

Morphology of Immature Stages, Chromatic Polymorphism of Adults and Natural History of *Oplomus catena* (Heteroptera: Pentatomidae: Asopinae)

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Abstract

The predatory stink bugs are well known by their behavior, but the knowledge of the immature morphology and their natural history are scarce. Studies on predatory stink bugs are important to better understand their evolution and their use as biological controllers. Here, we describe the morphology of egg and the five nymphal instars of *Oplomus catena* (Drury, 1782), using optical and scanning electron microscopy. In general, *O. catena* immatures are very distinctive from other Asopinae species already studied. The egg is black, with short aero-micropylar processes and similar to those described for *Stiretrus* species. The nymphs can be diagnosed by the abdominal plates very large and bright blue. The color polymorphism of adults is fully illustrated, and four color patterns are proposed. The natural history of the species is described based on field and laboratory observations. The known prey of the species is reviewed and new preys are reported. The morphological and biological traits here described are discussed in order to better understand the biological role of predatory stink bugs.

Introduction

Predatory stink bugs (Asopinae) are well known as important agents in biological control programs, with several species used in all continents of the world (De Clercq 2000, Richman & Mead 2001, Zanuncio *et al* 2014, Grazia *et al* 2015). The use of alternative methods to control pests in crops is increasingly important to reduce the use of pesticides and keep a balance between agricultural and ecological systems. However, the biology and morphology of immature stages of these predators are poorly studied which makes their application in biological control and pest management programs difficult (Cohen 2000, Schaefer & Panizzi 2000, Brugnera & Grazia 2018).

Oplomus Spinola, 1837 is a genus of predatory stink bugs, composed of 12 species distributed through the American Continent from the USA to Argentina and remarkable by the

bright and aposematic colorations and intraspecific color variation (Thomas 1992). Species of *Oplomus* can be recognized by the frenal margin of scutellum shorter than the postfrenal and presence of foretibial expansions and abdominal glandular patches in males (Thomas 1992, Brugnera *et al* 2019).

Oplomus catena (Drury, 1782) was one of the first asopines studied in Brazil by Silva (1933) who provided information about the life cycle and the morphology of immature and adults (see Pires *et al* 2015). The species is widely distributed in Austral South America, easily recognized by the angulated apex of mandibular plates (Thomas 1992), and it is known to prey on Lepidoptera (Silva 1933, Paluch *et al* 1999) and Coleoptera (Silveira *et al* 2002, Gomes 2012). Among *Oplomus* species, the morphology of immatures of *O. cruentus* Burmeister, 1835 was studied by Saini (1990), and the biology of *O. dichrous* Herrich-Schäffer, 1838 by Drummond *et al* (1987).

Here we describe the external morphology of the egg and the five nymphal instars of *O. catena*, emphasizing the post-embryonic changes. Additionally, we describe the chromatic polymorphism of adults proposing color patterns, provide details on the natural history, and review the known preys of the species.

Material and Methods

Adults were collected in São Domingos do Sul, RS, Brazil (28°33′51.1″S 51°49′56.8″W). Specimens were reared in laboratory in mesh boxes (12 × 12 cm) composed of a structure made of wood (floor and four lateral pilaster), surrounded by a fine and translucent tulle; the door is also made with tulle and coupled to the box with velcro (Fig 4d). The boxes were maintained in a control chamber at 25 ± 1°C, 70 ± 10 RH, and 12 L:12Dh photoperiod. The boxes were sprayed daily with water and the specimens fed with larvae of Lepidoptera and larvae and pupae of *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae) *ad libitum* (see “Discussion”). Additionally, leaves of *Eugenia uniflora* (L.) (Myrtaceae) were offered once a week.

Pinned adult specimens used in this study were obtained from the following institutions (Supplementary 1):

EMG Entomologisches Museum Geyer, Insekten Dauerausstellung, Geyer, Germany.

NMPC National Museum, Prague, Czech Republic.

MNRJ Museu Nacional, Universidade Federal do Rio de Janeiro, Brazil.

MPEG Museu Paraense Emílio Goeldi, Belém, Brazil.

MZUSP Museu de Zoologia, Universidade Estadual de São Paulo, São Paulo, Brazil.

UFRG Universidade Federal do Rio Grande do Sul, Departamento de Zoologia, Porto Alegre, Brazil

Immature and adults were photographed in multiple focal planes with a Nikon AZ100M scope coupled with a DS-Fi2 digital camera. Pictures were stacked in a single focused image using the NIS Elements software. Alive specimens were photographed with a Nikon D3100 digital camera. Photographs were processed in Photoshop® CS5 software.

Measurements and morphological data were obtained from 10 eggs and 10 nymphs of each instar, fixed in 70% ethanol, and maintained in freezer. The description of color patterns was carried out *in vivo*. Measurements are presented in millimeters (mean ± standard deviation [maximum–minimum]) and were obtained according to Brugnera & Grazia (2018) (Table 1). Voucher specimens were deposited at the Entomological Collection of the Department of Zoology, Federal University of Rio Grande do Sul (UFRG).

For scanning electron microscopy (SEM) analysis, two eggs and two specimens of each instar (preserved in 70% ethanol) were dehydrated in an increasing concentration of

ethanol series (70%, 80%, 90%, and 99.9%) for 10 min each, and transferred to 100% acetone. Samples were critical point dried (10 cycles), mounted in stubs, sputter-coated with gold, and observed and photographed in a scanning electron microscope (JEOL JSM 6060).

