Research Article

Taxonomic Study of Sub-Family Epilachninae (Coleoptera: Coccinellidae) of Gilgit-Baltistan

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Abstract | Sub-family Epilachninae (Coleoptera: Coccinellidae) of Gilgit-Baltistan was explored during 2006 to 2011. The examination of the specimens collected during the survey showed that there are three species of epilachnine beetles in two genera including one species in Genus *Henosepilachna* (*H. vigintioctopunctata* (Fabricius)) and two species in Genus *Afidetula* (*A. bisquadripunctata* (Gyllenhal) and *A. maderstgernae* (Mulsant)). All the species, reported here, are new records from the studied territory. Specification of first coxal line and genitalia are provided. Remarks containing taxonomic history, identification key up to species level and the dispensation of each species in the studied area are also provided.

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Introduction

Epilachninae is one of the six sub-families within family Coccinellidae. Contrary to the other sub-families, members of Epilachininae are phytophagous and commonly known as Epilachnine ladybird beetles or Hadda beetles. These beetles are almost exclusive leaf feeders of the plant species that belong to family Cucurbitaceae and Solanaceae while some species cause damage to plants belonging to family Fabaceae and Compositae. The sub-family Epilachninae is therefore considered as economically one of the most important sub-families of Coccinellidae (Dieke, 1947; Li and Cook, 1961). Total devastation of potato, tomato, brinjal, squash, bitter gourd, tobacco, nightshades, melon and cotton crops in China, Australia and the United States of America have been reported (Reh, 1913; Froggatt, 1923; Richards, 1983).

Infestation period of epilachnine beetles vary from season to season but the peak is generally found in July to August (Rajgopal and Trivedi, 1989). Both the larvae and adults cause damage. Terminal instars of larvae and adults feed randomly on the leaf surface creating irregular holes in the leaf (Hashmi, 1994). In case of severe infestation, the plant ends up in a completely dried form. Growth and development of the plant is greatly impaired and final yield is

markedly reduced (Alam, 1969).

Epilachninae species occur in almost all the tropical and sub-tropical regions of the world, with nearly all species found between 30° line of latitude. Maximum species are widespread with an obvious tolerance to climatic extremes and wide host range (Anand *et al*., 1988). Kuznetsov (1997) provided details of the differences between phytophagous and predatory coccinellids. Jawdwiszczak and Wegrzynowicz (2003) summarized the world fauna of Epilachnine beetles where 1051 species were reported in 22 genera from all over the world.

In Pakistan, the fauna of this sub-family is very poorly explored, and the available information is fragmentary and scattered. Ashrafi (1966) was the first to report two species of epilachnine beetles from Karachi namely *Epilachna sparsa* and *Epilachna chrysomelina*. Similarly, Chaudhry (1966) and Chaudhry *et al*., (1970) reported three species viz. *Epilachna ocellata*, *Afidentula gradaria* (now called *Afidenta miera*) and *Afidentula manderstjernae*.

The first comprehensive work was attempted by Commonwealth Institute for Biological Control (CIBC, 1982) where it reported seven species from the group in Pakistan. Fursch (1984) described *Afidentula erberi* as a new species from Battal, Hazara, Pakistan. Later on, Hashmi (1994) mentioned that there are three major species of Epilachninae in Pakistan namely *Epilachna vigintioctopunctata, E. dodecastigma* and *E. dermurili*. Naz *et al*. (2012) recently provided comprehensive notes on the host plants and distribution pattern of *Henosepilachna vigintioctopunctata* and *E. septima*.

Despite comprehensive work by CIBC on the sub-family in Pakistan, the fauna of Gilgit-Baltistan (Northern Pakistan) has remained unexplored. Many valuable contributions by these workers, there is still confusion in the taxonomy of epilachninae. Furthermore, this group is poorly explored in Pakistan and only 7 species have been reported so far.

Gilgit-Baltistan lies in the Palearctic region (Hindu Kush, Western Himalaya, Karakuram). The study area is further associated to the dry mountain sub-ecological zone of Pakistan. The area has diversified flora and fauna because of varied climatic conditions and unique ecology. Still limited information is available on insect diversity in this region. Considering the facts about its economic importance and the poor attention to this important economic group from the scientific community in the past, comprehensive surveys were undertaken to explore the epilachnine ladybird beetle fauna of Gilgit-Baltistan.

Materials and Methods

The study area

The Gilgit-Baltistan (formerly the Northern Areas of Pakistan), a newly designated province, is an area of about 72498 km^2 which is segregated into seven districts on administrative basis: namely, Skardu, Gilgit, Ghizer, Diamer, Hunza, Ghanche, Nahgar and Astore. The province coordinates between 72° and 77° east longitude and 34.5° and 37° north latitude. This area is bordered by China, India and Afghanistan while narrowly separated from Tajikistan by the Wakhan strip.

