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Apertochrysa smitzi (Navás, 1914) (Neuroptera: Chrysopidae), a novel chrysopid biocontrol agent for Pakistan

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Abstract. Biological control is a key component of Integrated Pest Management, however, the use of green lacewings as a biocontrol agent has not received much attention in Pakistan, although *Chrysoperla carnea*, a generalist predator against a variety of soft-sucking pests, has received much attention in Pakistan. Predatory insects always come out on top when it comes to biological pest control. In addition to minimizing losses caused by pests, applying biocontrol agents helps reduce the ecosystem from being affected by excessive chemical use. The present study proposes a new combination and confirms the newly recorded potential predatory species of green lacewings, *Apertochrysa smitzi* (Navás, 1914) **comb. nov.**, found in Pakistan, belongs to the *Apertochrysa venosa* group based on male genitalia having gonocristae, which is only present in the *A. venosa* group. The diagnostic features of adult, as well as the photographs of dorsal habitus and genitalia, both male and female wings, and the complete life stages of *A. smitzi* are provided. We reared the larvae and adults of *A. smitzi* on the larval and nymphal stages of *Sitotroga cerealella* (Lepidoptera: Gelechiidae) and *Pyrilla perpusilla* (Hemiptera: Lophopidae), respectively, under controlled conditions. *Apertochrysa smitzi* is active most of the year and collected from sugarcane, wheat, and cotton crops from February to March, September and October. Both the larval stages and adults of *A. smitzi* were reared and released as a pest management tactic against various harmful sucking sugarcane insects for the first time in the Sindh province of Pakistan.

Keywords: Biological control, Integrated Pest Management (IPM), green lacewing, Sindh, Pakistan

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Introduction

Chrysopidae is a nearly cosmopolitan family as the second largest group of lacewings, after Myrmeleontidae, with 1,415 described species in three extant subfamilies: Apochrysinae (5 genera, 26 species), Chrysopinae (~80 genera, more than 1000 species), and Nothochrysinae (9 genera, 27 species) (Brooks & Barnard, 1990; Winterton & Brooks, 2002; Breitkreuz *et al.,* 2017, 2021, 2022; Hassan & Liu, 2022; Oswald, 2023). Among the subfamilies, Chrysopinae are widely distributed and hold the highest number of species, containing over 97% of described species in the family (Winterton & de Freitas, 2006; Tauber *et al.,* 2009), which belong to five tribes including Nothancylini, Belonopterygini, Chrysopini, Leucochrysini and Ankylopterygini (Winterton *et al.,* 2019; Breitkreuz *et al.,* 2021, 2022). Currently, Chrysopinae consist of two tribes, six genera, and 16 extant described species in Pakistan: Belonopterygini has only a single species, and Chrysopini with five genera and 24 species (Hassan *et al.,* 2019; Hassan & Liu, 2022).

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Despite being the second-largest family of Neuroptera in Pakistan with 24 species, the larval information on Chrysopidae from this country is poorly known (Hassan & Liu, 2022). So far, only *Chrysoperla carnea* (Stephens, 1836), a common and widespread lacewing across the Holarctic, has been extensively studied as a potential biocontrol agent due to its outstanding polyphagous predatory nature, high consumption rate, relative species abundance, year-round accessibility, and ease of laboratory rearing on soft-bodied insects or on artificial diets (Mirabzadeh *et al.,* 1998; Fathipour *et al.,* 2004; Sattar *et al.,* 2007, 2011; Sarwar, 2014; Hassan & Liu, 2022). Among the chrysopid species in Pakistan, *Chrysoperla carnea* s.l. is distributed in almost all agroecological zones and has been extensively studied as a potential biocontrol agent against various notorious agricultural pests on oilseed crops, fruits, vegetables, fruit trees, and forest trees in Pakistan (Hassan & Liu, 2022).

In the recently published catalogue of the Pakistani Neuropterida, the green lacewing species currently recorded in this country are listed (Hassan *et al.,* 2019). However, the chrysopid fauna of Pakistan has been recently revised and so far, 24 green lacewing species are known to occur in Pakistan (Hassan & Liu, 2022). Before this study, the genus *Apertochrysa* was only known from northern Pakistan; however, we found the representative of this genus for the first time in southern parts and reported *Apertochrysa smitzi* as a new country record.

