

ORIGINAL ARTICLE

Bionomic notes of *Isyndus reticulatus* Stål (Hemiptera: Reduviidae)

Yisheng Zhao, Fan Song, Hu Li, Wanzhi Cai*

Department of Entomology and MOA Key Lab of Pest Monitoring and Green Management, College of Plant Protection, China Agricultural University, Beijing 100193, China

*Corresponding author, E-mail: caiwz@cau.edu.cn

Abstract The biological characteristics of the assassin bug *Isyndus reticulatus* Stål, 1868 (Hemiptera: Heteroptera: Reduviidae: Harpactorinae), especially its life history are provided in detail. And the assassin bug is redescribed here.

Key words Heteroptera, Reduviidae, Harpactorinae, bionomics, life cycle.

1 Introduction

The assassin bug *Isyndus reticulatus* Stål, 1868 is a large diurnal reduviid, perhaps best known for its potential use as a biocontrol agent against important agricultural and forestry pests, such as the lepidopterans *Dendrolimus punctatus* (Walker, 1855), *Bupalus piniarius* (Linnaeus, 1758), *Pieris rapae* (Linnaeus, 1758), and *Cinara formosana* (Takahashi, 1924) (Yi, 2004). The first insights into the biology of this species were provided by Hoffmann (1935), who reared this species in laboratory in Guangdong, southern China. He recorded its life history, described and illustrated the morphological characters of adults and nymphs, but did not mention other details, such as the number of generations and ecologically important behaviors. In present study, we describe and illustrate diagnostic characters of adult, and provide photos of different instars, which can be used for identifying any stages of this species in the field. The bionomics of this species is described in detail, primarily based on laboratory-reared specimens.

2 Materials and methods

Laboratory colonies were established in Beijing in 2013, based on specimens captured in Yunnan Province, China. The colonies were reared in plastic containers, under a largely constant temperature of $23\pm 2^{\circ}\text{C}$ and RH of $50\pm 7\%$. All instars were fed with yellow mealworms *Tenebrio molitor* Linnaeus, 1758. After eclosion, 20 first instar specimens were separated individually into plastic cups with an absorbent cardboard at the bottom, and they were offered food every third day. Each bug was maintained under a light incubator at $25\pm 2^{\circ}\text{C}$ and $60\pm 5\%$ RH, and were checked daily for ecdysis or death. The following instars were reared under similar conditions. Any special behavior was observed and noted.

Male genitalia of the reduviid were macerated in hot 10% KOH solution for approximately 5 minutes to remove soft tissue, subsequently rinsed in distilled water, and then dissected under a Motic binocular dissecting microscope. Dissected genitalia were preserved in vials with glycerol and pinned under the corresponding specimens. All drawings were made with a camera lucida. Morphological terminology follows Lent & Wygodzinsky (1979) and Davis (1966). Measurements were using a calibrated micrometer. Body length was measured from the apex of the head to the hemelytron in resting position. The maximal width of the pronotum was measured across the humeral angles. All measurements are in millimeters (mm).

3 Taxonomy

Isyndus reticulatus Stål, 1868 (Figs 1–14)

Isyndus heros Stål, 1858 (nec Fabricius): 445.

Isyndus reticulatus Stål, 1868: 101; Stål, 1874: 21; Lethierry & Severin, 1896: 187; Hoffmann, 1935: 145; China, 1940: 254; Hoffmann, 1944: 67; Hsiao & Ren, 1981: 495; Maldonado-Capriles, 1990: 221; Cai & Wang, 1998: 171.

Euagoras reticulatus: Walker, 1873: 121.

Isyndus reticulatus reticulatus: Dispons, 1969: 71; Putshkov & Putshkov, 1996: 242.

Coloration. Body dark brown to black. Most of anteocular portion, annulations on first antennal segment, basal half of second antennal segment, apices of third antennal segment, apical portion of fourth antennal segment, markings on pronotum, annulations on legs, markings on corium and connexiva orange to red brown (Fig. 1).

