

THE ENTOMOLOGICAL SOCIETY OF INDIA

New Delhi, 110012

ESI- BEST Ph.D. THESIS AWARD 2021

1. Name of the applicant: **ABHISHEK RANA**
2. Year of the award: **2021**
3. Date of birth: **13/12/1992**
4. Field of specialization: **Entomology**
5. Mode of award collection: **In person**
6. Postal address (with Phone/Fax/Email etc.): **Dr. Abhishek Rana, Junior Research Fellow, Department of Entomology, CSK HPKV Palampur, District Kangra, Himachal Pradesh 176062; 9459687827 / 7018856682; Email: abhir1392@gmail.com**

7. Educational qualifications beginning with the first-degree or equivalent (in a tabular form)

S.No.	Degree	Institution	Year
1	10 th Class	HPBOSE Dharamshala, Himachal Pradesh	2008
2	10+2 (12 th)	HPBOSE Dharamshala, Himachal Pradesh	2010
3	Bachelor's degree	Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, H.P.	2014
4	Master's degree	Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, H.P.	2016
5	Ph.D. degree	Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, H.P.	2021

8. Date on which qualifying examination passed: **01/04/2021**
9. Transcript of the course work done, if any, as part of the Ph.D. degree programme. (enclose photocopy): **(Copy enclosed)**
10. Was the thesis submitted in partial fulfillment of the requirement of the Degree? **Yes**
11. Date on which successfully completed thesis viva voce examination. **01/04/2021**
12. Date of the Degree/provisional certificate **06/04/2021**
13. Have you received best thesis award earlier? **No**
14. Details of the thesis

- (i) Title of the thesis

Bioecology and Management of *Melolontha* spp. (Scarabaeidae: Coleoptera) in Himachal Pradesh

- (ii) Department/Division/Institution where research work was done
Department of Entomology, College of Agriculture, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176 062 (HP) India
- (iii) Institution/University to which thesis was submitted as a part of the Ph.D. degree programme
Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176 062 (HP) India
- (iv) Name and designation of the thesis supervisor
Dr. R.S. Chandel, Professor and Head, Department of Entomology, CSK HPKV Palampur- 176062 (visitrvchandel@yahoo.com Mob No. 9418019747)
- (v) Research problem: Hypothesis, brief description, scientific, technological, socio-economic relevance and priority (limit to 1 page) **(Copy enclosed)**
- (vi) What standard methods and modern procedures were used in the experimental work? (limit to one page) **(Copy enclosed)**
- (vii) Briefly describe the significant results obtained? (Not more than 3 pages) **(Copy enclosed)**
- (viii) In what way these results have made an original contribution to science and have impact

No information was available on biology and management of *Melolontha* species, neither in Himachal Pradesh nor from other hilly states of India where this genus is mainly confined. The biology of all four species has been studied for the first time and the larval/pupal descriptions are totally original contribution. Similarly, molecular analysis has been carried out for the first time in the world for these species. This study is going to have an impact on the management of the *Melolontha* species in their endemic areas throughout Himalayan regions. The white grubs are causing serious damage in several crops like cabbage, rajmash and potato which are the main cash crops of Himachal Pradesh and no control measures are actually available for their management. There are general recommendations for the management of white grubs which are based on the studies conducted on *Holotrichia consanguinea*, *Holotrichia serrata* and *Brahmina coriacea* which are having annual life cycle. Our study revealed that *Melolontha* species have biannual

life cycle and the grubs, they remain in the soil for about 20 months and their nature of damage also differs from other white grub species having annual life cycle. Therefore, the observations recorded on biology can be utilized scientifically for an effective management of *Melolontha* white grubs in different crops.

- (ix)** Publications (in journals/along with NAAS rating as on 1.1.2021, Seminar proceedings, University/Institute Newsletter, Lecture delivered, Book chapter etc.(publications only from the thesis to be included, only those publications in published/accepted/proof stage will be considered, no marks will be given for other publications not related with thesis works)

Research articles

- Rana Abhishek, Chandel RS, Sharma KD, Chandel SS and Verma KS. 2021. Phylogenetic analysis of *Melolontha* and *Polyphylla* beetles (Scarabaeidae: Coleoptera) from north-western Himalaya, India. *Phytoparasitica* <https://doi.org/10.1007/s12600-021-00945-5>. (NAAS: 7.14)
- Rana Abhishek, Chandel RS and Verma KS. 2021. Morphological description and identification of *Melolontha cuprescens*, *Melolontha furcicauda* and *Melolontha indica* (Coleoptera: Scarabaeidae): serious root feeding and defoliating pests in India. *Phytoparasitica* <https://doi.org/10.1007/s12600-021-00961-5> (NAAS: 7.14)
- Rana Abhishek, Chandel RS and Verma KS. (2020). Efficacy of insecticides as seed treatment for the management of white grubs. *Himachal Journal of Agricultural Research* 46 (2) : 169-172 (NAAS: 3.44)
- Chandel RS, Soni S, Vashisth S, Pathania M, Mehta PK, Rana Abhishek, Bhatnagar A and Agrawal VK. 2018. The potential of entomopathogens in biological control of white grubs. *International Journal of Pest Management* 65(4): 348-362 (NAAS: 7.09)
- Chandel RS, Rana Abhishek, Sanjta S. 2018 and Mehta P.K. 2018. Potential of Entomopathogens in Managing Potato White grubs in Himachal Pradesh. *Indian Journal of Ecology* 45(1): 210-213 (NAAS: 5.79)

Bulletins

Chandel RS, Mehta PK, Sanjta S, Sharma M, Rana Abhishek and Agrawal VK. 2017. *Himachal Pradesh mein Kurmula Keet ka samekit prabandhan*. Published by Department of Entomology, CSK HPKV, Palampur 6p.

Folder

Chandel RS, Verma KS, Baloda AS, Sanjta S and Rana Abhishek. 2018. Kaise karein faslon ka kurmula keet se bachaw. Published by Department of Entomology, CSK HPKV, Palampur 3p.

Abstract

- Chandel RS, Verma KS and Rana Abhishek. 2019. *Melolontha virescens* Brenske: An emerging pest of wheat in high hills and dry temperate zone of Himachal Pradesh. Proceedings of Conference on “4th International Group Meeting (4 IGM-2019) on Wheat Productivity Enhancement through Climate Smart Practices. p71, 14-16 February 2019. CSKHPKV, Palampur, Himachal Pradesh.
- Chandel RS, Rana Abhishek, Sanjta S and Mehta PK. 2017. Potential of Entomopathogens in Managing Potato White grubs in Himachal Pradesh. Proceedings of National Conference on “Alternate Farming Systems to Enhance Farmer’s Income. p201, 19-21 September, 2017. YSP University of Horticulture & Forestry, Nauni, Solan, Himachal Pradesh.
- Rana Abhishek, Chandel RS, Verma KS and Sanjta Suman. 2020 Evaluation of entomopathogenic nematode, *Heterorhabditis indica* against potato white grubs in Himachal Pradesh. Proceedings of “Global Potato Conclave 2020; Roadmap for a better world”. p156, 28-31 January, 2020. Organized by CPRI, Shimla, ICAR, New Delhi and Indian Potato Association, Shimla at Gandhinagar, Gujarat, India.

(x) Soft copy in PDF format needs to be submitted for the above including whole thesis
(Copy enclosed)

15. Whether any patents have been taken/applied for, if yes, attach evidence : **No**
16. Professional recognition, awards, fellowships received (In a separate sheet give full particulars such as the agency/ organization which gave the award, purpose, the nature of the award, etc.). (Copy enclosed)
17. A concise statement (about 150 words) highlighting the most significant aspects of the research work done that you would like to see in your citation of award, if chosen.

White grubs are considered as national pests in India. The cockchafer of the genus *Melolontha* rank amongst the serious pests in agriculture and forestry. The work carried out

on *Melolontha* species is totally a new piece of work in India and the information generated on their biology will be very useful in their management. The description of the larvae of different species will be very useful in their identification. The pictures and diagrammes given in the thesis are of very good quality and very informative. The *Cox1* gene has been found to be a better DNA barcode for identification of *Melolontha* species. The grubs are present in soil at a depth of about 10 cm and it is very difficult to translocate lethal doses of insecticides in the soil. Therefore, seed treatment seems to be safe and economical method for the control of white grubs in cash crops like rajmash as discussed in the thesis.

18. Certificate by the student that all the details given are true (**Copy enclosed**)

I, **Abhishek Rana** son of **Late Sh. Kamlesh Kumar** hereby declare that all the statements and entries made in this application are true, complete and correct to the best of my knowledge and belief.



Signature

19. Certificate by the supervisor/guide that the information submitted has been verified and true (**Copy enclosed**)

Signature

20. Forwarding note by the Head of the Department/professor (**Copy enclosed**)

Name and Designation

Seal

21. Annexures

Sr. No.	Documents
1	Matriculation certificate (Age proof)
2	Plus two certificate
3	B.Sc. degree certificate
4	M.Sc. degree certificate
5	Ph.D. PDC
6	Ph.D. transcript
7	Research problem: Hypothesis, brief description, scientific, technological, socio-economic relevance and priority
8	What standard methods and modern procedures were used in the experimental work?
9	Briefly describe the significant results obtained?
10	Professional recognition, awards, fellowships received (In a separate sheet give full particulars such as the agency/ organization which gave the award, purpose, the nature of the award, etc.).
11	Certificate by the student that all the details given are true
12	Certificate by the supervisor/guide that the information submitted has been verified and true
13	Forwarding note by the Head of the Department/professor
14	Passport size photograph
15-24	Research papers, abstracts, bulletins etc.

List of self-attested scanned documents to be enclosed:

1. Publications (**First page alone**)
2. Photo copy of PhD degree
3. Age proof
4. Best passport size photograph
5. Thesis (soft copy)
6. Completeness of the application rests with applicant and no further additions/alterations will be entertained.
7. Complete nominations are to be submitted online at www.entosocindia.com awards section by using member login and password on or before **11.59 PM of the 21 October 2021**. No correspondence will be entertained in this regard.
8. This year we are also considering applications through email **in single PDF (not more than 50 pages)** including application form and with all enclosures must reach by email: esiaward@gmail.com (hardcopy will not be entertained) on or before **11.59 PM of the 21 October 2021**. No correspondence will be entertained in this regard.

1301489

Roll No. 1702820

HIMACHAL PRADESH BOARD OF SCHOOL EDUCATION**MARCH 2008**

This is to certify that

ABHISHEK RANA

Sex : Male/Female

Father's Name Shri

KAMLESH KUMAR

Mother's Name Smt.

DAYA DEVI

Born on

(13/12/92) THIRTEENTH DECEMBER

One thousand nine hundred and

NINETY TWOhas passed the **MATRICULATION EXAMINATION** of this Board

from/as

GOVT HIGH SCHOOL DRAMAN

District

KANGRA

and placed in

FIRST

Division

DETAIL OF MARKS

Sr.No.	Subjects	Marks Obtained	Max. Marks
1.	English	78 *	100
2.	Mathematics	71	100
3.	Hindi	60	100
4.	Social Science (His. Civ. Geog)	86 *	100
5.	Science & Technology (Phy. Chem. Life Sci.)	87 *	100
6.	SANSKRIT	84 *	100
7.	ART (Elective)	79 *	100
8.	Grace Marks for Division Improvement, if any	XX	
	TOTAL	FIVE HUNDRED AND FORTY FIVE ONLY	700

* Denotes Distinction i.e. 75% marks or above.

'F' indicates Failure in the subject & marks not included in the Grand Total.

NON EXAMINATION SUBJECTS INTERNAL (FOR SCHOOL CANDIDATES ONLY)**GRADE**

i) Socially useful productive work and Community Service

A

ii) Physical and Health Education.

A

iii) Education for Human Values and Supervised Studies.

B

Note : For gradation of Non Examination Subjects, please see overleaf.

DHARAMSHALA

Dated : 08/05/2008



[Signature]
SECRETARY

[Signature] self attested

Sr. No. **543977**Roll No. **2982960****HIMACHAL PRADESH BOARD OF SCHOOL EDUCATION****SENIOR SECONDARY (PLUS TWO) EXAMINATION**

MARCH 2010

SCIENCE

Group

This is to certify that **ABHISHEK RANA**Sex : **MALE**Father's Name Shri **KAMLESH KUMAR**Mother's Name Smt. **DAYA DEVI**has passed the **PLUS TWO EXAMINATION** of this Boardfrom/as **A V M SR SEC SCHOOL PAHRA**District **KANGRA**and placed in **FIRST** Division**DETAIL OF MARKS**

Sr. No.	Subjects	Marks Obtained	Maximum Marks
1.	English	62	100
2.	MATHEMATICS	89 *	100
3.	PHYSICS W.64 P.23	87 *	100
4.	CHEMISTRY W.53 P.24	77 *	100
5.	PH. EDUCATION W.59 P.20	79 *	100
6.	//////////////////// (Additional)	XX	100
7.	//////////////////// (Additional)	XX	100
8.	Grace Marks for Division Improvement, if any	XX	
	TOTAL THREE HUNDRED AND NINETY FOUR ONLY	394	500

1. * Denotes Distinction i.e. 75% marks or above.
2. The Marks of Additional Subject(s) have not been included in the aggregate.
3. Where the candidate has failed in the Elective Subjects the marks of Additional Subject(s) have been accounted towards aggregate and division.

**DHARAMSHALA**Dated : **20/04/2010****SECRETARY**

[Signature]

[Signature] self attested

प्रवेशांक/Admission No. A-2010-01-01



विश्वविद्यालय के प्रतीकाधीन प्रदत्त
Given under the seal of the University

चौ० सनवण कुमार

हिमाचल प्रदेश कृषि विश्वविद्यालय

विद्वत् परिषद् की संस्तुति पर

अभिषेक नाणा

को

कृषि विज्ञान स्नातक(सम्मान)

की उपाधि के लिए अपेक्षित पाठ्यक्रम का जून, २०१४ में सफल पानण करने
७.७१/१०.०० समग्रकोटि मध्यमान अंक अर्जित करने पर उक्त उपाधि प्रदान की जाती है।

Ch. Sarwan Kumar

Himachal Pradesh Krishi Vishwavidyalaya

on the recommendation of the

Academic Council hereby confers upon

Abhishek Rana

the degree of

Bachelor of Science (Hons.) Agriculture

having successfully completed in June, 2014 all the requirements for the degree
with an overall credit point average of 7.71/10.00.

पालमपुर (हिमाचल प्रदेश)

Palampur (Himachal Pradesh)

दिनांक

The 12th Sept., 2014

Rathan Paulam
कुल सचिव
Registrar

K. Lal
कुलपति
Vice-Chancellor

[Signature]
Self attested



विश्वविद्यालय के प्रतीकाधीन प्रदत्त
Given under the seal of the University

चौ० सनवण कुमार

हिमाचल प्रदेश कृषि विश्वविद्यालय

विद्वत् परिषद् की संस्तुति पर

अभिषेक राणा

को

कृषि विज्ञान प्रवीण

(कीट विज्ञान)

की उपाधि के लिए अपेक्षित पाठ्यक्रम का १६.०७.२०१६ में सफल पानण करने
८.३९/१०.०० समग्रकोटि सध्यमान अंक अर्जित करने पर उक्त उपाधि प्रदान की जाती है।

Ch. Sarwan Kumar

Himachal Pradesh Krishi Vishwavidyalaya

on the recommendation of the
Academic Council hereby confers upon

Abhishek Rana

the degree of

Master of Science in Agriculture

(Entomology)

having successfully completed on 16.07.2016 all the requirements for the
degree with an overall grade point average of 8.39/10.00.



