First record of *Megaselia scalaris* (Loew) as a potential facultative parasitoid of *Apis mellifera* in India

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Abstract

Aims: In 2016, suddenly a pile of dead honey bees, *Apis mellifera* with unfolded wings had been noticed adjacent to the Langstroth bee boxes on the daily interval for a consecutive period of 2-3 months. The present study was an attempt to investigate the reason for this abnormal death.

Place and duration of study: Department of Agricultural Entomology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India between May 2016 and February 2018.

Methodology: Forty-eight numbers of sick bees with stiff-unfolded wings and 50 healthy bees were collected and maintained at a temperature of 30 ± 2° C and 70 ± 5% relative humidity. Maggots developed in the unfolded winged bees and adult flies emerged from pupae were examined. Duration of larval and pupal emergence was recorded at a fixed atmospheric condition. Relative mortality and parasitization rate of *A. mellifera* was also investigated for a period of one year.

Results: Parasitoid larvae emerged from the stiff-unfolded winged dead bees were identified as being *Megaselia scalaris* and further observation revealed that the parasitized bees contained empty body cavities while; normal honey bees were found to be non-parasitized. Matured phorid maggots emerged from the dead honey bees on an average 6 days after collection with the range of 1-14 larvae per honey bee. Maximum 12 pupae per honey bee were found after 18 days of the collection with the emergence of adults at 22nd day. Infestation rate was highest in May and lowest in January.

Conclusion: From this study, *M. scalaris* can be considered as one of the facultative endo-parasitoids of *A. mellifera* and further research should be done about the impact of phorids in Indian commercial beekeeping.

Keywords: Honey bee, India, parasitization, phorid fly, relative abundance

1. **Introduction**

Recently, it has been known that the handful of fly species responsible for global infestations derive from mainly five families *viz*. Sarcophagidae, Phoridae, Conopidae, Tachinidae, and Calliphoridae [1]. The family Phorid (Diptera) has diverse larval food requirements and adaptable feeding behaviours such as phytophagous, scavenger and entomophagous [2]. Phorids are small flies (1-6 mm lengths) often called "scuttle fly" due to their movement in rapid bursts with short pauses [3]. In the tropical belts of America, about eight different species of the genus *Melaloncha* (Diptera: Phoridae) have been found to cause a significant damage to honey bee populations [4]. This particular genus has been known as regional parasitoid of meliponid bees [5] but passed to *Apis mellifera* thereafter, and until recently, all the identified species and *Melaloncha ronnai* in particular, were considered as being the only known parasitoids of honey bees belonging to the Dipteran family Phoridae [6]. In fact, this devastating infestation of honey bee by these Phorid flies was named "autumn disease" due to their regular occurrence [7]. But, recent detection of *Apocephalus borealis* Brues, 1924, a phorid fly native to North America, previously known to parasitize bumble bees and paper wasps [8,9], on the honey bee is now considered as one of the causes of the "Colony Collapse Disorder" [10]. The larvae of *A*. *borealis* have wreaked heavily on honey bee population by causing disorientation, hive abandonment during night time followed by early death; leading thus to the huge destruction of commercial beekeeping in North America [6].

The genus *Megaselia* comprises about 1400 species thoroughly distributed in the tropics [3, 11]. Members of the genus *Megaselia* are mainly dependent on pollen grains and other organic matters in nature [12] where, the most common *Megaselia scalaris* (Loew) is very much distinct for its brown spots on humped thorax, dark eyes and the white eggs presenting upper side covered by thorns [13]. It is Catholic and synanthropic [3] with a wider ordination [14]. Due to the consumption of a broader range of organic remains from both of plant and animal origin, the larval stages of *M. scalaris* have been recognized as facultative as well as an obligatory parasite, parasitoid, detritivore etc. [15, 12]. But, the adult stage generally acts on various living and dead materials as polyphagous [3]. Several documentation revealed that *M*. *scalaris* is able to feed on several living arthropods including the members of different orders like Orthoptera [16], Lepidoptera [17], Coleoptera [18], Hymenoptera [19], Diptera [20] etc. Rocha et al. [21] reported about the infestation of well-fed cattle-tick (*Boophilus microplus*) females by *M*. *scalaris* and at the same time accentuate the probability of this Phorid fly to act as a parasitoid in the colonies of *A*. *mellifera* L. (Hymenoptera: Apidae). Later, *M*. *scalaris* has been identified as a scavenger of *A*. *mellifera* in Spain [22]. Although it attacks living cells inside the larvae of *A*. *mellifera* or dead adults, *M*. *preacuta* has not been considered as honey bee parasite but, behaves like a scavenger in sick colonies [23]. Dutto and Ferrazzi [6] recently demonstrated the facultative parasitoidism in *A*. *mellifera* by *M*. *rufipes* in Italy. In India, two documentations of *M*. *scalaris* *viz*. intestinal myiasis of a 32 years old woman in Manipur [24] and infection in oyster mushroom [25] has been documented till date. However, from the point of the economic importance of honey bee in India, both *A. mellifera* and *A. cerana indica* play the pivotal role in commercial apiculture [26, 27].

