



Morphotaxonomy and external genitalic attributes of a biological control agent: *Perillus bioculatus* (Fabricius) (Heteroptera: Pentatomidae: Asopinae) with new distributional records from India

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Abstract

Perillus bioculatus (Fabricius) was collected during the faunistic survey from various localities of North India. The morphotaxonomic details along with the male and female external genitalia of *Perillus bioculatus* (Fabricius) have been studied and elaborated with well labeled and colored illustrations. A review of the species is also cited. New distributional localities from India (Punjab & Himachal Pradesh) have also been added to the previous literature.

Keywords: *Perillus bioculatus* (Fabricius), external genitalia, morphotaxonomic, North India, illustrations

Introduction

The genus *Perillus* was first proposed by Stål (1862) [24] based on type species *Asopus confluens* Herrich-Schaeffer. It is represented by 7 predatory species from the world viz., *Perillus bioculatus* (Fabricius, 1775) [9], *Perillus circumcinctus* Stål, 1862 [24], *Perillus confluens* (Herrich-Schaeffer, 1839) [11], *Perillus exaptus* (Say, 1825) [21], *Perillus lunatus* Knight, 1952 [15], *Perillus splendidus* (Uhler, 1861) [28] and *Perillus strigipes* (Herrich-Schaeffer, 1853) [12]. All the members are exclusively predaceous and are used as biological control agents in agricultural fields especially for the control of severe damage done by various lepidopteran and coleopteran larvae.

Palmer and Knight (1924) [16] did experimental work on *Perillus bioculatus* (Fabricius) (Family: Asopinae) to study its red and yellow polymorphic forms. Their study revealed the presence of carotin as the principal cause of varied colored forms, and its biological origin from the lymph of potato-beetle *Leptinotarsa decemlineata* (Say) on which it was feeding. Knight (1952) [15] revised the genus *Perillus* in order to study the inheritance of color patterns in this species and included the detailed biological study. He concluded that *Perillus bioculatus* Fab. is highly variable in color pattern, ranging from the white and black *clanda* Say to nearly solid black males in the extreme dark forms.

Furth, D.G. (1974) [10] provided a check list of the 53 taxa of pentatomidae present in Ohio and in the surrounding states. He provided the key to the 4 species of *Perillus* from Ohio that also included a variety of *Perillus bioculatus* (Fabricius). The species were, *Perillus exaptus* (Say), *Perillus circumcinctus* Stål, *Perillus bioculatus* (Fabricius) and *Perillus bioculatus* var. *clanda* (Say). The existing synonymy along brief description was also provided.

Rojas *et al.*, (2000) [20] studied the effect of two artificial diets (devoid of insect components) for the predator *Perillus bioculatus* (Fabricius). The diets were based on chicken liver and tuna fish to approach the nutritional characteristics of the primary insect prey, *Leptinotarsa decemlineata* (Say) eggs. Their study revealed longer developmental time and

preovipositional period, and egg viability, survival from egg to adult, and fecundity were significantly lower in *P. bioculatus* individuals reared on artificial diets than in those reared on *L. decemlineata* eggs.