पालमपुर (हिमाचल प्रदेश)
Palampur (Himachal Pradesh)

दिनांक
The 8th September, 2017

[Signature]
कूल सचिव
Registrar

[Signature]
कुलपति
Vice-Chancellor

[Signature]
Self attested

Serial Number 3123

Admission Number: A-2016-40-007

CSK HIMACHAL PRADESH KRISHI VISHVAVIDYALAYA
PALAMPUR, H.P. 176062
Provisional Degree Certificate

This is to certify that Abhishek Rana Son/Daughter of Smt. Daya Devi and Shri Kamlesh Kumar has successfully completed the requirements for award of Degree in Doctor of Philosophy (Entomology) of this Krishi Vishvavidyalaya on 01.04.2021 His/Her Overall Grade Point Average is 8.12 out of 10.00 with 81.20 percent marks.



PALAMPUR

Dated: 06 APR 2021

(Signature)
Self attested

(Signature)
REGISTRAR
Registrar
CSK HPKV, Palampur (H.P.)

CSK HIMACHAL PRADESH KRISHI VISHVAVIDYALAYA, PALAMPUR-176062



TRANSCRIPT OF ACADEMIC RECORD (POSTGRADUATE STUDENT)



Name: **Abhishek Rana**
 Aadhar No: **2488 2409 2935**
 Programme: **Doctor of Philosophy**
 Department: **Entomology**
 Year of Admission: **July, 2016**
 Major Subject: **Entomology**

Father's Name: **Sh Kamlesh Kumar**
 Mother's Name: **Smt. Daya Devi**
 College of Agriculture

Date of successful completion: **01.04.2021**
 Minor Subject (i) **Plant Pathology**
 (ii) **Agricultural Biotechnology**

1. (a) Semester (s), if any, during which dropped from the University
 (b) Reason (s) for dropping/Date of Re-admission, if any
 2. Eligibility for Honour Certificate

No
 No
 No

Title of thesis: **"Bioecology and management of Melolontha spp. (Scarabaeidae: Coleoptera) in Himachal Pradesh"**

Semester	Course No.	Course Title	Credit Hours	Grade point	Credit points
2016-2017 1st Semester					
	ENT 604	Advanced Insect Ecology	2	8.2	16.4
	ENT 606	Recent Trends in Biological Control	2	7.7	15.4
	ENT 611	Molecular Approaches in Entomological Research	2	7.9	15.8
	PL PATH 507	Diseases of Field and Medicinal Crops	1	8.6	25.8
	VSC 501	Principles of Vegetable Production	3	8.2	24.6
2nd Semester					
	ENT 603	Advanced Insect Physiology	2	7.1	14.2
	ENT 607	Advanced Insecticide Toxicology		8.2	24.6
	ENT 612	Advanced Integrated Pest Management	2	8.7	17.4
	ENT 613	Plant Biosecurity and Biosafety	2	8.2	16.4
	ENT 699	Doctoral Research	(2)	5	-
	BIOTECH 562	Genetic Engineering	2	7.4	14.8
	BIOTECH 563	Lab on Genetic Engineering	2	8.6	17.2
	PL PATH 509	Diseases of Vegetable & Spice Crops	3	8.0	24.0
2017-2018 1st Semester					
	ENT 699	Doctoral Research	(16)	S	
	BIOTECH 591	Master's Seminar	1	8.4	8.4
2nd Semester					
	ENT 699	Doctoral Research	(15)	S	-
	ENT 691	Doctoral Seminar	1	8.6	8.6
2018-2019 1st Semester					
	ENT 699	Doctoral Research	(12)	S	-
2nd Semester					
		Thesis Writing	-	-	-
2019-2020 1st Semester					
	-	Thesis Writing	-	-	-
2nd Semester					
	-	Thesis Writing	-	-	-
2020-2021 1st Semester					
	-	Thesis Writing and Viva-Voce	-	-	-

Total Credit Hours **30** Total Credit Points **243.6**
 Research Credit Hours **45**
 OVERALL GRADE POINT AVERAGE **8.12**
 Percentage of Marks **81.20**

PALAMPUR

Dated **06 AUG 2021**

REGISTRAR
 Registrar
 CSK HPKV, Palampur (H.P.)

self attested

(v) Research problem: Hypothesis, brief description, scientific, technological, socio-economic relevance and priority (limit to one page)

The scarabaeid beetles are the most common leaf chafers, whereas the larvae are most destructive soil pests. The family Scarabaeidae represents about 2,500 species from the Indian subcontinent having Melolonthinae as the largest subfamily. The Scarabaeidae family is divided into 13 subfamilies, of which Melolonthinae is the largest and is widely distributed throughout the world. The beetles in tribe Melolonthini are comparatively larger, take comparatively longer period to complete their larval stages, hence the pests in the tribe Melolonthini usually cause greater economic damage. The genus *Melolontha* is a phytophagous scarab group constituting the type genus of the subfamily Melolonthinae and tribe Melolonthini. The *Melolontha* are often recorded as crop pests as foliage feeding adults and root damaging larvae. The cockchafer of the genus *Melolontha* rank among the serious pests in agriculture and forestry, but the larvae cause greater damage than adult beetles. The grubs show a distinct preference for a variety of tree roots and are able to migrate as far as several meters through the soil towards their host plants, while adult beetles migrate kilometers and defoliate several tree species. In India, the genus *Melolontha* is mainly distributed in north western Himalaya, and eight species have been reported from different parts by earlier workers. In Himachal Pradesh, five species of *Melolontha* are known to occur. This group of scarab beetles is considered as one of the serious pests attacking potato, rajmash, cole crops, cereals, pulses and fruit/forest nurseries in Himachal Pradesh, but this group in India has not been critically studied as in other countries, and little is known of the identity, distribution, biology and food habitats of their grubs and adult beetles. The larval identification plays a significant role in pest management. The grubs and beetles exhibit typical behaviour, which makes them less vulnerable to the control strategies. Understanding the biology and behaviour of scarab larvae, including their interactions with host plants and the soil environment is an essential component to enabling effective management and control both in India and at a global scale. Keeping these points in view, the present study was undertaken to i) To study the distribution, relative abundance and population build up of *Melolontha* spp. in Himachal Pradesh; ii) To generate the information on bionomics of predominant *Melolontha* spp. and to assess genetic diversity using molecular markers; iii) To find effective chemicals against predominant *Melolontha* spp.

(vi) What standard methods and modern procedures were used in the experimental work (limit to one page)?

The population of *Melolontha* beetles was monitored with the help of UV light traps installed at seven locations in six districts of Himachal Pradesh. Host trees including apple, plum, peach, persimmon, walnut, wild rose, *bathua*, and Indian siris were also sampled for beetle collection at dusk during June-September in 2017 and 2018. In alpha diversity studies, Shannon index (H'), Simpson's index of diversity (D) and Pielou's evenness index (J') were used to work out species richness, species abundance and species evenness, respectively. In beta diversity studies, Sorensen's similarity index, Jaccard's similarity index and Bray-Curtis similarity index were used to estimate species similarity between two habitats, whereas Bray-Curtis distance index was calculated to find dissimilarity among the beetle assemblages. Food preference studies of *Melolontha* beetles were carried out in multi-choice and no-choice tests under laboratory conditions using different twigs of rose, plum, apple, peach and pecan nut.

The data on abundance and infestation of *Melolontha* grubs were recorded on potato, cabbage, rajmash, onion and apple at seven locations in five districts of Himachal Pradesh using pit sampling method.

Comparative biology and morphometrics of *Melolontha* species were studied under lab conditions. Detailed observation were recorded on mating, incubation, larval and pupal periods, larval characters (mouthparts, raster, spiracles and thoracic legs); pupal characters (antennae and caudal appendages); adult characters (antennae, pronotum, scutellum, meso-metasternal process, legs, pygidium and male genitalia).

The *COXI* and *28S rRNA* genes of these species were sequenced for the identification of *Melolontha* species. For sequencing analysis, methods followed were:

- Extraction of genomic DNA: CTAB method; Estimation of quality and quantity of DNA
- Amplification of *Cox1* and *28S rRNA* genes and cloning of PCR Products
- Isolation of plasmid DNA by alkali-lysis method and sequencing

For the management of grubs, six insecticides viz., chlorantraniliprole 18.5 SC, bifenthrin 10 EC, clothianidin 50 WDG, thiamethoxam 25 WDG, imidacloprid 17.8 SL and chlorpyrifos 20 EC were tested against the second and third instar grubs of *Melolontha* species by mixing with soil and as seed treatment under lab conditions.

(vii) Briefly describe the significant results obtained? (Not more than 3 pages)

At Palampur, *M. furcicauda* and *M. indica* were recorded to be the predominant species on light trap constituting 48.91-50.19 and 48.67-50.0 per cent of total *Melolontha* beetle catch. At Chhatanseri, *M. cuprescens*, *M. furcicauda* and *M. indica* were collected and *M. cuprescens* constituted 69.17 and 73.33 per cent of total *Melolontha* beetle catch during both years. At Badagaon, *M. cuprescens* (27.93-36.11%) and *M. virescens* (= *Polyphylla fullo*) (23.89-32.40%) were found to be the predominant species, whereas at Janjehli, *M. cuprescens* was predominant during both years. The catch for *M. cuprescens* was 43.62-48.11 per cent higher at Ahla as compared to other *Melolontha* species. In Sangla area, *P. fullo* constituted 72.73 and 80 per cent of total *Melolontha* beetle catch during 2017 and 2018, respectively. The Shannon index ($H' = 1.35-1.36$), Simpson index of diversity ($D = 0.73-0.76$) and Pielou's evenness index ($J' = 0.97-0.98$) were calculated to be maximum at Badagaon. The species richness and abundance showed total similarity among *Melolontha* species between Badagaon and Janjehli as indicated by the values of Sorensen's index (1.0) and Jaccard's index (1.0). Maximum dissimilarity was recorded among *Melolontha* assemblages from Palampur and Sangla as indicated by the values of Bray Curtis distance index (0.93-0.94). At Palampur, Chhatanseri and Badagaon, 54.55-100 per cent beetles of *M. cuprescens*, *M. furcicauda* and *M. indica* were taken on apple, whereas *M. cuprescens* and *P. fullo* at Badagaon displayed distinct preference for persimmon (56.25-80%). At Ahla, maximum collection for different species was done on wild rose (25-100%) and Indian siris (66.67-100%). In food preference studies, both in multi and no-choice tests, rose was found to be the most preferred host for *M. indica* and *M. furcicauda*, and apple for *M. cuprescens*.

At Badagaon, the maximum plant mortality was observed on potato (42.50-47.50%), cabbage (26.25-28.75%) and Rajmash (18.7-20.0%). At Chhatanseri, 6.25-8.75 per cent onion bulbs were found to be infested by the *Melolontha* grubs. At Sangla and Pangna, fully established apple trees were found to be attacked by grubs of *M. indica* and *P. fullo*.

The average length x width of newly laid eggs of *M. cuprescens*, *M. furcicauda*, *M. indica* and *MP. fullo* was recorded to be $4.58 \pm 0.06 \times 3.33 \pm 0.04$, $3.94 \pm 0.06 \times 2.90 \pm 0.06$, $3.63 \pm 0.02 \times 2.60 \pm 0.09$ and $3.88 \pm 0.07 \times 2.81 \pm 0.06$ mm, respectively. After about three weeks, the average length x width of eggs increased to $5.11-5.50 \times 3.61-3.80$, $5.23 \pm 0.06 \times 3.81 \pm 0.02$, $4.49 \pm 0.05 \times 3.20 \pm 0.05$ and $4.86 \pm 0.03 \times 3.23 \pm 0.03$ mm in all four species. The incubation period varied from 19-22, 22-25, 24-30 and 16-18 days in *M. cuprescens*, *M. furcicauda*, *M.*

indica and *P. fullo*, respectively. Fecundity was recorded to be 19, 26, 23 and 21 eggs/female in all four species.

The fully fed first instar grubs of *M. cuprescens*, *M. furcicauda*, *M. indica* and *P. fullo* averaged 15.33 ± 0.66 , 11.15 ± 0.37 , 13.49 ± 0.70 and 12.08 ± 0.78 mm, respectively. The width of head capsule of first instar grubs was measured to be 3.65 ± 0.22 , 3.21 ± 0.19 , 2.37 ± 0.05 and 2.72 ± 0.05 mm, respectively. The duration of first instar grubs of *M. cuprescens*, *M. furcicauda*, *M. indica* and *P. fullo* ranged from 56-64, 54-56, 53-58 and 46-51 days, respectively. The mean body length of second instar grubs of *M. cuprescens*, *M. furcicauda*, *M. indica* and *P. fullo* was recorded to be 22.99 ± 0.32 , 23.20 ± 0.36 , 22.61 ± 0.35 and 25.89 ± 0.71 , respectively, and the width of head capsule averaged 4.88 ± 0.17 , 5.04 ± 0.09 , 4.63 ± 0.20 and 4.68 ± 0.17 mm, respectively. The duration of second instar grubs of *M. cuprescens*, *M. furcicauda*, *M. indica* and *P. fullo* was recorded to be 226-230, 218-224, 234-237 and 214-229 days, respectively. The third instar grubs of *M. cuprescens*, *M. furcicauda*, *M. indica* and *P. fullo* varied from 51.12-58.20, 50.11-53.39, 40.13-50.03 and 48.33-54.17 mm in body length, and 7.68-8.99, 6.89-7.13, 6.98-7.58, and 7.92 ± 8.48 mm in head capsule width, respectively. The duration of third instar grubs ranged from 325-329, 299-311, 310-312 and 309-320 days in *M. cuprescens*, *M. furcicauda*, *M. indica* and *P. fullo*.

In case of *M. cuprescens* and *M. indica*, there were 11 sensilla basiconica, whereas in *M. furcicauda* and *P. fullo*, only nine sensilla basiconica were present in apical sensory area of antennae. The epipharynx haptomerum of *M. cuprescens* had 18 heli, and six minute sensilla basiconica. In *M. indica*, the haptomerum had 15 heli and eight sensilla basiconica. In *P. fullo*, 12 heli and five sensilla basiconica were present, whereas in *M. furcicauda*, there were 07 heli and 03 sensilla basiconica. In mandibles, the width of scissorial area was recorded to be maximum in *M. indica* (1.58 mm), and minimum in *M. cuprescens* (1.04 mm). In raster pattern, the paired palidia consisted of 28 or 29 pali in *M. cuprescens*, 26 pali in *M. furcicauda*, 31 or 33 pali in *M. indica* and 15 pali in *P. fullo*. The anal slit was Y-shaped in *M. cuprescens*, *M. furcicauda* and *M. indica*, but it was V-shaped in *P. fullo*.

The average pupal period was 20.3 ± 0.7 , 30.0 ± 0.47 , 25.1 ± 0.67 and 18.6 ± 0.82 days in *M. cuprescens*, *M. furcicauda*, *M. indica* and *P. fullo*, respectively and the mean body length x width of pupa of these species was recorded to be $32.22 \pm 0.37 \times 14.56 \pm 0.29$,

32.47±0.11x14.55±0.13, 31.97±0.15x12.71±0.22, and 32.72±0.41x14.23±0.01 mm, respectively. The clear-cut differences existed in caudal appendages in male and female pupae.

The caudal processes of pygidium were elongated and bifurcated into two lobes in males of *M. cuprescens* and *M. furcicauda*, whereas in females, the caudal processes were short and widely separated. In *M. indica* males, the caudal processes were less projected. In females of *M. indica*, the caudal extremity was slightly bifid without any further extension. In *P. fullo*, the pygidium lobe was narrow in females as compared to males. The antennal club was 7-segmented in males and 6-segmented in females in all *Melolontha* species. In legs, the differences in male and female beetles were evident in tibial spine and tarsal claws. The male genitalia showed clear-cut difference among different species. In *M. cuprescens*, the phallobase was broader anteriorly, slightly narrow at the base, both parameres symmetrical, separated widely and lobe-like. In *M. furcicauda*, the phallobase tapered gradually from anterior region to the base and the parameres look elongated and sickle shaped. In *M. indica*, the phallobase was broader at the base. Parameres typically joined in the centre in the form of two sub-triangular plates. In *P. fullo*, the phallobase was shorter than parameres. The attachment of phallobase to parameres showed concavity towards the base of phallobase.