Materials and methods

Taxonomy

The adults and egg clusters of *A. smitzi* were collected from different localities in District Shaheed Benazirabad (26°21'25.79"N, 68°14'2.13"E) and Naushahro Feroze (26°50'46.66"N, 68°7'30.92"E), Sindh province, Pakistan. The adults and eggs of *A. smitzi* were collected in two seasons during 2020: February and March, and September and October, from sugarcane, wheat, and cotton crops. Collections were done through hand picking and sweeping net. Field collected adults and eggs were reared in the Biological Control Laboratory at Al-Noor Sugar Mills Limited, Shahpur Jahania, Sindh, Pakistan. The specimens were examined under Stereo Zoom microscope with DSLR Camera mounted (Model SZM405; HT Company United Kingdom) and identified at genus level by using the following source of literature: Breitkreuz *et al.* (2021) and Hassan & Liu (2022). The species was confirmed by Dr. Peter Duelli (Zurich Switzerland) and the examined specimens are deposited in the Entomological Collection of Sindh Agriculture University, Tandojam, Pakistan. The morphometric analyses of each larval instar and adults (males and females) were performed with images taken by Fujifilm Finepix S8100fd DSLR camera. The illustrations were further improved using Adobe Photoshop graphic software (ver. 7.0). All the measurements were done in a software generated program image-scope 9.0 (H9D).

Biology

The mass rearing of the Angoumois grain moth, *Sitotroga cerealella* (Olivier, 1789) (Lepidoptera: Gelechiidae) has been carried out under controlled conditions. First, wheat grain was treated with hot water at 100°C for 5-10 minutes to protect the wheat grain from pathogens and predatory mites, which are harmful for *S. cerealella*. After hot water treatment, the grains were exposed to sunlight for 24 h to maintain the optimal moisture content. After that, a cold treatment was applied to eliminate any remaining pathogens. The treated wheat grains were then filled into ½ kg per bottle jars, with 4-5 ml *S. cerealella* eggs added to each jar. After 3-4 days, larvae emerged and fed on wheat grain. After 8-9 days, pupation occurred, and adults emerged after another 8-9 days. The adults were collected using an electronic sucking pump and released into small plastic jars. The base of the plastic jar was fixed with 40-mesh net, and starch was provided through the net for egg laying purpose. After 24 hours, the eggs were sieved and collected through 80-mesh nets.

The egg culsters of *A. smitzi* were kept in black cloth for the larval emergence, while *Sitotroga cerealella* eggs were kept for larval feeding. Tiny alligator like larvae emerged from the eggs. After the first instar larvae emerged, each larva was placed individually in transparent straw tubes, and eggs of the host (*Sitotroga cerealella*) were provided as food for larvae. Six larval samples of each larva were taken for microscopic study, including length of larvae and head capsule. *A. smitzi* larvae were reared and maintained at 28 ± 2°C with relative humidity (RH) of 60 to 70 %. Approximately, 1200-1500 eggs of *Sitotroga cerealella* were provided to a single larva in a transparent straw (tube size 3 inches). One side of the tube was sealed, and larvae were kept inside and provided with host eggs as their diet. The sealed side was punched with a stapler and these tubes were kept on trays, with the larvae spending their whole developmental period inside these tubes. The larval period lasted 8-14 days, involving 3 larval instars. Full-grown larvae spun circular cocoons and pupated in a whitish and circular ball in the transparent plastic straw tubes. Subsequently, the tubes were cut from both sides, allowing the adults to easily emerge from the puparium. These pupal tubes were then placed in Petri dish, and after 6-9 days, adults emerged out from the pupa inside the cage.