Structure. Body large, covered by dense, yellowish, short, appressed setae, intermixed with long erect setae. Interocular sulcus broad, ocelli-bearing area of postocular slightly protruding. Anterior pronotal lobe with 1+1 processes bilaterally; posterior lobe with distinct transverse rugosities, humeri ending into an acute spine with an obtuse denticle posteriad of its base. Posterior pronotal angles prominent backwards, posterior margin slightly concave. Scutellum equilaterally triangular, with a strongly impressed “Y”-shaped ridged, its subapical portion ending into a small cone-shaped process (Fig. 2). Hemelytron of female slightly surpassing tip of abdomen, that of male extending distinctly beyond tip of abdomen. Fifth and sixth connexival segments significantly expanded, abdominal sternum with glabrous spots (Figs 2–3). Median process of pygophore prominent, biramous, each ramus faintly subdivided into an outer small process and an inner large one (Figs 4–5). Paramere club-shaped, curved, with long setae (Figs 6–7); apical portion of phallosoma slightly raised (Fig. 8);

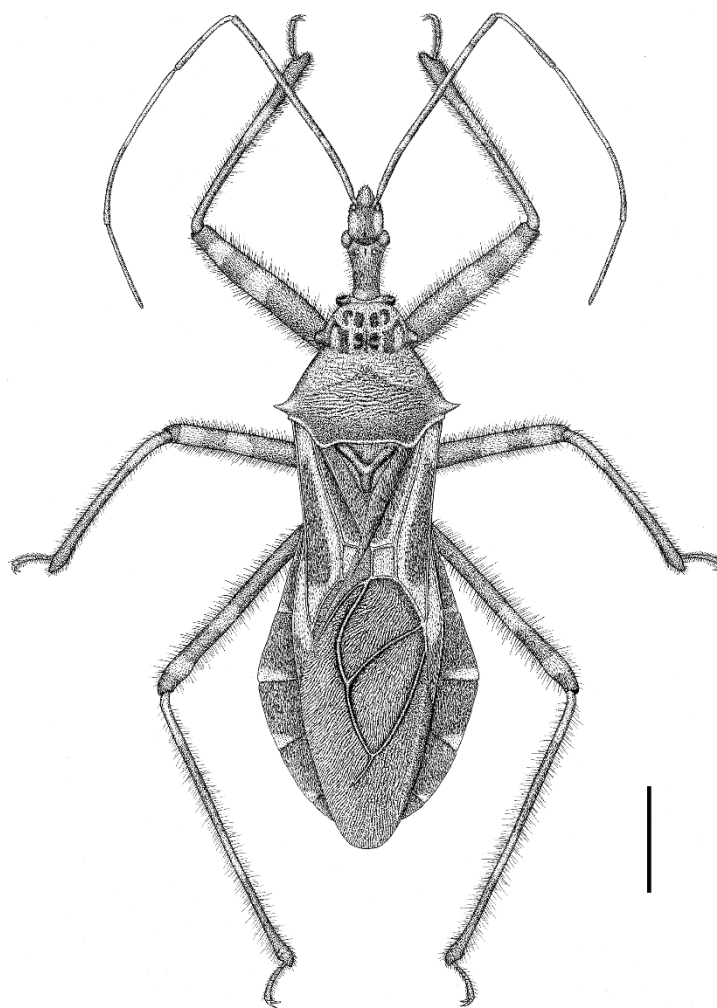
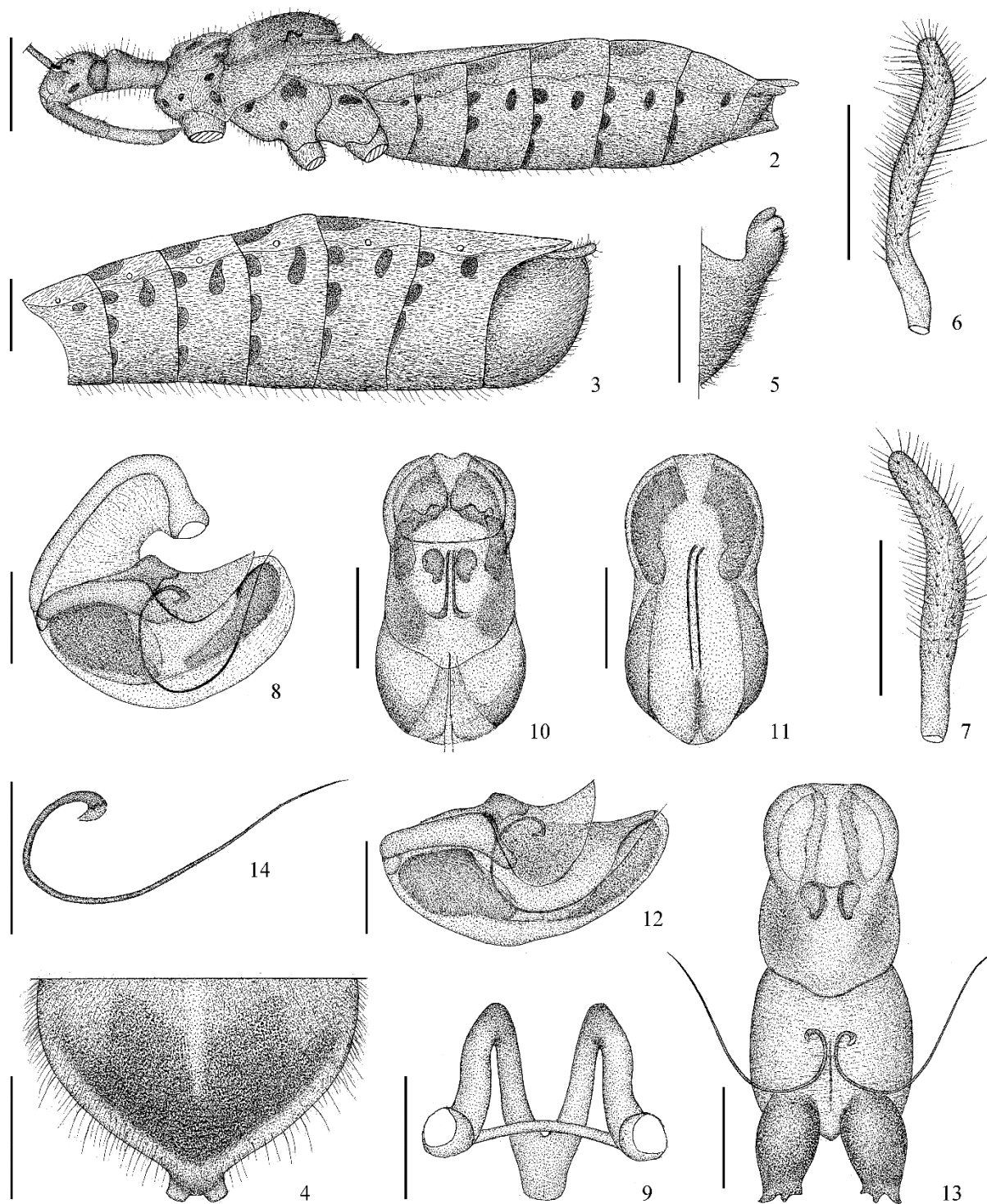