Mitochondrial gene (*Cox1*) and nuclear gene (*28s rRNA*) from *M. cuprescens*, *M. furcicauda*, *M. indica* and *P. fullo* were amplified and sequenced. The *Cox1* sequence from *M. cuprescens* showed 99.89 per cent similarity to *P. fullo*, whereas *Cox1* sequence from *M. furcicauda* showed 89.47 per cent similarity to *M. indica*. Among retrieved *Cox1* sequences from NCBI database, *M. hippocastani* and *M. hippocastani* were more close to *M. furcicauda* (86.02-86.51 %) and *M. indica* (84.15-84.69 %) as compared to *M. cuprescens* (81.47-81.69 %) and *P. fullo* (81.47-81.58 %). Based on the *28s rRNA* gene sequence, *M. indica* showed maximum identity of 99.42 per cent to *M. melolontha* as compared to *M. cuprescens* and *M. furcicauda* which were distantly related.

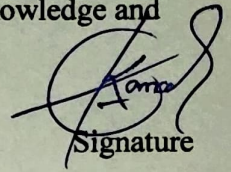
The order of toxicity for third instar grubs of *Melolontha* species was chlorantraniliprole (3.85)>bifenthrin (3.59)>clothianidin (1.33)>thiamethoxam (1.22)>chlorpyrifos (1.16). Seed treatment in rajmash also indicated better efficacy of chlorantraniliprole 18.5 SC (2.5 ml/kg seed), followed by chlorpyrifos 20 EC (5.0 ml/kg seed).

16. Professional recognition, awards, fellowships received (In a separate sheet give full particulars such as the agency/ organization which gave the award, purpose, the nature of the award, etc.):

1. Awarded university merit scholarship during Ph.D. (Entomology) by CSK HPKV Palampur
2. Awarded Merit scholarship during Matric by Government of Himachal Pradesh, India.
3. Awarded appreciation certificate by Himveer Wives Welfare Association, Indo Tibetan Border Police on the occasion of International Youth Day (12th August, 2008) for securing good marks during matriculation.
4. Awarded certificate of honour with B.Sc. and M.Sc. degree certificate by CSK HPKV Palampur
5. Best paper award by Agriculture and E-Food Newsletters for article entitled “*Bioecology, Incidence and Management of Deudorix epijarbas Moore in India*”
6. Best paper award by Agriculture and E-Food Newsletters for article entitled “*Soil Health and Insect Herbivore Population Dynamics*”
7. Awarded *certificate of excellence in reviewing* by Journal “Annual Research and review in Biology”
8. Awarded *certificate of excellence in reviewing* by Journal “Uttar Pradesh Journal Of Zoology”
9. Awarded *certificate of excellence in reviewing* by Journal “Journal of Experimental Agriculture International”

18. Certificate by the student that all the details given are true

I, Abhishek Rana son of Late Sh. Kamlesh Kumar hereby declare that all the statements and entries made in this application are true, complete and correct to the best of my knowledge and belief.



Signature



CSK Himachal Pradesh Krishi Vishvavidyalaya

College of Agriculture

Department of Entomology

PALAMPUR-176062 H.P.



No. QSD. CSKHPKV/Ento/2021/- 1877

Dated, Palampur the 06-11-2021

TO WHOM IT MAY CONCERN

Certify that Dr. Abhishek Rana has completed his Ph.D. degree on the topic "Bioecology and Management of *Melolontha* spp. (Scarabaeidae: Coleoptera) in Himachal Pradesh" on 01.04.2021 under my chairmanship. It is further certified that the information submitted by Dr. Abhishek Rana for ESI- Best Ph.D. Thesis Award 2021 is verified and correct.

(R.S. Chandel)

Major advisor & Head,

Dept. of Entomology

CSKHPKV, Palampur
CSKHPKV, Palampur



CSK Himachal Pradesh Krishi Vishvavidyalaya
College of Agriculture
Department of Entomology
PALAMPUR-176062 H.P.



No. QSD. CSKHPKV/Ento/2021/- 1876

Dated, Palampur the 06-11-2021

To

The Chief Editor
 Entomological Society of India
 New Delhi, 110012

Subject: Application of Dr. Abhishek Rana for ESI- Best Ph.D. Thesis Award 2021

Sir,

Dr. Abhishek Rana is submitting his application for ESI- Best Ph.D. Thesis Award 2021.
 All documents are attached as per your proforma. I strongly recommend his thesis for this award.

Thanking you

Yours faithfully

Head,
 Dept. of Entomology
 CSKHPKV, Palampur
 CSKHPKV, Palampur



Phylogenetic analysis of *Melolontha* and *Polyphylla* beetles (Scarabaeidae: Coleoptera) from north-western Himalaya, India

Abhishek Rana · Ravinder Singh Chandel · Kamal Dev Sharma ·
 Surender Singh Chandel · Kuldeep Singh Verma

Received: 5 May 2021 / Accepted: 8 July 2021
 © The Author(s), under exclusive licence to Springer Nature B.V. 2021

Abstract White grub species, *Melolontha cuprescens*, *Melolontha furcicauda*, *Melolontha indica* and *Polyphylla fullo* are serious pests of different crops in north-western Himalaya. None of these species are, however, characterized at molecular level including DNA barcodes. Here we provide DNA barcodes for these species. The adults of these species were collected from different locations of Himachal Pradesh and described by molecular characterization. The mitochondrial *Cytochrome c oxidase I* (*COX1*) and 28S ribosomal RNA (28S rRNA) genes of these species were amplified and sequenced for molecular identification and phylogenetic analysis. The sequences of these genes from other species of these genera and other related genera were also retrieved from DNA databases and included in the analysis. The *COX1* sequences of *M. cuprescens* were 99.88% similar to *P. fullo*, whereas those of *M. furcicauda* were 84.91% similar to *M. indica*. Among retrieved *COX1* sequences, *Melolontha hipocastani* (HM120757 & KX087316) were closer to

M. furcicauda (86.14–86.50%) and *M. indica* (85.74–86.04%) as compared to *M. cuprescens* (81.44–81.47%) and *P. fullo* (81.32–81.47%). Based on 28S rRNA gene sequences, *M. indica* showed 99.42% similarity to *Melolontha melolontha* (EU084231) as compared to *M. cuprescens* and *M. furcicauda* which were distantly related. The *COX1* was a better DNA barcode for the identification of *Melolontha* and *Polyphylla* species. The 28S rRNA has a limited utility in DNA barcoding of *Melolontha* species.

Keyword *Melolontha* spp. · *Polyphylla* spp. · *COX1* · 28S rRNA · Phylogenetic analysis

Introduction

The genus *Melolontha* is a phytophagous scarab group constituting the type genus of the subfamily Melolonthinae and tribe Melolonthini. There are about 60 species/subspecies within the genus *Melolontha* which have been discussed worldwide (Keith & Li, 2005). The *Melolontha* are often recorded as crop pests as foliage feeding adults and root damaging larvae (Liu et al., 1997). Defoliation caused by beetles is usually serious when they feed on the young one or two-years old broad-leaved plantings (Svestka, 2007). On the other hand, white grubs feeding in forest plantations leads to dieback of trees causing serious damage to forest management and may even restrict forest regeneration. In Germany, *M.*

A. Rana (✉) · R. S. Chandel · K. S. Verma
 Department of Entomology, CSK Himachal Pradesh
 Agricultural University, Palampur, Himachal Pradesh,
 India
 e-mail: abhir1392@gmail.com

K. D. Sharma · S. S. Chandel
 Department of Agricultural Biotechnology, CSK
 Himachal Pradesh Agricultural University, Palampur,
 Himachal Pradesh, India

Published online: 16 July 2021

Springer

Handwritten signature
 self attested



Morphological description and identification of *Melolontha cuprescens*, *Melolontha furcicauda* and *Melolontha indica* (Coleoptera: Scarabaeidae): serious root feeding and defoliating pests in India

Abhishek Rana · Ravinder Singh Chandel ·
 Kuldeep Singh Verma

Received: 3 July 2021 / Accepted: 14 September 2021
 © The Author(s), under exclusive licence to Springer Nature B.V. 2021

Abstract *Melolontha* is an important genus of the subfamily Melolonthinae and the tribe Melolonthini. It is a major polyphagous pest of different agricultural crops and forest trees. The adults of this species are defoliators and grubs are root feeders. It is essential to acquire a better understanding of the correct identity of these species to implement the most suitable pest management options. In this study, three *Melolontha* species viz., *Melolontha cuprescens*, *Melolontha furcicauda* and *Melolontha indica* were investigated using light microscopy, in order to generate detailed information on morphological characters for the pest identification. A detailed comparative description of the larval characters (mouthparts, raster, spiracles and thoracic legs); pupal characters (antennae and caudal appendages); adult characters (antennae, pronotum, scutellum, meso-metasternal process, legs, pygidium and male genitalia) is given with the help of suitable microscopic pictures and line diagrams. The larvae of these species can easily be distinguished based on mandibles, epipharynx, raster pattern and shape of anal opening. The caudal extremity of pupae also allows differentiation among *Melolontha* species. In beetles, shape and size of antennae, pronotum and pygidium can be used to separate sexes and to differentiate *Melolontha* species. Male genitalia is the most

stable character for differentiation of species in the adult stage.

Keyword *Melolontha* spp. morphometrics. male genitalia. raster pattern. taxonomic characters

Introduction

Scarabaeidae is the largest family of Scarabaeoidea represented by 30,000 species of beetles worldwide (Erler & Ates, 2015). It is divided into 13 subfamilies, of which Melolonthinae is the largest subfamily which is widely distributed throughout the world (Kumar et al., 2017a, 2017b). The majority of the phytophagous species of scarabs belong to this subfamily. The scarabaeids are polyphagous pests both in grub and adult stage and inflict heavy damage to different agricultural crops, fruits, vegetables, ornamental plants, plantation crops, pastures, meadow grasses, and forest trees in different parts of the world (Chandel & Kashyap, 1997; Potter et al., 1992).

The genus *Melolontha* Fabricius, 1775 is a phytophagous scarab group constituting the type genus of the subfamily Melolonthinae and tribe Melolonthini. The type genus, *Melolontha* is a representative genus, with regard to the Scarabaeidae family. There are about 60 species/sub species within the genus *Melolontha* which have been discussed worldwide (Keith & Li, 2005). The beetles belonging to genus *Melolontha* are cosmopolitan and some of the species

A. Rana (✉) · R. S. Chandel · K. S. Verma
 Department of Entomology, CSK Himachal Pradesh
 Agricultural University, Palampur, Himachal Pradesh,
 India

self attested



Efficacy of insecticides as seed treatment for the management of white grubs

Abhishek Rana*, R.S. Chandel and K.S. Verma

Department of Entomology

CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176 062, India

*Corresponding author: abhir1392@gmail.com

Manuscript received: 20.10.2020; Accepted: 06.11.2020

Abstract

The efficacy of six insecticides namely chlorpyrifos 20 EC (5.0 ml/kg seed), imidacloprid 17.8 SL (5.0 ml/kg seed), clothianidin 50 WDG (3.5 g/kg seed), thiamethoxam 25 WG (3.5 g/kg seed), bifenthrin 10 EC (2.5 ml/kg seed) and chlorantraniliprole 18.5 SC (2.5 ml/kg seed) as seed treatment was tested against grubs of *Melolontha indica* Hope under laboratory conditions. Rajmash, *Phaseolus vulgaris* L. was used as test crop. The experiment was conducted in plastic trays filled with soil and farm yard manure (FYM) in 3:1 ratio. In each tray, ten third instar grubs of *M. indica* were released and 20 treated rajmash seeds were sown at a depth of about 5 centimeter. The results revealed that plants from chlorantraniliprole 18.5 SC (2.5 ml/kg seed) treated seeds registered least mortality (20.0 %), followed by chlorpyrifos 20 EC (5 ml/kg seed) (21.67 %) at 10 days after sowing (DAS). The grub mortality recorded in seeds treated with chlorantraniliprole 18.5 SC (2.5 ml/kg seed) and chlorpyrifos 20 EC (5 ml/kg seed) was 56.67 and 46.67 per cent, respectively at 20 DAS. Therefore, seed treatment with chlorantraniliprole 18.5 SC (2.5 ml/kg seed) could be another alternative in addition to chlorpyrifos 20 EC (5 ml/kg seed) for effective management of *M. indica* grubs infesting rajmash under field conditions in high hills of north western Himalaya.

Key words: Insecticides, Rajmash, seed treatment, white grubs.

In Himachal Pradesh, white grubs are the most destructive and troublesome soil pests affecting production of many crops especially in high hills. Rajmash, *Phaseolus vulgaris* L. in particular is attacked by a large number of insect-pests including white grubs at different crop growth stages from germination to harvest (Sood *et al.* 2003). The economic importance of white grubs is primarily due to root damage caused by feeding activity of third instar grubs resulting in plant mortality and subsequent yield losses (Chandel *et al.* 2015). The grubs of *Melolontha* sp. have been observed to damage roots of rajmash causing sudden wilting and death of plants in different regions of the state. The plants at 5-6 leaves stage are more prone to the attack of white grubs causing considerable plant mortality at this stage (Mehta *et al.* 2010). In Sangla valley of Kinnaur, Himachal Pradesh, white grubs potentially caused 8-10 per cent plant mortality in rajmash (Sood *et al.* 2007). Rajmash, is an important pulse crop grown in different states of India including Himachal Pradesh for grains as well as for vegetable purposes (Singh and Singh 2015). Nationally, it is cultivated on an area of approximately

228 thousand hectares with a production of nearly 2277 thousand tones. In Himachal Pradesh, it is cultivated in high hills of Chamba, Kinnaur, Kullu, Lahaul & Spiti, Mandi, Shimla and Sirmour districts on about 3.80 thousand hectares with an annual production of 50.87 thousand tones (Anonymous 2018). Therefore, it is necessary to manage the menace of white grubs in this important pulse crop. White grubs are generally managed by soil application of synthetic insecticides either through drenching or by broadcasting after mixing with sand or fine soil. However, soil application of insecticides is usually ineffective because insecticides are not readily trans-located through soil to the root zone in lethal concentrations. Moreover, *Melolontha* grubs are generally present in the soil at a depth of about 5-10 centimeters, and it is very difficult to manage them with soil treatment. Therefore, the present study was undertaken to evaluate the efficacy of some new insecticides as seed treatment against grubs of *Melolontha indica* Hope.

Materials and Methods

The study was carried out in the White Grub Laboratory of the Department of Entomology, CSK

[Handwritten signature]
Self attested

The potential of entomopathogens in biological control of white grubs

Ravinder S. Chandel^a, Saurbh Soni^a, Sumit Vashisth^b, Mandeep Pathania^c, Pawan K. Mehta^a, Abhishek Rana^a, Ashok Bhatnagar^d and V. K. Agrawal^e

^aDepartment of Entomology, Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh, India; ^bDivision of Entomology, ICRISAT, Patancheru, Hyderabad, Telangana, India; ^cPunjab Agricultural University, Regional Research Station, Abohar, Punjab, India; ^dRajasthan Agricultural Research Institute, Durgapura, Jaipur, Rajasthan, India; ^eSchool of Agriculture, JECRC University, Jaipur, Rajasthan, India

ABSTRACT

White grubs are highly polyphagous and most destructive soil pests inflicting damage to a wide variety of crops. In India, more than 1000 species of white grubs are known of which over 40 species attack wide range of plants. White grubs are naturally infected by various entomopathogens which include fungi, bacteria and nematodes. Entomopathogenic fungi offer great potential and members of genera *Beauveria* and *Metarhizium* are widely used against white grubs. Several commercial products of entomopathogenic fungi like Bio Green, ORY-X, Grub X 10G, Betel, Biotrol FMA and Meta-Guard have been developed for the control of white grubs. In India, good control of white grubs in paddy, ginger and sugarcane has been achieved with different entomofungi. Among EPNs, *Heterorhabditis bacteriophora* is moderately effective against *Popillia japonica* and *Rhizotrogus majalis*. *H. indica* and *H. bacteriophora* are effective against potato white grubs in India. *Paenibacillus popilliae* cause milky disease in *P. japonica* grubs. The bacterium is pathogenic to *Holotrichia consanguinea*, *H. serrata* and *Leucopholis lepidophora*. In north-western Himalaya, *B. cereus* is highly toxic to the grubs of *H. seticollis* and *Anomala dimidiata*.