The adults of *Apertochrysa smitzi* were reared under laboratory conditions at 28 ± 2°C with relative humidity (RH) of 60 to 70 %. The adults were fed an artificial diet, a liquid solution prepared from yeast, sugar, honey, water, and protein-carbohydrates. After the maintenance of temperature and humidity, a cage was selected for keeping the adults. The cages were made from transparent plastic sheets, sealed on the sides with copper angles, and fitted with a 32-mesh net on the front site. In the center of the mesh, a hole was provided which was covered with white cloth, and that hole was used for sanitation and for providing diet and other activities. At the bottom of the cage, a wooden stand was provided, from feet of that stand water troughs were provided to save adults from crawling organisms. 100-150 mature pupae per cage were provided from which adults emerged inside the cage. After placing the adults in cages, the cages were checked daily, and the diet was provided three times in a day. A black cloth was placed at the top of the cage for egg laying purpose and changed daily to collect eggs.

Results

Taxonomy

Family Chrysopidae Schneider, 1851

Subfamily Chrysopinae Schneider, 1851

Genus *Apertochrysa* Tjeder, 1966

Checklist of *Apertochrysa* Tjeder, 1966 from Pakistan

Apertochrysa murreensis (Tjeder, 1963)

Distribution in Pakistan

Punjab province: Rawalpindi (Murree) (Tjeder, 1963; Hassan *et al.,* 2019; Hassan & Liu, 2022).

Global distribution

India: Uttarkashi (Gangotri) (Ghosh, 1985; Oswald, 2023).

Apertochrysa vartianorum (Hölzel, 1973)

Distribution in Pakistan:

Khyber Pakhtunkhwa province: Madyan (Hölzel, 1973; Brooks & Barnard, 1990; Hassan *et al.,* 2019; Hassan & Liu, 2022; Oswald, 2023).

Global distribution:

This species is so far only known from Pakistan.

Apertochrysa venosa (Rambur, 1838)

Distribution in Pakistan

Gilgit-Baltistan: Gilgit, Nagar, Hunza; Punjab province: Attock (Hölzel, 1967; Oswald, 2023).

Global distribution

Afghanistan, Algeria, Anatolia, Egypt, France, Georgia, Iran, Israel, Lebanon, Mongolia, Morocco, Oman, Pakistan, Portugal, Russia, Saudi Arabia, Sinai Peninsula, Spain, Sudan, Turkey, Tunisia, Yemen (Rambur, 1838; Navás, 1934; Steinmann, 1965; Hölzel, 1967, 2002; Aspöck et al. 1980, 2001; Duelli et al. 2015; Dobosz et al. 2016; Hassan & Liu, 2022; Oswald, 2023).

***Apertochrysa* sp. 1**

Distribution in Pakistan

Punjab province: Islamabad Capital territory, Margalla Hills (Hassan & Liu, 2022).

***Apertochrysa* sp. 2**

Distribution in Pakistan

Azad Kashmir: Poonch, Rawalakot; Khyber Pakhtunkhwa province: Swat valley (Hassan & Liu, 2022).

***Apertochrysa* sp. 3**

Distribution in Pakistan

Gilgit-Baltistan: Skardu, Sermik valley (Hassan & Liu, 2022).

***Apertochrysa* sp. 4**

Distribution in Pakistan

Azad Kashmir: Poonch, Rawalakot (Hassan & Liu, 2022).

***Apertochrysa* sp. 5**

Distribution in Pakistan

Azad Kashmir: Bagh, Rawalakot (Hassan & Liu, 2022).

Apertochrysa smitzi (Navás, 1914) **comb. nov.**

Chrysopa smitzi Navás, 1914: 426. Type locality: India (Maharashtra: Mumbai).

Material examined

Sindh province: Shaheed Benazirabad (26°21'25.79"N, 68°14'2.13"E), 1 February to 31 March, 2020, 1 September to 31 October, 2020, 5♂, 5♀, leg. J.M. Dahri; Naushahro Feroze (26°50'46.66"N, 68°7'30.92"E), 1 February to 31 March, 2020, 1 September to 31 October, 2020, 5♂, 5♀, leg. J.M. Dahri.

Distribution in Pakistan

Sindh province: Shaheed Benazirabad, Naushahro Feroze (present study). New country record.

Global distribution

India: Maharashtra, Karnataka (Navás, 1914; Ghosh & Sen, 1977; Oswald, 2023) and Pakistan (present study).