Collection

Comprehensive collection expeditions were launched for epilachnine beetles, where all seven districts of the province were thoroughly surveyed from 2006 to 2011. Due to severe cold and harsh weather conditions in the greater part of the areas of Gilgit Baltistan, the surveys mostly took place in the months of May through September. Standard collection procedures were applied. About 120 specimens of lady bird beetles were collected as a result of multiple surveys. Besides fresh collections, the preserved collection of National Insect Museum (NIM) at National Agricultural Research Centre, Islamabad was also studied and the findings were incorporated.

The taxonomic key provided by (Dieke, 1947; Kapur, 1958) was used for the identification of the specimens up to genus level. The species were also cross checked and confirmed by their comparison with already identified species in reference collections. The species were confirmed based on genitalia study. For the extraction of genitalia, the methodology provided by Majerus and Kearns (1989) with some modification given by Naz *et al*. (2012) was followed.

To minimize prolixity, only the characteristics anomalous to species level taxon are provided in its description while the literature of Kapur (1958), Li and Cook (1961), Kuznetsov (1997), Katakura *et al*., (2001) and Rafi *et al*. (2005) have been followed for

the description of the species.

Adult specimens and their genital characteristics were thoroughly examined under Olympus SZX-16 stereoscope. The specimens and their genitals were photographed with digital DP2-BSW (CCTV) camera mounted to the stereoscope. All the identified specimens were relinquished to the National Insect Museum (NIM), NARC, Islamabad.

Results and Discussion

The present study revealed the occurrence of three species in two genera of Epilachninae in Gilgit-Baltistan.

Key to the genera of sub-family Epilachninae of Gilgit-Baltistan

- Sixth visible abdominal sternite of female longitudinally split; yellowish setae on upper side of median lobe in male genitalia…. *Henosepilachna*
- Sixth visible abdominal sternite of female entire; no yellowish setae on upper side of median lobe in male genitalia…… *Afidentula*

Genus Afidentula Kapur 1958
Type of the genus: *Afidentula manderstjernae* (Mulsant)
Genus *Afidentula* was separated from the genus *Afidenta* by Kapur (1958).

Remarks: The genus *Afidentula* Kapur is most closely related to genus *Afidenta* Dieke, from which it is separated on the basis of three unserrated mandibular teeth, claw with sub-triangular teeth and well-developed male genitalia. This genus was erected by Kapur (1958) and designated *Afidentula manderstjerae* as its type species. The world catalogue by Jawdwiszczak and Wegrzynowicz (2003) contains 18 species of this genus from the world. In Pakistan, the genus is represented by three species including *Afidentula manderstjernae, A. bisquadripunctata* and *A. erberi* (Naz, 2012). The species occurring in Gilgit-Baltistan can be separated by the following key.

Key to the species of Genus Afidentula Kapur from Gilgit-Baltistan

- Pronotum spotless, elytra with four spots …… *A. bisquadripunctata*
- Pronotum spotted. Elytral spot 3 and 4 oval, spot 5 not touching suture *A. manderstjernae*

Afidentula bisquadripunctata (Gyllenhal, 1808) (Figure 1A, B)
Material Examined: Pakistan – Gilgit-Baltistan Prov. 5♂ 4♀, Astore, Rama, 5 Jun. 2007, Falak Naz leg. (NIM); 1 ♂ 2 ♀, Astore, 10 Jul. 2006, Ashfaque leg. (NIM); 2 ♀, Astore, Rama, 21 May 2009, Falak Naz leg. (NIM).
Size: Male (N=6) 5.17 mm ± 0.12; Female (N=8), 5.85 mm ± 0.2

A: Adult

B: Male genitalia

Figure 1: *Afidentula bisquadripunctata. A: Adult; B: Male genitalia.*

Description: Ground colour brownish red; prontum spotless, Elytra each with 4 persistent spots , the first one near the base between shoulder and suture, spot 2 in front of the middle, spot 3 close to margin, spot

4 near to apex. All spots rounded and big in size. First coxal line rounded and subcomplete, mandible uniquely thick as their length and width, siphonal tip of male genitalia compressed and with dense short hairs.

Male Genitalia (Figure 1B)
Phalobase, median lobe straight, thickened just before apex, appearing to end in a sharp, upturned point. The apex seems bi-lobed from the lower side. Paramera is very thin, long, bent down slightly at base, only slightly widened at apex, and apex sparsely clothed with hairs. Siphon bent 180° near base, slightly widened at apex, compressed and then tapering sharply towards end. The apex is clothed uniformly with short hairs.

