercles flat, distal tubercles elongate placed in a single and irregular row nearer lateral margin of, from base to median region of corbiculum. Abdomen entirely whitish-yellow, lateral margins with sparse hairs, denser at apex; the three pairs of dorsal abdominal scent gland openings are placed between abdominal terga III–IV, IV–V, and V–VI, first pair almost hyaline, second and third pairs slightly darkened; spiracles on urosternites II to VIII; trichobothria (1+1) from urosternites III to VII, placed medially to spiracles near anterior margins of each segment.

Second instar (Fig 1b and Table 1). Body elliptical, color whitish-yellow with light brown areas. Head elliptical, lateral margins more sclerotized. Thorax whitish-yellow with symmetrical light brown spots; mesonotum posterior margin convex at middle denoting the scutellum formation; legs from whitish-yellow to light brown. Abdomen whitish-yellow with darker spots along median line and lateral margins. Spiracles, trichobothria, and other characteristics as in first instar.

Third instar (Fig 1c and Table 1). Body ovate, color whitish-yellow with light brown areas. Head sloping ventrally, with seven transversal grooves, margins darkened from fourth groove. Thorax darker than the remaining body; pronotum with light brown irregular spots; mesonotum and metanotum posterior margins with lateral slight projections denoting the wing pads formation; metathorax shorter than pro- and mesothorax. Abdomen globose, mostly whitish-yellow. Other characteristics as described for previous instars.

Fourth instar (Fig 1d and Table 1). Body ovate, strongly convex, color whitish-yellow with light brown areas. Head light brown with transversal curved grooves from apex to basal third, near eyes; tylus longer than juga, with apex almost twice as wide as an eye diameter; ocelli present. Thorax light brown; pronotum subdivided in two lobes, the anterior with darker, small spots irregularly distributed; lateral projections on posterior margins of meso- and metanotum well defined denoting the wing pad formation. Abdomen with median dorsal plates juxtaposed the posterior margins of tergites III–V; urotergites VII–IX with longer and denser setae; posterior margins of urosternites with a row of setae. Other characteristics as described for previous instars.

Fifth instar (Fig 1e and Table 1). Head with transversal curved grooves surpassing the line of the eyes. Thorax and legs with varying color from whitish-yellow to light brown. Scutellum well developed on mesonotum. Wing pads well developed on meso- and metanotum, reaching or surpassing posterior margin of urotergites II or III. Abdomen urosternites III to V with 1+1 pre-trichobothria, additional to trichobothria, placed near posterior margins of the segments, posteriorly and almost along the spiracles row. Abdomen with sparse setae on median ventral surface, denser at apex (segments VII–IX), surrounding anal tube. Other characteristics as described for previous instars.

Discussion

The importance of identifying immature insects and the increased need for morphological and biological studies of heteropterans have been recently emphasized by different authors (Martins & Campos 2006, Matesco *et al* 2009). These studies tend to not only increase knowledge on the target species, but also bring solutions to many of the problems related to taxonomy or applied entomology (Decoursey & Esselbaugh 1962, Richter 1972, Brailovsky *et al* 1992).

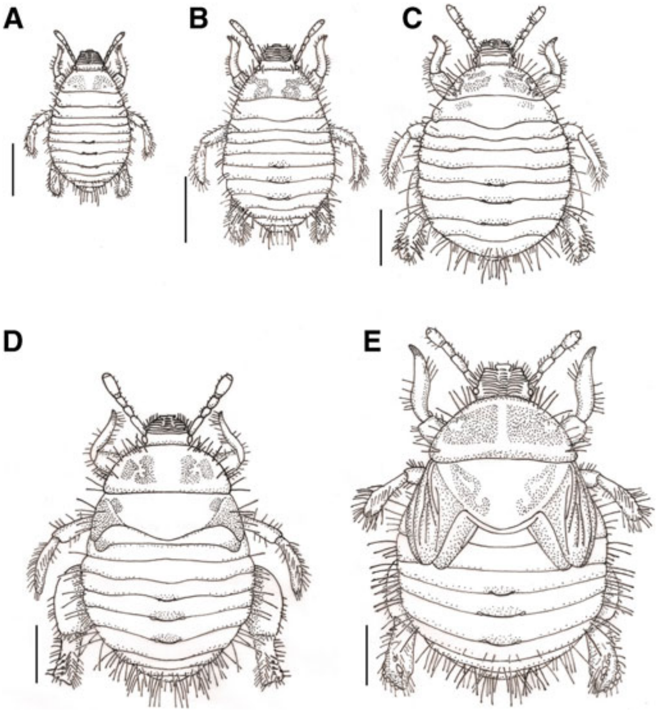

Fig 1 Immature stages of *Scaptocoris carvalhoi*. (A) First instar; (B) Second instar; (C) Third instar; (D) Fourth instar; (E) Fifth instar (illustrated by Patricia Milano) Scale bar=1mm.