Figure 1. *Isyndus reticulatus* Stål, ♂, habitus. Scale bar = 3.0 mm.

articulatory apparatus with basal plate and basal plate bridge thin, pedicel short (Fig. 9); dorsal phallosomal sclerite with two small elliptical processes (Figs 10–11); dorsal conjunctiva processes whip-like, bent, apically tapering (Figs 12–14); basal portion of ventral processes concealed beneath dorsal phallosomal sclerite when endosoma is partly uneverted, dorsal and lateral endosoma processes podgy, apically provided with three small processes (Fig. 13).

Measurements (σ ($n=5$)/ φ ($n=6$)). Body length 18.40–20.10/23.40–25.50; maximum width of abdomen 4.64–5.01/



Figures 2–14. *Isyndus reticulatus* Stål. 2. Body of female, antennae and legs partly removed, lateral view. 3. Abdomen of male, lateral view. 4. Apical part of pygophore, ventral view. 5. Apex of pygophore, lateral view. 6–7. Different views of paramere. 8. Phallus, uneverted condition, lateral view. 9. Phallobase, dorsal view. 10–13. Phallosoma, partly everted condition. 14. Dorsal conjunctiva process, dorsal view. 10, 13. Dorsal view; 11. ventral view; 12. lateral view. Scale bars: 2–3=3.0 mm; 4, 6–14=1.0 mm; 5=2.0 mm.