The Phorid and focus of this paper, *M*. *scalaris* (Loew), has been detected accidentally from stiff-unfolded winged honey bees as a potential agent of facultative parasitoidism of commercial beehives in India. The seasonal incidence of *M. scalaris* on *A. mellifera* with stiff-unfolded wings along with its parasitization rate has also been studied, which may develop a transparent idea regarding their infestation and survivability throughout the year.

2. Material and Methods

2.1. Identification of the parasitoid:

During May 2016, Langstroth beehive boxes near the faculty centre of our University campus (Nadia, West Bengal, India) at an elevation of 9.75 m above sea level (22.9452° N, 88.5336° E) were inspected. Forty-eight live worker honey bees (*A. mellifera*) with markedly stiff-unfolded wings were collected during the evening hours from the concrete floor mostly near a light source adjacent to the hives. At the same time, forty living worker honey bees those capable to flight were also collected from those particular hives with the help of an electric vacuum cleaner [6]. The specimens were placed within test tubes properly plugged with absorbent clinical cotton in order to maintain sufficient air exchange and transported to the laboratory. The stereomicroscopic examination was done and then each specimen was introduced into a Petri plate and maintained at a temperature of 30 ± 2 °C and 70 ± 5% relative humidity. The living honey bees were sustained by a wad of cotton piece soaked in sucrose solution and monitored daily. For systematic determination, maggot developed in the bees with unfolded wings along with adult flies after emergence from pupae were examined under a stereo zoom binocular (Olympus SZ 40, Japan) microscope at 10x magnification. The taxonomic identification of adult flies was done based on the key of Disney [28]. After the death of living honey bees, both the apparently healthy ones and those with unfolded wings were dissected in order to take an observation of their internal organs. Photographs of the Phorid maggot, pupa and adult were captured by Kodak Easy-Share Z740 camera mounted on Olympus SMZ-2T microscope.

2.2. Duration of larval and pupal emergence under laboratory condition:

Date of larval emergence for a subset of 212 parasitized bees and duration of pupal instar for a subset of 80 pupae were examined at the previously mentioned atmospheric conditions in Department of Agricultural Entomology of Bidhan Chandra Krishi Viswavidyalaya (BCKV), Nadia, West Bengal, India [10].

2.3. Relative abundance and infestation rate of the parasitoid:

Relative abundance of *M. scalaris* during different months was also investigated from March 2017 to February 2018. Stiff-unfolded winged bees found near the light sources of the adjacent beehive boxes were collected twice in a week throughout the year and brought at room temperature in individual *Drosophila* rearing containers. Containers of each of the collected lot were checked daily for a period of two weeks and recorded the rate of infestation caused by *M. scalaris*. Percent infestation was calculated on monthly basis. Daily meteorological data of the experimental location were also collected from the automated weather station of Department of Agro-meteorology & Physics, BCKV, Kalyani throughout the study period in order to conduct the correlation study.

2.4. Statistical interpretation:

The mean values were subjected to statistical analysis by ANOVA using SPSS (version 18.0: Inc., Chicago, IL, USA) software after making necessary transformations whenever required. Mean values were separated by Duncan's Multiple Range Test (DMRT) as per Gomez and Gomez [29] at $p<0.05$for interpretation of the results.