Taxonomic notes

Apertochrysa smitzi is similar to the undetermined *Apertochrysa* sp. 1 in Hassan & Liu (2022) by external body morphology with lack of distinct markings on pronotum but can be distinguished among the known *Apertochrysa* species in Pakistan by the following characters: body green, without markings, except antennal scape and compound eyes at the dorsal margin below the antennae with the red marking (Figs 1A, 2A, 4A); male genitalia with gonocristae (which is only present the *venosa* group) present, gonapsis butterfly-shaped (Fig. 3A-B).

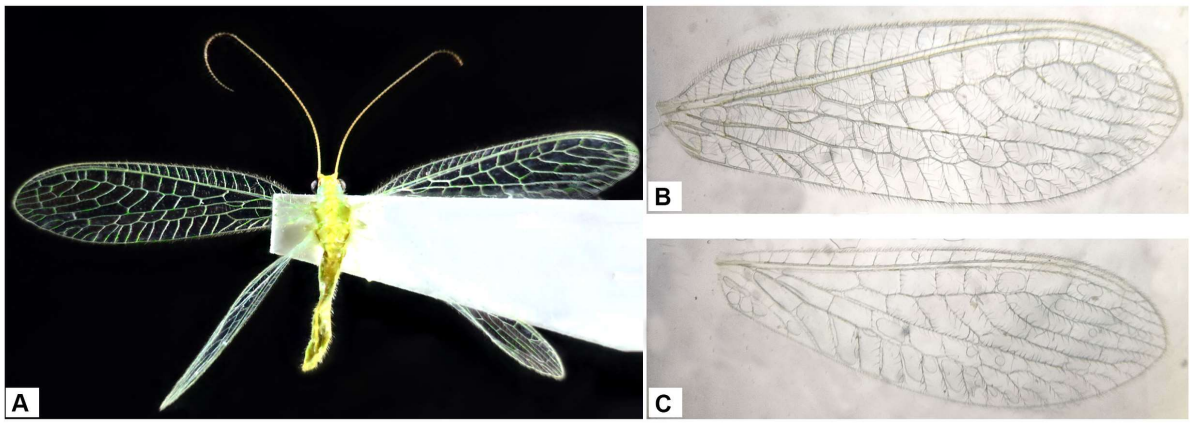

Fig. 1. *Apertochrysa smitzi* (Navás, 1914) (Neuroptera: Chrysopidae). **A.** Male, dorsal habitus; **B-C.** Right, fore- and hind wing.

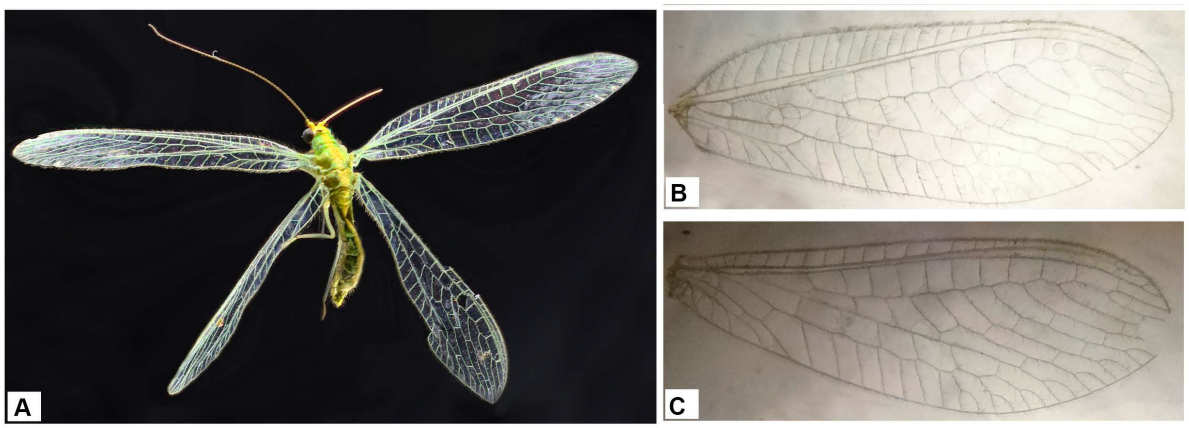

Fig. 2. *Apertochrysa smitzi* (Navás, 1914) (Neuroptera: Chrysopidae). **A.** Female, dorsal habitus; **B-C.** Right, fore- and hind wing.