7.87–8.97. Length of head 3.56–3.84/3.97–4.26; length of anteocular portion 1.40–1.49/1.70–1.99; length of postocular portion 1.51–1.60/1.64–1.80; length of interocellar space 1.09–1.23/1.27–1.46; length of synthlipsis 0.50–0.57/0.48–0.57; length of antennal segments I–IV= 6.83–8.22/7.94–9.94, 1.50–2.27/2.01–2.61, 5.36–7.84/6.94–7.86, 2.64–3.59/2.57–3.13; length of visible rostral segments I–III=1.73–1.93/1.71–2.37, 1.30–1.40/1.54–1.60, 0.48–0.71/0.71–0.73. Length of anterior pronotal lobe 1.44–2.14/1.76–2.11; length of posterior lobe 2.31–2.70/2.93–3.31; width of pronotum 5.21–5.71/6.84–7.40. Length of hemelytron 12.83–12.95/15.85–16.80.

Material examined. China, Yunnan, Mengla, 3♀5♂, 10.V.2009, leg. Hu Li; China, Guangxi, Nanning, 1♀, 16.V.1982, leg. Fasheng Li; China, Guangxi, Sanmeng, 1♀, 4.VI.1982, leg. Chi-kun Yang; China, Guangdong, Shixing, 1♀, 20.IV.1991, leg. Fasheng Li.

Distribution. China (Zhejiang, Jiangxi, Sichuan, Guizhou, Fujian, Guangdong, Guangxi, Yunnan, Hong Kong), India, Sri Lanka, Vietnam, Malaysia and Indonesia.

Notes. This species is a common harpactorine reduviid in southern China and is also broadly distributed in the Oriental Region. Dispons (1969) synonymized *I. pilosipes* Reuter, 1881 and *I. modestus* Distant, 1919 with this species, but the validity of Dispons’ opinion is still debated (Cai & Wang, 1998).

4 Bionomics

4.1 Life history

Isyndus reticulatus is a bivoltine assassin bug, living on trees, with five instars (Yi, 2004). Under laboratory conditions in Beijing, mature adults mate from late April to early May, and females lay eggs in early May to late June. From late May to August, nymphs of the first generation develop from eggs to fifth instar (Figs 15–21). The peak of emergence of new adults (Fig. 22) occurs in late July to early August. The first generation mates and lays eggs from late September to October. About twenty days later, the nymphs of the second generation appear and develop from early October to late December. Fifth instar appears in early December (Table 1). The development durations of the same instar of the two generations are very similar, with the exception that adults of the second generation overwinter from January to March and live longer than those of the first (Table 2).

Table 1. Life history of *Isyndus reticulatus* Stål (2013–2014, Beijing).

Month	1–3	4	5	6	7	8	9	10	11	12
	★★★	☆☆☆	☆☆☆ ⊙⊙⊙ Δ	⊙⊙⊙ ΔΔΔ	ΔΔΔ ☆	ΔΔΔ ☆☆☆	☆☆☆ ⊙	⊙⊙⊙ ΔΔΔ	ΔΔΔ	ΔΔΔ ☆☆★

⊙—Eggs; Δ—nymphs; ☆—adults; ★—overwintering adult

Table 2. The development duration of *Isyndus reticulatus* Stål.

Stage	First generation (days)			Second generation (days)		
	Minimum	Maximum	Average	Minimum	Maximum	Average
Egg	19	32	24.21±4.67	17	21	18.36±0.18
1st instar	11	28	17.27±4.92	12	19	18.54±0.31
2nd instar	8	17	12.30±2.84	10	22	15.00±1.03
3rd instar	9	22	14.07±3.98	12	23	15.82±1.06
4th instar	10	23	15.21±4.32	16	22	18.50±0.88
5th instar	16	24	20.21±2.80	22	28	24.40±1.03
Adult	41	58	50.17±2.92	95	108	103.25±1.03

4.2 Predatory behavior

Nymphs and adults of this reduviid mainly feed on the larvae of Lepidoptera or other insects (Yi, 2004). The process of prey capture follows a similar pattern with that of *Agriosphodrus dohrni* (Signoret, 1862) and *Platymeris biguttatus* (Linnaeus, 1767), involving the following steps: exciting, prey location, approaching, paralyzing, sucking, releasing and cleaning (Li *et al.*, 2010; Luo *et al.*, 2010). Similar to other reduviids, they always choose an intersegmental membrane of their prey to stab and inject a toxic salivary secretion (Fig. 23). If interrupted in this period, the assassin bug will drag the prey with its rostrum and remove it to a safer place. Feeding on a single prey item is usual in nymphs but not in adults, and it depends on prey size.