Adams, T.S. (2001) [1] studied the internal reproductive systems of male and female *Perillus bioculatus* in which the transfer of male and female products was analysed in detail during mating process. The closure and opening of valves of spermatheca at the time of mating and passage of male secretions into the spermathecal pouch was revealed. Further, the post mating events showed the variation in volume of male ejaculatory pouch volume which was decreased to 86%, was completely recharged within 24 h of the completion of mating. Similarly, spermathecal pouch volume increased more than twofold after mating for 0.5 h. Their ejaculatory system also showed presence of three soluble glycoproteins, pi, p2, and p3, with molecular masses of 101.9, 86.3, and 72.8 kDa, respectively, which were transferred to the female spermathecal pouch during mating. Coudron & Kim (2004) [4] worked on the life history and Cost Analysis for Continuous Rearing of *Perillus bioculatus* (Heteroptera: Pentatomidae) on a Zoophytogenous Artificial Diet. They examined the impact of a zoophytogenous, insect-free artificial diet on the rate of developmental, life history parameters, and fertility on over 11 consecutive generations for domesticated *Perillus bioculatus* (F.) (Heteroptera: Pentatomidae). Their study showed that insect-free artificial diet prolonged developmental times during nymphal and adult stages whereas the net reproductive rates (R_0) and the intrinsic rates of increase (r_m) were significantly found lower than when fed with larval *Trichoplusia ni*. Their study also revealed that cost to rear *P. bioculatus* on the artificial diet approached 1.1 times the cost of rearing *P. bioculatus* on natural prey. Kivan (2004) [14] recorded *Perillus bioculatus* (Fabricius) for the first time from Turkish fauna as an accidental introduction which is a predator on Colorado potato beetle, *Leptinotarsa decemlineata* (Say), native to North America. Rivard *et al.*, (2004) [19] showed jasmonate and arachidonate as potential

inducers of providing partial resistance to the potato beetle in potato, which suggested that digestive compensation in herbivorous insects is determined, at least in part, by defense-related compounds found in the plant in response to different stress stimuli or as a result of ectopic expression in transgenic plants.

Sites *et al.*, (2012) [23] reported the distributional records of *Perillus bioculatus* (Fabricius), *Perillus circumcinctus* Stål, 1862, *Perillus exaptus* (Say, 1825), and *Perillus strigipes* (Herrich-Schaeffer, 1853) from Missouri. Prasad & Pal (2015) [17] investigated the species composition of two spotted stink bug, *Perillus bioculatus* (Fab.) from Meerut (Uttar Pradesh), India. They studied the life cycle of the bug in laboratory which was found to complete its life cycle on an average in 22.33 days. The feeding potential of different stages of bug was also studied that showed a single bug consumed 22.0 grubs (ranged 20-22 grubs) during its life period. Tarla and Tarla (2018) [27] reported *Perillus bioculatus* on a new host i.e. Poplar leaf beetle in Anatolia (Asian portion of Turkey). Chandra *et al.*, (2019) [3] faunistic surveys revealed the presence of four morphs showing color polymorphism and reported new distributional records from Madhya Pradesh. The present paper includes for the first time, the detailed and colored illustrations of morphological structures viz., Head, pronotum, scutellum, osteolar peritreme, ventral structures of abdomen along with photographs of well labelled male and female external genital structures of *Perillus bioculatus* (Fabricius).

Materials and Methods:

Individuals belonging to this genus were collected from different localities of North Indian states i.e. Punjab, Haryana, Himachal Pradesh, Uttarakhand, NCR region of Delhi and parts of Jammu and Kashmir. The collection was identified using available literature and the current taxonomic status of studied species was finalized as per Distant's (1902, 1908, 1918) [6, 7, 8] faunistic works and updated World catalogue of Pentatomidae by Rider (2006-2012) [18]. The methodology for studying external genitalia was adopted as per procedure given by Kaur (2012) [13]. The study has also reported the new distributional records of the species from North India. The photographs of diagnostic external morphological and genital structures were taken with the help digital camera installed in the research laboratory.

Observations and Discussion

Genus *Perillus* STÅL

Perillus, Stål (1862) [24] *Stettin. ent. Ztg.*, 23(1): p. 88.

Mineus, Stål (1867) [25] *Öfv. Vet.-Ak. Förh.*, p. 498.

Gordonerius, Distant (1887) [5] *Tr. E. S.*, p. 343.

Perilloides, Schouteden (1907) [22] *Genera Insectorum Fasc.*, 52: p. 37.

Perillus, Knight (1952) [15] *Ann. Entomol. Soc. Am.*, 45: p. 229.

