Papéis Avulsos de Zoologia
Museu de Zoologia da Universidade de São Paulo

ARTICLE

Biological notes of *Harpactor angulosus* (Lepeletier & Serville, 1825) (Hemiptera: Reduviidae)

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Abstract. Assassin bugs are one of the most diverse families of cimicomorphan Heteroptera and are known from all biogeographic regions. Except for the kissing bugs (Triatominae) that are hemathophagous, reduviids show a predatory behavior and are usually study as potential biocontrollers of crop pests. In this way, Harpactorines have been specially studied as they are mostly diurnal and live on the vegetation. In this contribution, the complete life cycle of *Harpactor angulosus* (Lepeletier & Serville, 1825) is described and illustrated. Specimens were collected in the field in El Manantial, San Miguel de Tucumán, Argentina, and were reared in laboratory to obtain all immatures (eggs and nymphs) and to observe its feeding habits, molting, mating, and oviposition. Collection specimens as well as online resources such as iNaturalist records were also studied to know the current distribution of the species. This resulted in the expansion of *H. angulosus* distribution in Argentina to Tucumán and Buenos Aires provinces and to the recording of the species in Ecuador, Peru and Paraguay for the first time. Our findings reveal that *H. angulosus* would be studied as a potential biocontroller of *Agraulis* sp., an important pest of maracuyá crops in several South American countries.

Key words. Assassin bug; Immature; Behavior; Distribution; Harpactorini.

INTRODUCTION

Harpactorinae is the most specious subfamily of the Reduviidae, with ca. 2,800 described species, and more than 300 genera (Maldonado Capriles, 1990; Weirauch *et al.,* 2014). They are mostly diurnal predators that are frequently found on the vegetation (Schuh & Weirauch, 2020). Noteworthy, some species have been observed using other resources such as extrafloral nectary secretions as a food source (Alvarez *et al.,* 2019; Gil-Santana & Keller, 2022), and plant resins as sticky material for prey capture (Forero *et al.,* 2011).

Harpactor Laporte, 1833 was created to include three species, although the only one that persisted in the genus is the type species: *H. angulosus* (Lepeletier & Serville, 1825). Nowadays, it includes another three species all from the Neotropics: *H. tuberculosus* Stål, 1872 known from Argentina, Bolivia, Brazil, and Uruguay; *H. distinguendus* (Stål, 1859) from Brazil; and *H. rhombeus* (Erichson, 1848) from Brazil, Colombia, Guiana, Mexico and Venezuela (Wygodzinsky, 1946; Maldonado Capriles, 1990; Gil-Santana & Forero, 2009). They are large reduviids (ca. 20 mm) with inconspicuous coloration, mostly grey by a dense waxy secretion of the cuticle covering all over the body.

Harpactor angulosus was described from Brazil, and later, its known distribution expanded to northern Argentina (Melo *et al.,* 2023). It can be recognized by the posterior lobe of pronotum with 1+1+1 distinct tubercles, the emarginate humeral angles with spiniform process at the anterior border of the emargination; the strongly dilated abdomen, and the postero-lateral angles of connexival segments with a short dentiform process (Wygodzinsky, 1946).

In this contribution, we describe and illustrate the complete life cycle of *Harpactor angulosus,* and give biological observations of their feeding, mating and oviposition behavior under laboratory conditions. We also give an accurate distribution of the species.

MATERIAL AND METHODS

Specimens of *Harpactor angulosus* (Fig. 1A) were collected in the locality of El Manantial, in

Pap. Avulsos Zool., 2024; v.64: e202464020
https://doi.org/10.11606/1807-0205/2024.64.020
https://www.revistas.usp.br/paz
https://www.scielo.br/paz
Edited by: Cristiano Feldens Schwertner
Received: 07/12/2023
Accepted: 01/05/2024
Published: 02/07/2024