4.3 Mating

Sexually mature males of *I. reticulatus* become excited when encountering females, swaying their antennae and lifting their fore legs. The male then tries to excite the female, by touching her body with the apex of his fore tibiae while both sexes touch each other with their antenna intensely (Fig. 24). These actions are repeated several times and last for a few minutes.

After courting, the male will try to climb on the back of the female. The female always flees away and the male has to try to climb several times. After successful mounting, the male inserts his rostrum into the transverse sulcus of the pronotum of the female to fasten his body onto her (Fig. 25). The male's legs will hold the body of the female. This "clasping and riding" behavior always lasts for a long time (Fig. 26). Finally, the male's body inclines to one side and curves the tip of his abdomen to secure his pygophore with the female's genitalia and start the copulation. After mating, the couples separate. Following copulation, the adults will clean their body, including legs, antennae and genitalia, in a manner similar to that after predation.

4.4 Oviposition

Gravid females of *I. reticulatus* generally attach a cluster of eggs (Figs 27–28) on the stems or leaves of trees. While placing the eggs, the females work from the base to the tip of the eggs mass, sticking the eggs vertically with copious secretions from their accessory glands, thus providing their egg masses with a protective layer. The whole egg mass contains 40–60 eggs (Figs 29–30).

4.5 Hatching, molting, and emergence

Eggs will turn dark after three weeks following oviposition, and finally hatch. During hatching, the head of the nymphs emerges first, by completely removing the egg cap (Fig. 31). Then the fore legs stretch out (Figs 32–34), followed by the mid legs (Fig. 35), then the rostrum and the antennae (Fig. 36). Finally, after stretching its appendages, the nymph sway its body until it leaves the chorion completely (Figs 37–38). The newly hatched nymphs are initially overall yellow, and a few hours later they become darker as their cuticle hardens. More than 20 hours later, their bodies become black and orange (Fig. 17).

Before molting, the nymphs become satiated and stop preying, they usually cling on a cardboard and hang upside down. At first, the ecdysial cleavage line splits open on the pronotum, increasing in size from the front edge of the eyes to the first abdominal segment, then the head and the pronotum emerge. After that, the appendages are stretched out of the exuvium.

The process of emergence is similar to that of molting. The difference is that the wings emerge directly from the longitudinal crevice of the pronotum of the nymph exuviae, and then fore-legs, mid-legs, hind-legs and antennae emerges orderly.

4.6 Aggregation and cannibalism

Aggregation occurs in the immature stages of this species. The nymphs hatching from the same egg mass usually remain together, and sometimes they attack together and share one prey. The adults are active and live alone. By laboratory observation, when the population density is high, the newly hatched or molted individuals are frequently cannibalized by other nymphs and adults. It was once believed that the cause of such phenomenon is the lack of food (Luo *et al.*, 2010).



Figures 15–22. Life history of *Isyndus reticulatus* Stål. 15–16. Egg mass. 17–21. Nymphs, first to fifth instars. 22. Adult.



Figures 23–30. Behaviors of *Isyndus reticulatus* Stål. 23. Predation. 24–26. The clasping and riding process of mating behavior. 27–30. Oviposition.

5 Discussion

Isyndus reticulatus and most other species in the subfamily Harpactorinae, are diurnal and commonly found on plants,



Figures 31–38. Hatching process of *Isyndus reticulatus* Stål.

but some species are ground-dwelling or bark-inhabiting (Weirauch *et al.*, 2014). Oviposition and mating behavior of *Isyndus reticulatus* is much like that of other insects in Harpactorinae (Luo *et al.*, 2010; Ambrose & Ganesh-Kumar, 2016). Compared with other subfamilies, the harpactorines lay the highest number of eggs which are laid in clusters and cemented to each other and the substratum (Ambrose & Ambrose, 2009). They also have the shortest incubation period, higher hatchability, lower nymphal mortality and a higher number of generations (e.g., bivoltine or multivoltine) than other subfamilies (Srikumar *et al.*, 2014; Ambrose & Ganesh-Kumar, 2016). Some special behaviors are also found in Harpactorinae, such as the maternal care (Tallamy *et al.*, 2004), specialized feeding on termites (Hwang & Weirauch, 2012), and the use of endogenous sticky secretions or plant resins to aid in prey capture (Forero *et al.*, 2011; Zhang & Weirauch, 2014).