ARTICLE HISTORY

Received 23 February 2017

Revised 15 July 2018

Accepted 7 September 2018

KEYWORDS

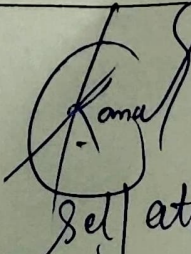
Biological control; entomopathogenic bacteria; entomopathogenic fungi; entomopathogenic nematodes; white grubs

1. Introduction

The superfamily Scarabaeoidea contains a large number of species whose larvae live in the soil and are commonly known as white grubs. As many species of white grubs are root feeders, they are also known as rootgrubs, but not all white grubs are rootgrubs (Gardner 1935). The white grubs are found chiefly in grasslands feeding on roots of various plants. Others grow on decaying organic matter (Misra and Chandel 2003). They live concealed and sudden increase in their population takes up in places having enough food and least soil-disturbance (Chandel and Kashyap 1997). The white grubs cause extensive damage to the roots of grasses, legumes, small fruit plants, shrubs and trees in many parts of the world (Pathania 2014). Larvae of greatest economic importance belong mainly to the tribe Melolonthini (Ritcher 1958). The first known white grub damage to crops in India is that by Stebbing (1902) from Punjab. More than 1000 species of white grubs are known from the Indian sub-continent of which over 40 species attack a wide range of crop plants (Veeresh et al. 1991). First major epidemic of white grubs in India was reported in sugarcane from Bihar during 1956 (Gupta and Avasthy 1956). They have now been included in the

category of five national pests (Misra and Chandra 1989), and are reported from every state causing damage to a wide variety of cultivated crops. Yadava and Sharma (1995) reported that *Holotrichia serrata* Fab., *Holotrichia consanguinea* Blanch., *Holotrichia longipennis* Blanch., *Brahmina coriacea* (Hope), *Holotrichia seticollis* Moser, *Anomala dimidiata* (Hope), *Holotrichia reynaudi* Blanch., *Leucopholis lepidophora* Blanch., *Leucopholis coneophora* Brum., *Melolontha* spp and *Lepidota* spp are the key pest species that attack different plants in different regions of the country.

The current method for the control of white grubs is through the use of chemical pesticides. However, concern about safety, environmental contamination and poor efficacy of recommended insecticides has increased the need to develop IPM approaches for these pests (Kumawat 2001). The naturally occurring entomopathogens (fungi, nematodes, bacteria and viruses) are important components in the soil ecosystem which brings about effective suppression of soil pests in nature. We provide an overview of microbial control of white grubs in different crops with special reference to fungi, nematodes and bacteria and their future prospects in white grub management.


Self attested



Potential of Entomopathogens in Managing Potato Whitegrubs in Himachal Pradesh

R.S. Chandel, Abhishek Rana, Suman Sanjta and P.K. Mehta

Department of Entomology, CSKHPKV Palampur-176 062, India
E-mail: visitrvchandel@yahoo.com

Abstract: *Brahmina coriacea* (Hope) is the most widespread and destructive species of whitegrubs having potential to inflict 40 to 50 per cent yield losses to potato in Himachal Pradesh. The entomopathogens constitute a potential group of biocontrol agents against whitegrubs, therefore *Beauveria bassiana* Vuill., *Metarhizium anisopliae* (Metchnikoff), *Heterorhabditis indica* Poinar and *Bacillus cereus* Frankland & Frankland were evaluated in potato at Kheradhar. *H. indica* @ 10 kg ha⁻¹ was highly effective with tuber damage ranging from 12.5 to 12.7 per cent in treated plots. Mixed application of *H. indica* + *B. bassiana* or *M. anisopliae* showed additive or synergistic effect. There was 11.4 to 12.2 per cent tuber damage in *H. indica* + *B. bassiana* or *M. anisopliae* treatments. Maximum reduction in tuber damage was observed in *H. indica* + *B. bassiana*, followed by *H. indica* + *M. anisopliae* treatment. There was 48.75 to 55.38 per cent reduction in tuber damage when *Galleria mellonella* cadavers infected with *H. indica* were applied in soil. Direct application of *G. mellonella* cadavers infected with *H. indica* showed higher efficacy which may be attributed to greater activity of infective juveniles in soil.

Keywords: Whitegrubs, *Brahmina coriacea*, *Beauveria bassiana*, *Heterorhabditis indica*, *Metarhizium anisopliae*

The problem of whitegrubs is quite serious in hilly states where potatoes are grown during summer season as rain-fed crop under long day conditions (Chandel et al 2015). In most parts of the north western Himalaya, potato cultivation is done on sloppy lands with light and loose textured soil and high rainfall. The potato fields are normally situated in the vicinity of fruit orchards in Himachal Pradesh and other hilly parts of India. These conditions are conducive for the development and multiplication of whitegrubs. In India, 24 species of whitegrubs damage potato, and *B. coriacea* is most widespread and destructive species in Himachal Pradesh (Pathania and Chandel 2017). The life cycle of *B. coriacea* is annual with three larval stages (Chandel et al 2003), and damage is caused by second and third instar grubs. The economic importance of chafers is primarily due to the feeding activity of third instar grubs. There exists a highly positive correlation between the population of the third instar grubs and tuber damage (Anonymous 2004). Chandel et al (2008) reported that with unit increase in population of third instar grubs of *B. coriacea*, the tuber damage increases by 12.17 per cent. It is very difficult to control whitegrubs by applying conventional insecticides owing to their poor efficacy, high cost and associated environmental pollution. Biological control is an important component of IPM which implies the utilization of ecologically safe and effective methods for the management of whitegrubs. Different entomopathogens such as *Metarhizium anisopliae*, *Beauveria bassiana* and *Heterorhabditis indica* are of

paramount importance in managing whitegrubs, as they sustain themselves in soil, after proper establishment and are commercially labeled for whitegrubs in India. In the present investigation, the field efficacy of entomopathogenic fungi, bacteria and nematodes for the control of *B. coriacea* grubs in potato was studied.

MATERIAL AND METHODS

Field evaluation of talc based formulations of different entomopathogens against whitegrubs in potato: *M. anisopliae*, *B. bassiana* and *Bacillus cereus* formulated as dusts and *H. indica* as semi solid formulation were evaluated against grubs of *B. coriacea* in potato. The experiment was conducted in summer planted potatoes grown in whitegrub endemic area at PDS Kheradhar on variety Kufri Jyoti. All recommended package of practices were adopted except for whitegrub's management. There were seven treatments which were replicated thrice in RBD with plot size of 12 m² (Table 1). The formulations of *M. anisopliae*, *B. bassiana* and *H. indica* were obtained from NBAIR, Bangalore, and the dust formulation of *B. cereus* was supplied from Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS), Almora. Each formulated product was mixed thoroughly with FYM in plastic trough, and added into the furrows near the base of plants. After addition of FYM+ biopesticide mixture, it was gently mixed in the soil using hand hoe. All the treatments were applied at the time of earthing up in first week of July.

Self attested

हिमाचल प्रदेश में कुरमुला कीट का समेकित प्रबन्धन

रवीन्द्र सिंह चन्देल
पवन कुमार मैहता
सुमन सांजटा
मोनिका शर्मा
अभिषेक राणा
विजय कुमार अग्रवाल



ऑल इंडिया नेटवर्क प्रोजेक्ट ऑन सॉईल ऑर्ग्रोपोड पैस्ट्स
कीट विज्ञान विभाग, चौ. स. कु. कृषि विश्वविद्यालय
पालमपुर - 178082 हिमाचल प्रदेश



[Signature]
Sd/- attested

कैसे करें फसलों का कुरमुला कीट से बचाव



डा. रवीन्द्र सिंह चंदेल
विभागाध्यक्ष, कीट विज्ञान विभाग
डा. कुलदीप सिंह वर्मा
प्राध्यापक, कीट विज्ञान विभाग
डा. अर्जुन सिंह बलौदा
नेटवर्क समन्वयक, ए.आई.एन.पी.
ऑन सॉअल ऑर्थोपोड पैस्ट्स, जयपुर

सुमन साँजटा,
पी. एच. डी. स्कॉलर, कीट विज्ञान विभाग
अभिषेक राणा
पी. एच. डी. स्कॉलर, कीट विज्ञान विभाग



कीट विज्ञान विभाग

कृषि महाविद्यालय
चौ. स. कु. हि. प्र. कृषि विश्वविद्यालय
पालमपुर (हि. प्र.)



Self attested

2.26

***Melolontha virescens* Brenske: An emerging pest of wheat in high hills and dry temperate zone of Himachal Pradesh**

R.S. Chandel, K.S. Verma and Abhishek Rana

Department of Entomology, CSK HPKV Palampur, H.P. 176062

ksverma_hpau@rediffmail.com

White grubs are highly polyphagous pest both in grub and adult stage and inflict heavy damage on various fruit/ forest trees, their nurseries, vegetables, lawns and field crops. The scarabaeid beetles are most common chafers, whereas larvae are among the most destructive soil pests feeding on roots of various plants. Almost all field crops grown during rainy season are damaged by the white grubs. In Himachal Pradesh, white grubs were reported earlier on forest/ fruit plants and potato; however, no record exists on any white grub damage to wheat until 2010. *Brahmina coriacea* (Hope) and *Holotrichia longipennis* Blanchard were found to be the predominant species of white grubs causing damage to different crops in Himachal Pradesh. Recently, in response to changing climatic conditions, *Melolontha virescens* Brenske was recorded in alarming numbers causing damage to several crops including wheat. In surveys conducted in Himachal Pradesh, *M. Virescens* was found to be fairly common in Chuhar valley and Sangla valley (2268-2588 m amsl) of the state. Few adults were also recorded on light traps at Palampur. In wheat, the damage of *M. virescens* grubs was recorded in Sangla valley. All underground parts of wheat were susceptible to grub feeding. The symptom of damage was root pruning by grubs showing varying degrees of wilting, yellowing, browning and eventually death of plant. There was appearance of patches of dry and wilted plants in the infested fields. There was browning and drying of leaves, followed by death of the plants. When the plants were uprooted, the roots were found to be totally pruned and the grubs were generally observed in root zone of the plants. In Barot valley, the adults of *M. Virescens* were collected on wheat spikes on first week of July. Although scarab beetles are generally nocturnal in behaviour and come out of soil at dusk, but *M. Virescens* beetles were also recorded on plants during day time. The observations on the biology of *M. Virescens* were recorded under laboratory conditions. After about one week of adult collection from the field, the egg laying was recorded in soil. Eggs hatched within two weeks of laying. In Himachal Pradesh, the pest was found to have two years life cycle. The third instar grubs occupied about 300 days. Full fed third instar grubs move deep into the soil and constructed an earthen cell and overwintering occurred inside these earthen shells. The third instar grubs averaged 55 mm in length and the head capsule width was approximately 8.4 mm. The grubs cause damage mainly in July; therefore the management strategies need to be targeted accordingly to check timely infestation of the pest.

[Handwritten signature] Self attested

IBS/AFS/2017/3083

Potential of Entomopathogens In Managing Potato White Grubs In Himachal Pradesh

RS Chandel, Abhishek Rana*, Suman Sanjta and PK Mehta

CSK HPKV (Entomology), Palampur 176 062 Kangra, Himachal Pradesh

*Corresponding author's email: abhir1392@gmail.com

Keywords: Entomopathogens, White grubs, *Brahminacoriacea*

Introduction

White grubs are quite serious problem in hilly states where potatoes are grown during summer season as rain-fed crop (Chandel *et al.* 2015). In these areas rainfall is high and potato cultivation is done on sloppy lands with light and loose textured soils which are highly conducive for multiplication of white grubs. In Himachal Pradesh, *Brahminacoriacea* (Hope) is the most wide spread and destructive species (Pathania and Chandel, 2017) and the tuber damage often exceeds 50 per cent in endemic areas. The white grubs are difficult to control with conventional insecticides; therefore studies on field efficacy of different entomopathogens having potential against white grubs were undertaken.

Material and methods

The experiment was conducted in potato fields at Kheradhar in plots of 12m². The formulations of *Metarhiziumanisopliae*, *Beauverlabassiana*, *Heterorhabditisindica*, and *Bacillus cereus* were mixed with FYM and added into furrows at earthing up. In a separate experiment, *Galleria mellonella* cadavers infected with *H. indica* supplied by FARMER Ghaziabad were tested. Total 14 cadavers were used per plot by digging 3-4 cm deep hole near the plant, and in each hole one cadaver was gently placed and covered with soil. Data on per cent tuber damage and number of grubs per 10 plants/ plot were recorded at harvest.

Results and conclusion

H. Indica @ 10 kg/ha was highly effective with tuber damage ranging from 12.5 - 12.7 per cent. In *M. anisopliae* and *B. bassiana* treatments, 15.74 and 14.7 per cent damage was recorded, respectively. Dual application of *H. indica*+*B. bassiana* or *M. anisopliae* showed comparatively better results. The grub population was almost half (2.1 grubs/ feet²) in this treatment in comparison to control (4.7 g/ feet²). When *G. mellonella* cadavers infected with *H. Indica* were put in soil, there was 44.62 to 51.25 per cent reduction in tuber damage over control. The grub population was recorded 1.71 - 2.34 times higher in untreated plots. The results clearly indicate that EPN saved greater potential to control infestation of white grubs. The combination of *B. bassiana*+*H. indica* has proven to be more effective method for decimating white grub population. The technology involving direct use of *G. mellonella* cadavers infected with nematodes has shown better efficacy compared to powder formulation. When cadavers are used, the infective juveniles show greater activity dispersing uniformly in all directions to seek their host.

References

- Chandel RS, Pathania M, Verma KS, Bhattacharyya Badal, Vashist, Sumit and Kumar V. 2015. The ecology and control of potato white grubs of India. *Potato Research* 58:147-164.
- Pathania M and Chandel RS 2017. Life history strategy and behaviour of white grub, *Brahminacoriacea*(Hope) (Coleoptera: Scarabaeidae: Melolonthinae) an invasive pest of potato and apple agro-ecosystem in north western India. *Oriental Insects*, 51(1): 46-69.

