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First Taxonomic Note on *Rodolia ruficollis* Mulsant (Noviini: Coccinellidae) and its Predatory Role against Scale Insect Pest, *Icerya aegyptiaca* (Dgl.) from Sindh, Pakistan

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ABSTRACT

The coccinellid predator, *Rodolia ruficollis* Mulsant is treated taxonomically for the first time from Sindh Province of Pakistan. This may be a unique contribution to the genus *Rodolia* with the description of this species on the basis of important diagnostic characters including male and female genitalia. It is an important predator of scale insect pest, *Icerya aegyptiaca* (Douglas, 1890) which is dangerous insect pest upon different fruit trees including date palm, mango, mulberry and other important fruit trees in this region. This study also investigates the predatory role both adults and 4th instar larvae of this coccinellid predator in the biological control of *I. aegyptiaca* comparatively in the laboratory and under field conditions. The 4th instar larvae were found to be more voracious than adults.

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INTRODUCTION

The genus *Rodolia* of tribe Noviini Mulsant, 1846 was first time established with *R. ruficollis* Mulsant as the type species (Mulsant,1850). The constant generic changing of *Rodolia* of Mulsant (1850) and Weise (1895) were briefed and included the tribe Noviini within subfamily Coccidulinae and divided it into three genera *Anovia* Casey, *Novius* Mulsant and *Rodolia* (Mulsant) (Gordon, 1972). The two *Rodolia* and *Anovia* were reported virtually indistinguishable in the adult stage, but can be differentiated based on number of larval antennomeres and proposed that some species which placed in *Novius* must be transferred to *Rodolia* (Gordon, 1985). The Australian species were transferred from the genus *Novius* to *Rodolia* of the tribe Noviini under the subfamily Coccinellinae (Ślipiński, 2007). A new classification was proposed based on the cladistic analysis and synonymized the *Anovia* and *Novius* in the genus *Rodolia* (Mulsant) and as a result, this tribe is now comprised of the single genus *Rodolia* in subfamily Coccidulinae and now the genus *Rodolia* comprises 46 species world widely (Forrester, 2008). Recently *R. shuiro* (Kitano) was reported as a new species from Japan (Kitano, 2014). Five species were reported from West Bengal (Chakraborty *et al*., 1996).

From Pakistan only three species have been reported, viz., *R guerini* Korschefsky, 1931, *R. fumida* Mulsant, 1850 and *R. ruficollis* Mulsant, 1850 by (Rasheed *et al*., 986; Poorani, 2002; Rafi *et al*., 2005; Hayat and Khan, 2013; Ali *et al*., 2014; Hayat *et al*., 2017). A comprehensive taxonomic study on 29 species of the family Coccinellidae, was made possible for the first time from Sindh province of Pakistan including the species *R. ruficollis* Mulsant, 1850 feeding upon *I.* aegyptiaca on date palm (*Phoenix dactylifera*), *Parkinsonia aculeata* and rose (*Rosa indica*) (Ali *et al*., 2014, 2018).

I. aegyptiaca (Douglas) is a highly dangerous polyphagous and widespread scale insect. According to the scale insect database, Scale Net (Ben-Dov and Gibson, 2009), this insect pest is found in the Afro-tropical, Australasian, Indo-Malayan and Palearctic regions, and it has been reporting feeding upon 123 species of plants