Terminologies for immature follow Matesco *et al* (2007, 2009, 2014) for general morphology, and Vilimová & Kotalová. (2012), Bottega *et al* (2015), and Bianchi *et al* (2016) for dorsal abdominal glands. Terminologies for adult morphology follow Thomas (1992).

Results

Morphology of immature

Egg

Barrel-shaped (Figs 1a and 2b); chorion thick, pale after oviposition, becoming dark brown few minutes later; egg color becoming reddish with the development of the embryo; lateral wall of chorion reticulated, with rounded projections (Figs 1a and 2b); operculum with a projected circle medially, sometimes with tiny projections in the center; lateral of operculum smooth; 15 to 23 translucent aero-micropylar processes surrounding the operculum (Figs 1b and 2a, b); openings of aero-micropylar processes subapical and directed inward (Fig 2d); aero-micropylar processes dilated at base (Fig 2c); distance between processes almost equal to one process length. Measurements: egg length (1.56 ± 0.04 [1.64–1.48]); egg width (1.17 ± 0.02 [1.2–1.12]).

First instar

Head and thorax dark brown to black (except intersegmental areas of antennae and discal region of thorax that are red). Abdomen red; abdominal plates dark brown to black; spiracles and trichobothria dark brown (Fig 1c, d).

Body oval; surface smooth, without punctures; lateral margin of the body, antennae, and legs with setae. Head rounded, declivent; clypeus longer than mandibular plates; apex of labium slightly surpassing the metacoxae; proportion of labial segments I < II > III < IV, fourth segment longest; proportion of antennal segments I < II > III < IV, fourth segment longest. Seven dorsal median plates in abdomen; first two plates slender, shorter than the III plate; II–IV plates with the openings of dorsal abdominal glands (DAGs). Eight pairs of semicircular lateral plates in abdomen, first plate smaller than the following, XI segment entirely covered by a plate; ventral median plates not visible (Fig 1c, d); 1 + 1 spiracles on II–VII abdominal segments and 1 + 1 pre-trichobothria and 1 + 1 trichobothria on III–VI abdominal segments (Fig 2g). Ventral plates covered by comb-like projections (Fig 2h). Ornamentation of

Table 1 Measurements (in millimeters) (mean \pm standard deviation [maximum–minimum]) of the morphometric characters of nymphs of *Oplomus catena*.

Character	First instar	Second instar	Third instar	Fourth instar	Fifth instar
Total length	1.75 \pm 0.04 (1.80–1.70)	3.59 \pm 0.11 (3.76–3.40)	4.74 \pm 0.24 (5.28–4.24)	7.84 \pm 0.67 (8.83–6.83)	9.84 \pm 0.58 (11.08–9.25)
Head length	0.55 \pm 0.01 (0.58–0.54)	0.85 \pm 0.04 (0.88–0.80)	0.98 \pm 0.04 (1.08–0.92)	1.32 \pm 0.15 (1.58–1.08)	1.69 \pm 0.07 (1.75–1.42)
Head width	0.82 \pm 0.03 (0.88–0.76)	1.06 \pm 0.02 (1.08–1.04)	1.32 \pm 0.02 (1.36–1.28)	1.80 \pm 0.03 (2.20–1.79)	2.10 \pm 0.05 (2.28–2.00)
Interocular distance	0.61 \pm 0.02 (0.64–0.58)	0.75 \pm 0.02 (0.80–0.72)	0.80 \pm 0.01 (0.84–0.76)	1.04 \pm 0.08 (1.25–0.92)	1.26 \pm 0.06 (1.32–1.16)
Rostral length	0.89 \pm 0.02 (0.92–0.86)	1.49 \pm 0.03 (1.52–1.46)	2.01 \pm 0.05 (2.12–1.92)	2.97 \pm 0.18 (3.42–2.58)	1.69 \pm 0.08 (1.75–1.42)
Rostral width of I segment	0.16 \pm 0.01 (0.16–0.14)	0.26 \pm 0.03 (0.28–0.20)	0.32 \pm 0.01 (0.36–0.32)	0.46 \pm 0.03 (0.48–0.40)	0.59 \pm 0.01 (0.60–0.52)
Length of I antennal segment	0.14 \pm 0.00 (0.14–0.14)	0.23 \pm 0.01 (0.24–0.22)	0.36 \pm 0.04 (0.40–0.30)	0.52 \pm 0.04 (0.60–0.44)	0.62 \pm 0.03 (0.72–0.60)
Length of II antennal segment	0.31 \pm 0.02 (0.34–0.28)	0.72 \pm 0.01 (0.74–0.70)	0.92 \pm 0.03 (1.00–0.90)	1.50 \pm 0.12 (1.84–1.28)	2.00 \pm 0.01 (2.04–2.00)
Length of III antennal segment	0.25 \pm 0.01 (0.26–0.24)	0.52 \pm 0.00 (0.54–0.52)	0.70 \pm 0.01 (0.72–0.68)	1.05 \pm 0.04 (1.20–0.96)	1.40 \pm 0.00 (1.40–1.40)
Length of IV antennal segment	0.52 \pm 0.01 (0.54–0.52)	0.80 \pm 0.00 (0.80–0.80)	0.92 \pm 0.02 (0.98–0.90)	1.28 \pm 0.06 (1.40–1.12)	1.47 \pm 0.04 (1.56–1.40)
Pronotal length	0.18 \pm 0.01 (0.20–0.16)	0.45 \pm 0.04 (0.52–0.40)	0.63 \pm 0.03 (0.68–0.60)	1.22 \pm 0.13 (1.67–1.00)	1.74 \pm 0.03 (1.83–1.67)
Pronotal width	1.10 \pm 0.02 (1.14–1.06)	1.61 \pm 0.02 (1.64–1.60)	2.20 \pm 0.00 (2.20–2.20)	3.57 \pm 0.33 (4.42–2.83)	4.58 \pm 0.13 (4.96–4.42)
Abdominal width	1.40 \pm 0.02 (1.44–1.34)	2.49 \pm 0.11 (2.68–2.36)	3.66 \pm 0.16 (4.00–3.33)	5.13 \pm 0.33 (5.67–4.50)	6.22 \pm 0.20 (6.75–5.83)