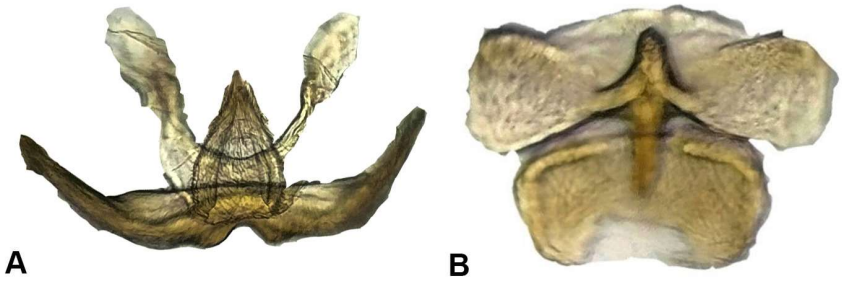

Fig. 3. Male genitalia of *Apertochrysa smitzi* (Navás, 1914) (Neuroptera: Chrysopidae). **A.** Gonapsis; **B.** Wing shapes.

Measurements (♂ n=10, ♀n=8)

Body length: ♂ 9.0 ± 0.05 mm, ♀ 10.4 ± 0.15 mm; forewing length: ♂ 11.2 ± 0.25 mm; hindwing ♂ 9.8 ± 0.84 mm, ♀ 10.0 ± 1.0 mm; pupal length: 1.41 ± 0.30 mm; larval head capsule width: L1 0.52 ± 0.01 mm, L2 0.58 ± 0.01 mm, L3 0.84 ± 0.02 mm; larval body length: L1 3.2 ± 0.05 mm, L2 4.6 ± 0.03 mm, L3 5.6 ± 0.12 mm.

Biological notes

The eggs are laid in clusters, resembling a bunch of grapes protected by a stalk, which protect them from hyperpredators. A single egg cluster contains 15 ± 2 eggs. The development involves three larval instars. The newly hatched larva is called 1st instar larva, it lasts about 3-4 days, and has a redish brown color. The second larval instar is creamy white, having more setae on its body than the first instar. The third larval instar is more active than the first and second instars, with a bunch of setae on the pleuron. Before the third larval instar starts pupation, the abdomen thickens and becomes more prominent. The color of this instar is creamy white. The larvae have setae on their bodies, resembling a tiny alligator in shape, and feed on almost all the developmental stages of the host. They carry debris such as dead insects when feeding on the eggs or nymphs of the host. After feeding, they lift those leftover materials onto their bodies, utilizing this mechanism to protect themselves from hyperpredators. As they mature, they become voracious feeders. The total larval length was 13.2 ± 2 mm, with the length of head capsule ranging from 0.52 to 0.836 ± 0.05 mm. The larval period lasts for 8-14 days. The fully grown larvae excrete waxes from their salivary glands and spin a whitish circular cocoon which is called pupa. The radius of the pupa was measured as 1.406 ± 0.3 mm.

Discussion

Apertochrysa smitzi (Navás), a little-known but excellent predator of numerous sucking pests of valuable crops that was previously known from its type locality in India and is now found in Pakistan after its original description by Navás in 1914.