The lack of research on the forms of young Cydnidae is evident since few species had their immatures described in detail. Immature Amnestinae were studied by Froeschner (1960) and Mayorga & Cervantes (2001), who emphasized that many of these insects do not present fossorial legs as it commonly occurs in other species in this family. For Sehirinae, the first descriptions were done for immatures of *Tritomegas bicolor* (Linnaeus) (as *Sehirus bicolor*) (Southwood 1949, Southwood & Hine 1950, Stokes 1950, Paul 1953). Later, McDonald (1968) and Sites & McPherson (1982) described the immatures of *Sehirus cinctus albonotatus* Dallas and *Sehirus cinctus cinctus* (Palisot de Beauvois), respectively, both North American subspecies. Cobben (1968) briefly characterized the egg of *Adomerus biguttatus* (Linnaeus) (as *Sehirus biguttatus*), mentioning that as in other members of the subfamily, this species protects its egg clusters which are deposited onto the soil. In turn, immature Cydnidae were described by Garcia & Bellotti (1980), who stressed the presence of tarsi in the three pairs of legs and tibia with spines in *Cyrtomenus bergi* Froeschner. For Cephalocteinae, only two descriptions of immatures were done: one by Ayyar (1930), who briefly characterized the eggs and the first instar of *Schiodtella tabulata* (Schiödte) (as *Stibaropus tabulate*), and another by Lis (1991), who characterized the fifth instar of *Schiodtella secunda* Lis.

The tarsi are absent in *S. carvalhoi* and the prototarsal insertion can be visualized from the fourth instar and on. According to the taxonomy proposed by Grazia *et al* (2004), the presence of prototarsal insertion in adults is one of the characteristics that differentiate *Scaptocoris* from *Atarsocoris*. Considering that the tarsal insertion is evident also in immatures of *S. carvalhoi*, this characteristic can be of great importance for differentiating insects in this life stage. Since other *Scaptocoris* species have well developed tarsi in the adult stage, we suggest that additional studies should be done in order to verify the presence of tarsi or tarsal insertions in immatures to enable species recognition at the immature stage.

The trichobothria observed in segments III to VII near the anterior margin interiorly to the spiracles of *S. carvalhoi* may play a sensorial role as described in other body regions (head, legs, and abdomen) of Pentatomoidea (Schaefer 1975, Cobben 1968, Schuh & Slater 1995). In Cephalocteinae, the distribution of trichobothria in adults is one of the main characteristics used to define a few genera and is considered an important trait for phylogeny and taxonomy (Lis & Hohol-Kilinkiewicz 2001). According to Lis & Kilinkiewicz (2001), there are three different types of trichobothria distribution in Cephalocteinae adults on abdominal segments III to VII. According to these authors, fifth instars of *Scaptocoris australis* L. present these structures with the same distribution as the adults. We observed that the trichobothria of *S. carvalhoi* are already evident in first instars. From the first to the fourth instars, 1+1 trichobothria were observed anteriorly in urosternites III to VII, and 1+1 pre-trichobothria were observed in urosternites III to V posteriorly to the spiracles in fifth instars. Considering that *S. carvalhoi* is an underground insect with intense physical contact with soil, it is possible that trichobothria have an important role in sensorial perception and in the way these insects move. According to Keil (1997), these structures may be involved in the perception of air flow or sound as demonstrated in other soil organisms (Haupt 1970, 1978, Bareth & Juberthie-Jupeau 1986), including heteropterans (Draslar 1973).

Biological studies on burrower bugs are very limited especially due to the difficulties in rearing and breeding these insects in laboratory conditions. Sales Junior & Medeiros (2001) described the bioecology of the burrower bug *S. carvalhoi* and estimated the duration of the egg, nymph, and adult stages, but their biological data are only an estimate and were obtained from field observation. Consequently, the present morphological study contributes to the knowledge of the biology and laboratory rearing of *S. carvalhoi* especially in instar recognition is necessary. Furthermore, our data may help in the recognition of organisms collected from soil as well as support future comparative studies with other species of this genus.

Acknowledgments Special thanks are due to CNPq/FAPESP and "INCT—Semioquímicos na Agricultura" for financial support and for the PhD fellowship granted to LMV (CNPQ no. 152291/2007-5).

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