evaporatorium of anterior dorsal abdominal glands (DAG1) with wrinkles located anteriorly and posteriorly to the ostiole; ostioles slit-like (Fig 2e); ostioles of median (DAG2) and posterior (DAG3) attended by cuticular fold; surface of evaporatorium with spiked projections (Fig 2f).

Second to fifth instar

From second to fifth instar, the morphology of the nymphs is very similar, differing only in the size (Table 1) and some aspects of the antennae, mandibular plates, and legs that are detailed below.

Head, thorax and dorsal abdominal plates somewhat bright blue. Intersegmental areas of antennae, discal region of thorax and abdomen red (Fig 1e–n).

Body oval (Fig 1e–j) slightly elongated in the fifth instar (Fig 1i, j); dorsal surface slightly punctured on the second instar, becoming more dense and deep in the following instars (Figs 1e–j and 2n, o), each puncture with a basiconic sensilla (Fig 2n, o); clypeus slightly longer than mandibular plates on second instar (Fig 1k), subequal on third instar (Fig 2l); and shorter than mandibular plates on fourth and fifth instars (Fig 1m,n); apex of labium surpassing the metacoxae on second instar (Fig 1f), becoming

increasingly shorter along the development, slightly surpassing the mesocoxae on fifth instar (Fig 1j). II and IV antennal segments equally in length in the second instar (Fig 1e); III antennal segment longest from that of the third instar (Fig. 1g, h, j). Lateral margins of pronotum serrated. Development of wing pads, femoral spine and foretibial expansion visible from the third instar (Fig 1e, f), and abdominal spine in the fifth instar (Fig 1j). Dorsal and lateral plates of abdomen almost touching each other; abdomen with six dorsal plates, plate I not distinctly separated from thorax; plate II long and slender, slightly shorter than III; six dorsal median plates and five ventral median plates present; IX segment entirely covered by a plate. A pair of slender ventral plates on the posterior margins of II and III abdominal segments; a pair of mesolateral plates on the III abdominal segment; openings of dorsal abdominal scent glands on II–IV dorsal median plates (Fig 1e, g–i); 1 + 1 spiracles on II–VIII segments and 2 + 2 trichobothria on III–VII ventral abdominal segments (Fig 2m). Ostiole of DAG1 slit-like surrounded by cuticular wrinkles and reticulated evaporatorium with shallow alveoli composed of low bridges and trabeculae (Fig 2i). DAG2 (Fig 2j) and DAG3 bearing spout peritreme, surrounded by a reticulate evaporatorium; peritreme lip and postostiolar fold present (Fig 2j–l).