ISSN On-Line: 1807-0205
ISSN Printed: 0031-1049
ISNI: 0000-0004-0384-1825

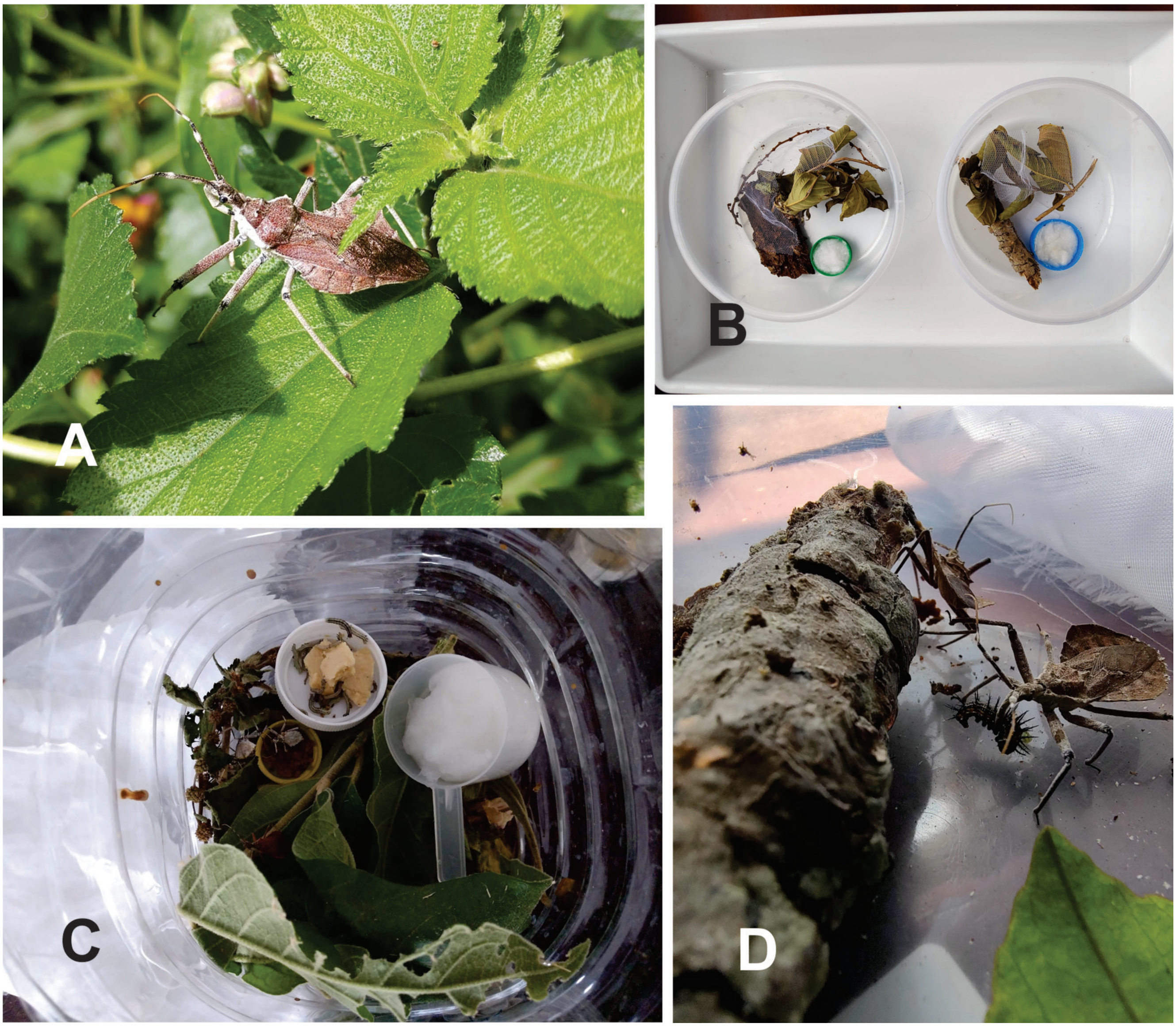

Figure 1. (A) *Harpactor angulosus* (Lepeletier & Serville), female in the field; (B) small plastic boxes used to breed the specimens; (C) large plastic boxes used to breed specimens; (D) adults feeding on Lepidoptera larva of *Agraulis vanillae* (Linné).

the province of Tucumán, Argentina (26°50'S, 65°19'W) during 22/XII/2022 and 10/V/2023 by NM. They were found on native vegetation, with predominance of *Solanum mauritianum* Scopoli, *Phenax hirtus* (Sw.) Wedd., *Ipomoea alba* L., and *Passiflora edulis* Sims. Specimens collected were reared in the laboratory in plastic cages of 20 × 7 cm (Fig. 1B) and in big plastic bottles of 5 lts (Fig. 1C). The main open of both kinds of cages were covered with voile to allow ventilation. For the study of the "Developmental stages" we separate the egg clutches into plastic cages of 22 cm diameter and 7 cm high (Fig. 1B). The specimens from the clutch we obtained by a female collected in the field were fed with different arthropods (Coleoptera, Lepidoptera, Hemiptera, etc.), and the ones obtained by a female raised in the laboratory were fed with third instar larvae of *Diatraea saccharalis* (Fabricius, 1794), in both cases the feeding was *ad libitum.* We registered daily the state of development of each specimen.

The statistical analysis were conducted using InfoStat version 2020 (Di Rienzo *et al.,* 2020). The response variables by development time, defined as the relationship between the number of days that pass in each stage/instars, and the total number of individuals, were analyzed using Wilcoxon (Mann-Whitney U) with egg stage/nymphal instars, and origin of the female as factors.