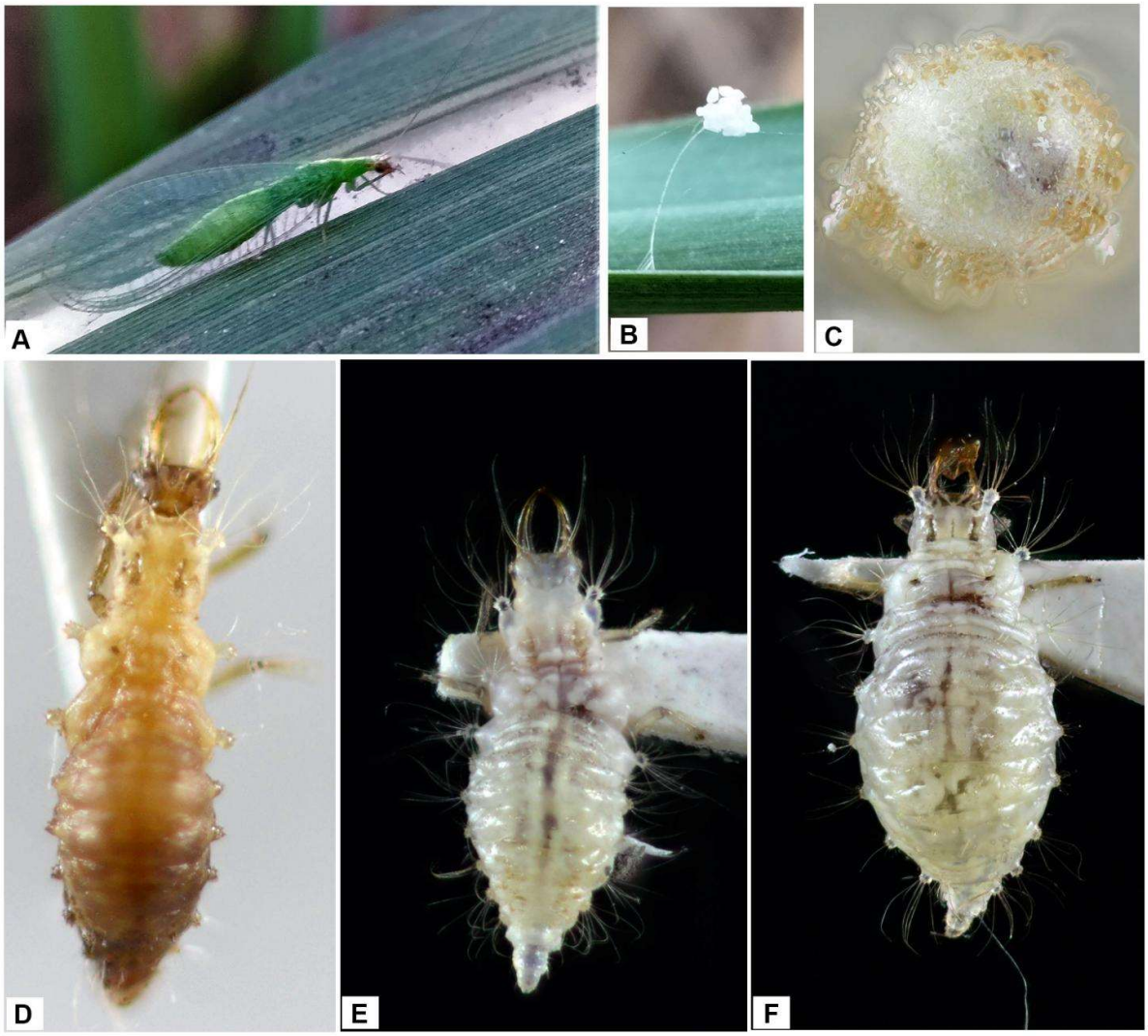

Fig. 4. A. Living habitus of *Apertochrysa smitzi* (Navás, 1914) (Neuroptera: Chrysopidae); **B.** Eggs; **C.** Cocoon; **D-F.** Dorsal habitus of larval instars; **D.** 1st larval instar; **E.** 2nd larval instar; **F.** 3rd larval instar.

Apertochrysa currently includes eight species in Pakistan, with five undetermined species described by Hassan & Liu (2022). Before this study, the *Apertochrysa* species were only known from northern Pakistan; however, we found *Apertochrysa smitzi* from the southern parts, Sindh province of Pakistan. Among the known *Apertochrysa* species in Pakistan, *A. smitzi* is unique by its butterfly-shaped gonapsis and the presence of gonocristae in male genitalia, lack of markings of head and thorax, except a red mark around the eyes and on the antennal scape and the larave with debris carrier.