Type species: *Asopus confluens* Herrich-Schaeffer

Diagnosis of the Genus: Labium is crassate and reach upto mesocoxae; IInd labial segment is longer, IIIrd is the shortest. IIIrd and IVth segments combined together are longer than or equal to IInd segment; bucculae are closed from behind; paraclypei and clypeus are subequal in length; anterolateral pronotal margins are obtuse and rectilinear; humeral angles are not produced; frenal margin of scutellum is slightly

longer than postfrenal part; scutellar apex is as wide as corium; scent gland osteolar peritreme is flat and extends about half way to metapleural margin; base of abdomen is with short and stoutly forwardly directed tubercle; prefemora is armed with preapical tubercle; protibiae is prismatic; males are with a pair of glandular patches of silky hairs on abdominal venter.

Perillus bioculatus (Fabricius)

(Figs. 1)

Cimex bioculatus, Fabricius (1775) [9] *Syst. Ent.*, p. 715.

Pentatoma clanda, Say (1825) [21] *J. Acad. Nat. Sci. Phila.*, 4: p. 312.

Perillus claudus, (sic) Uhler (1876) [29] *Bull. Geol. & Geogr. Surv. Terr.*, 1: p. 281.

Oplonus virgatus, Stål (1862) [24] *Stett. Entomol. Zeit.*, 23: p. 89.

Perillus bioculatus, Stål (1872) [26] *Kongl. Svensk. Vet.-Akad. Handl.*, 10: p. 129.

Mineus bioculatus, Uhler (1886) [30] *Brooklyn Entomol. Soc. New York*: p. 4.

Perilloides bioculatus, Schouteden (1907) [22] *Genera Insectorum Fasc.*, 52: p. 37.

Perillus bioculatus var. *claudus*, Caesar (1912) [2] *Annu. Rpt. Entomol. Soc. Ontario.*, 42: p. 33.

Perillus bioculatus: Knight (1952) [15] *Ann. Entomol. Soc. Am.*, 45: p. 229.