Images and videos were taken in the field and in the lab with a cellphone (Motorola Edge 30 Fusion), and to take close up images in the lab the cellphone was attached to a Leica EZ4 microscope. These data is deposited in the https://ri.conicet.gov.ar.

Specimens studied are deposited in the entomological collection of La Plata Museum, La Plata, Argentina (MLP), and voucher specimens in the Zoología Agrícola Lab, Facultad de Agronomía, Zootecnia y Veterinaria, Universidad Nacional de Tucumán, Argentina (FAZyV, UNT). Additional material studied belongs to the entomological collection of the MLP.

Material studied: Argentina: *Buenos Aires:* 1 male, Carmen de Patagones, ex coll. Berg (MLP); *Misiones:* 1 female, Puerto Peninsula, 25°44.142S, 54°32.124W, 271 m, 08/III/2010, P. Dellapé col. (MLP); 1 male 1 female, Loreto, Dr. A.A. Ogloblin (MLP); 1 male, Pto. Bemberg, VI/1942, H.S. (MLP); *Salta:* 2 females, de Rio Cañas a Astillero, VI/[19]33, ex coll. Denier (MLP); *Tucumán:* 1 male, 3 females, 2 first instar (N1), 2 second instar (N2), 3 third instar (N3), 1 fourth instar (N4), 3 fifth instar (N5), El Manantial, Lules, 26°49′49.31″S, 65°19′21.68″W, 494 m, XII/2022-III/2023, Maza col. (MLP); 3 males, 6 females, 1 second instar, 1 third instar, 2 fourth instar, 1 fifth instar, same data (FA-ZyV, UNT). **Brazil:** 1 female, Rio, 19/XI/1901, P.G.B. (MLP). **Paraguay:** 1 female, San Pedro, Koslovasky col. (MLP); 1 female, Col. Independencia, II-1957, Foerster, ex coll. Carcavallo (MLP); 1 female, Paso Yobay, XI/1957, Foerster, ex coll. Carcavallo (MLP).

Current distribution was determined by bibliographic records, specimens from MLP collection, and specimens identified by MCM in the citizen science platform iNaturalist (https://www.inaturalist.org).

RESULTS AND DISCUSSION

Feeding behavior: Nymphs and adults were mostly fed with larvae (Figs. 1D, 2A-B) of *Spodoptera cosmioides* (Walker, 1858), *Rachiplusia nu* Guenée, 1852 (Lepidoptera, Noctuidae), and *Diatraea saccharalis* (Fabricius, 1794) (Lepidoptera, Crambidae) raised in the laboratory with an artificial diet; occasionally, they were also fed with adults and pupae of these Lepidoptera. In the field, they were observed eating small curculionidae (Coleoptera), *Agraulis vanillae* (Linné, 1758) larvae (Lepidoptera, Nymphalidae), adults and nymphs of Tropiduchidae (Hemiptera, Auchenorrhyncha), adults of *Epilachna paenulata* Germar, 1924 (Coleoptera, Coccinellidae) (Fig. 2F), and small unidentified coleopteran which were collected and offered under captivity.

As *H. angulosus* specimens were sometimes found together with adults and nymphs of *Edessa rufomarginata* (De Geer, 1773) (Hemiptera, Pentatomidae), we also offered them as food; they accepted the prey but avoided the adults. They also avoid other large preys, such as crickets. Nevertheless, prey size was not a limitation considering the relative size of *D. saccharalis* larvae and the N1 of *H. angulosus,* which choose large larvae as preferred food source (Fig. 2B, and http://hdl.handle.net/11336/221277). On the other hand, *H. angulosus* III-IV and V instar nymphs were observed praying on nymphs and adults of stick insects of 22 cm of length by piercing on the membranous areas such as sutures and joints (Costa *et al.,* 2022). These observations were made in captivity, and the authors also observed the assassin bugs feeding on the leaves of guava and powder-puff branches, leaving a small drop of excrement near the point of piercing. Some species of Harpactorinae have been reported feeding on the sap of plants or other sugary substances (Haviland, 1931; Wygodzinsky, 1947; Parker, 1965, 1969; Gil-Santana & Keller, 2022), but some breeding experiments on various plants were negative (Stoner *et al.,* 1975). In addition, the particular case of *Zelus araneiformis* was observed to complete its life cycle on a *Cecropia* sp. (Cecropiaceae) in French Guiana (Bérenger & Pluot-Sigwalt, 1997). Many authors also postulated that it is likely that most of the species seek to hydrate themselves more than to feed. In this work, we offered apples' halves leaving the mesocarp exposed but also cotton balls sunken in water inside small plastic containers (Figs. 1B-C, 2D) as sources of hydration; the bugs preferred the apples instead of the cotton balls, but they hydrated from both as well (Figs. 2C-D).