3. Results

Microscopic results revealed that 3 specimens (6%) among the stiff-unfolded winged bees had some eggs adhering to their abdomens at the point of inter-segmental membranes. Following the placement of Petri plate, 41 honey bee specimens (85%) had died and started to release Dipteran maggots after 48-72 hours. 5 specimens survived 2-3 days more than the others among the stiff-unfolded winged population as those were not infested by *M. scalaris*. The larval parasitoid those developed on dead honey bees were classified as belonging to the family Phoridae (Plate 1). The pupal metamorphosis took place near about 13 days (Plate 1) after the hatching of eggs, followed by 9-11 days later adult flies emerged. Identification of the fly, *M. scalaris* was confirmed by examining the adult male and female (Plate 1) under the light microscope. Microscopic examination of the dissected abdomen of dead bees indicated that maggots developed in the bee body cavity by consuming the internal organs. Investigation in the parasitized bees clearly revealed that the thorax and abdomen had been fully emptied and dismembered. No other parasitoid was detected from the dead honey bee specimens.

Matured phorid larvae emerged from the junction between the bee's head and thorax and from 8^{th} or 9^{th} abdominal segments of ventral side (Plate 1), on an average six days after collection (n = 375, Range = 2-13, SD = 1.52) (Figure 1) and moved away from the bee to pupate. All larvae emerged from worker bees successfully pupated under laboratory conditions. Production rates from dead bees ranged from 1 to 14 mature larvae per infected honeybee (n = 837, Mean = 5.4, SD = 2.8) (Figure 2). Pupal production with 12 pupae produced by a single bee was also observed under laboratory condition (Figure 3). Adult flies emerged on an average 22 days after hatching (n = 76, Range = 17-29, SD = 1.6).

Relative abundance of *M. scalaris* was found throughout the year but, the rate of infestation to *A. mellifera* varied significantly during different months (Table 1). Highest mean percent infestation was encountered in May ($F_{11,22}$= 11.16, P = 0.03) while, lowest per cent infestation was recorded in the month of January ($F_{11,22}$ = 15.25, P = 0.01). Summer months registered relatively more infestation of this phorid fly than that of winter and monsoon months which is very much clear from Table 1. Table 2 revealed that mean per cent parasitization on *A. mellifera* by *M. scalaris* significantly and positively correlated with maximum and minimum temperature while, non-significant but positive correlation was registered by rainfall and minimum relative humidity respectively. In contrast, maximum relative humidity showed non-significant and negative correlation on the relative abundance of *M. scalaris*.

4. Discussion

In case of the infestation of *M. scalaris*, dead honey bees served itself as a suitable resource to exploit after deposition of eggs on their abdomen for ensuring embryonic development. First instar larvae after hatching penetrated the inter-segmental membranes and after completion of the larval stage they had left their host and emerged outside the host body in search of a favourable pupation site. Similar behaviour among many members of the genus *Megaselia* has been documented under laboratory condition against Hemipterans [3], cockroach species [12], Noctuid moths [30], blowflies (Diptera: Calliphoridae) [20]. Parasitoid activity of *M. rufipes* was detected only in bees with deformed wings by Dutto and Ferrazzi [6] and concluded the phorid fly as a facultative parasitoid of *A. mellifera*, fully supports the findings of the present study. However, an infestation of *M. scalaris* was recorded from honey bee in Spain by Farnendez et al. [22]. Robinson [31] observed similar behaviours in *M. scalaris* in both the laboratory cockroach culture and in the field. On the other hand, the suggestions of Macieira et al. [32] and Rocha et al. [21] indicated the possibility of *M. scalaris* to be an optional parasitoid of honey bees.

The life cycle of *M. scalaris* is very short and generally completes in about three weeks with strong temperature dependent stadial duration [33].Temperature significantly played the most important role on developmental times of *M. scalaris* has been documented by Trumble and Pienkowski [34] and concluded that the rate of development increased with increasing temperature; corroborates the findings of the present authors. On the other hand, Disney [12] mentioned the relative abundance of *M. scalaris* is higher than that of the lower range of temperature. Average rainfall does not affect significantly and had played a very minor role on the seasonal incidence of the phorid fly.

Conclusions

M. scalaris can be considered as a facultative parasitoid of *A*. *mellifera* under Indian climatic condition and its detection from honey bees with stiff-unfolded wings opens a new pavement in the role of Phoridae in honey bee colony health apart from *Varroa destructor*. All these information require a thorough investigation of the role of Phoridae against honeybee. It also focuses the requirement of further inquiry into the action of *M. scalaris* on healthy and infested bees and its incidence in other commercial apiaries outside West Bengal.