Predatory behavior of assassin bugs is similar and can be categorized as arousal, approach, capture, rostral probing, paralyzing, sucking, and postpredatory behavior (e.g., releasing and cleaning) (Ambrose, 1999; Li *et al.*, 2010; Luo *et al.*, 2010; Evangelin *et al.*, 2014). They have developed a wide variety of prey capture strategies and exhibit prey and stage preferences (Zhang & Weirauch, 2011; Ambrose & Ganesh-Kumar, 2016). The harpactorines usually feed on soft prey, such as caterpillars, grubs, and termites, and some species are explored as natural enemies (e.g., Grundy & Maelzer, 2000). Despite the rich diversity of Reduviidae in China, the knowledge of their biological information is limited. The conservation and utilization for integrated pest management (IPM) can be achieved with a comprehensive understanding of their biology and ecology in the future studies.

Funding This work was supported by grants the National Natural Science Foundation of China (31420103902).

Acknowledgements We are particularly grateful to the valuable comments and constructive suggestions from the editor and two reviewers.

References

- Ambrose, D.P. 1999. *Assassin Bugs*. Oxford & IBH Publishing Company Private Limited, New Delhi, India and Science Publishers, Incorporation, New Hampshire, USA. 337 pp.
- Ambrose, D.P., Ambrose, A.D. 2009. Predation, copulation, oviposition and functional morphology of tibia, rostrum and eggs as tools in the biosystematics of Reduviidae (Hemiptera). *Indian Journal of Entomology*, 71(1): 1–17.
- Ambrose, D.P., Ganesh-Kumar, A. 2016. Reduviid predators. In: Omkar, O. (ed.). *Ecofriendly Pest Management for Food Security*. Academic Press, Cambridge, Massachusetts. pp. 217–257.
- Cai, W.Z., Wang, Y.B. 1998. A review of Chinese *Isyndus* Stål (Hemiptera: Reduviidae: Harpactorinae). *Acta Entomologica Sinica*, 41: 163–179.
- China, W.E. 1940. Key to the subfamilies and genera of Chinese Reduviidae with descriptions of new genera and species. *Lingnan Science Journal*, 19: 205–255.
- Davis, N.T. 1966. Contributions to the morphology and phylogeny of the Reduivoidea (Hemiptera: Heteroptera). Part III. The male and female genitalia. *Annals of the Entomological Society of America*, 59: 911–924.
- Dispons, P. 1969. Note sur le genre *Isyndus* Stål. *L'Entomologiste*, 25: 66–72.
- Evangelin, G., Bertrand, H., Muthupandi, M., William, J.S. 2014. Feeding behaviour of the predatory reduviid, *Rhynocoris kumarii* (Hemiptera: Reduviidae). *International Journal of Life Sciences*, 3(2): 64–69. doi: 10.5958/2319-1198.2014.01087.2.
- Forero, D., Choe, D.H., Weirauch, C. 2011. Resin gathering in neotropical resin bugs (Insecta: Hemiptera: Reduviidae): functional and comparative morphology. *Journal of Morphology*, 272: 204–229. doi: 10.1002/jmor.10907.
- Grundy, P.R., Maelzer, D.A. 2000. Assessment of *Pristhesancus plagipennis* (Walker) (Hemiptera: Reduviidae) as an augmented biological control in cotton and soybean crops. *Australian Journal of Entomology*, 39: 305–309. doi: 10.1046/j.1440-6055.2000.00182.x.
- Hoffmann, W.E. 1935. The bionomics and morphology of *Isyndus reticulatus* Stål (Hemiptera, Reduviidae). *Lingnan Science Journal*, 14: 145–153.
- Hoffmann, W.E. 1944. *Catalogue of Reduviidae of China*. Lingnan University Science Bulletin, No.10, Chanton. 80 pp.
- Hsiao, T.Y., Ren, S.Z. 1981. Reduviidae, In: Hsiao, T.Y., Ren, S.Z., Zheng, L.Y., Jing, X.L., Zou, H.G., Liu, S.L. (eds). *A Handbook for the Determination of the Chinese Hemiptera-Heteroptera (II)*. Science Press, Beijing. pp. 390–538.
- Hwang, W.S., Weirauch, C. 2012. Evolutionary history of assassin bugs (Insecta: Hemiptera: Reduviidae): insights from divergence dating and ancestral state reconstruction. *PLoS ONE*, 7(9): 1–12. doi: 10.1371/journal.pone.0045523.
- Lent, H., Wygodzinsky, P. 1979. Revision of the Triatominae (Hemiptera, Reduviidae) and their significance as vectors of Chagas' disease. *Bulletin of the American Museum of Natural History*, 163: 125–520.