We observed that last instar nymphs (N5) show preference for Fulgoridae (Hemiptera, Auchenorrhyncha), when other preys were offered such as Lepidoptera larvae (Fig. 2D). Adults of *S. cosmioides* were observed to be hunted by two nymphs, showing a collaborative way of preying. This behavior was also observed in the nymphs that fed from the same larvae even when there were more than one available in the cage (http://hdl.handle.net/11336/221277).

Major susceptibility was observed during nymph molting, in some occasions Lepidoptera larvae bred with artificial diet take the chance to attack and feed on the molting assassin bugs.

Our observations and previous studies under laboratory conditions, that also feed *H. angulosus* on Tenebrionidae pupae and Noctuidae larvae (Pereira *et al.,* 2009), show the generalist feeding behavior of this species.

Adults and nymphs prefer enriched spaces (with leaves and sticks) (Figs. 1B-D), although they often stay at the upper part of the cages. We also observe that overcrowding leads to cannibalism.

Mating: Multiple events of mating were observed in the laboratory during ca. 22 days (Fig. 3A). After the oviposition began, no more mating events were registered and after 8 days, the male died. For coupling, the male gets on the back of the female, attaching himself to the thorax and anterior region of the abdomen of the female by his anterior and posterior legs, and juxtaposing his genital capsule to the female terminalia. This behavior is common among reduviids and many other insects (Manrique & Lazzari, 1994; Huber, 2010).

Oviposition: Eggs were always laid aligned (Figs. 3B-D), mostly upside down on the voile of the cages, but also at the bottom of the handle of the container with water of the cage where a female was kept (Fig. 3B). The number of eggs ranged from three to 11 (ten, once; five, once; four, twice; eleven, twice; two, once and six, twice). These observations are mostly in accordance with previous ones, as Pikart *et al.* (2012) reported egg masses of one to seven eggs. Eggs were adhered to the substrate without a great amount of a viscous and sticky yellowish substance between them (Fig. 3C), after the emergence of the nymphs, they become opaque and covered with fungi. Pereira *et al.* (2009) studied specimens from Minas Gerais (Brazil) and documented the oviposition in the laboratory, where the eggs were aligned on the abaxial side of the guava leaves (Myrtaceae). The position where the eggs were laid suggests their arboreal habits, which