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belonging to 49 families. *I. aegyptiaca* was originally described in the genus *Crossotosoma* Douglas as *C. aegyptiacum* Douglas, 1890, and later on it was transferred by Riley and Howard (1890) to the genus *Icerya* as *I. aegyptiacum* (Douglas). Maskell (1893) changed the name as *I. aegyptiaca* (Douglas). The genus *Icerya* comprise 35 species worldwide that are commonly known as fluted scales due to the fluted appearance of the ovisac. The Egyptian fluted mealybug, *I. aegyptiaca* (Douglas) (Hemiptera: Monophlebidea) is considered as a cosmopolitan inest pest with numerous host plants. It is commonly known as Egyptian *Icerya*, because it was originally described from Egypt. Egyptian fluted mealybug, *I. aegyptiaca* (Douglas) was reported as a serious insect pest of navel orange trees, *Mangifera indica*, citrus tree and tropical fruit trees and causes a severely considerable damage (El-Sobky, 2020; Moghaddam *et al*., 2015). Body of adult female is orange-red, antennae and legs black, covered in white wax, the margin covered with a fringe of elongate waxy processes, giving the ovisac to a fluted appearance. *I. aegyptiaca* has 2 pairs abdominal spiracles, while *I. purchasi* has 3 pairs abdominal spiracles. From Pakistan, *I. aegyptiaca* (Dgl.) was reported from Karachi and Thatta infesting date palm (*Phoenix dactylifera*), *Parkinsonia aculeata*, rose (*Rosa indica*), mulberry (*Morus alba*) and *Erythrina* sp., citrus, mango, guava, *Ficus benghalensis* and *F. religiosa* (Muzaffar, 1970).

Biological Control, especially using insects to control other insects, is an ancient pest control strategy (Parra, 2014). Biological control method effectively begun in 1888, when Australian vedalia ladybeetle, *R. cardinalis* (Mulsant) (Coleoptera: Coccinellidae) was introduced to control *I. purchasi* Maskell (Hemiptera: Monophlebidae) infesting orchards in California, USA. Impressive results for pest control were being obtained already by 1889, through the participation of entomologists from the University of California's Berkeley and Riverside campi in the form of a symposium in Riverside, CA, USA to celebrate the centenary anniversary of biological control, entitled "*Vedalia* symposium of biological control: A century of success" (Leppla and Williams, 1992). Predaceous coccinellid beetles feed on various soft bodied insect pests, viz., aphids, scale-insects, mealy bugs, mites, white flies, thrips, etc. and are considered as potential important biocontrol agents (Omkar and Pervez, 2002a). Over the last 120 years, *R. cardinalis* (Mulsant) (Coleoptera: Coccinellidae) has successfully and effectively suppressed populations of *I. purchasi* in many countries (Caltagirone and Doutt, 1989).

From Pakistan the parasite *Tetrastichus* sp. and the predator *Scymnus* (Pullus) *coccivora* Ram. Ayyar were found at both localities whereas the predator *R. ruficollis* Muls. and the parasite *Cryptochetum grandicorne* Rond. at Karachi only; *Tetrastichus* and *Scymnus* were found on all the food-plants except *Parkinsonia*, and the other two natural enemies on *Erythrina*, *Morus*, *Phoenix* and *Rosa*. In spite of their considerable activity, *Icerya* infestation was still heavy on some of its food-plants in the coastal region (Muzaffar, 1970).

Collection, identification and ecological studies are prerequisites to their augmentative release (Pervez, 2004). To seek natural enemies or transfer from one region to another in biological control programmes, the correct identification of both the pest and the natural enemy species is of great importance (Narendran, 2003). In biological control, workers may commit several mistakes without the help of taxonomists (Schauff and LaSalle, 1998; Narendran, 2001, 2003, 2006).

The present investigation aims to present a detailed taxonomic study upon the coccinellid predator, *R. ruficollis* and exploring its important role in the biological control of *I. aegyptiaca*. The another aim of this study is to reflect the importance and impact of the taxonomy of coccinellid predator (*R. ruficollis*) upon its valid and authentic role as biocontrol agent of *I. aegyptiaca*. The last focus of the study to divert the potential of entomologists in Pakistan mainly upon the taxonomy with reference to their role as predators in the field of biological control necessary according to future perspectives related with the economy, agribusiness and bright future of agriculturists in Pakistan.

MATERIALS AND METHODS

Study area

The study area of the present study includes Thatta, Sajawal, Landi, Malir, Korangi and different research fields of University of Karachi. This study was conducted during 2009-2013 in the laboratory of Department of Zoology, University of Karachi, Karachi.