Seasonal Occurrence: June to September.

Present Record: Astore (Gilgit-Baltistan).

Earlier Record from Pakistan: Changa Manga (Kasur); Swabi; Muzaffarabad; Rawalakot.

World Distribution: China, India, Nepal, Sri Lanka, Vietnam.

Afidentula manderstjernae (Mulsant, 1853)
(Figure 2A, B)
Material examined: Pakistan – Gilgit-Baltistan Prov. 4 ♂ 3 ♀, Gilgit, 10 May 2006, Israr leg. (NIM); 2 ♂ 2 ♀, Gilgit, Danyor, 15 Jul. 2008, Falak Naz leg. (NIM); 4 ♂ 2 ♀, Gilgit, 21 Apr. 2009, Ashfaque leg. (NIM).
Size: Male (N=10) 4.42 mm ± 0.07; Female (N=7), 4.75 mm ± 0.17

Description: Body small sized; upper side red, a transverse median spot present on pronotum. Each elytra with six spots. Spot No. 1 not touching the suture, No. 5 on the suture, No. 6 has a typical shape and shows tendency to split into two. In some specimens it is completely split into two. Post coxal line is complete.

Male Genitalia (Figure 2B)
Phalobase; Median lobe gradually widens towards the apex from base. Maximum width is observed just before the apex. The apex converges sharply to the slightly tipped up point. Parameres, sparsely covered with hairs near apex, are thread like and robustly thin in structure. Siphon; an exceptional bent of 180° found near base, then slightly curved in the same direction, turn outward sharply at right angle near apex and then gradually diminishing in thickness and tapering off into a very fine point.

A: Adult

B: Male genitalia

Figure 2: *Afidentula manderstjarne, A: Adult; B: Male genitalia.*

Seasonal occurrence: July-September

Present Record from Gilgit-Baltistan: Gilgit

Earlier Record from Pakistan: Swat and Abbottabad.

World Distribution: China, India, Nepal, Vietnam.

Genus Henosepilachna (Li and Cook, 1961)
Type species: *Henosepilachna sparsa* (Harbst) by original designation

Remarks: This genus was created by Li and Cook (1961) while studying the Epilachninae of Taiwan and designated *Epilachna sparsa* as its type species. They distinguished this new genus from genus *Epilachna* Chevrolat (Type species: *Epilachna borealis* (F.). The new designated type species was based on a claw with basal tooth and a visibly split 6th abdominal sternite in both female and male genitalia with an apical thorn on the parameres, a basal knife edge and setae on the median lobe.

The genus in Gilgit-Baltistan is only represented by a single species *H. vigintioctopunctata.*

Henosepilachna vigintioctopunctata (Fabricius, 1775) (Figure 3A, B)

Material examined: Pakistan – Gilgit-Baltistan Prov. 3 ♂ 2 ♀, Gilgit, Jaglot, 7 May 2006 (Israr) (NIM); 2 ♂ 4 ♀, Diamer, Chilas, 15 Jul. 2007, Falak Naz leg. (NIM); 5 ♂ 3 ♀, Diamer, Chilas, 21 Jun. 2009 (Ashfaque leg. (NIM); 2 ♂ 1 ♀, Gilgit, Jaglot, 23. Jun. 2009, Ashfaque leg. (NIM).

Description: Ground color pale brown or reddish brown. Elytral apex angled. Post coxal line sub-complete. Elytral spots vary between 12 and 28 but majority with 26 spots. Spot cb3d usually lie on a straight line. Exact diagnosis can be made by examining male genitalia which is with a well-developed basal knife edge and apical thorn on median lobe, siphonal tip tapering on one side.

Size: Male (N=12), 6.0 ± 0.13 mm; Female (N=10), 6.5 ± 0.08 mm.

Male Genitalia (Figure 3B)
Phalobase: Median lobe has basal knife edge beginning at the foot of paramera and a buldge beyond the middle, after which it curves up into an apical hook. Second half with two rows of hairs. Paramera with an apical thorn and covered with hairs shorter than those of median lobe. Siphon gently curved near the base, then straight, ending in a point.

A: Adult

B: Male genitalia

Figure 3: *Henosepilachna vigintioctopunctata. A: Adult; B: Male genitalia.*

Seasonal occurrence: May to August.

Present Record from Gilgit-Baltistan: Chilas (Diamer) and Jaglot (Gilgit).