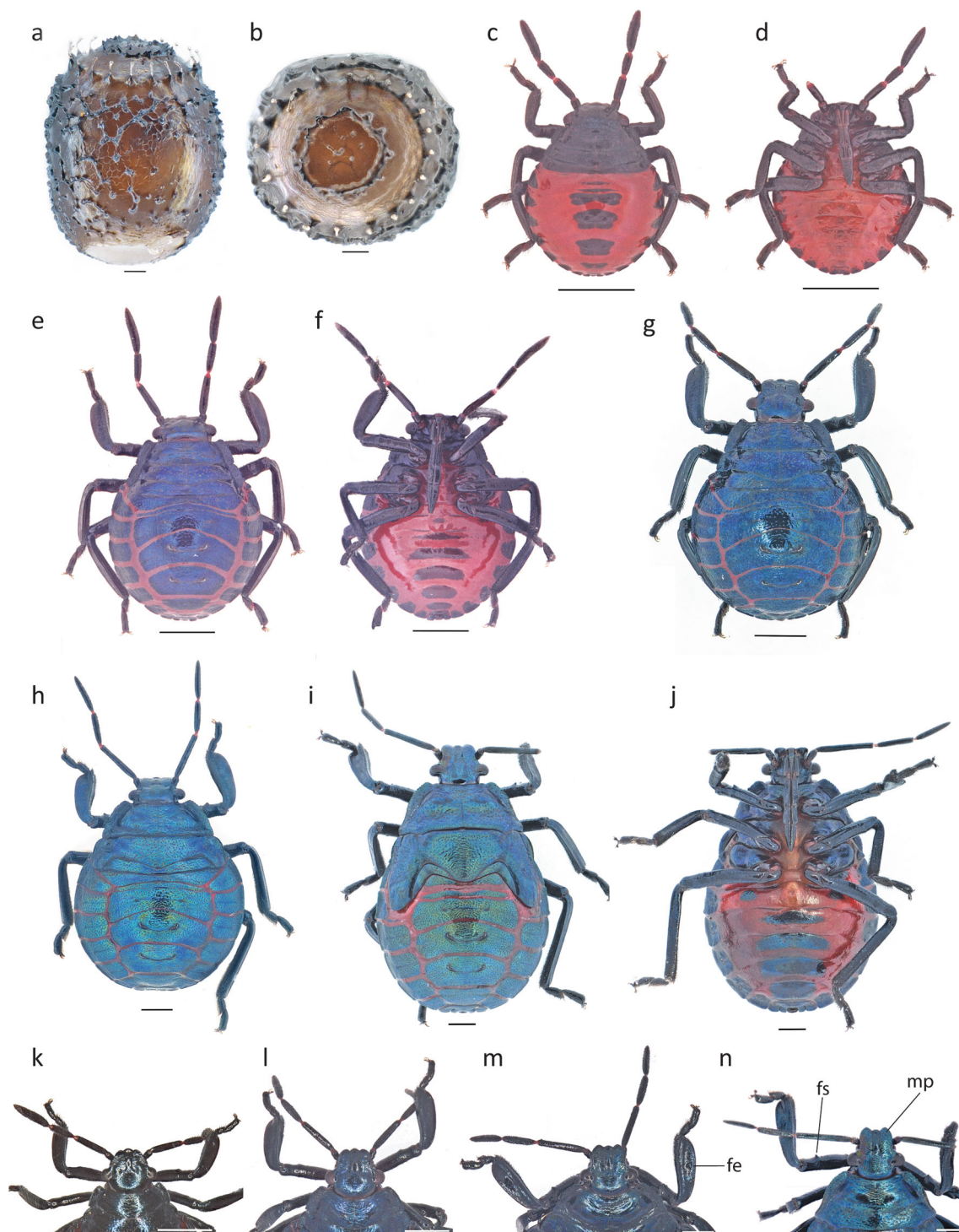


Fig 1 Immature stages of *Oplomus catena*. **a, b** egg; **c, d** first instar; **e, f, k** second instar; **g, l** third instar; **h, m** fourth instar; **i, j, n** fifth instar. fe, foretibial expansion; fs, foretibial spine; mp, mandibular plate. Scale bars = **a, b** 0,1 mm; **c-n** 1 mm.

Chromatic polymorphism of adults

Seventy-four adults of *Oplomus catena* were analyzed from different localities of Brazil. Four color patterns were observed among males and females, with drab and bright

variations (Fig 3). Each specimen analyzed was classified in one of the patterns described below (Supplementary 1).

Color patterns: (1) entirely bright blue, sometimes with the abdominal spine and the apex of abdomen yellow (Figs 3a, b and 4h). (2) Head drab black with the apex of