IBS AFS 2017 YSPUHF

201

Scanned by CamScanner

Self attestation



GPC2020:PPM_17

Evaluation of entomopathogenic nematode, *Heterorhabditis indica* against potato white grubs in Himachal Pradesh

Abhishek Rana*, R.S. Chandel, K.S. Verma and Suman Sanjta

Department of Entomology, Chaudhary Sarwan Kumar Himachal Pradesh Agricultural University,
Palampur, HP, 176 062, India

*email id: abhir1392@gmail.com

Whitegrubs are regular pests of potato in higher hills of Himachal Pradesh. In entire North Western Himalaya, the grubs of *Brahmina coriacea* and *Melolontha indica* cause wide spread damage to potato. Adults of *B. coriacea* cause heavy defoliation of fruit and forest trees while the grubs are categorized as national pest of potato. *M. indica* grubs produce a single large cavity in the tubers. Both species are difficult to control with insecticides; therefore, in present study, the efficacy of Grub Nash (*Heterorhabditis indica*) was tested under lab conditions. The formulation was tested @ 1-6 g/ kg of soil against third instar grubs of *B. coriacea* and *M. indica*. The formulated product was mixed with soil and filled in pots. In each treatment 5 grubs were released. The data on grub mortality were recorded after 48 hours of treatments. *H. indica* @ 6 g/kg of soil was found highly effective showing 100 per cent mortality in grubs of *B. coriacea*. This was followed by *H. indica* @ 4g/ kg and 2g/ kg of soil showing 86.67 and 73.33 per cent mortality in grubs, respectively. *H. indica* @ 1.5 g/kg and 1g/ kg of soil produced mortality to the tune of 13.33. Whereas in case of *M. indica*, no mortality was recorded in tested doses. It might be due to the presence of small hairs around the spiracles of grubs which can act as a trap for entomopathogenic nematodes. The results clearly indicate that *H. indica* is having more specificity towards *B. coriacea* grubs and can have better potential for its utilization under field conditions.

[Handwritten signature]
Self attested



Application Form For ESI Best Ph.D. Thesis Award

Personal Information

Applicant's Name : Abhishek Rana

Present Position/Designation : Junior Research Fellow, Entomology, CSK HPKV Palampur

Applicant's Address : Dr. Abhishek Rana, Junior Research Fellow, Department of Entomology, CSK HPKV Palampur, District Kangra, Himachal Pradesh 176062; 9459687827 / 7018856682; Email: abhir1392@gmail.com

DOB : 1992-12-13

Subject of Specialization : Entomology



Recommendations

Academic Record and Professional Attainments

Academic Record				
SN	Degree	University/Institution	Year	Distinction
1.	Bachelor of Science (Hons.) Agriculture	Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, H.P.	2014	
2.	Master of Science in Agriculture (Entomology)	Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, H.P.	2016	
3.	Ph.D. (Entomology)	Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, H.P.	2021	

Professional Attainments			
SN	Position Held	Institution	Period of appointment

Special attainments of the nominee

maximum 250 words

Publications

Print/Save as PDF

List 20 most important research publications in chronological order. For each publication, give name(s) of the author(s), indicate the corresponding author by '*' after his/her name, year of publication, full title of the paper, name of the journal, volume No. and page Nos. and NAAS rating during the 2021 for ESI senior scientist award

SN	Details of Publications in the format given above	NAAS Journal ID and (Score)	Number of Citations
1.	Rana Abhishek*, Chandel RS, Sharma KD, Chandel SS and Verma KS. 2021. Phylogenetic analysis of Melolontha and Polyphylla beetles (Scarabaeidae: Coleoptera) from north-western Himalaya, India. Phytoparasitica https://doi.org/10.1007/s12600-021-00945-5.	P084 (7.14)	-
2.	Rana Abhishek*, Chandel RS and Verma KS. 2021. Morphological description and identification of Melolontha cuprescens, Melolontha furcicauda and Melolontha indica (Coleoptera: Scarabaeidae): serious root feeding and defoliating pests in India. Phytoparasitica https://doi.org/10.1007/s12600-021-00961-5	P084 (7.14)	-
3.	Rana Abhishek*, Chandel RS and Verma KS. (2020). Efficacy of insecticides as seed treatment for the management of white grubs. Himachal Journal of Agricultural Research 46 (2) : 169-172	H013 (3.44)	2
4.	Chandel RS*, Soni S, Vashisth S, Pathania M, Mehta PK, Rana Abhishek, Bhatnagar A and Agrawal VK. 2018. The potential of entomopathogens in biological control of white grubs. International Journal of Pest Management 65(4): 348-362	I312 (7.09)	7
5.	Chandel RS*, Rana Abhishek, Sanjta S. 2018 and Mehta P.K. 2018. Potential of Entomopathogens in Managing Potato White grubs in Himachal Pradesh. Indian Journal of Ecology 45(1): 210-213	I056 (5.79)	-

List of 5 most important Products/Technologies/Discoveries/Patents by the nominee
(Please see item 1 (b) in the Guidelines for Evaluation and list the products, technologies and software in the order scoring maximum to minimum score)

SN	Details of Product/Technology/Discoveries/Software	Patent No./Country of patent Release/ notification details Commercialization	List according to category (I,II) as given in 2 of the guidelines for evaluation
----	---	--	--

Externally Funded Projects

Projects funded by ICAR, CSIR, DST, DBT, other government and private agencies.

SN	Title of project
----	------------------

Involvement in Teaching

Print/Save as PDF

Involvement in Extension Activities

SN	Activity
----	----------

I certify that the information given in the form are correct.

Name:	Abhishek Rana
-------	---------------

Address:	Dr. Abhishek Rana, Junior Research Fellow, Department of Entomology, CSK HPKV Palampur, District Kangra, Himachal Pradesh 176062; 9459687827 / 7018856682; Email: abhir1392@gmail.com
----------	---

Date of Submission:	24th November 2021
---------------------	--------------------

Print/Save as PDF



**Bioecology and Management of *Melolontha* spp.
(Scarabaeidae: Coleoptera) in Himachal Pradesh**

THESIS

By

**ABHISHEK RANA
(A-2016-40-007)**

Submitted to



**CHAUDHARY SARWAN KUMAR
HIMACHAL PRADESH KRISHI VISHVAVIDYALAYA
PALAMPUR – 176062 (H.P.) INDIA**

in

**Partial fulfilment of the requirements for the degree
of**

**DOCTOR OF PHILOSOPHY IN AGRICULTURE
(DEPARTMENT OF ENTOMOLOGY)
(ENTOMOLOGY)**

2021

Dr. R.S. Chandel
Professor and Head

Department of Entomology,
CSK Himachal Pradesh Krishi Vishvavidyalaya,
Palampur-176 062 (HP) India

CERTIFICATE - I

This is to certify that the thesis entitled “**Bioecology and management of *Melolontha* spp. (Scarabaeidae: Coleoptera) in Himachal Pradesh**” submitted in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy (Agriculture)** in the discipline of **Entomology** of CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur is a bonafide research work carried out by **Mr. Abhishek Rana (Admission No. A-2016-40-007)** son of **Sh. Kamlesh Kumar and Smt. Daya Devi** under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged.



Place: Palampur
Dated: 16 February, 2021

(R.S. Chandel)
Major Advisor

CERTIFICATE-II

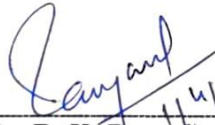
This is to certify that the thesis entitled “**Bioecology and management of *Melolontha* spp. (Scarabaeidae: Coleoptera) in Himachal Pradesh**” submitted by **Mr. Abhishek Rana (Admission No. A-2016-40-007)** son of **Sh. Kamlesh Kumar** to the CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur in partial fulfilment of the requirements for the degree of **Doctor of Philosophy (Agriculture)** in the discipline of **Entomology** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

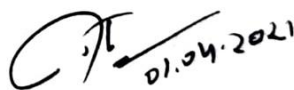

01/04/2021
(Dr. R. S. Chandel)
Chairperson
Advisory Committee


11/4/21
(Dr. R. N. Barwal)
External Examiner


01.04.2021
(Dr. K. S. Verma)
Member


01.04.2021
(Dr. K. D. Sharma)
Member


11/4/21
(Dr. D. K. Banyal)
Member


01.04.2021
(Dr. P. K. Dogra)
Dean's nominee


Head of the Department
Department of Entomology
CSKHPKV, Palampur

Dean, Postgraduate Studies

ACKNOWLEDGEMENTS

First and foremost, immeasurable gratitude and appreciation to my mother **Smt. Daya Devi** and father **Sh. Kamlesh Kumar** whose blessings and encouragement has enabled me to dream and achieve. Sincere gratitude to my brother **Avinesh Rana** for his continuous support. I thank **God almighty** for bestowing me with strength and blessings to overcome every hurdle and fulfill my dream.

I would like to express my special thanks to my advisor **Dr. R.S. Chandel**, Professor & Head, Department of Entomology, who gave me the golden opportunity to do this wonderful and interesting research work on one of the important species of white grubs, and also helped me a lot during the research work. I got to explore and learn so many new things. I am very much thankful to him for encouraging me during the entire journey.

I am extremely indebted to Dr. P.K. Mehta for his precious guidance and consistent support. He always encouraged me with his motivational words and constant guidance. I am grateful to the members of my advisory committee, Dr. K.S. Verma, Dr. K.D. Sharma, Dr. D.K. Banyal and Dr. P.K. Dogra for their kind cooperation.

A special thanks to Dr. Surender Chandel for his invaluable help. Without his help, this research work would have been incomplete. He was always there during my molecular work and guided a lot. I feel privileged to be associated with him. “Thank you Sir”.

A special thanks to Dr. K. Sreedevi for the identification of white grub species.

I would like to extend my sincere thanks to Dr. Saurbh Soni, Ritesh K. Gupta Sir, Dr. Suman Sanjta, Dr. Kalpana, Dr. Mandeep Pathania, Dr. Swaroop Thakur and Dr. Vikas Tandon and my juniors, Manishkumar Joshi, Yamini Chandel, Vasu Mehta, Himanshu Thakur, Titiksha, Radha Koranga and Mayur who have helped in one way or another in completing this research. I also extend my thanks to my friends Dr. Gourav, Dr. Anudeep, Dr. Sanchit, Rajneesh (AFO), Vineet (PO) and Rimpay (ADO) who helped me a lot. My sincere thanks to Anjali Thakur and her parents, who helped me during beetle collection surveys from their native place. I am highly thankful to Mr. Jagbir Singh, Anil bhai Munish Bhae, Rajesh Bhae and M.S. Pathania Sir who helped me during my surveys.



(Abhishek Rana)

Place: Palampur
Dated: 16 February, 2021

TABLE OF CONTENTS

Chapter	Title	Page
1.	INTRODUCTION	1-4
2.	REVIEW OF LITERATURE	5-31
3.	MATERIALS AND METHODS	32-49
4.	RESULTS AND DISCUSSION	50-230
5.	SUMMARY AND CONCLUSIONS	231-237
	LITERATURE CITED	238-262
	BRIEF BIODATA OF THE STUDENT	

LIST OF ABBREVIATIONS USED

Sr. No	Abbreviation/Symbol	Meaning
1.	'	Minutes
2.	"	Seconds
3.	%	Per cent
4.	*	Asterisk
5.	/	Per
6.	<	Less than
7.	=	Equal
8.	>	More than
9.	±	Plus or Minus
10.	Σ	Summation
11.	≥	More than equal to
12.	♀	Female
13.	♂	Male
14.	a.i	Active ingredient
15.	amsl	Above mean sea level
16.	CD	Critical Difference
17.	cm	Centimeter
18.	conc.	Concentration
19.	DNA	Deoxyribonucleic Acid
20.	dNTP	Deoxynucleotide triphosphate
21.	EC	Emulsifiable concentrate
22.	et al.	Et alii (and others)
23.	etc.	Etcetera
24.	Fig.	Figure
25.	FYM	Farmyard Manure
26.	ft.	Feet
27.	g	Gram
28.	i.e.	Id est (that is)
29.	KOH	Potassium hydroxide
30.	L	Litre
31.	LC50	Lethal concentration
32.	m	Meter
33.	ml	Mililitre
34.	mm	Milimeter
35.	N	North
36.	no.	Number
37.	°	Degree
38.	p	Page
39.	ppm	parts per million
40.	SC	Suspension concentrate
41.	SE	Standard Error
42.	SL	Soluble liquid
43.	sp.	Species
44.	sq.	Square
45.	UV	Ultraviolet

Sr. No	Abbreviation/Symbol	Meaning
46.	<i>viz.</i>	Vi delicet (namely)
47.	WDG	Water Dispersible Granule
48.	MgCl ₂	Magnesium Chloride
49.	mM	Millimolar
50.	NaCl	Sodium Chloride
51.	μl	Microlitre
52.	RNase	Ribonuclease
53.	Rpm	Revolutions per minute
54.	Taq polymerase	Thermus aquaticus DNA polymerase
55.	TE	Tris EDTA buffer
56.	Tris	Tris (hydroxy methyl) amino methane
57.	TAE	Tris Acetate EDTA
58.	PVC	Polyvinyl chloride
59.	CTAB	Cetyltrimethylammonium Bromide
60.	PCR	Polymerase Chain Reaction

LIST OF TABLES

Table no.	Title	Page
3.1	Sampling locations for <i>Melolontha</i> beetles on light traps and host trees	36
3.2	Description of locations surveyed for the collection of <i>Melolontha</i> grubs in Himachal Pradesh	40
3.3	PCR conditions for different primers	45
3.4	List of insecticides tested against grubs of <i>M. virescens</i> (= <i>P. fullo</i>)	47
4.1	Checklist of <i>Melolontha</i> beetles collected on light trap during 2017-18 in HP	51
4.2	Abundance of <i>M. cuprescens</i> beetles on light trap in HP during 2017 and 2018	52
4.3	Abundance of <i>M. furcicauda</i> beetles on light trap in HP during 2017 and 2018	55
4.4	Abundance of <i>M. indica</i> beetles on light trap in HP during 2017 and 2018	59
4.5	Abundance of <i>P. fullo</i> beetles on light trap in HP during 2017 and 2018	62
4.6	Diversity indices calculated for light trap catches of <i>Melolontha</i> beetles during 2017 and 2018	65
4.7	Beta diversity showing similarity among <i>Melolontha</i> species in different locations of HP	68
4.8	Bray Curtis distance index of <i>Melolontha</i> species in different locations of HP	69
4.9	Abundance of <i>Melolontha</i> beetles on different hosts in HP during 2017	69
4.10	Abundance of <i>Melolontha</i> beetles on different hosts in HP during 2018	70
4.11	Leaf area consumption by <i>Melolontha</i> beetles in multi-choice test	74
4.12	Leaf area consumption by <i>Melolontha</i> beetles in no-choice test	75
4.13	Abundance and infestation of <i>Melolontha</i> grubs in potato fields during 2017 and 2018	77
4.14	Abundance and infestation of <i>Melolontha</i> grubs in cole crops during 2017 and 2018	79
4.15	Abundance and infestation of <i>Melolontha</i> grubs in Rajmash during 2017 and 2018	81
4.16	Abundance and infestation of <i>Melolontha</i> grubs in onion at Chhatanseri	83
4.17	Distribution and abundance of <i>Melolontha</i> grubs in apple during 2017 and 2018	85
4.18	Size of eggs of <i>M. cuprescens</i> , <i>M. furcicauda</i> and <i>M. indica</i>	90
4.19	Incubation period of <i>M. cuprescens</i> , <i>M. furcicauda</i> and <i>M. indica</i>	90
4.20	Body length and head capsule width for grubs of different <i>Melolontha</i> spp.	98
4.21	Larval period of <i>M. cuprescens</i> , <i>M. furcicauda</i> and <i>M. indica</i>	101
4.22	Morphometric measurements of pre-pupae and pupae	136
4.23	Duration of pre-pupa, pupa and total life cycle	140
4.24	Morphometric measurements of <i>Melolontha</i> adults	156
4.25	Pre-oviposition, oviposition and post oviposition periods of <i>Melolontha</i> spp.	170
4.26	Adult longevity of <i>M. cuprescens</i> , <i>M. furcicauda</i> and <i>M. indica</i>	170
4.27	Duration and morphometrics of different stages of <i>M. virescens</i> (= <i>P. fullo</i>)	201