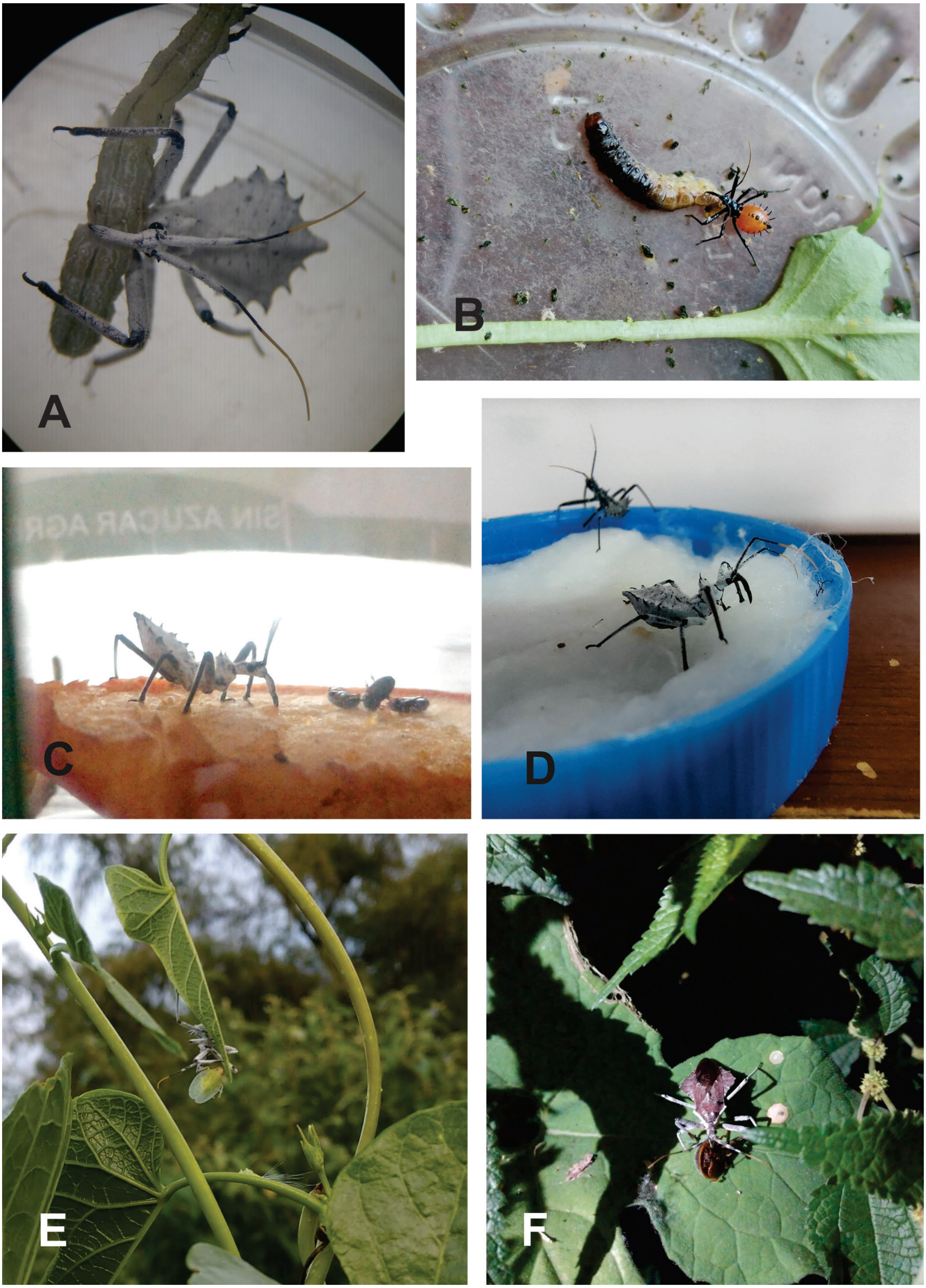

Figure 2. *Harpactor angulosus* (Lepeletier & Serville). (A) N4 feeding on larva; (B) N1 feeding on larva; (C) N4 feeding on the mesocarp of an apple; (D) N2 hydrating from sunken cotton; (E) N5 feeding on fulgoridae (Auchenorryncha) in the field; (F) adult feeding on *Epilachna paenulata* Germar (Coleoptera, Coccinellidae).

is in accordance with observations made by Vennison & Ambrose (1990) in Harpactorinae Indian species. Our observations in the field agree with this hypothesis as the specimens were collected over the leaves of plants, mostly Solanaceae, where they fed. All studies agree that the eggs are dark brown to black, with a yellowish operculum (Fig. 3C).

Developmental time: From the first clutch of four eggs we obtained by a female collected in the field, and after nine days of oviposition, nymphs hatched. Ecdysis was synchronized from N1 to N4 (Table 1). A male and a female raised in the laboratory copulated and left fertile offspring. This female laid 13 egg clutches with between two and 11 eggs. We could follow the development of eight individ-