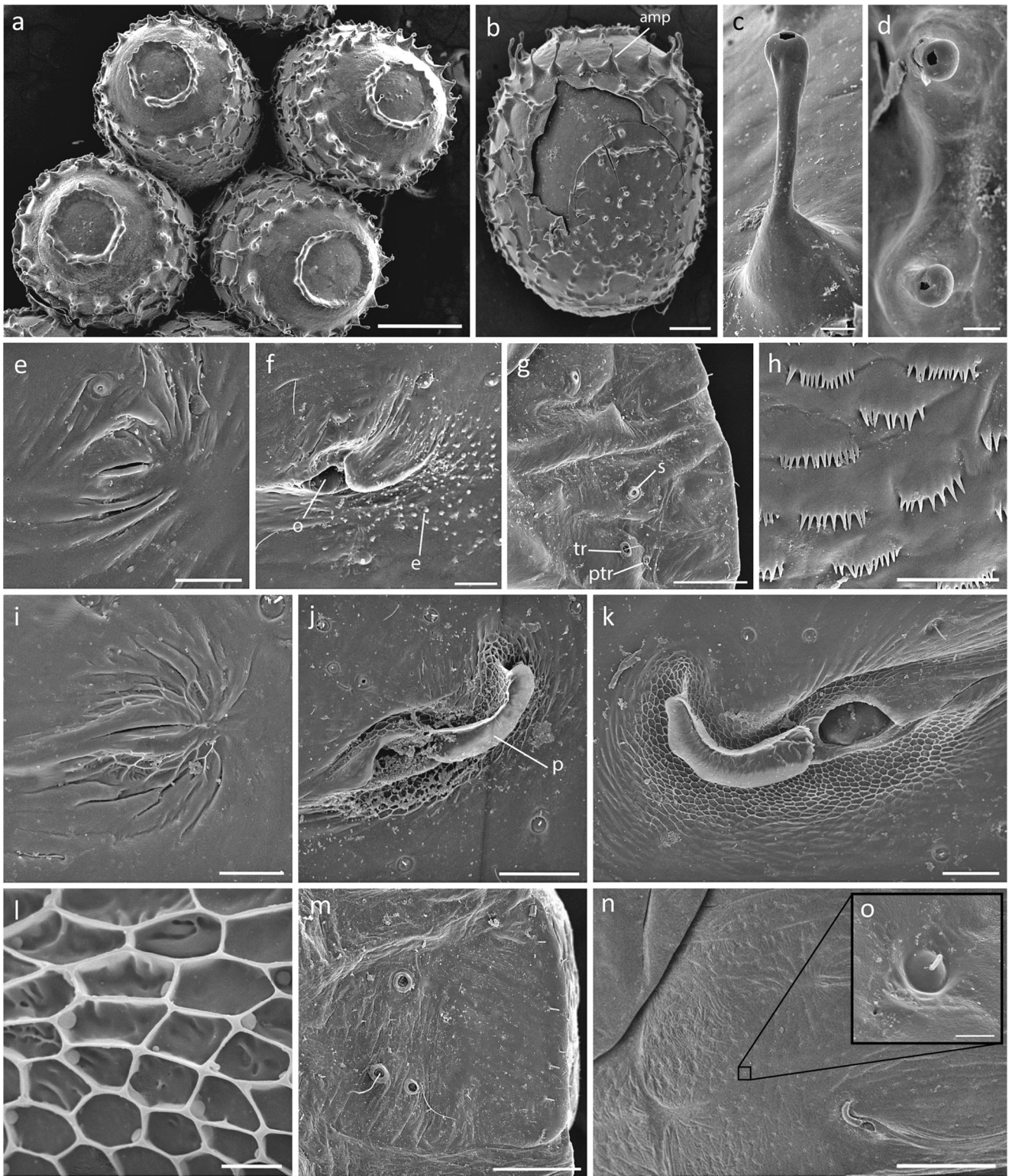


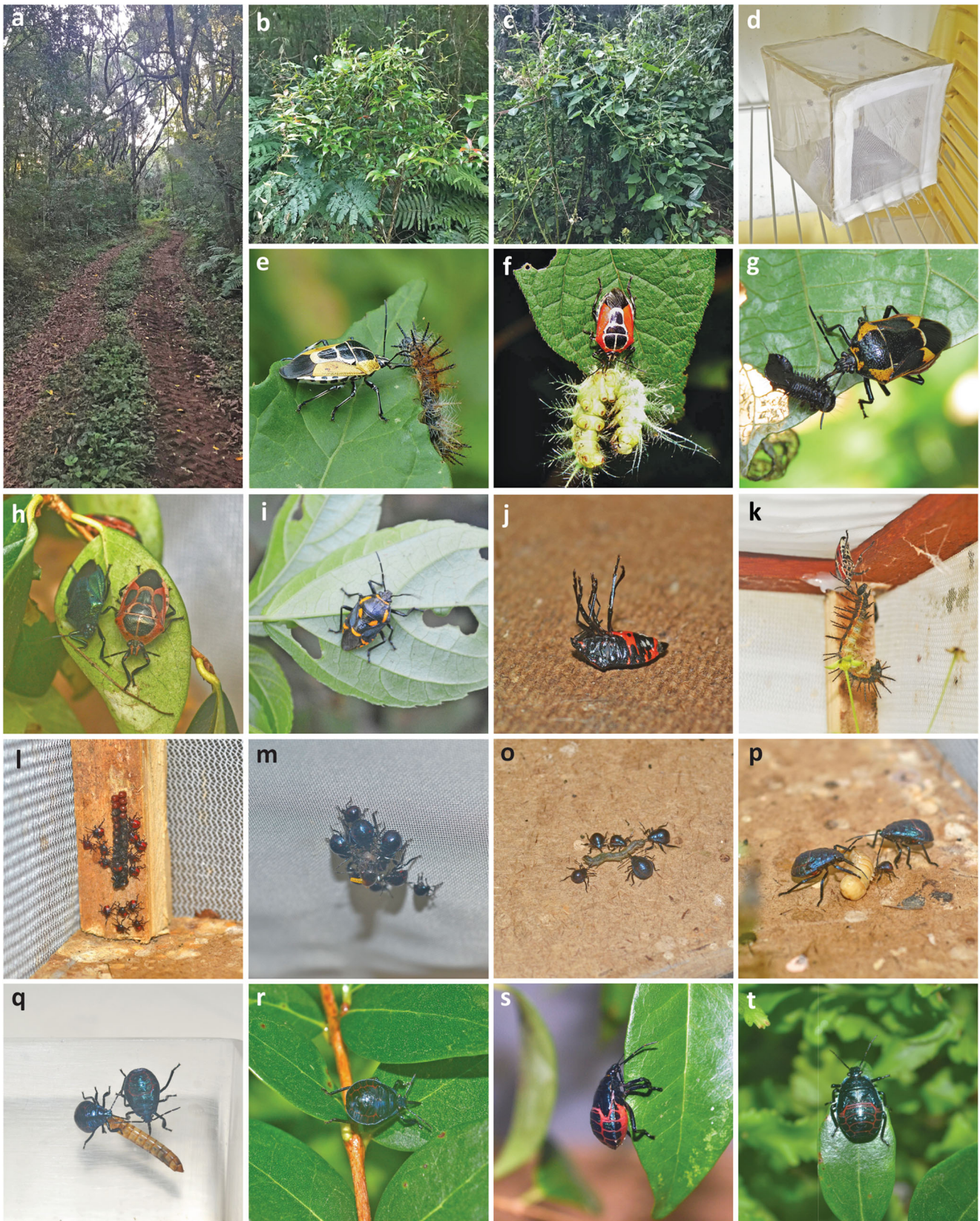
Fig 2 Immature stages of *Oplomus catena* under scanning electron microscopy. **a–d** egg; **a** dorsal view; **b** lateral view; **c**, **d** aero-micropylar process; **e–h** first instar; **e** DAG1; **f** DAG2; **g** spiracle and trichobothria of II and III ventral abdominal segments; comb-like projection on VI abdominal segment; **i**, **j**, **m**; second instar; **i** DAG1; **j** DAG2; **k**, **l**, **n**, **o** fifth instar; **k** DAG2; **l** evaporatorium; **m** spiracle and trichobothria of II ventral abdominal segment; **n**, **o**