Table no.	Title	Page
4.28	Sequences of amplified products of <i>Cox1</i> gene of <i>M. cuprescens</i> , <i>M. furcicauda</i> , <i>M. indica</i> and <i>M. virescens</i> (= <i>P. fullo</i>)	205
4.29	Sequences of amplified products of 28S rRNA gene of <i>M. cuprescens</i> , <i>M. furcicauda</i> and <i>M. indica</i>	206
4.30	Concentration-mortality response of chlorantraniliprole against second instar grubs of <i>M. virescens</i> (= <i>P. fullo</i>)	217
4.31	Concentration - mortality response of bifenthrin against second instar grubs of <i>M. virescens</i> (= <i>P. fullo</i>)	218
4.32	Concentration - mortality response of clothianidin against second instar grubs of <i>M. virescens</i> (= <i>P. fullo</i>)	218
4.33	Concentration - mortality response of thiamethoxam against second instar grubs of <i>M. virescens</i> (= <i>P. fullo</i>)	219
4.34	Concentration - mortality response of chlorpyrifos against second instar grubs of <i>M. virescens</i> (= <i>P. fullo</i>)	220
4.35	Concentration - mortality response of imidacloprid against second instar grubs of <i>M. virescens</i> (= <i>P. fullo</i>)	220
4.36	Concentration - mortality response of chlorantraniliprole against third instar grubs of <i>M. virescens</i> (= <i>P. fullo</i>)	222
4.37	Concentration - mortality response of bifenthrin against third instar grubs of <i>M. virescens</i> (= <i>P. fullo</i>)	223
4.38	Concentration - mortality response of clothianidin against third instar grubs of <i>M. virescens</i> (= <i>P. fullo</i>)	223
4.39	Concentration - mortality response of thiamethoxam against third instar grubs of <i>M. virescens</i> (= <i>P. fullo</i>)	224
4.40	Concentration - mortality response of chlorpyrifos against third instar grubs of <i>M. virescens</i> (= <i>P. fullo</i>)	225
4.41	Concentration - mortality response of imidacloprid against third instar grubs of <i>M. virescens</i> (= <i>P. fullo</i>)	225
4.42	Relative toxicity of insecticides against second instar grubs of <i>M. virescens</i> (= <i>P. fullo</i>)	228
4.43	Relative toxicity of insecticides against third instar grubs of <i>M. virescens</i> (= <i>P. fullo</i>)	229
4.44	Mortality of rajmash plants due to attack of <i>M. indica</i> grubs	230
4.45	Mortality response of <i>M. indica</i> grubs to different seed treatments	231

LIST OF FIGURES

Fig. No.	Title	Page
3.1	Locations selected for beetle sampling on light trap and host trees in Himachal Pradesh	35
3.2	Locations selected for <i>Melolontha</i> grubs sampling in Himachal Pradesh	39
4.1	Month-wise occurrence of <i>M. cuprescens</i> beetles on light trap during 2017	52
4.2	Month-wise occurrence of <i>M. cuprescens</i> beetles on light trap during 2018	53
4.3	Cumulative month-wise catch of <i>M. cuprescens</i> beetles during 2017 and 2018	53
4.4	Month-wise occurrence of <i>M. furcicauda</i> beetles on light trap during 2017	56
4.5	Month-wise occurrence of <i>M. furcicauda</i> beetles on light trap during 2018	57
4.6	Cumulative month-wise catch of <i>M. furcicauda</i> beetles during 2017 and 2018	57
4.7	Month-wise occurrence of <i>M. indica</i> beetles on light trap during 2017	60
4.8	Month-wise occurrence of <i>M. indica</i> beetles on light trap during 2018	60
4.9	Cumulative month-wise catch of <i>M. indica</i> beetles during 2017 and 2018	61
4.10	Month-wise occurrence of <i>M. virescens</i> (= <i>P. fullo</i>) beetles on light trap during 2017	62
4.11	Month-wise occurrence of <i>M. virescens</i> (= <i>P. fullo</i>) beetles on light trap during 2018	63
4.12	Cumulative month-wise catch of <i>M. virescens</i> (= <i>P. fullo</i>) beetles during 2017 and 2018	63
4.13	Overall diversity of <i>Melolontha</i> beetles in different locations of HP	63
4.14	Occurrence of <i>Melolontha</i> beetles on host trees during 2017 and 2018 at Palampur	70
4.15	Occurrence of <i>Melolontha</i> beetles on host trees during 2017 and 2018 at Chhatanseri	71
4.16	Occurrence of <i>Melolontha</i> beetles on host trees during 2017 and 2018 at Badagaon	72
4.17	Occurrence of <i>Melolontha</i> beetles on host trees during 2017 and 2018 at Ahla	72
4.18	Clypeus and labrum (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	104
4.19	Grub antennae (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	106
4.20	Epipharynx (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i>	109
4.21	Epipharynx of <i>M. indica</i>	110
4.22	Mandibles (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	113
4.23	Maxillae (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	116
4.24	Labium (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	118
4.25	Hypopharynx (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	121
4.26	Pro thoracic legs (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	126
4.27	Mesothoracic legs (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	126
4.28	Metathoracic legs (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	126
4.29	Prothoracic and abdominal spiracles (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	128
4.30	Raster pattern (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	131
4.31	Pupal tips (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	139
4.32	Scutellum (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	146
4.33	Pronotum (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	149
4.34	Pygidium (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	155
4.35	Antennae (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	158
4.36	Forelegs (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	161

Fig. No.	Title	Page
4.37	Midlegs (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	162
4.38	Hindlegs (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	163
4.39	Male genitalia of <i>M. cuprescens</i> (a) Dorsal view; (b) Ventral view; (c) Lateral view; (d) Spiculum gastrale	166
4.40	Male genitalia of <i>M. furcicauda</i> (a) Dorsal view; (b) Ventral view; (c) Lateral view; (d) Spiculum gastrale	166
4.41	Male genitalia of <i>M. indica</i> (a) Dorsal view; (b) Ventral view; (c) Lateral view; (d) Spiculum gastrale	169
4.42	Clypeus and labrum of <i>M. virescens</i> (= <i>P. fullo</i>)	178
4.43	Antenna <i>M. virescens</i> (= <i>P. fullo</i>) grub	178
4.44	Epipharynx <i>M. virescens</i> (= <i>P. fullo</i>)	178
4.45	Mandibles <i>M. virescens</i> (= <i>P. fullo</i>)	180
4.46	Maxilla <i>M. virescens</i> (= <i>P. fullo</i>)	180
4.47	Labium <i>M. virescens</i> (= <i>P. fullo</i>)	184
4.48	Hypopharynx <i>M. virescens</i> (= <i>P. fullo</i>)	184
4.49	Legs <i>M. virescens</i> (= <i>P. fullo</i>) grubs (a) Prothoracic leg; (b) Mesothoracic leg; (c) Metathoracic leg	186
4.50	Prothoracic and abdominal spiracles of <i>P. fullo</i>	186
4.51	Raster pattern <i>M. virescens</i> (= <i>P. fullo</i>)	186
4.52	Pupal tips <i>M. virescens</i> (= <i>P. fullo</i>)	190
4.53	Scutellum of <i>P. fullo</i>	193
4.54	Pronotum of <i>P. fullo</i>	193
4.55	Pygidium of <i>P. fullo</i>	196
4.56	Antennae of <i>P. fullo</i> adult	196
4.57	Legs of <i>P. fullo</i> adult (a) Forelegs; (b) Midlegs; (c) Hindlegs	198
4.58	Male genitalia of <i>P. fullo</i>	200
4.59	Phylogenetic relationships between five <i>Melolontha</i> and three <i>Polyphylla</i> species based on <i>Cox1</i> gene sequences. The tree was constructed using maximum likelihood method. The Boot Strap values are given on the branches	215
4.60	Phylogenetic relationships between four <i>Melolontha</i> and nine different white grub species based on 28s <i>rRNA</i> gene sequences. The tree was constructed using maximum likelihood method. The Boot Strap values are given on the branches	216
4.61	Dose - mortality response of different insecticides tested in soil against second instar grubs of <i>M. virescens</i> (= <i>P. fullo</i>)	221
4.62	Dose - mortality response of different insecticides tested in soil against third instar grubs of <i>M. virescens</i> (= <i>P. fullo</i>)	226

LIST OF PLATES

Plate No.	Title	Page
3.1	UV light traps used for monitoring of beetles (a) Close-up view; (b) In operation during night	34
3.2	Glass jars used for mating and feeding of adult beetles	42
3.3	Maize seedlings used for rearing of first instar grubs of <i>Melolontha</i> species	42
3.4	Rajmash seedlings used for rearing of first instar grubs of <i>M. virescens</i> (= <i>P. fullo</i>)	42
3.5	Insecticides tested against <i>Melolontha</i> grubs	48
4.1	Infestation by <i>M. cuprescens</i> grubs in potato tubers (a) Tuber damaged by second instar grubs; (b) Tubers damaged by third instar grubs	78
4.2	Infestation by <i>M. virescens</i> (= <i>P. fullo</i>) grubs in cabbage (a) Wilted plant; (b) Roots pruned by grubs	80
4.3	Infestation by <i>M. virescens</i> <i>P. fullo</i> grubs in rajmash (a) Wilted plant; (b) Grubs feeding on roots	82
4.4	(a) Apple plant damaged by <i>P. fullo</i> ; (b) Apple plant damaged by <i>M. indica</i> ; (c) <i>M. cuprescens</i> grubs feeding on apple roots	84
4.5	Mating pairs (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	86
4.6	Eggs (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	89
4.7	First instar grubs (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	92
4.8	Second instar grubs (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	94
4.9	Third instar grubs (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	97
4.10	(a) <i>M. cuprescens</i> grub infected with <i>B. brongniartii</i> ; (b) <i>B. brongniartii</i> isolated on PDA; (c) Conidia of <i>B. brongniartii</i>	100
4.11	Clypeus and labrum (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	103
4.12	Antennae of grub (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	105
4.13	Epipharynx (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	108
4.14	Mandibles (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	112
4.15	Maxillae (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	115
4.16	Labium (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	117
4.17	Hypopharynx (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	120
4.18	Prothoracic legs (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	123
4.19	Mesothoracic legs (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	124
4.20	Metathoracic legs (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	125
4.21	Spiracles (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	127
4.22	Raster pattern (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	130
4.23	Pre-pupae (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	133
4.24	Pupae (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	135
4.25	Pupal antennae (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	137
4.26	Pupal tips (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	138
4.27	Adults (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	142
4.28	Head capsule (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	144
4.29	Scutellum (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	145
4.30	Pronotum (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	148
4.31	Pubescens on meta-sternum (a) <i>M. cuprescens</i> (b) <i>M. furcicauda</i> (c) <i>M. indica</i>	150
4.32	Meso-metasternal process (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	151

Plate No.	Title	Page
4.33	Triangular patches on abdomen (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	152
4.34	Pygidium (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	154
4.35	Antennae (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	157
4.36	Forelegs (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	160
4.37	Midlegs (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	160
4.38	Hindlegs (a) <i>M. cuprescens</i> ; (b) <i>M. furcicauda</i> ; (c) <i>M. indica</i>	160
4.39	Genitalia of <i>M. cuprescens</i> male	165
4.40	Genitalia of <i>M. furcicauda</i> male	165
4.41	Genitalia of <i>M. indica</i> male	168
4.42	Mating pair of <i>M. virescens</i> (= <i>P. fullo</i>)	172
4.43	Eggs of <i>M. virescens</i> (= <i>P. fullo</i>)	172
4.44	Different larval stages of <i>M. virescens</i> (= <i>P. fullo</i>)	175
4.45	Clypeus and labrum of <i>M. virescens</i> (= <i>P. fullo</i>)	177
4.46	Antenna of <i>M. virescens</i> (= <i>P. fullo</i>)	177
4.47	Epipharynx of <i>M. virescens</i> (= <i>P. fullo</i>)	177
4.48	Mandibles of <i>M. virescens</i> (= <i>P. fullo</i>)	179
4.49	Maxilla of <i>M. virescens</i> (= <i>P. fullo</i>)	179
4.50	Labium of <i>M. virescens</i> (= <i>P. fullo</i>)	183
4.51	Hypopharynx of <i>M. virescens</i> (= <i>P. fullo</i>)	183
4.52	Legs of <i>M. virescens</i> (= <i>P. fullo</i>) grubs (a) Prothoracic legs; (b) Mesothoracic legs; (c) Metathoracic legs	185
4.53	Spiracles of <i>M. virescens</i> (= <i>P. fullo</i>) (a) Prothoracic spiracle; (b) Abdominal spiracle	185
4.54	Raster pattern of <i>M. virescens</i> (= <i>P. fullo</i>)	185
4.55	Prepupa and Pupa of <i>P. fullo</i>	187
4.56	(a) Pupal antennae; (b) Pupal tips of <i>P. fullo</i>	189
4.57	Adults of <i>P. fullo</i>	191
4.58	Head capsule of <i>P. fullo</i>	192
4.59	Scutellum of <i>P. fullo</i>	192
4.60	Pronotum of <i>P. fullo</i>	192
4.61	Pygidium of <i>P. fullo</i>	195
4.62	Antennae of <i>P. fullo</i> adult	195
4.63	Legs of <i>P. fullo</i> adults	197
4.64	Genitalia of <i>P. fullo</i>	199
4.65	Agarose gel electrophoresis (1%) showing quality of DNA; Lane 1: <i>M. indica</i> , Lane 2: <i>P. fullo</i> , Lane 3: <i>M. cuprescens</i> , Lane 4: <i>M. furcicauda</i>	203
4.66	Agarose gel electrophoresis (1%) showing amplified product of <i>Cox 1</i> gene; Lane 1: <i>M. indica</i> , Lane 2: <i>P. fullo</i> , Lane 3: <i>M. cuprescens</i> , M: 100 bp DNA ladder	203
4.67	Agarose gel electrophoresis (1%) showing amplified product of 28S <i>rRNA</i> gene; Lane 1: <i>M. indica</i> , Lane 2: <i>P. fullo</i> , Lane 3: <i>M. cuprescens</i> , M: 100 bp DNA ladder	203
4.68	Agarose gel electrophoresis (1%) showing amplified product of 28S <i>rRNA</i> and <i>Cox 1</i> genes; M: 100 bp DNA ladder, Lane 1 and 2: <i>M. furcicauda</i> (28s <i>rRNA</i> gene), Lane 3 and 4: <i>M. furcicauda</i> (<i>Cox1</i> gene)	203
4.69	Transformed bacterial cells	204

Department of Entomology
CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur-176062 (HP)

Title of thesis : Bioecology and Management of *Melolontha* spp. (Scarabaeidae: Coleoptera) in Himachal Pradesh
Name of the student : Abhishek Rana
Admission number : A-2016-40-007
Major discipline : Entomology
Minor discipline : (i) Plant Pathology (ii) Agricultural Biotechnology
Date of thesis submission : 16 February, 2021
Total pages of the thesis : 263
Major Advisor : Dr R.S. Chandel