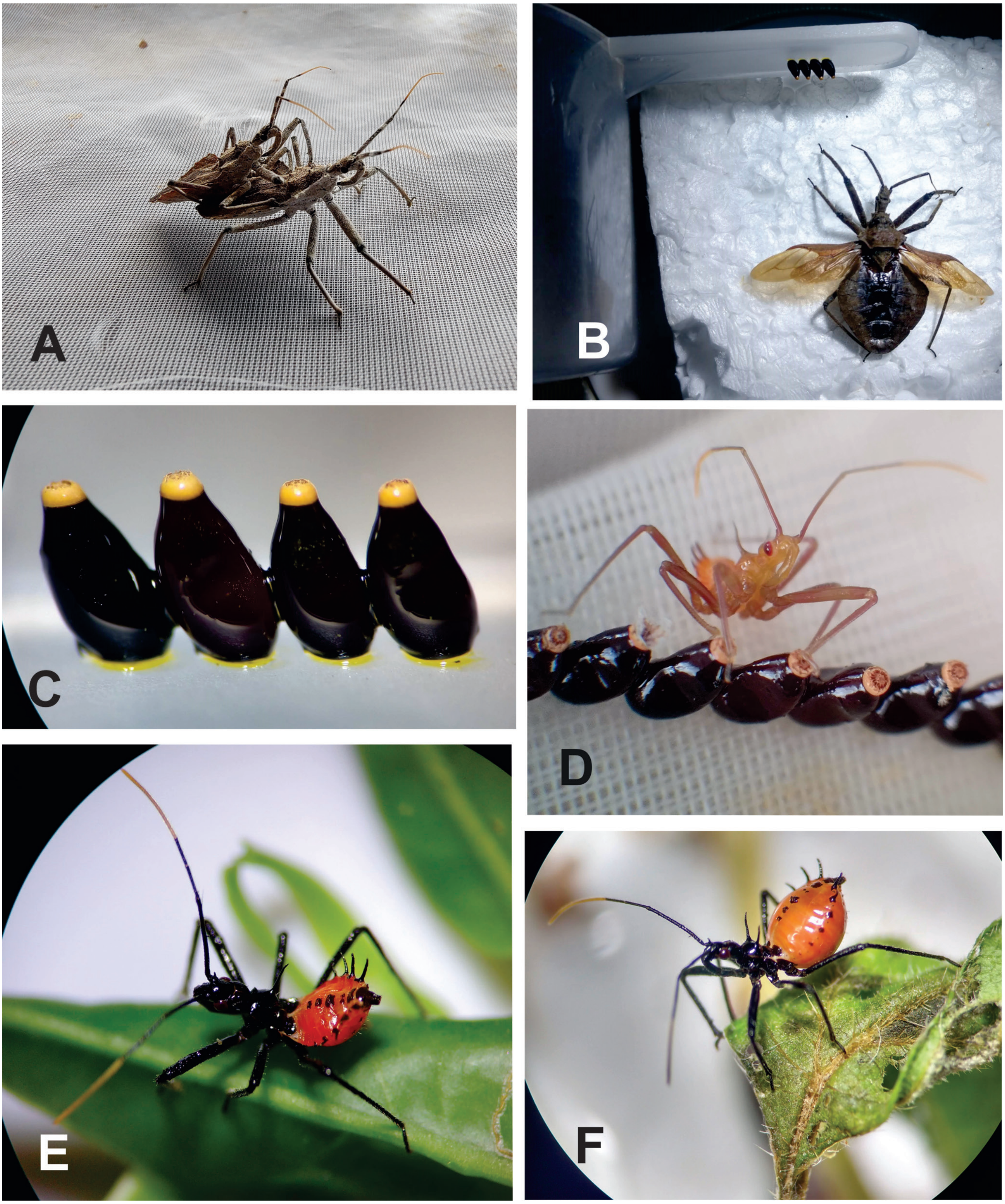

Figure 3. *Harpactor angulosus* (Lepeletier & Serville). (A) mating couple; (B) female and eggs; (C) detail of eggs; (D) newly hatched N1; (E) well sclerotized and starved N1; (F) N1 after feeding, with enlarged abdomen.

Table 1. Development time of the immature stages/instars obtained by a female collected in the field and a female raised in the laboratory.

	Stage/instar	Egg	N1	N2	N3	N4	N5	Adult (♂/♀)
Duration (days)	Female from the field	9 (n = 4)	9 (n = 2)	8 (n = 2)	10 (n = 2)	16 (n = 2)	49 ± 4 (n = 2)	90 ± 30 (n = 2)
	Female raised in laboratory	11.2 ± 0.5 (n = 8)	11.7 ± 0.8 (n = 6)	11 ± 1.6 (n = 5)	16 ± 1 (n = 2)	—	—	—

uals until N4 (Table 1). Unfortunately, the cycle of these nymphs could not be completed because of the voracity of the nymphs and the decline of *D. saccharalis* breeding.

The development time of the egg stage and the nymphal instars (N1, N2 and N3) recorded from a clutch obtained by the female collected in the field compared to a clutch obtained from the female raised in the laboratory were not significant (egg p = 0.26; N1 p = 0.07; N2 p = 0.28; N3 p = 0.33).

Morphology of nymphs:

N1: (Figs. 3D-F) Immediately after hatching N1 shows a pale orange coloration (Fig. 3D) that rapidly turns to black and brightly orange/bright red (Figs. 3E-F). It shows all body polished and black with the distiflagellomere and the abdomen orange; on the abdomen, it shows a black central macula from tergite 3 to the last one, and on the sides, a small black macula is present from segment 2 to 7. From these lateral maculae, appears a black lateral acute projection that increases its size to the back (Fig. 3E). The head shows a short acute spine posteriorly of the antennal tubercles. The pronotum shows a pair of long acute erect spines. After feeding, the abdomen is remarkably much enlarged (Fig. 3F).

N2: (Figs. 4A-B). Similar to N1, but the abdomen is paler, pale yellow to orange and the body starts to be covered with a dense white secretion. This secretion is more abundant on the dorsum, and it is patchy on the legs. The spines of the abdomen are less elongate but more stout.

N3: (Fig. 4C) Body mostly black, except for the apical half of basi- and the entire distiflagellomere, and the abdomen that are pale orange. The waxy secretions are more abundant on legs and labium than in previous instar, but it is absent on the most apical part of femora and most basal part of tibiae, and it appears but scarcely on scape and pedicel. Wing pads are short (not exceeding abdominal segment 2), but conspicuous, also covered with waxy whitish secretions.