Earlier Record from Pakistan: Abbotabad, Mingora, Haripur (Khyber Pakhtunkhwa); Wah, Rawalpindi (Punjab); Muzaffarabad (AJK) and Karachi (Sindh).

World Distribution: Australia, Bhutan, Burma, China, Fiji, India, Indonesia, Pakistan, Japan, Nepal, New Guinea, Philippine, Sri Lanka, Taiwan, Thailand, Vietnam.

The taxonomic study of the collected specimens resulted in three species of two genera of sub-family Epilachninae in the region. The identification was done mainly on the basis of male genitalia which is a safe character for the separation of closely related species. The identified species occurring in Gilgit-Baltistan are *Afidentula bisquadripunctata, A. manderstjernae* and *Henosepilachna vigintioctopunctata.* All these three species are new records for the Gilgit-Baltistan region.

Afidentula bisquadripunctata is small to medium sized beetle and can be found on grasses. together with *A. manderstjernae* but not so abundant like the later one. It is a little bit larger than *A. manderstjernae*. It has 4 elyral spots which are away from suture and margins while *A. manderstjernae* has 6 spots, in which spot 5 is on suture. It has a unique mandible structure in the whole sub-family Epilachninae. *A. bisquadripunctata* has uniform male genitalia in which the siphonal tip is more tapering, compressed, elongated and clothed with short hairs.

A. manderstjernae is a beetle of small size which can be collected by sweeping grasses in hilly areas. Elytral spot 6 split into two parts, short parameres in phalobase and highly pointed siphonal tip of male genitalia are its distinguishing characters. The type locality of the species is Asia and was also reported from India (Dieke, 1947) and Nepal (Kapur, 1955).

Dieke (1947) while working on the group placed it under genus *Affisa*, to which Kapur (1958) disagreed on the basis of bifid claw with basal tooth. He further emphasized that it cannot even be placed under *Epilachna* due to the undivided terminal abdominal sternite in the female nor can it be placed under genus *Afidenta* due to the reasons already given under genus *Afidentula.* Therefore in 1958 new genus *Afidentula* was described by Kapur for this species where this species was designated as the type species of this newly described genus.

Henospilachna vigintiocpunctata is a large sized beetle and highly variable in both pronotum and elytral spots pattern, ground colour and size. Therefore, it has been misidentified in the past by many taxonomists. Considering these variations, (Dieke, 1947 segregated this species into four subspecies based on their geographical presence under its old synonym *sparsa.* However, Li and Cook (1961) considered them as variable forms of a same species. Similiarly, Richards in 1983 studied the same complex and separated it from two other closely related species *H. vigintisexpunctata* and *H. cucurbitae* in Australia. In our collection every succeeding specimen mostly varies from the previous one in spot number, spot size, spot intensity and ground colour.

It was reported under the name *H. sparsa* from solinaceous plants from Pakistan by CIBC (1982) and under the name *H. vigitioctopunctata* by Hashmi (1994). CIBC (1982) stated that the species can be found in Pakistan from coastal areas to 2,000 m in the northern hills and was not found in Quetta and Parachinar. But during this study the species was also found above 2,000 m in Gilgit.

Conclusions and Recommendations

Previously epilachnine ladybird beetles have not been recorded or reported from the high altitude dry mountains areas (Gilgit-Baltistan) of Pakistan. The results of present exploration of the Gilgit Baltistan region for the species of Sub-family Epilachninae and its taxonomic study have established the occurrence of at least two genera with three species. This group of insects is first time reported from the area. Further the study is based on the morphology of genitalia while previously such group was taxonomically dealt with only by its external morphology in Pakistan which may have resulted in misidentification. The results are notable with three new records of Epilachninae. The results could prove to be a baseline study for many other studies that deals with ecosystem, ecology, insect taxonomy and taxonomic algorithm (in future) of Gilgit-Baltistan region. Further exploration and efforts may yield more species of the sub-family in the region.

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Novelty Statement

The contents and the discovery of the facts and information pertaining to the specified geography in this article have never been published before both locally and abroad and are being published for the first time.

Author's Contribution

Muhammad Ashfaque: Planned the study, collected specimens, conducted taxonomic analysis and genital dissection, and prepared the manuscript.
Mian Sabahatullah: Assisted with taxonomic identification, species description, and specimen photography.
Farman Ullah:Conceptualization, Planning, and finalized the manuscript.
Falak Naz: Assisted with fieldwork, laboratory tasks, and in species description and manuscript preparation.
M. Ather Rafi: Supervised the work, provided necessary literature, arranged logistics and secured financial support for collection expeditions.

Conflict of interest
The authors have declared no conflict of interest.

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