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Competing interests

Authors have declared that no competing interests exist.

Authors' contributions

PD identified the parasitoid species and performed the laboratory experiments related to duration of stadial duration. DR conducted the last part of the study of relative abundance along with the statistical analysis. Both the authors read and approved the final manuscript.

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Table 1

Mean per cent infestation of *M. scalaris* on *A. mellifera* during March, 2017 to February, 2018

Months	Mean % infestation
March	31.13 (33.91)*de
April	47.14 (43.36)c
May	74.28 (59.53)a
June	73.05 (58.73)a
July	69.51 (56.48)ab
August	59.33 (50.38)bc
September	66.70 (54.76)ab
October	52.81 (46.61)c
November	39.52 (38.95)d
December	24.67 (29.78)e
January	12.32 (20.55)f
February	15.30 (23.03)f
SEM(±)	0.60
LSD (0.05)	2.52

*Data in these parentheses are Sin-1 transformed values

Mean values followed by different letters are significantly different (otherwise statistically at par) at *P*<0.005 by Duncan's multiple range test

Table 2

Correlation coefficients (*r*) for meteorological factors affecting per cent parasitization of *A. mellifera* by *M. scalaris* during March, 2017 to February, 2018

Abiotic factors	Pearson's *r*
Maximum Temperature	0.856**
Minimum temperature	0.703*
Rainfall	0.273
Maximum relative humidity	-0.191
Minimum relative humidity	0.349

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

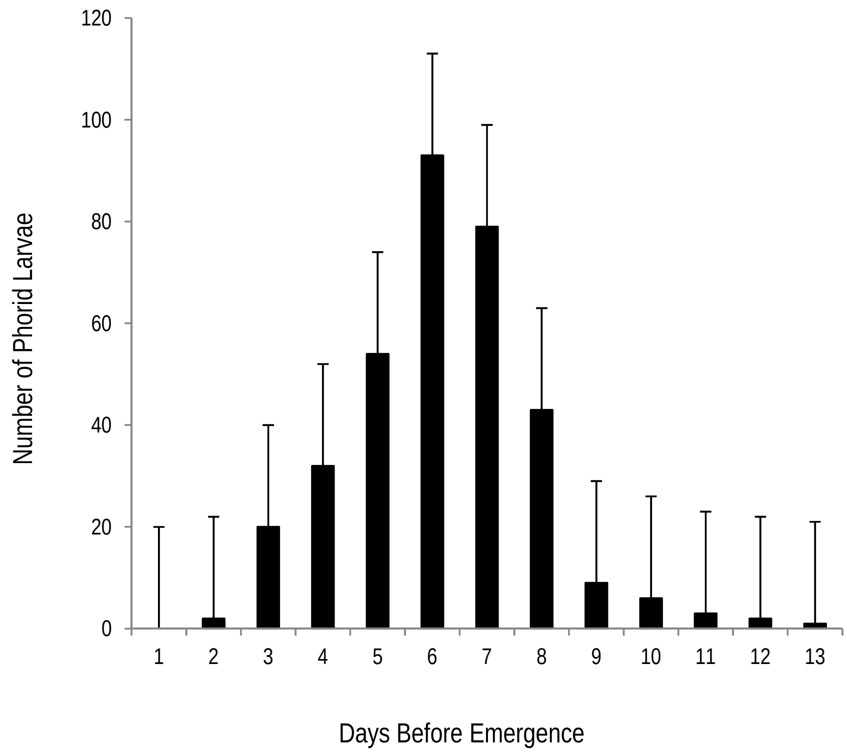

Fig. 1
Length of time after sample collection until phorid larvae emerged from honey bees (n = 375, Mean = 6.22 days, SD = 1.52)