Collection

The ladybird specimens were collected with the help of insect nets and hand picking methods whereas the specimens of *I. aegyptiaca* by using camel brushes of different size. Collection was done during morning and evening time because in heavy sunlight they hide themselves under stones and shading parts of plants. The ladybird specimens were collected from date palm trees feeding upon the scale insect pest, *I. aegyptiaca* and shifted into plastic jars of different size. Collected specimens were brought to the laboratory for mounting, pinning and identification.

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Identification

The specimens were identified by the author following the checklists, descriptions, terminologies and keys given by Innayatullah (1980), Gordon (1985), Poorani (2002), Rafi *et al*. (2005) and Forrester (2008) and with the help of the following websites: Atlas of ladybirds (Coccinellidae) of Russia, Bug Guide, Coccinellidae of Indian Subcontinent, CSIRO, NBAII and NIDP. Identifications of ladybird was confirmed by Dr. Claudio Canepari, an authority on the family Coccinellidae from Italy, Mealybugs were identified and confirmed by Dr Gillian Watson, Senior Insect Biosystematist, California Department of Food and Agriculture, Plant Pest Diagnostic Center, Sacramento, U.S.A for holotypes, lectotypes and distribution of taxa, the checklist made by Poorani (2004), book written by Rafi *et al*. (2005) were followed.

Stereoscope binocular microscope was used for specimen study during identification. Micro-millimeter scale was used for the measurement of the body and various body structures using at least five males and five females of the taxon.

For the study of external morphology of head, thorax and abdomen, the entire specimen was boiled in 10% KOH for about 15 min then washed in to tap water, later all the parts of head, thorax and abdomen were dissected and drawn where necessary. At the end of the taxonomic study and drawings, these dissected parts were preserved in microvial with glycerine.

For the study of male and female genitalia, the whole abdomen was removed from the base and warmed in to 10% KOH on an electric heater for about 5 to 10 min. The whole male and female genitalia were detached from the terminal segments of abdomen very carefully and then dissected out in water. Before the examination of structures of genitalia, the genitalial parts were washed out in 70% Ethanol and then the structures of genitalia were examined in a drop of glycerin sometime a piece of cotton dipped in glycerin to avoid the movement. After the examination of these structures, both male and female genitalia were illustrated and then dissected parts were preserved in microvial with a drop of glycerine, pinned with specimens.

For the study of predatory potential of predatory Coccinellids, the fourth instar larvae and adults of *R. ruficollis* were selected for experimentations against the scale insect pest, *I. aegyptiaca* in the laboratory as well as under field conditions. The fresh ladybird specimens were collected and reared in laboratory by providing the specific pests with their host plant leaves. For laboratory experimentations, Petri dishes were used separately for both fourth instar larvae and adults. For field experimentations, a group of ten adults and a group of ten 4th instar larvae were released on date palm trees repeatedly for ten days for 24 h. The numbers of dead and alive pests were counted and collected data on daily basis. The predatory potentials of both larvae and adult in the laboratory and under field conditions were studied comparatively. The obtained results were showed in the form of tabulations and histograms for both the laboratory and under field conditions. Eview 6 Qauntitative Micro software was used for the common statistical analysis.

Genus *Rodolia* Mulsant

Rodolia Mulsant, 1850: 901, 902; Crotch, 1874: 280; Kapur, 1949: 531; 1950: 1-7; Gordon, 1985; Poorani, 2004; Rafi *et al*., 2005.

Type species. *Rodolia ruficollis* Mulsant, 1850.

Diagnosis

Dorsum pubescent; black to redish-brown, sometimes with spots. Adult length 4–5mm; width 3–4mm; oval to nearly rounded, broadly convex. Antennae with first segment broadly lobed, second segment globose, third cylindrical and about twice as long as wide, fourth and fifth progressively shorter and each wider apically than the preceding, sixth to eighth forming club, eighth rounded at apex; mandible moderate. Prosternal process with carinae; mesosternum never notched anteriorly; elytral outer margin narrow, abruptly reflexed; epipleura concave, strongly descending externally and not foveate; tibial spurs absen; claws sexually dimorphic, split (male) or with a broad basal tooth (female). Postcoxal line complete, sixth sternum in male with apical strong emargination.