ABSTRACT

In Himachal Pradesh, five species of *Melolontha* beetles viz., *Melolontha cuprescens*, *Melolontha furcicauda*, *Melolontha indica*, *Melolontha* sp.1 and *Melolontha virescens* (= *Polyphylla fullo*) were collected on light traps and from host trees. *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*) comprised 30.0-32.84, 32.23-33.33, 24.30-24.88 and 10.62-11.79 per cent of total *Melolontha* beetle catch during 2017-18. The Shannon index ($H' = 1.35-1.36$), Simpson index of diversity ($D = 0.73-0.74$) and Pielou's evenness index ($J' = 0.97-0.98$) were calculated to be maximum at Badagaon. The species richness and abundance showed total similarity among *Melolontha* species between Badagaon and Janjehli. Maximum dissimilarity was recorded among *Melolontha* assemblages from Palampur and Sangla. In food preference studies, rose was found to be the most preferred host for *M. indica* and *M. furcicauda*, and apple for *M. cuprescens* both in multi and no-choice tests. The data on white grub infestation were recorded at seven locations in potato, cabbage, rajmash, onion and apple. Maximum tuber damage (42.50-47.50 %) in potato due to *M. virescens* (= *P. fullo*) was recorded at Badagaon, and in onion, 6.25-8.75 per cent bulb infestation by grubs of *M. cuprescens* was recorded at Chhatanseri. In cabbage and rajmash, the grubs of *M. virescens* (= *P. fullo*) inflicted 26.25-28.75 and 18.7-20.0 per cent damage at Badagaon. The biology of four species was studied in laboratory at Palampur. The average length x width of newly laid eggs of *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*) was recorded to be $4.58 \pm 0.06 \times 3.33 \pm 0.04$, $3.94 \pm 0.06 \times 2.90 \pm 0.06$, $3.63 \pm 0.02 \times 2.60 \pm 0.09$ and $3.88 \pm 0.07 \times 2.81 \pm 0.06$ mm, respectively. There were three larval instars, and the fully fed first instars of *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*) measured 15.33 ± 0.66 , 11.15 ± 0.37 , 13.49 ± 0.70 and 12.08 ± 0.78 mm in body length and 3.65 ± 0.22 , 3.21 ± 0.19 , 2.37 ± 0.05 and 2.72 ± 0.05 mm in head capsule width, respectively. The fully fed second instar grubs measured 22.99 ± 0.32 , 23.20 ± 0.36 , 22.61 ± 0.35 and 25.89 ± 0.71 mm in body length, and the width of head capsule averaged 4.88 ± 0.17 , 5.04 ± 0.09 , 4.63 ± 0.20 and 4.68 ± 0.17 mm, respectively. In third instar grubs of *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*), the body length measured 51.12-58.20, 50.11-53.39, 40.13-50.03 and 48.33-54.17 mm, and the head capsule width was 7.68-8.99, 6.89-7.13, 6.98-7.58, and 7.92-8.48 mm, respectively. The total larval period varied between 608-619, 572-590, 598-605, and 574-594 days in *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*), respectively. A detailed description of the mouth parts, raster, spiracles, thoracic legs of the third instar larva and last abdominal segment of pupa is given with the help of suitable diagrams. The raster consisted of paired palidia represented by 28 or 29 pali in *M. cuprescens*, 26 in *M. furcicauda*, 31 or 33 in *M. indica* and 15 in *M. virescens* (= *P. fullo*). The body length x width of pupa measured 32.22 ± 0.37 mm x 14.56 ± 0.29 mm, 32.47 ± 0.11 mm x 14.55 ± 0.13 mm, 31.97 ± 0.15 mm x 12.71 ± 0.22 mm, and 32.72 ± 0.41 mm x 14.23 ± 0.01 mm, and the pupal period ranged from 17-23, 28-32, 22-28 and 14-22 days in *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*), respectively. The adult formation occurred during second week of May to second week of July in different species. All species had biennial life cycle ranging from 619-685 days. The female beetles ($24.69 \pm 0.38-33.37 \pm 0.39$ mm) were slightly larger than male beetles ($23.35 \pm 0.46-32.68 \pm 0.62$ mm). The antennal club was seven segmented in males, and six segmented in females. The male genitalia showed clear-cut differences among the species. The *Cox1* sequence from *M. cuprescens* showed 99.89 per cent similarity to *M. virescens* (= *P. fullo*), whereas *Cox1* sequence from *M. furcicauda* showed 89.47 per cent similarity to *M. indica*. Based on LC_{50} values, chlorantraniliprole was found highly effective against grubs of *M. virescens* (= *P. fullo*).



(Abhishek Rana)
Student
Dated: 16 February, 2021



(R. S. Chandel)
Major Advisor
Dated: 16 February, 2021



Head of the Department

1. INTRODUCTION

The superfamily Scarabaeoidea contains an immense number of species whose larvae live in the soil and are commonly known as white grubs. It constitutes a large distinct group of highly specialized beetles, which could easily be identified by their lamellate antennae (Gardner 1935). The scarabaeid beetles are the most common leaf chafers, whereas the larvae are most destructive soil pests (Ritcher 1958; Jackson and Klein 2006). The Scarabaeoidea is one of the largest superfamily of order Coleoptera (Johnson et al. 2004), and it comprises a very diverse and cosmopolitan group constituting of nearly 31,000 species worldwide, of which the family Scarabaeidae is represented by 27,800 species representing about 91 per cent of the scarab beetles worldwide (Ratcliffe and Jameson 2004). Scarabaeidae is the largest family of Scarabaeoidea and it is divided into 13 subfamilies, of which Melolonthinae and Rutelinae are the largest that are widely distributed throughout the world (Kumar et al. 2017), and majority of the phytophagous scarabs belong to these two subfamilies.

Ali (2001) reported that the family Scarabaeidae represents about 2,500 species from the Indian subcontinent having Melolonthinae as the largest subfamily with 750 genera and 11,000 species known to occur in the world, and 75 genera with 932 species occurring in India (Houston and Weir 1992). The bodies of adults in tribe Melolonthini are comparatively larger, take comparatively longer period to complete their larval stages (Ritcher 1958), and because of their larger size and longer larval stages, the pests in the tribe Melolonthini usually cause greater economic damage (Britton 1978).

The genus *Melolontha* is a phytophagous scarab group constituting the type genus of the subfamily Melolonthinae and tribe Melolonthini. The *Melolontha* are often recorded as crop pests as foliage feeding adults and root damaging larvae (Liu et al. 1997). There are about 60 species/subspecies within the genus *Melolontha* which have been described worldwide (Keith and Li 2005). The cockchafers of the genus *Melolontha* rank among the serious pests in agriculture and forestry, but the larvae cause greater damage than adult beetles (Svestka 2006). The grubs show a distinct preference for a variety of tree roots and are able to migrate as far as several meters through the soil towards their host plants, while adult beetles migrate kilometers and defoliate several tree species (Weissteiner et al. 2012). The larvae of Melolonthini have been described for only 58 species, of which four belong to the genus *Melolontha*.

The beetles belonging to genus *Melolontha* are cosmopolitan and some of the species of international importance are *Melolontha melolontha* (L.), *Melolontha hippocastani* Fabricius, *Melolontha incana* (Motschulsky), *Melolontha pectoralis* Germar and *Melolontha guttigera* Sharp. The common European cockchafer, *M. melolontha* is widely distributed throughout temperate Europe and continental United States (Fraval 1988). The forest cockchafer, *M. hippocastani* occurs in the forest and forest-steppe regions of Russia from the Primorije westward up to central and northern Europe (Lisov 1984). In Czech Republic, grubs of *M. hippocastani* feed on roots, and cause considerable damage to plants of forest trees, especially pine, oak, linden etc. and sometimes destroying as much as 100 per cent (Svestka 2007). In Black Sea region of Turkey, the common cockchafer, *M. melolontha* is one of the most important insect pests of hazelnuts, causing 15-20 per cent damage annually (Sezen et al. 2007). In Germany, *M. melolontha* and *M. hippocastani* were found to damage large areas of forest (Keller 1984), whereas in Austria, both *M. melolontha* and *M. hippocastani* feed on leaves of many forest and fruit trees like oak, hornbeam, beech, birch, walnut, mountain ash and stone fruits (Kratochvil et al. 1953). According to Tsai (1965), the large chestnut chafer, *M. melolontha* is one of the serious pests of agriculture and forestry in Szechwan (China). In Czechoslovakia, *M. melolontha* has been reported to cause serious damage to forest and fruit trees (Muska 1975). In United States, *M. melolontha* inflicts damage to fruit, ornamental, and forest trees like strawberry, grape, apple, pear, plum, cherry, pea, bean, onion, lettuce, potato, crucifers, oats, barley, corn, soybean, tobacco, alfalfa, and clover (Woodruff 1978). In Switzerland, adults of *M. melolontha* feed preferentially on oak and beech trees, and cause defoliation mainly along the borders of the forests (Keller et al. 1999).

In India, the genus *Melolontha* is mainly distributed in north western Himalaya, and eight species viz., *Melolontha indica* Hope, *Melolontha cuprescens* Blanchard, *Melolontha furcicauda* Ancey, *Melolontha virescens* (Brenske), *Melolontha aeneicollis* Bates, *Melolontha nepalensis* (Blanchard), *M. melolontha*, *M. guttigera* have been reported from different parts (Chandra 2005; Pathania 2014; Kumar et al. 2007; Chandra and Gupta 2012). *Melolontha indica* and *M. furcicauda* occur in Uttarakhand (Shah and Garg 1988), Jammu & Kashmir (Bhat et al. 2005; Raina and Khan 2007) and Himachal Pradesh (Chandra 2005), whereas *M. nepalensis* has been reported to occur in Uttarakhand (Mishra and Singh (1999) and Himachal Pradesh (Kumar et al. (2007). Raina and Khan (2007) have recorded the presence of *M. melolontha* during June-August from Kashmir valley. *M. cuprescens* is commonly found in mid hill areas in north-western Himalaya, but

Kumar and Vishvendra (2018) have also reported this species from Indo-Gangetic plains in Meerut, Muzaffarnagar, Baghpat and Saharanpur districts of western Uttar Pradesh. Chandra and Gupta (2012) have reported *M. guttigera* from Arunachal Pradesh, and this species is also distributed in Sikkim, West Bengal and eastern Nepal. Deka (2014) has recorded the presence of *Melolontha* sp. nr. *furcicauda* Ancey in large cardamom ecosystem from Sikkim.

In north Kashmir, *M. furcicauda* is an important pest of oats during the seedling stage (Kumar et al. 2019). *Melolontha furcicauda* has been reported as a key pest of apple, apricot and cherry, whereas *M. indica* was feeding only on apple (Gupta et al. 1977). In Kumaon hills, beetles of different *Melolontha* species defoliate different fruit plants like apple, apricot, walnut and Cherry (Joshi and Joshi 1980). According to Chandra (2005), the adults of *M. furcicauda* feed on stone fruits and fig, and grubs damage potato tubers. Bhat (2007) said that *Melolontha* grubs damage potato tubers by making circular tunnels, thus render them unfit for consumption in Kashmir.

In Himachal Pradesh, five species of *Melolontha* viz; *M. indica*, *M. furcicauda*, *M. cuprescens* and *M. aeneicollis* are known to occur (Chandra 2005). *M. cuprescens* is more commonly found in Holi area of Chamba district (Pathania 2014). Chandra (2005) reported this species to occur from Chamba, Kinnaur and Kullu districts in Himachal Pradesh. *Melolontha virescens* is preponderant in Badagaon area of Kangra district situated at an altitude of 2268 m amsl, and cause damage to almost all crops grown during summer season in Chuhar valley of Himachal Pradesh. *Melolontha furcicauda* occurs mainly in association with coniferous forests up to an altitude of about 3000 amsl. The adults are on wings during second half of June and feed on leaves of stone fruits and fig. The grubs damage potato and other vegetable crops. They also feed on the roots in seed beds of deodar nurseries (Mehta et al. 2008). *Melolontha furcicauda* has been recorded as an important pest of poplar nurseries in India by Singh and Singh (1986). In Kullu valley of Himachal Pradesh, *M. furcicauda* and *M. nepalensis* have been recorded to cause damage in maize and paddy crops (Kumar et al. 2005). In high altitude areas, the grubs of *Melolontha* species feed on the roots of rajmash causing sudden wilting and death of the plants. The plants at 5-6 leaf stage are more prone to their attack causing 8-10 per cent plant mortality (Mehta et al. 2010)

This group of scarab beetles is considered as one of the serious pests attacking potato, rajmash, cole crops etc. in Himachal Pradesh, but the group in India has not been

critically studied as in other countries, and little is known of the identity, distribution, biology and food habitats of their grubs and adult beetles. There are several conflicting reports on the identification of these beetles in Himachal Pradesh. Different entomologists have cited same species using different names adding puzzle to the *Melolontha* complex not only at species, but also at genus level in north western Himalaya. The larval identification plays a significant role in pest management. The grubs and beetles exhibit typical behaviour, which makes them less vulnerable to the control strategies. Understanding the biology and behaviour of scarab larvae, including their interactions with host plants and the soil environment is an essential component to enabling effective management and control both in India and at a global scale. Keeping these points in view, proposed problem was planned to undertake the present study with the following objectives:

Objectives:

- i) To study the distribution, relative abundance and population build up of *Melolontha* spp. in Himachal Pradesh
- ii) To generate the information on bionomics of predominant *Melolontha* spp. and to assess genetic diversity using molecular markers
- iii) To find effective chemicals against predominant *Melolontha* spp.

2. REVIEW OF LITERATURE

The scarab beetles are found across tropical, subtropical and temperate regions in Indian sub continent in a broad range of ecosystem types including agro-ecosystems. There are 35 species of *Melolontha* described from east and south-east Asia. The greatest species diversity of *Melolontha* in the region is in south-western China and northern India where habitats are significantly differentiated from sea level to ≥ 3600 m in elevation. However, most species are found in forest of mid-elevation montane areas. The Palaearctic and Oriental *Melolontha* generic groups are in need of comprehensive study to ascertain their phylogenetic relationship (Li et al. 2010). Herein, we provide an overview of relevant literature pertaining to important species of *Melolontha* present in the world.

2.1 Relative abundance and diversity of *Melolontha* beetles on light trap

Light affects insect behaviour in a variety of ways and one of the most typical responses to light is phototaxis (Jander 1963). The impact of light on insect behaviour varies both qualitatively and quantitatively depending on the light source (light bulb) and material i.e. light reflecting plate (Johansen et al. 2011; Honda 2011). The attraction of insects to light has been analysed in various behavioural experiments both in the field and in the laboratory. Radiation in the near ultraviolet range of the spectrum, with wavelengths of 340 to 380 nm, is the most effective in attracting insects with few exceptions (Mikkola 1972). Szentkiralyi (2002) reported that light trapping is most common and regular sampling technique and the light traps have been widely used for collection of nocturnal insects (Chandel et al. 1995; Pathania 2014). In recent times, the light sources which produce large amounts of UV radiations, have revolutionized the light trap sampling (McGavin 2007). Light trap data provides valuable information about the distribution, abundance and seasonal activity of insects (Sheikh et al. 2016). Holloway et al. (2001) reported that light trap captures large number of insect specimens with minimum efforts. The spectral sensitivities of photoreceptors determine the visible light wavelength for insects, which often expands into the ultraviolet (UV) region, which is invisible to humans (Shimoda and Honda 2013).

The long-wave radiation inhibits the flight of *Melolontha* and short-wave radiation promotes it. The attractiveness of a mercury vapour lamp often increases if the lamp is equipped with an UV-transmitting filter (p. 247) indicating the flight inhibitory action of long-wave radiation Couturier (1967). Couturier and Jacky (1970) investigated flight

stimulation and sensitivity of *Melolontha melolontha* (L.) to the spectrum of light of various wave-lengths in laboratory in France. They demonstrated that beetles are stimulated to fly only when light of a wave-length below 475 mμ is visible.

Tadic (1976) worked with several kinds of light traps in different parts of Yugoslavia during 1972-74, and reported use of ultraviolet light traps for monitoring the flight and activity of *M. melolontha* to be an accurate and practical method for forecasting purposes. The beetles were collected only in the month of May. Devi et al. (1994) monitored the population of scarab beetles with the help of light trap at Palampur, Himachal Pradesh. More than 20 species of beetles were trapped during different months, and *M. melolontha* was recorded during July to August. In Uttarakhand, Mishra and Singh (1997) monitored scarab beetles on light trap using 20 W fluorescent tube at Saraikhet in district Almora. They collected 14 species of scarabaeids with *Melolontha furcicauda* Ancy and *Melolontha nepalensis* (Blanchard) constituting 0.42 and 0.53 per cent of total scarab catch, respectively. Both these species were prevalent from June to August. Tripathi and Gupta (1985) conducted studies on the collection of scarabaeid beetles on light trap from June 1983 to September 1985 at Chaubattia in Almora district of Uttarakhand. During their three years of survey, 33 species of beetles were collected, however, total catch ranged from 1-18 beetles per night per light trap. *M. furcicauda* was reported to be one of the predominant species constituting 73.66 of total beetle catch.