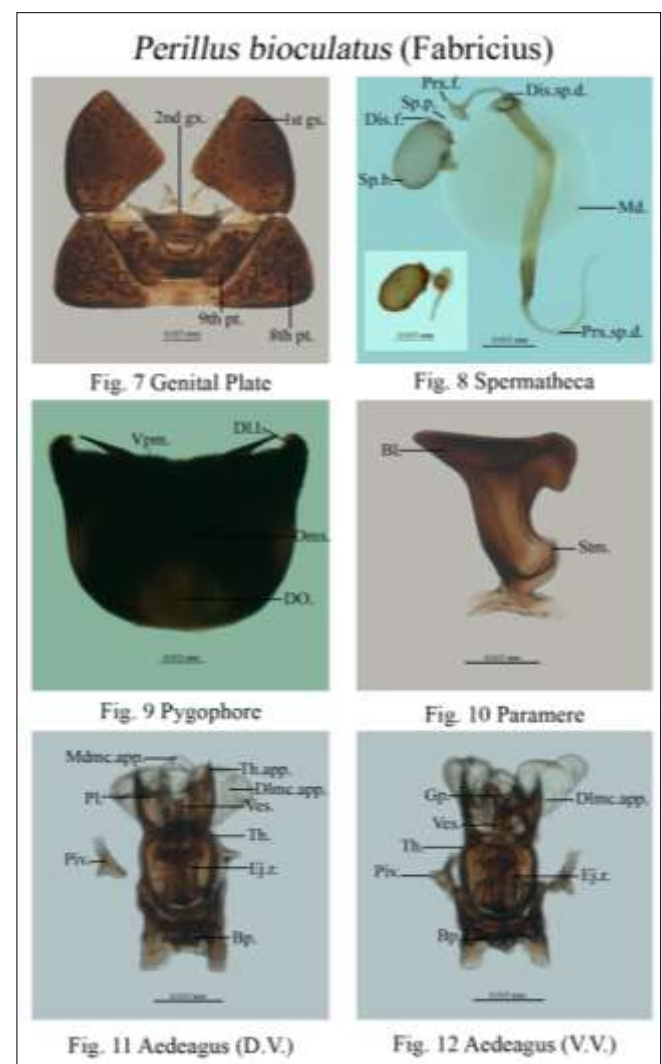


Fig 1

Male

Measurements (in mm): Head length: 1.80; width across eyes: 2.09; interocular space: 1.20; interocellar space: 0.70; preocular distance: 0.80; length of posterior head including eyes: 1.0; antennal segments lengths: Ist: 0.40, IInd: 1.0, IIIrd: 1.02, IVth: 1.10, Vth: 1.10; pronotal length: 3.04; width across humeral angles: 6.40; scutellar length: 4.0; width: 4.25; length of abdomen: 4.50; maximum width of abdomen: 5.94; length of rostral segments: Ist: 0.92, IInd: 1.10, IIIrd: 0.50, IVth: 0.60; Distance - base scutellum-apex clavus: 2.30, apex clavus-apex scutellum: 1.70, apex scutellum-apex abdomen including membrane: 2.80; length of abdominal spine: 0.80; total body length: 10.50

Dorsal Coloration: Head is pitch black with punctures; eyes are dark brownish; ocelli dark red; antennal segments are black; pronotum is sparingly punctate, bicolorous with anterior three-fourth portion reddish, rest is black; anterior pronotal area is with two centrally broken broad transverse black punctate callosities; scutellum is reddish except medial Y-shaped black fascia that do not reach extreme ends, lateral margins of scutellum, clavus and corium are black and finely punctured; anterior most area of costal margin is reddish, rest area is black; membrane is black; connexiva with lateral margins and last segment is impunctate and reddish in color, inner margins are black and punctate.

Ventral Coloration: Labium, ventral area of head, lateral area of sternum, metathoracic scent gland peritreme, area between thoracic legs are black; pleural margins are reddish, except ventroposterior area of prosternum with incomplete brownish-yellow transverse fascia that reach just half way towards pleural margin; abdominal tubercle is red; abdomen is reddish in color with broad continuous fascia on entire Vth, VIth and anterior half of VIIth abdominal segments. IVth segment is with two large fascia on lateral sides and a small on mediolateral sides; spiracles are black; legs are black and pubescent, mid and hind tibia with paler areas.

Structure

Head (Fig. 2): Head is broader than long, rugulose with irregular callosities, strongly declivent, rectilinear at apex; paraclypeus and clypeus are equal in length, lateral margins of former are slightly sinuous above eyes, reflexed upwards, clypeus of head is slightly raised medially; antennal length formula is: I<II<III<IV=V; labium is robust reaching mesocoxae, IIIrd labial segment is the smallest and broad, labial length formula is: III<IV<I<II. Thorax: Pronotum (Fig. 3) is deflected downwards, distinctly broader than long, its anterior margin is concave, depressed and truncated behind eyes, produced into small obtuse tubercles that are directed anterolaterad in position; anterolateral margins of pronotum are slightly crenulated, smooth and medially sinuous; humeral angles are subprominent and obtusely angulated; scutellum is as long as broad; frenal region is slightly longer than post frenal area, beyond frena, the margins are slightly sinuous and are produced as long narrow apical impunctate lobe; metathoracic scent gland ostiole peritreme (Fig. 4) is broad, medially concave, curved and rounded at apex, reaching halfway to metapleural margin; evaporatorium is completely surrounding the peritreme; legs with protibia are flattened with antepical small tubercle; prefemora is with broad and blunt antepical tubercle; Abdomen: Venter is moderately ampliate; abdominal tubercle arise from base of IIIrd abdominal segment, reaching metacoxae in opposition to

labium; connexiva is exposed at repose dorsally; ventroanterior and ventroposterior margins of VIIth abdominal sternite is medially concave, somewhat an inverted U-shaped (Fig. 5); hemelytral membrane passes beyond the apex of abdomen.