N4: (Fig. 4D) Body black, except for the basal ⅓ of the basi- and the entire distiflagellomere. Body densely covered with waxy secretions all over. The lateral projections of the abdomen are shorter than in previous instar, as well as the two projections of the pronotum. The most basal part of the basi- and the entire distiflagellomere remain uncovered of the waxy whitish secretion. Wing pads are longer, exceeding the anterior margin of abdominal segment 3.

N5: (Fig. 4E) Body black, setose, covered with waxy whitish secretions but with some parts on pro- and mesonotum uncovered. Wing pads are longer than in previous instar, extending to abdominal segment IV.

Pikart *et al.* (2014) showed that the waxy secretion appears in N3, but we observed that it appears in N2. We have hypothesized two options that could explain the difference in wax appearance between studies. One explanation could be the different food used in both studies that could affect the epidermal cellular activity. Another option could be related to the need to protect the body from extreme temperatures (and subsequent water loss), as those registered when our study was conducted. One particular difference we observed with Pikart *et al.* (2014) study is that the N2 shows a bright red head and all the nymphs we studied show a black head.

It should be noted that nymphs raised with different preys did not present differences in coloration with the different diets.

Ecdysis: After each molt, the specimens covered their body with the waxy secretion, when turning into adults, the individuals are brightly orange (Fig. 4F), then turning darker and well covered by the waxy secretions.

Potentiality as a biocontrol agent: The voracity of *H. angulosus* to Lepidoptera larvae, and considering that it was observed feeding on larva of *Agraulis vanillae* in the field and in captivity, make us consider this assassin bug as a potential biological controller of *Agraulis* spp. Among other Lepidoptera, this Heliconiid is considered as an important pest of maracuyá crops (*Passiflora edulis* Sims) in Brazil (Fancelli & Martins Mesquita, 1998; Martins de Oliveira & Frizzas, 2014), Colombia (Hernández *et al.,* 2011), Mexico (Ruiz-Coronado *et al.,* 2020), and Venezuela (Dominguez-Gil & McPheron, 1992). The maracuyá is widely distributed in tropical and subtropical South America and it is an important crop in many countries (Ocampo *et al.,* 2007), so the potentiality of *H. angulosus* as a biocontroller of the Lepidoptera larvae that affect the normal development of the plant and the posterior commercialization of the fruit, would be an interesting topic to research.

Distribution: *Harpactor angulosus* was described from Brazil, and has been reported from Misiones (Berg, 1879; Pennington, 1920) and Salta (Coscarón & Martin-Park, 2011) in Argentina (Melo *et al.,* 2023). Here we reported the species for the first time from Buenos Aires and Tucumán provinces in Argentina, and Paraguay from specimens studied and deposited in the Entomological collection of the Museo de La Plata, La Plata, Argentina; and from Ecuador and Peru from observations from the iNaturalist platform.

Additional records from the citizen science platform iNaturalist, with our confident identification are included.