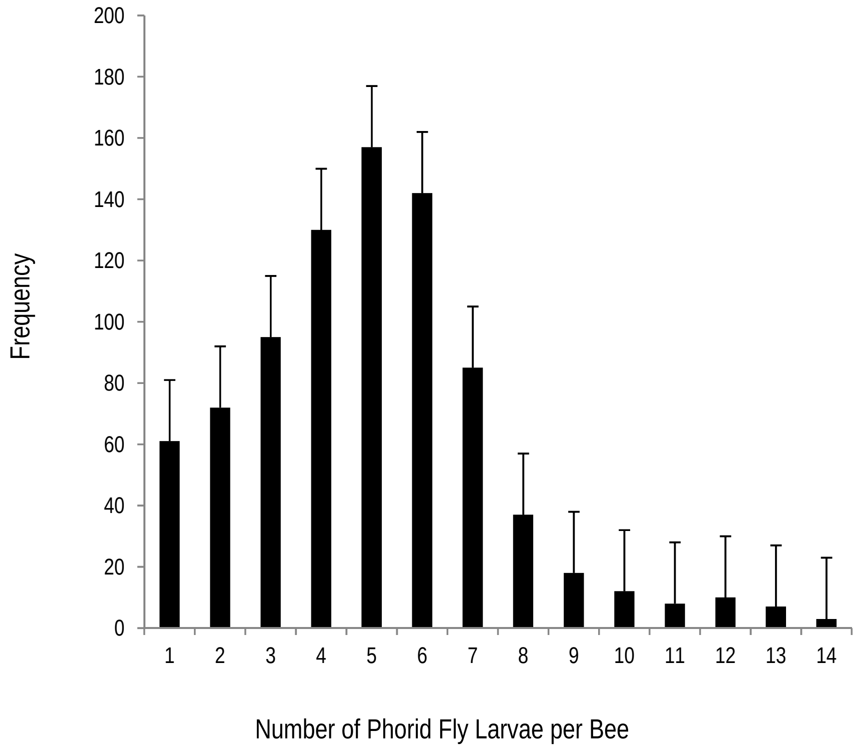

Fig. 2
Number of phorid fly larvae per infected bee for samples from main study hive (n = 837, Mean = 5.4, SD = 2.8)

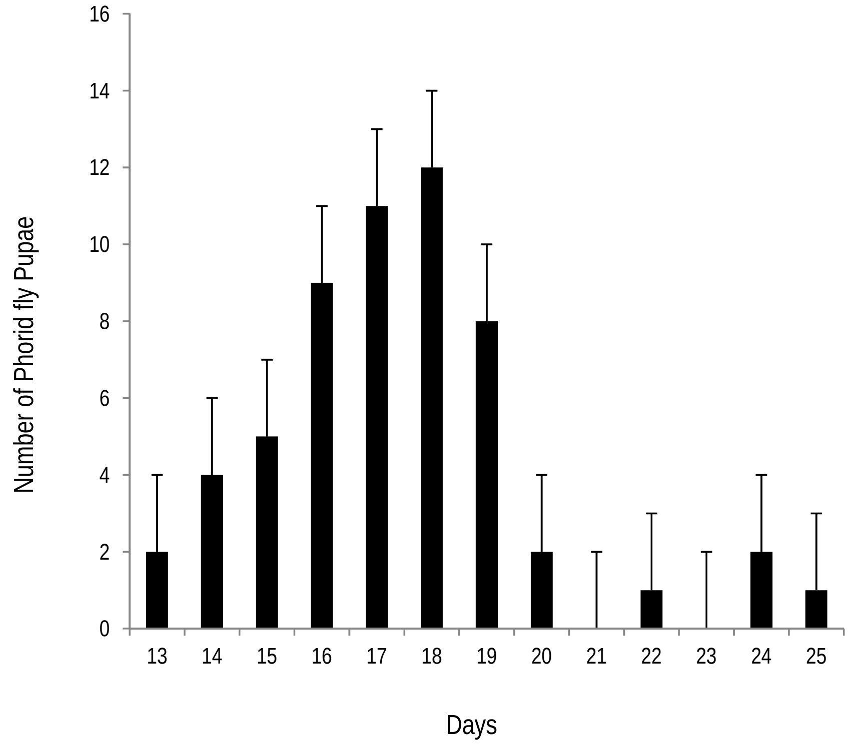

Fig. 3
Length of pupal period (n = 70, Mean = 18.2 days, SD = 1.6)

Plate 1

Megaselia scalaris (Loew). A1: Third instar larvae of *M. scalaris*. A2: Anterior end of larval mouthpart. B: Pupa, dorso-lateral habitus. C1: Adult male, lateral habitus. C2: Adult female, lateral habitus. D1 & D2: Mature *M. scalaris* larva partially emerging from the thoracic segment (D1) and abdominal segment (D2) of an *Apis mellifera* worker.