Fig 3 Patterns of chromatic polymorphism in adults of *Oplomus catena*. a–d pattern 1; e–h pattern 2; i–l pattern 3; m–p pattern 4. Scale bars = 1 mm.

mandibular plates reddish or yellow; thorax drab reddish or yellow dorsally, with two black and semicircular maculae in the center of pronotum, and one macula in the center of

each corium (sometimes not present or very reduced). Scutellum red or yellow, with a black macula at base and a black macula in the middle of postrenal portion. Ventral



◀ Fig. 5 Habitat and photos of *Oplomus catena*. **a** general aspects of site of collection (São Domingos do Sul, Brazil); **b** shrub of *Eugenia uniflora*; **c** climbing plants tangles; **d** mesh box; **e–j** habit of live adults; **l–t** habit of live immature; **j** male displaying thanatosis behavior; **l** egg cluster and first instar; **m** second and third instar preying *Heraclides* sp. larva; **n** second and third instar preying cf. Noctuidae larva; **p** fifth and second instar preying *Tenebrio molitor* pupa; **q** fourth and fifth instar preying *Tenebrio molitor* larva; **r** fourth instar feeding on *Eugenia uniflora* leave; **s** lateral view of fifth instar; **t** dorsal view of fifth instar. Photos **e–f** by Antonio C. F. Junior. Photo **g** by Diogo J. Luiz.

drab red or yellow, with several black maculae. Legs brown, with red or yellow areas on femur and tibia (Figs 3e–h and 4e, f, h, j). (3) Head generally totally black; dorsal and ventral bright blue or drab black; apex of corium and posterolateral portion of pronotum red or yellow, apical margin of scutellum pale or concolor with scutellum. Ventral bright blue or drab black, sometimes with pale spots; abdominal spine pale. Legs bright blue or drab black (Figs 3i–l and 4g). (4) Dorsal and ventral drab black; base and lateral of pronotum and apex of corium red or yellow; basal angles and apex of scutellum red or yellow. Ventral red or yellow at the apex of abdomen, abdominal spine, and medial part of the IV segment (frequently with a black spot in the middle). Legs drab black with red or yellow spots in the femur (Figs 3m–p and 4i, k).

Among the patterns proposed, 1 and 2 were observed in both sexes, pattern 3 was present only in females and 4 observed only in males (Supplementary 1). Pattern 2 is the most common variation, observed in 38 (51, 3%) individuals. Pattern 3 was observed in four individuals from Paraná and São Paulo states. Among the adults obtained from our rearing (bold individuals in Supplementary 1) were observed all the patterns except 3, and pattern 2 was observed only in females. No yellow forms of pattern 2 were obtained from our rearing; we observed that the red pigmentations tend to fade with the age of adult, becoming pale/yellow (Supplementary 1).

Natural history

Several immature and adults were found among December and February in a relative small portion of native Atlantic Rain Forest (see “Material and Methods”) in different plants (Fig 4a). Frequently these bugs were collected in small shrubs of *Eugenia uniflora* (L.) (Fig 4b), but also in climbing plants tangles (Fig 4c). Usually larvae of Lepidoptera and Coleoptera were found in the same plant.

Eggs are laid in two rows, with about to 24 eggs per cluster, but sometimes with less than 10 eggs (Fig 4l). After hatching, the first instar nymphs stay around the egg cluster, feeding on water until the molt. Few hours after molting to second instar, nymphs start to walk around the box, usually alone, apparently to find food. When a larva is found, the nymph made fast movements with the antennae and

immediately tries to insert the stylets on the prey. Usually the prey try to escape but the bugs are successful in almost all the cases. Right after the nymphs start to feed, more nymphs come and share the prey (Fig 4m–p). Generally, in second and third instars, the nymphs do not avoid to share their prey, even if it is small. In fourth and fifth instars, the nymphs try to escape with the prey when other nymphs come closer. This behavior was also observed in adults (Fig 4k). After feeding, the nymphs stay a long time motionless, frequently until the next molt.