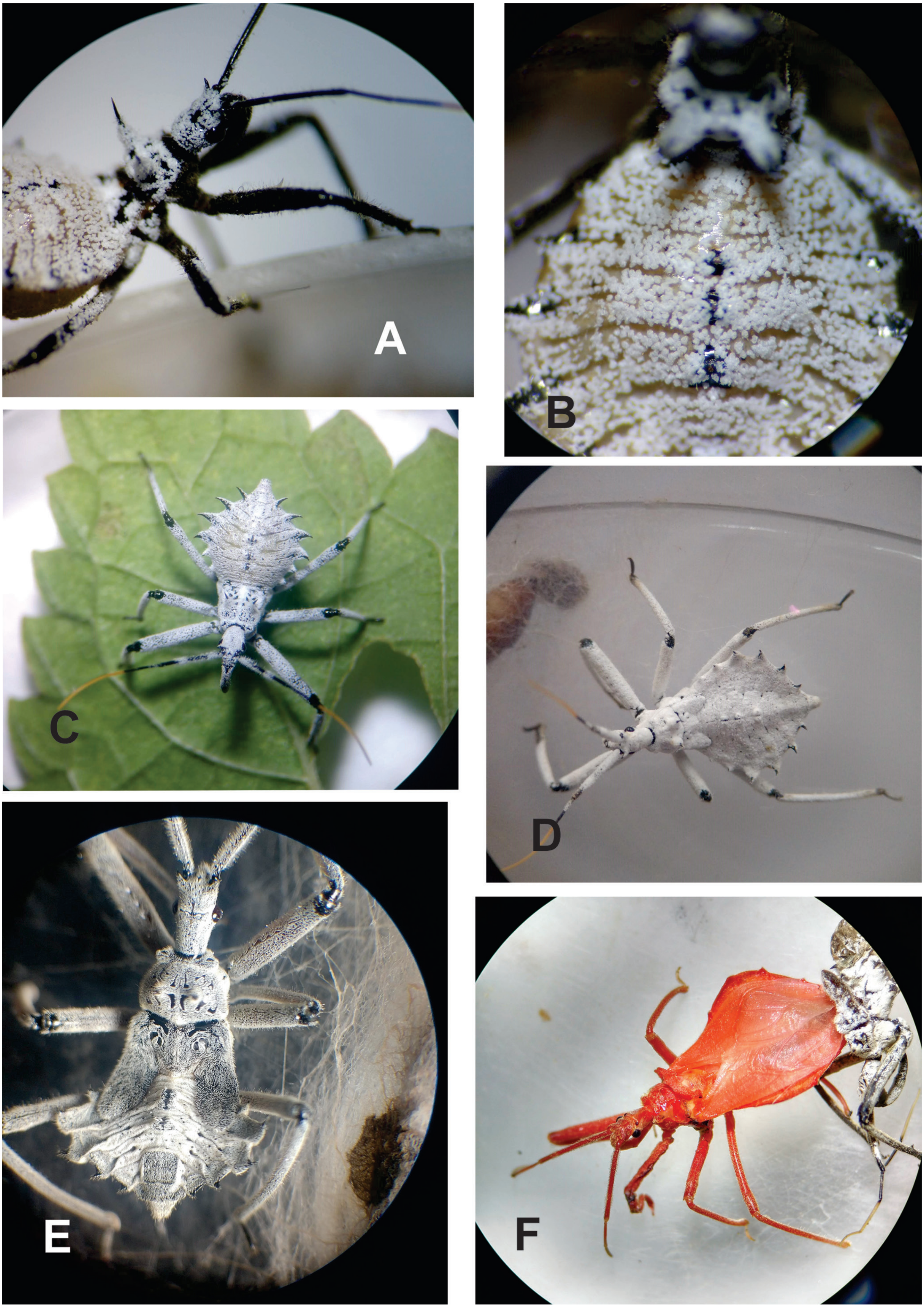

Figure 4. *Harpactor angulosus* (Lepeletier & Serville). (A) N2; (B) N2 abdomen; (C) N3; (D) N4; (E) N5; (F) molting adult.

COUNTRY	STATE/PROVINCE	iNaturalist RECORD
ARGENTINA	Misiones	https://www.inaturalist.org/observations/143461746
	Salta	https://www.inaturalist.org/observations/94058419
BRAZIL	Espirito Santo	https://www.inaturalist.org/observations/105509882
		https://www.inaturalist.org/observations/158772803
	Rio de Janeiro	https://www.inaturalist.org/observations/94647548
		https://www.inaturalist.org/observations/65014403
		https://www.inaturalist.org/observations/32760718
		https://www.inaturalist.org/observations/45043462
	Rio Grande do Sul	https://www.inaturalist.org/observations/104550028
		https://www.inaturalist.org/observations/17197177
	Paraná	https://www.inaturalist.org/observations/96519889
	Santa Catarina	https://www.inaturalist.org/observations/36712564
		https://www.inaturalist.org/observations/28186028
ECUADOR	Napo	https://www.inaturalist.org/observations/76351498
	Sucumbios	https://www.inaturalist.org/observations/107656276
	Zamora Chinchipe	https://www.inaturalist.org/observations/57673226
PERU	Cusco	https://www.inaturalist.org/observations/161116203
	Huánuco	https://www.inaturalist.org/observations/45152819
	Pasco	https://www.inaturalist.org/observations/103898395
	San Martin	https://www.inaturalist.org/observations/142124838
		https://www.inaturalist.org/observations/146362697

AUTHORS' CONTRIBUTIONS: MCM: Supervision; NM, MCM: Conceptualization, Methodology, Data Curation, Formal Analysis, Writing – original draft, Visualization, Investigation, Writing – review & editing, Funding Acquisition. All authors actively participated in the discussion of the results; they reviewed and approved the final version of the paper.
CONFLICT OF INTEREST: Authors declare there are no conflicts of interest.
FUNDING INFORMATION: This work was funded by the Secretaría de Ciencia, Arte e Innovación Tecnológica (SCAIT) PIUNT A721, and Facultad de Ciencias Naturales y Museo, UNLP by PI N904 and PI N905.
ACKNOWLEDGMENTS: We would like to thank Dra. Ing. Agr. Paula Gabriela Páez Jerez (FAZyV, Universidad Nacional de Tucumán, Argentina) for providing the Lepidoptera larvae for feeding the assassin bugs in the lab.

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Published with the financial support of the "Programa de Apoio às Publicações Científicas da Universidade de São Paulo"