- Lethierry, L., Severin, G. 1896. *Catalogue Général des Hémiptères. Tome III. Hétéroptères*. R. Friedländer & Fils, Berlin. 275 pp.
- Li, H., Zhao, G.Y., Cao, L.M., Xu, K., Cai, W.Z. 2010. Taxonomic and bionomic notes on the white spot assassin bug *Platyeris biguttatus* (Linnaeus) (Hemiptera: Reduviidae: Reduviinae). *Zootaxa*, 2644: 47–56. doi:10.5281/zenodo.198670.
- Luo, X.Y., Zhou, D.K., Li, H., Cheng, W., Cai, W.Z. 2010. Taxonomic and bionomic notes on *Agriosphodrus dohrni* (Signoret) (Hemiptera: Reduviidae: Harpactorinae). *Zootaxa*, 2358: 57–67. doi: 10.5281/zenodo.193604.
- Maldonado-Capriles, J. 1990. *Systematic Catalogue of the Reduviidae of the World (Insecta: Heteroptera)*. Caribbean Journal of Science, Puerto Rico. 694 pp.
- Putshkov, P.V., Putshkov, V.G. 1996. Family Reduviidae Latreille, 1807, assassin-bugs. In: Aukema, B., Rieger, C. (eds.). *Catalogue of the Heteroptera of the Palearctic Region, volume 2 Cimicomorpha I*. The Netherlands Entomological Society, Amsterdam, pp. 148–265.
- Srikumar, K.K., Bhat, P.S., Raviprasad, T.N., Vanitha, K., Saroj, P.L., Ambrose, D.P. 2014. Biology and behavior of six species of reduviids (Hemiptera: Reduviidae: Harpactorinae) in a cashew ecosystem. *Journal of Agricultural and Urban Entomology*, 30: 65–81. doi: 10.3954/JAUE14-14.1.
- Stål, C. 1858. Hemipterologiska bidrag. *Öfversigt af Kongliga Vetenskapsakademiens Förhandlingar*, 15: 433–454.
- Stål, C. 1868. Hemiptera Fabriciana. *Kongliga Svenska Vetenskapsakademiens Handlingar (N.F.)*, 7: 1–148.
- Stål, C. 1874. Enumeratio Hemipterorum, IV. *Kongliga Svenska Vetenskapsakademiens Handlingar (N.F.)*, 22: 1–182.
- Tallamy, D.W., Walsh, E., Peck, D.C. 2004. Revisiting paternal care in the assassin bug, *Atopozelus pallens* (Heteroptera: Reduviidae). *Journal of Insect Behavior*, 17: 431–436.
- Walker, F. 1873. *Catalogue of the Specimens of Hemiptera Heteroptera in the Collection of the British Museum. Part VII*. British Museum, London. 123 pp.
- Weirauch, C., Bérenger, J.M., Berniker, L., Forero, D., Forthman, M., Frankenberg, S., Freedman, A., Gordon, E., Hoey-Chamberlain, R., Hwang, W.S., Marshal, S.A., Michael, A., Paiaro, S.M., Udah, O., Watson, C., Yeo, M., Zhang, G., Zhang, J. 2014. An illustrated identification key to assassin bug subfamilies and tribes (Hemiptera: Reduviidae). *Canadian Journal of Arthropod Identification*, 26: 1–115. doi:10.3752/cjai.2014.26.
- Yi, G. 2004. Observation on biological characteristics of *Isyndus reticulatus* Stål. *Guangdong Forestry Science and Technology*, 20(2): 44–46.
- Zhang, G., Weirauch, C. 2011. Sticky predators: a comparative study of sticky glands in harpactorine assassin bugs (Insecta: Hemiptera: Reduviidae). *Acta Zoologica*, 94(1): 1–10. doi: 10.1111/j.1463-6395.2011.00522.x.
- Zhang, G., Weirauch, C. 2014. Molecular phylogeny of Harpactorini (Insecta: Reduviidae): correlation of novel predation strategy with accelerated evolution of predatory leg morphology. *Cladistics*, 30(4): 339–351. doi: 10.1111/cla.12049.