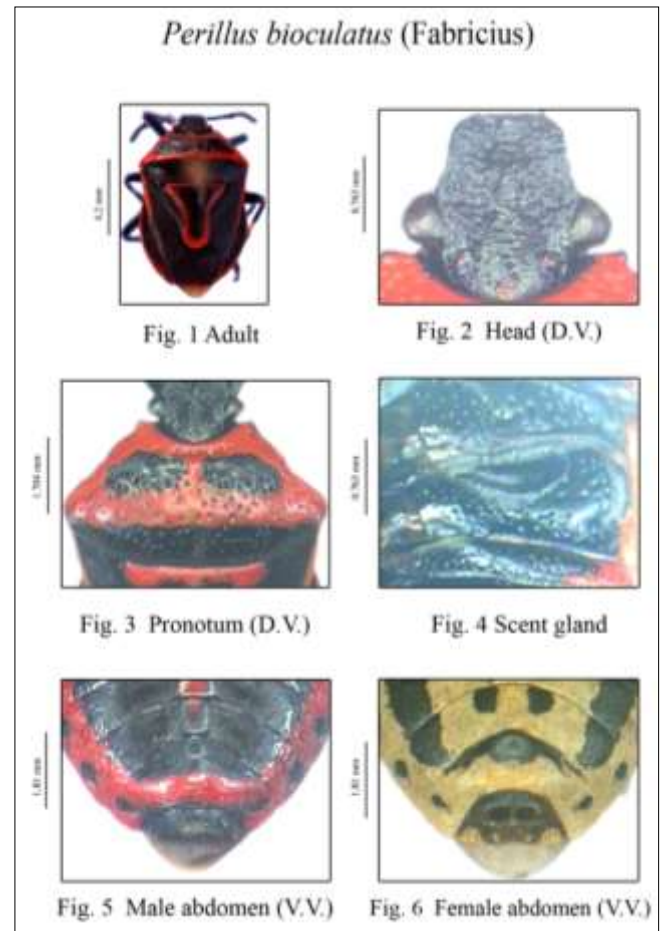


Fig 2

Female

Measurements (in mm): Head length: 1.80; width across eyes: 2.09; interocular space: 1.20; interocellar space: 0.75; preocular distance: 0.80; length of posterior head including eyes: 1.0; antennal segments lengths: Ist: 0.40, IInd: 0.90, IIIrd: 1.05, IVth: 1.15, Vth: 1.15; pronotal length: 3.4; width across humeral angles: 6.40; scutellar length: 4.0; width: 4.25; length of abdomen: 4.80; maximum width of abdomen: 5.94; ; length of abdominal spine: 0.80; length of rostral segments: Ist: 1.0, IInd: 1.10, IIIrd: 0.50, IVth: 0.60; Distance - base scutellum-apex clavus: 2.12, apex clavus-apex scutellum: 1.88, apex scutellum-apex abdomen including membrane: 3.20; total body length: 10.50.

Coloration and Structure: Dorsal coloration in females is similar to males except some variation on ventral surface: abdomen venter is reddish with double series of black fascia from segments IInd to VIth; a broad punctate continuous black fascial spot is present on anterior margin of VIIth abdominal segment; genital capsule is black with golden pubescence; legs are also black except large yellow patch on metatibial area and smaller patch in mesotibia; ventroanterior and ventroposterior margins of VIIth abdominal sternite is medially concave, somewhat an inverted broad U-shaped (Fig. 6).

Female Genitalia: 1st gonocoxae is triangular with convex

outer margin, posterior margin is slightly sinuous and irregular; 8th paratergites are broadly triangular and subrounded at outer margins; 9th paratergites are narrow at apex and are distinctly shorter than posterior margin of fused 8th paratergites (Fig. 7); spermatheca (Fig. 8) is with distinct distal and proximal flanges; pump region is membranous and short; spermathecal bulb is longer than broad, somewhat rectangular in shape with subrounded margins; proximal spermathecal duct is 2X longer than distal spermathecal duct.