Male genitalia

Sipho mostly thick, terminally broadly to strongly sharped, siphonal capsule adjacent arm mostly elongated than opposite arm; median lobe mostly longer than paramere; trabes longer but sometime very short.

Female genitalia

Genital plates elongated and triangular; spermatheca without nodulus, ramus but cornu narrow to broadly thick; infundibulum absent; sperm duct short moderate.

***Rodolia ruficollis* Mulsant**

(Fig. 1**)**

Rodolia ruficollis Mulsant, 1850: 903; Korschefsky, 1931: 102; Kapur, 1949: 535; Poorani, 2004; Rafi *et al*., 2005. *Vedalia ruficollis*: Crotch, 1874: 281.

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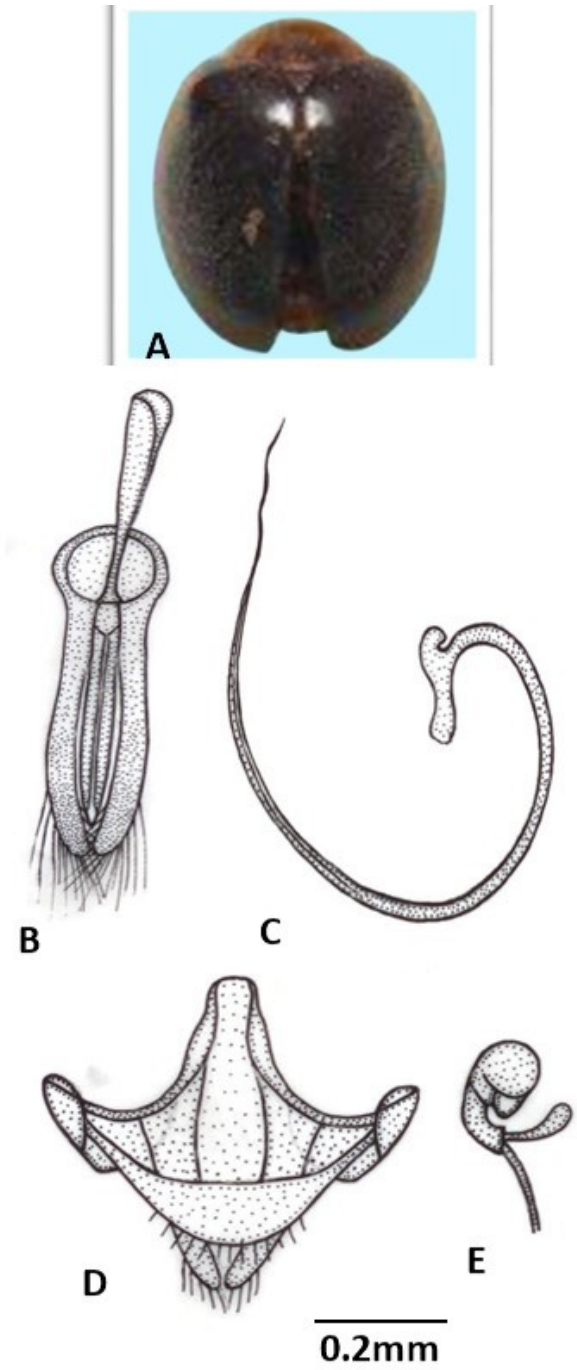

Fig. 1. *Rodolia ruficolis* Mulsant: A, Adult; B, Tegmen; C, Sipho; D, Genital plates; E, Spermatheca.

Diagnosis

Dorsal surface pubescent, uniformly reddish brown to brown except the margin of elytra; head, pronotum, legs and lower part of body densely covered with yellow hairs; eyes black. Adult length 4mm-5mm; width 3mm-3.66mm; elongately oval. Eyes small with minute facets, ligula with straight anterior margin, labial; gula broader than long; submentum with anterior margin broader. Prosternum bifurcated anteriorly; prosternal process with carinae not clear; scuto-scutellar suture broader, less sclerotized anteriorly whereas narrow, highly sclerotized posteriorly; epipleuron with inner margin deeply concave. Postcoxal line broadly curved meeting the anterior margin of first sternite; terminal sternite bearing small hairs throughout.