Oplomus catena seems to be very generalist, having preference for soft body larvae. The adults are voracious and attack the prey holding it with the claws of forelegs (Fig 4f). Initially in laboratory, we have tried to feed adults and nymphs with larvae and pupae of *Tenebrio molitor* but apparently, they cannot pierce the external cuticle to access the internal tissues and feed. So we offered any Lepidoptera or Coleoptera larvae, daily collected in a remnant of Atlantic Rain Forest in Campus do Vale—UFRGS, Porto Alegre, Brazil. All the larvae offered to nymphs and adults were eaten. Because of the difficulty to frequently find and collect soft body larvae, we made small cuts on the sides of the body of *Tenebrio molitor* larvae and offered to the bugs. Apparently, with the help of antennae and apex of labium, they could find the cuts and feed on the larvae without difficulties (Fig 4p, q). Almost all the specimens reared for this study were fed with this method, and the mortality index was almost zero. Additionally, nymphs from second instar and adults were frequently observed feeding on leaves of *Eugenia uniflora* (Fig 4r).

Adults exhibit thanatosis behavior when they are perturbed, usually extending the legs and antennae and staying in that position for about 10 s. This behavior was not observed in the nymphs (Fig 4j).

Discussion

Morphology of immature

Only about 1% of the immature stages of predatory stink bugs that occur in the neotropics have been studied so far. This lack of knowledge makes it difficult the identification of most of immature stages collected and prevents to include immature characters in taxonomic diagnosis and phylogenetic analysis (Matesco *et al* 2014, Brugnera & Grazia 2018).

Taking into consideration the species of Asopinae whose morphology is known, the egg of *Oplomus catena* has similar characteristics with *O. cruentus*, *Stiretrus decastigmus* Herrich-Schäffer, 1838, and of *S. anchorago* Fabricius, 1775 [as *S. fimbriatus* (Germar)] (Oetting & Yonke 1971, Saini 1990, Richman & Mead 2001). According to unpublished phylogenetic hypotheses (Gapon 2008, Roell 2019), *Oplomus* is the sister group of the *Stiretrus* + *Discocera* clade. The similarities

Table 2 Review of the known prey of *Oplomus catena*. The scientific name of the species is written as in the references.

Family (order)	Genus/species	Reference
Nymphalidae (Lepidoptera)	<i>Actinote pellenea</i> Huebn.	Silva 1933, Grazia & Hildebrand 1987
Nymphalidae (Lepidoptera)	<i>Actinote pyrrha pyrrha</i> (Fabricius)	Rezende <i>et al</i> 2008
Nymphalidae (Lepidoptera)	<i>Actinote surima</i> (Schaus)	Paluch <i>et al</i> 1999
Nymphalidae (Lepidoptera)	<i>Actinote</i> sp.	This study (Fig 4e)
Papilionidae (Lepidoptera)	<i>Heraclides</i> sp.	This study (Fig 4m)
Papilionidae (Lepidoptera)	<i>Heraclides anchisiades capys</i> (Hübner)	Costa Lima 1968
Crambidae (Lepidoptera)	<i>Duponchelia fovealis</i> Zeler	Bischoff <i>et al</i> 2013
Saturniidae (Lepidoptera)	cf. <i>Dyrphia</i> sp.	This study (Fig 4e)
cf. Noctuidae (Lepidoptera)	–	This study (Fig 4o)
Chrysomelidae (Coleoptera)	<i>Coelomera lanio</i> (Dalman)	Silveira <i>et al</i> 2002
Chrysomelidae (Coleoptera)	<i>Stolas chalybea</i> Germar	Nogueira-de-Sá & Vasconcellos-Neto 2003
Chrysomelidae (Coleoptera)	–	This study (Fig 4g)

between the egg of *Oplomus* and *Stiretrus* and their hypothetical phylogenetic relationship reinforce the phylogenetic importance of the egg structure in Pentatomidae, as tested by Matesco *et al* (2014) for *Chinavia* Orian, 1965 and *Nezara* Amyot & Serville, 1843.

Besides the underexplored morphology of Asopinae eggs, some studies show their remarkable diversity in size, shape, chorion sculpture, and size of aeromicropylar processes. Eggs of predatory stink bugs are known by the aero-micropylar processes, generally much longer than in other subfamilies of Pentatomidae (De Clercq 2000, Matesco *et al* 2009, 2014), as observed in *Podisus* Herrich-Schäffer, 1851 (Oetting & Yonke 1971, Grazia *et al* 1985, De Sá *et al* 2013), *Brontocoris* Thomas 1992, *Supputius* Distant, 1889 (Dimaté *et al* 2014), *Tynacantha* Dallas, 1851 (Brugnera & Grazia 2018), *Conquistator* Gapon, 1992 (Costello *et al* 2002), *Troilus* Stål, 1867 (Hinton 1981) among others. However, as *Oplomus* and *Stiretrus*, many genera exhibit short aero-micropylar processes, as *Euthyrhynchus* Dallas, 1851 (Richman & Mead 2000), *Alcaeorrhynchus* Bergroth, 1891 (Mead & Richman 2000), *Picromerus* Amyot & Serville, 1841, and *Pinthaeus* Stål, 1867 (Rieger 2000) These egg patterns observed among Asopinae genera seems to be correlated with their phylogenetic relationship, which can be useful for the systematics of the group and should be more investigated..