Male Genitalia: Pygophore (Fig. 9) is broader than long, its lateral margins are largely sinuous; dorsolateral lobes are narrow and slightly curved on inner side; ventroposterior margin is irregularly sinuous; paramere (Fig. 10) is with short and broad stem and broad blade, squarish at one end, with distinct subacute spine at other end; aedeagus (Fig. 11, 12) is with oval theca that is spinosely produced with a pair of sclerotized thecal appendages and is directed anteriorly; conjunctiva is with a pair of dorsolateral membranous conjunctival appendages and a mediadorsal conjunctival appendage; vesica is long and do not pass beyond thecal appendages.

Population Variation: In the present survey, all the three-color morphs (Red, Dark yellow and black & white form) were obtained from different localities. Each specimen was studied for its external morphology and color variations. Coloration on pronotum, scutellum and connexival margins varied from reddish to pale yellowish-red or bright brownish-yellow to paler forms; posterior area of pronotum also showed blackish to chocolate brown color variation; thickness of punctures also varied in density in various regions of the body; lateral areas of sternum was found to be pitch black in red morphs, brownish -yellow in paler specimens; maculate fascia on lateral sides of abdomen were usually irregular; legs also showed color variations from being pitch black to brownish-yellow in populations, with reddish tinge in some collected from the same area.

Distributional records/Material Examined of the present study

Punjab: Hoshiarpur, 17. viii. 2009 - 24♀♀, 15♂♂, 11. iv. 2010 - 1♀; Pathankot, 20. viii. 2009 - 17♀♀, 13♂♂; Tarantaran, 8. iv. 2010 - 3♀♀, 1♂; Gurdaspur, 10. iv. 2010 - 1♀, 2♂♂; Ropar, 6. viii. 2010 - 5♀♀, 6♂♂; Patiala, 1. x. 2010 - 1♀, 1♂.

Himachal Pradesh: Nagwain, 22. vii. 2010 - 1♀, 5♂♂; Mandi, 23. vii. 2010 - 1♀, 4♂♂.

Worldwide Distribution: Czechoslovakia; France; Germany; Russia; Yugoslavia; India (Punjab, Himachal Pradesh).

Remarks: *Perillus bioculatus* (Fabricius) is commonly known as a two spotted stink bug. It is an American species, that was introduced in Europe as a biological control agent to control Colorado Potato Beetle (*Leptinotarsa decemlineata* Say), occurring throughout the Northwest and is also known to be predaceous on the larvae of *Crioceris asparagi*, *Lema trilineata*, *Trichoplusia ni*, *Disonycha xanthomelaena* and *Trirhabda canadensis*. This species shows distinct colour polymorphism ranging from red, yellow, white and black morphs. Earlier studies by Knight (1952)^[15] have proved that the food of this bug consists of the eggs and larvae of the potato beetle as well as the adult beetle itself, which is responsible for its colour variations.

Carotin has also been proved to be one of the reasons for the red and yellow color patterns in the hypodermis of this stink-bug.

Abbreviations: 8th pt. - Eight paratergites; 9th pt. - Ninth paratergites; Sp. b. - Spermathecal bulb; Dis. f. - Distal flange; Prx. f. - Proximal flange; Dis. sp. d. - Distal spermathecal duct; Prx. sp. d. - Proximal spermathecal duct; Md. - Median dilation; Sp. p. - Spermathecal pump; Vpm. - Ventroposterior margin; Dl. l. - Dorsolateral lobes; Dms. - Dorsomedial surface; DO. - Dorsal Opening; Bl. - Blade; Stm. - Stem; Bp. - Basal plate; Piv. - Pivot; Th. - Theca; Ves. - Vesica; Gp. - Gonopore; Pl. - Penial lobe; Vmc. app. - Ventral membranous conjunctival sspendages; Dlmc. app. - Dorsolateral membranous conjunctival appendages; Mdmc. App. - Median dorsal membranous conjunctival appendages; Th. App. - Thecal appendages; Ej. r. - Ejaculatory reservoir.

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