Male genitalia

Sipho terminally much narrow and thread like; siphonal capsule with an elongated adjacent arm whereas a short, curved slight pointed opposite arm; median lobe elongated, straight, narrowed anteriorly, slightly expanded medially, deeply pointed apically parameres distinctly elongated, narrowed anteriorly, broader, inwarded distally; trabes narrow proximally while expanded, curved distally.

Female genitalia

Genital plates elongated, trisngulsr, sharped distally; lateral plates elongated with slightly curved mesad; spermatheca with cornu ball-shaped, nodulus narrow, elongated, bent while ramus totally absent.

Holotype

BMNH

Material examined

5 males, 6 females, Pakistan; Sindh: Tandojam, Sukkar, Mirpur Khas, Hyderabad, Karachi, Punjab: Jhang, Lyalpur, 13.7.2009; 4. 9.2008 on *I. aegyptiaca* from cocconut, other wild plants, leg., Khan, M.I and Ali, M, lodged at Natural History Museum, Department of Zoology, University of Karachi.

Comparative note

This species resembles with *R. fumida* in size and shape but differs due to uniformly reddish brown to brownish black, margin of elytra reddish brown; head, pronotum, legs and lower part of body densely covered with yellow hairs; eyes black.

Distribution

Hyderabad, Sukkhar, Larkana, Mirpur Khas, Karachi coastal area; Baluchistan: Gawadir coastal area.

Population abundance of I. aegyptiaca

The occurrence of *I. aegyptiaca* started from the June to November. The huge infestation on date palm tree was recorded in the month of September and October during which the temperature of Karachi remains moderate

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between 20°C to 30°C. During the hottest months from June to August, its population remains under control due the predation of *R. ruficollis*. When summer season gradually ends the ultimately its population also decreases (Fig. 2). Therefore, increase or decrease in the population of *R. ruficollis* is totally dependent upon the population of *I. aegyptiaca*.

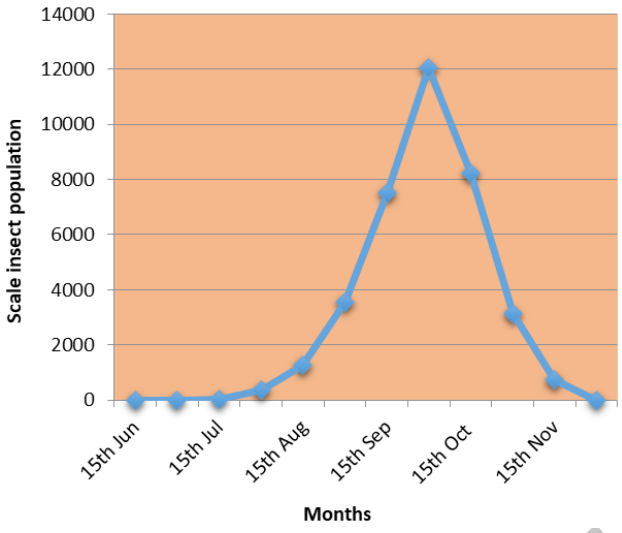

Fig. 2. Population abundance of *I. aegyptiaca* on date palm.

Biological control

In the laboratory and under field conditions, the predatory potentials of 4th instar larvae (56.8±2.8, 37.2±4.3) of *R. ruficollis* were found to be higher than the predatory potentials of adults (34.5±3.5, 22±2.2) (41.8±4.4, 26.1±5.2) (Table I, Fig. 3). The experiments in the laboratory and under field were carried out at temperature 25 ± 3°C, 35 ± 5°C and humidity 62 ± 6% and 78± 4%.