Among these nine species, only *Apertochrysa murreensis* (Tjeder) has been recorded feeding on *Adelges piceae* on *Abies pindrow* in Pakistan (Tjeder, 1963). *Apertochrysa smitzi* is a good biological control agent and is quite helpful for biological control in Pakistan and other neighbouring countries. It has the potential to survive in a wide range of environmental conditions and is particularly effective for biological pest management. It is different from other predators in its temperature survival power and defence mechanism and it can survive even in stressful conditions. It not only controls sucking pests, but also feeds on the honeydew secreted by many sucking pest species, so through this enormous creature we can also get rid of the diseases which are developed upon that honeydew. Its larvae are voracious predators of almost all the developmental stages of the pest such as egg, larvae, etc. The larva has hair on its body which provides defense against harmful organisms, this natural capability makes it dominant over other predators and has more prey-searching ability than other chrysopids. It is a very important insect in our agroecosystem and through these insects, we can eradicate the indiscriminate use of pesticides which are hazardous to the environment, soil, human beings and other living things on this planet. Climate change has the potential to bring about various impacts on pests, such as changing their global distribution and potentially

increasing their resistance to pesticides. As we know, pests are a biological problem which can be managed through methods like biological control, highlighting the importance of rearing relevant biological control agents. Before that, it is necessary to report such biocontrol agents and their complete life cycle as well as detailed photographs of larval and adult morphological diagnosis characters. Herein, we presented the detailed life cycle and integrated digital photographs showing morphology of both the larval stages and adults. This study will also provide a baseline for comparative studies of *Apertochrysa smitzi* with *Chrysoperla carnea* or any other chrysopid species in future studies in Pakistan. We may also hope to continue this experiment to explore potential egg parasitoids of *Apertochrysa smitzi*, which will be helpful to understand the trophic interactions of this biological agent and its natural parasitoids in the future studies.

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Apertochrysa smitzi (Navás, 1914) (Neuroptera: Chrysopidae)، عامل کنترل بیولوژیک جدید در پاکستان

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چکیده

کنترل بیولوژیک، یکی از اجزای کلیدی مدیریت تلفیقی آفات است، اگر چه استفاده از بالتوری سبز به عنوان یک عامل کنترل بیولوژیک در پاکستان نادیده گرفته شده، ولی *Chrysoperla carnea*، به عنوان یک شکارچی عمومی در برابر انواع آفات مکنده، توجه زیادی را در پاکستان به خود جلب کرده است. در مبحث کنترل بیولوژیک آفات، حشرات شکارچی همیشه در صدر قرار می گیرند. علاوه بر این که صدمات ناشی از آفات را کاهش می دهند، استفاده از عوامل کنترل بیولوژیک به حفظ اکوسیستم از استفاده بی رویه سموم شیمیایی نیز کمک می کند. مطالعه حاضر ترکیب جدیدی را از گونه‌های شکارچی بالقوه، بالتوری سبز، *Apertochrysa smitzi* (Navá, 1914) comb. nov. را پیشنهاد می‌کند که در پاکستان یافت شده و به تازگی ثبت شده است و بر اساس ژنیتالیای حشره نر متعلق به گونه *Apertochrysa venosa* است که دارای gonocristae بوده که فقط در گروه *A. venosa* وجود دارد. ویژگی‌های مورد استفاده در تشخیص حشرات کامل و همچنین عکس‌های habitus پشتی و اندام تناسلی، بال‌های نر و ماده و همه مراحل زندگی *A. smitzi* ارائه شده است. ما لاروها و بالغین *A. smitzi* را به ترتیب روی مراحل لاروی و پورگی *Sitotroga cerealella* (Lepidoptera: Gelechiidae) و *Pyrilla perpusilla* (Hemiptera: Lophopidae) تحت شرایط کنترل شده پرورش دادیم. *Apertochrysa smitzi* بیشتر سال فعال است و از روی محصولات نیشکر، گندم و پنبه از فوریه تا مارس، سپتامبر و اکتبر جمع آوری می شود. هم مراحل لاروی و هم بالغین *A. smitzi* به عنوان یک روش در مدیریت آفات علیه حشرات مکنده و مضر نیشکر برای اولین بار در استان سند پاکستان پرورش داده شده و رهاسازی شدند.

کلمات کلیدی: کنترل بیولوژیک، مدیریت تلفیقی آفات، بالتوری سبز، سند، پاکستان.

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