First instar nymphs of Asopinae generally show similar characteristics: red abdomen and black to dark

brown head and thorax (Grazia *et al* 1985, Brugnera & Grazia 2018, *this study*) which makes their identification difficult. From the second instar on, the nymphs show more evident differences between the species that allow distinguishing them. In *Oplomus catena* the most diagnostic characteristic is the dorsal and lateral abdominal plates that almost touch each other, occupying nearly the entire dorsal surface of abdomen (Fig 2e–i). These features are not observed in any other species of Asopinae whose morphology is known, presenting always much shorter plates (see Brugnera & Grazia 2018 for an example), except for *Stiretrus anchorago*. Although not described by Richman & Mead (2001), the nymphs of *S. anchorago* seem to have fused dorsal and lateral plates from the second instar on, forming a single plate that covers the entire dorsal surface of abdomen.

The femoral spine and the angulated apex of mandibular plates, features present in adults (Thomas 1992), can be recognized in the nymphs from the third instar, which progressively increases in size on fourth and fifth instar (Fig 2e–i). Additionally, *O. catena* nymphs present a pair of pre-trichobothria and a pair of trichobothria in III–VI abdominal segments on first instar (Fig 2g). Generally pentatomids exhibit only a pair of trichobothria on first instar, but some contrary examples can be found in species of *Chinavia* (Schaefer 1975, del Vecchio *et al* 1988, Schwertner *et al* 2002). This is the first time that a pair of pre-trichobothria is reported for the subfamily Asopinae.

Color polymorphism and natural history

The intraspecific color variation is a feature present in some bugs of the superfamily Pentatomoidea and can be expressed in nymphs and adults (Schwertner *et al* 2002, Sánchez-Soto *et al* 2004, Campos *et al* 2007). Among adults, the condition is observed in drab bugs such in the well-known green stink bug, *Nezara viridula* (Linnaeus, 1758) (Pentatomidae) (Harris *et al* 1984, Esquiavel *et al* 2018) and frequently observed among species that exhibit bright and/or aposematic coloration, as in *Pachycoris torridus* (Scopoli, 1772) (Scutelleridae) (Sánchez-Soto *et al* 2004), in Catechantini (Pentatomidae) species such as in the genus *Arocera* Spinola, 1837 (Rider 1992), and in many Asopinae (Pentatomidae) species (Thomas 1992, 1994, Paleari 2013, Roell *et al* 2019, *this work*), among others. From the second instar on, nymphs of *O. catena* exhibit a bright blue coloration on head, thorax, and abdominal plates, without intraspecific variation (Figs 1e–j). Similar condition was already reported for other asopines: *Tynacantha marginata* Dallas, 1851 (Brugnera & Grazia 2018), *Euthyrhynchus floridanus* Linnaeus, 1767 (Richmann & Mead 2000), and *Alcaeorrhynchus grandis* (Dallas, 1851) (Mead & Richman 2000). Among the possible biological roles of this condition, the defensive behavior, sexual selection, type of food and diapause have been studied so far, which directly influences the systematic studies on these taxa (Please, replace this citation by Schwertner *et al* 2002, Souza *et al* 2012, Musolin & Saulich 2018); however, the intraspecific color variation is not yet well understood in stink bugs.

The evolutionary implications and the biological role of this variation in Asopinae are yet unclear but were discussed by Schaefer (1996). The author suggests bright asopines prefer to feed on bright beetles compared with drab asopines, and may derive defensive allomones by feeding, mainly from chrysomelid and coccinellid beetles. Schaefer (1996) also suggested the bright condition of these bugs may trace an evolutionary progression from drab bugs that commonly feed on other insects besides beetles. However, in laboratory and natural conditions *O. catena* was frequently reported feeding on Lepidoptera larvae, especially of the genus *Actinote* (Fig 4, Table 2) which suggests that the species may not have a preference for beetles, as reported for *Stiretrus decemguttatus* (Paleari 2013) for example. It is suggested the species seems to be generalist and able to feed on any insect they can pierce, but more studies on the predatory behavior are needed to confirm this hypothesis.

As in other pentatomids, first instar nymphs of *Oplonus catena* do not feed and only need moisture to survive (Schuh & Slater 1995). From the second instar, the nymphs need to feed on other insects to continue the development and some species need to feed also on plants, probably to obtain water for saliva and to complete the development (De Clercq 2000,

2008, Grazia *et al* 2015). Early instar nymphs of *O. catena* seem to prefer group feeding; this behavior could improve the efficiency of the predation especially on large size preys, as reported for *Andrallus spinidens* (Fabricius) (Hyodo *et al* 2014).

Although yet underexplored, the knowledge about the predatory behavior and feeding strategies of asopines has been improved in the last decades. These stink bugs use essentially visual, chemical, and tactile senses to detect potential preys, which are then selected based on their nutritional quality and their defense capacity (Marston *et al* 1978, De Clercq 2000, Lemos *et al* 2003). However, the influences of remarkable features like body coloration and some body structures (e.g. foretibial apparatus and foretibial expansion) in the predation are yet poorly understood (Barão *et al* 2013, Brugnera *et al* 2019). In general, the knowledge about the biology of asopines is very scarce and focused only in species with economic importance. Studies in less known species are important to better understand the evolutionary traits and feeding strategies of these bugs.

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Authors' Contribution RB planned, designed, collected data, and executed experimental work of the study. RB, LAC, and JG wrote and approved the final manuscript.

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