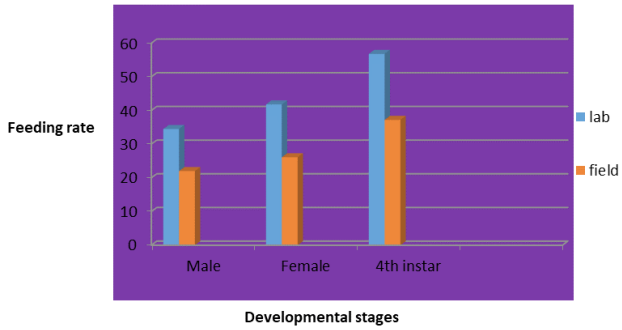

Fig. 3. Feeding potential of 4th instar larvae and adults of *R. ruficollis* on *I. aegyptiaca* on date palm fruit tree in the laboratory and under field conditions.

Table I. Feeding potential of 4th instar larvae and adults of *R. ruficollis* on *I. aegyptiaca* on date palm fruit tree in the laboratory and under field conditions.

Conditions	Male	Female	4th instar
Laboratory	34.5 ± 3.5	41.8 ± 4.4	56.8 ± 2.8
Field	22 ± 2.2	26.1 ± 5.2	37.2 ± 4.3

DISCUSSION

For the first time nine species of the genus *Rodolia* Mulsant were described from India with a key to the species (Kapur, 1951) but still many deficiencies were present regarding at the genital level. In this study the whole genital parts were not described except only spermathecal capsule. Information about their role as predators was never mentioned. Therefore, many deficiencies specially about this species were found in this finding which were totally cleared by the present study.

Five species of this genus were reported from the coleopteran fauna of West Bengal (Chackraborty *et al*., 1996) but this study was limited with the distribution of Coccinellids including the species, *R. ruficollis* as compared to the detailed study of the present investigation regarding taxonomy and predatory role of the species.

The species of the tribe Noviini of the world were described in detail (Forrester, 2008) but no any information related with the systematics and predatory role of the species, *R. ruficollis* was mentioned in this study. This may be a type of systematic negligence instead the available of the previous literature.

Thirty-two species of the family Coccinellidae were described from Azad Jammu and Kashmir including the species *R. ruficolis* (Hayat *et al*., 2017) but this species has been misidentified which may be *R. fumida* according the male and female genital structures given by Kapur (1951) and Ali, 2013. Therefore, the present study contributed to correct the misidentification of the genus *Rodolia* done by Hayat *et al*. (2017).

From Pakistan the species *R. ruficollis* was first time brought under the observations of Muzaffar (1970) but was not confirmed based on any diagnostic character till the time of present study. The present study for the first time described this species based on taxonomic characters including male and female genitalia reporting from Sindh Province of Pakistan in a valid, authentic and scientific way. Therefore, the present study may only be a unique and creditable scientific contribution to the genus *Rodolia* from Pakistan.

Two species of the genus *Rodolia* viz., *R. fumida* and *R. octoguttata*, were treated taxonomically from Punjab Province of Pakistan (Iqbal, 2018) but the species *R.*

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fumida was misidentified. This species may be *R. ruficollis* again according the genital characters given by Kapur (1951).

The other works related with diversity and pest-predator relationship (Muzaffar, 1970; Rasheed *et al*., 1986; Omkar and Pervez, 1999; Rafi *et al*., 2005; Bhuiyan, 2015; Kundoo and Khan, 2017; Kibar *et al*., 2017; Hayat *et al*., 2017; Pathan *et al*., 2018) provided the basic information related to the biodiversity and the relationship of the species of the genus *Rodolia* with mealybugs and scale insect pests but the present study explore their predatory role in the biological control of *I. aegyptiaca* for the first time from Pakistan with reference to the adults and 4th instar larvae of *R. ruficollis*. The present study also gives directions regarding the use of developmental stages with high efficacy needed for a successful biological control program. This study can also predict that the species of the genus *Rodolia* may play important role in the biological control of different mealybug pests infesting different agricultural crops including fruits trees throughout Pakistan.

CONCLUSION

The coccinellid predator, *R. ruficollis*, is first time treated taxonomically as well as with the report as a predator of a Scale insect, *I. aegyptiaca* from Sindh Province of Pakistan. This study helps the future studies related with the taxonomy and biological control against mealybug pests in Pakistan.

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