Barwal (1998) recorded the emergence of scarabaeids in north-western Himalaya at Katrain, and in north-eastern parts of India at Shillong. At Katrain, *M. furcicauda* and *M. nepalensis* were recorded from April to August with more predominance of *M. furcicauda* as compared to *M. nepalensis*. At Shillong, eight species of scarabaeid beetles were recorded, but *Melolontha* beetles were absent in trap catch. Shah and Garg (1988) carried out light trap studies from April-October to find the peak period of emergence of positively heliotactic scarabaeid beetles at Hawalbagh in Almora district of Uttarakhand. They reported *Melolontha indica* Hope and *M. furcicauda* beetles occurring in small numbers on light trap during August to September.

In Hungary, Toth (1998) noticed that beetles of *M. melolontha* respond positively to artificial light and therefore they can be collected easily using light traps. They were using a network of 25 light traps in Hungary since 1962 to monitor cockchafer population, and reported that *Melolontha hippocastani* Fabricius and *M. melolontha* occur simultaneously causing heavy damage. The other common and abundant cockchafer species are *Melolontha pectoralis* Germar and *Polyphylla fullo* (Linnaeus). Humayun and

Ali (1999) carried out investigation on important pests of filbert in Alamount area of Qazvin province using light trap for three years, and reported *Polyphylla olivieri* (Castelnau) as an important pest of filbert. Mishra and Singh (1999) identified 26 species of scarab beetles in Gharwal Himalaya using light trap with 20 W fluorescent tube at Ranichauri from April to September. They collected *M. furcicauda* and *M. nepalensis* during June to August with peak activity in July for both the species. The relative abundance of *M. furcicauda* and *M. nepalensis* has been reported to be 2.99 and 1.37 per cent, respectively.

Slater (2001) operated a Rothamsted moth light trap at Lysdinam Field Centre, Newbridge-on-Wye in mid Wales between 1975 to 2001. Although the trap catch period of *M. melolontha* varied from under two to over nine weeks owing to the vagaries of the weather in mid Wales in May and June, yet the number of active nights when catches actually took place were more stable with a mean of 13 and the number of active nights by which time 50 per cent of catch had been taken averaged five nights with a range of 2 to 9. Chandra (2005) reported four species of *Melolontha* beetles viz., *Melolontha aeneicollis* Bates, *M. indica*, *Melolontha cuprescens* Blanchard and *M. furcicauda* occurring in Kullu, Shimla, Solan, Chamba, Kinnaur and Mandi districts of Himachal Pradesh. *M. aeneicollis* has been reported to be prevalent in Kullu, *M. indica* in Kullu, Shimla and Solan, *M. cuprescens* in Chamba, Kinnaur and Kullu and *M. furcicauda* in Chamba, Kinnaur, Kullu, Mandi, Sirmaur and Solan districts. Bhat et al. (2005) collected beetles on light trap from April-September, 2004 in Srinagar district of Jammu and Kashmir. They captured nine species of beetles with *M. indica* as a new record from Kashmir.

Svestka (2006) explored the abundance and species spectrum of *Melolontha* beetles in four forest regions of Czech Republic using light traps. They reported that *M. hippocastani* and *M. melolontha* cause serious damage in forest stands of south east Moravia and central Bohemia. The emergence of beetles begins at the time when average daily temperature reaches more than 10-12°C, and the maximal swarming takes place in the days when the average daily temperature reaches 20°C. Svestka (2007) used light trap with a HQL 125 W discharge lamp in Czech Republic and reported emergence of *M. hippocastani* between 16 April to 02 June, 2003 when 19,510 cockchafer were trapped in light traps of which males and females constituted 61.78 and 38.22 per cent, respectively. Heavy intensive swarming has been reported between 28 April to 12 May when 18,062 cockchafer were trapped comprising 92.58 per cent of total number of the entire emergence period.

Sushil et al. (2006) installed five light traps for trapping the scarab beetles at research farm of Vivekananda Institute of Hill Agriculture located at Hawalbagh, Almora in Uttarakhand from May to October during 2002-2004. They collected more than 16 species on light trap, with *M. indica* constituting 1.35 per cent of total beetle catch. Kumar et al. (2007) carried out light trap studies at Bajaura, Katrain and Targali in Kullu valley of Himachal Pradesh. In total, 29, 19 and 18 species were collected at Bajaura, Katrain and Targali, respectively. *M. furcicauda* was recorded at all three locations, whereas *M. nepalensis* was collected at Targali, and *Melolontha* sp. at Bajaura only.

Raina and Khan (2007) recorded observations on seasonal activity of insects damaging agricultural crops, horticultural and forestry plantations by using light trap fitted with 12 W fluorescent light source in high altitude regions of Kashmir. They recorded 1,038 scarabaeid beetles during a period of two years. *M. furcicauda* comprised 1.73 per cent of total scarab beetle catch, and was active during July to August, whereas *M. melolontha* was recorded from June to August in Kashmir valley and its relative abundance was 1.93 per cent.

In hill and mountain agro ecosystems of Kashmir, total 10 species of scarabaeid beetles were collected on light trap from Srinagar, Pahalgam and Wadura Sopore areas. In Srinagar and Pahalgam areas, *M. furcicauda* was present in very low numbers constituting 2.42 to 4.05 per cent, however in Wadura Sopore, *M. furcicauda* was reported to be the leading species comprising 18.80 per cent of the total beetle catch with peak activity in the month of July (Bhat 2007). Chandra and Uniyal (2007) recorded observations on Pleurostict Scarabaeidae from Great Himalaya National Park situated in Kullu district of Himachal Pradesh. They collected total nine species of scarabaeid beetles during the month of April and May from light sources including *M. furcicauda* and *M. indica*. Daskocil (2007) captured scarab beetles using black light trap in Oklahoma, USA. *Polyphylla* sp. was found to be predominant species at Jenks and Woodward constituting 1.47 and 6.39 per cent of total beetle catch.

Nowinszky et al. (2010) determined the relationship between lunar phase and emergence of adult brood of insects in Hungary. Based on many years of light trap catch data by the Hungarian light trap network and other traps, they reported that broods of common cockchafer, *M. melolontha* start most often in the first or last quarter. On the other hand, broods begin significantly more often in the waxing than in the waning lunar phases. Adult broods emerge in the vicinity of a full moon less frequently than in any other quarter.

Svestka (2010) studied the changes in the abundance of *M. hippocastani* and *M. melolontha* in the Czech Republic during 2003-2009 using light trap. They observed three tribes of *M. hippocastani* appearing in a four year cycle and one tribe of *M. melolontha* appearing in a three year cycle. Adults of both species begin to swarm when the average daily temperature reaches 12-13°C, usually in the third decade of April and that mass swarming occurs when the average daily temperature reaches 15°C and more, i.e. from late April to the third decade of May. Puskas and Nowinszky (2011a) examined the relationship between height of tropopause and *M. melolontha* using light trap catch data obtained from national light trap network in Hungary. They indicated a close positive relationship between the height of tropopause and the number of *M. melolontha* beetles captured in the light trap. Nowinszky et al. (2017) also found a strong positive relation between trap catches of *M. melolontha* and the altitude of tropopause.

Puskas and Nowinszky (2011b) studied the efficiency of light trapping of *M. melolontha* in connection with ozone concentration of air in Hungary. They established that low ozone concentration reduces the success of catch and effectiveness of light trapping increases if the ozone concentration is high. Hinson (2011) investigated species diversity and seasonal abundance of Scarabaeoidea at four locations in south Carolina, and recorded 37 Melolonthine species on light trap. *Polyphylla comes* Casey and *Polyphylla occidentalis* (L.) were very low in numbers. In case of *P. comes*, all collected beetles were males, whereas in *P. occidentalis*, the male beetles constituted 62 per cent of the total beetle catch.

Chandra et al. (2012) carried out light trap studies to identify the scarabaeid beetles prevalent in Govind Wildlife Sanctuary, Garhwal, Uttarakhand. They reported 11 species of scarab beetles, and in genus *Melolontha*, only one species i.e. *M. cuprescens* was collected. Singh and Banyal (2013) studied insect fauna of Khajjiar Lake of Chamba District, Himachal Pradesh by using artificial light on white cloth sheet to collect different insects. They collected five species of scarabaeid beetles and reported *M. fuscicauda* to be a common species in this area. Trotus et al. (2013) followed the evolution of adult flight of *M. melolontha* with the help of light trap in Romania during 1993-2012. The records were made daily from 01 April to 31 October every year and reported that adult flight start in the second or third decade of April and lasts until the second or third decade of May in most of the years with few exceptions when the flight is extended upto first decade of June. The average duration of the flight was 39 days, and maximum flight curve was recorded every three years.

Wagenhoff et al. (2014) studied the spring phenology of *Melolontha* species by using light traps for a period of three years during early spring in forests of south-western Germany. They reported that massive swarming flights occur every third year for *M. melolontha* or every fourth year for *M. hippocastani*. Male cockchafer start leaving the soil first when temperature in the upper soil layer is approximately 10°C and peak emergence from the soil takes place quite synchronously 1-2 weeks after the first swarming flights have been detected. The number of female beetles attracted to the light trap peak at dusk and at the time when oviposition flights culminate, independently of ambient air temperatures. Hardly any female is attracted during the process of oogenesis, whereas the number of male cockchafer attracted to the light trap continuously increases after the termination of swarming flight activity when darkness fell. There was a strong correlation between the number of males trapped and ambient air temperatures during the light trap exposure.

Deka et al. (2014) installed different light traps of different colours in large cardamom ecosystem and recorded the presence of *Melolontha*. sp. nr. *furcicauda* Ancy in Sikkim. Feroz et al. (2015) studied rove beetles, scarabaeid beetles, dermestid beetles and weevil from Kargil district of Jammu and Kashmir. In subfamily Melolonthinae, they have reported *M. furcicauda* to be the predominant species. Pathania et al. (2015) surveyed scarabaeid beetles from eight landscapes of different zones of Himachal Pradesh during 2011 and 2012. The survey was conducted during four months between May to August by using light trap and a total of 13,569 scarab adults of 20 genera and 56 species belonging to subfamilies, Melolonthinae, Rutelinae, Cetoninae and Dynastinae were recorded. In genus *Melolontha*, four species viz., *M. cuprescens*, *M. furcicauda*, *M. indica* and *Melolontha virescens* (Brenske), have been reported and their cumulative catch was 7.17 to 6.98 per cent. In genus *Melolontha*, *M. furcicauda* was the most predominant species constituting 3.03 to 3.10 per cent of total catch, followed by *M. cuprescens* (2.71-2.80 %), *M. indica* (1.37-1.79 %) and *M. virescens* (0.07-0.10 %).

Sreedevi et al. (2017) used black light trap (wavelength 365 nm) to document species of white grubs in Himachal Pradesh, Uttarakhand, Uttar Pradesh and Rajasthan, and recorded high species diversity in Uttarakhand and Himachal Pradesh. In Himachal Pradesh and Uttarakhand, *M. nepalensis* and *M. indica* constituted 0.42 to 1.29 and 0.22 to 1.09 per cent of total beetle catch, respectively. *Melolontha furcicauda* was recorded only in Uttarakhand comprising 3.72 per cent of total beetle catch, whereas *M. cuprescens* was recorded only in Himachal Pradesh with relative abundance of 4.40 per cent.

Tartanus et al. (2017) utilized two kinds of light traps for field trials carried out on two locations in Poland. The light traps were placed in the strawberry plantation, and they reported much higher efficiency of trap having a large white screen to capture adults of *M. melolontha* compared to those formed only with light bulb. They noted that majority of the captures occurred at the beginning of flight period *i.e.* in early May, which gives an additional indication about the possibility to increase the efficiency of trap catch method. The trap with white screen attracted more males of *M. melolontha*, while in the other, a slightly higher number of females were found. Lal and Sharma (2017) studied the species richness and abundance of white grubs from forest ecosystem of Doon valley. They identified 19 species from different sites belonging to 12 genera under four subfamilies and nine tribes. *Melolontha furcicauda* and *M. indica* were collected at all four study sites and their relative abundance has been reported to be 4.12 and 3.37 per cent, respectively.

Kumar and Vishvendra (2018) conducted survey in the vicinity of sugarcane growing areas in Meerut, Muzaffarnagar, Baghpat and Saharanpur districts of western Uttar Pradesh by utilizing light traps. They identified 26 species of scarabaeid beetles belonging to five subfamilies. In genus *Melolontha*, only one species *i.e.* *M. cuprescens* has been reported. Kumar et al. (2018) estimated the population of different soil borne insect-pests in oat agro-ecosystem in north Kashmir by installing light trap to monitor the activity of adult beetles. They recorded four scarabaeid beetles with *M. furcicauda* as one of the predominant species in oat ecosystem.

Alekseev and Shapoval (2019) sampled beetles on the territory of national park “The Curonian Spit” in Moscow, and collected 10,719 specimens of Coleoptera belonging to 173 species. They specially analysed the material on the *P. fullo* and reported that number of males collected through light trap was much more than number of females. The males showed different frequency of two colour forms with the sharp and stable dominance of dark colouration under bright colouration being in the ratio of 2:1. The colour forms in females have been represented by equal ratio. Kumar et al. (2019) assessed the diversity of scarabaeid beetles at different elevations of Almora district in Uttarakhand during May-August. They identified 24 species of white grubs, and in genus *Melolontha* two species *viz.*, *M. furcicauda* and *M. indica* have been reported.

Arif et al. (2019) studied insect-pests of maize at different altitudes of north Kashmir in Jammu and Kashmir. They reported three species of white grubs at Wadura Sopore (1590 m amsl) and four species at Imberzal Wari (2120 m amsl). *Melolontha*

furcicauda was present at both the locations. *Melolontha furcicauda* has also been recorded on light traps installed at apple nurseries at Sopore in north Kashmir (Altaf and Ahad 2020). Malusa et al. (2020) utilized light traps to control *Melolontha* species in organic strawberry plantation in Poland. They reported that traps positioned at 4 m height were more efficient in attracting the beetles than those kept at 2 m height.

2.2 Relative abundance and diversity of *Melolontha* beetles on host trees

Host plant range is a key ecological characteristic for phytophagous insects as it defines their resource base, which in turn is an important factor influencing their population dynamics (Novotny et al. 2002). The phytophagous insects differ in their degree of specialization on host plants, and range from strictly monophagous species to extremely polyphagous species (Hafsi et al. 2016). For polyphagous insects, the selection of a suitable host plant is essential to ensure their development. Adult scarabaeid beetles are mostly phytophagous with a variety of different feeding habits. Scarab beetles belonging to sub families: Dynastinae feed on stems or roots; Cetoniinae feed on sap, fruit and flowers; Rutelinae feed on foliage, fruit and flowers; and Melolonthinae feed on foliage (Jackson and Klein 2006). Scarab beetles are found in habitats as varied as tropical rainforests and desert like grasslands and on all continents except Antarctica. They occupy many habitats, and infestations can often be recognized by signs of their feeding (Jackson and Klein 2006). In European countries, *Melolontha* spp. causes foliage damage in deciduous forests by eating leaves during the mating period. In Germany, *M. melolontha* and *M. hippocastani* occur in less frequent outbreaks where they totally strip foliage from large areas of forest (Keller 1984).

According to data from Austria, both species begin to swarm together, but swarming of *M. hippocastani* culminates and ends earlier. Males prevail at the beginning of swarming and only at the time of culmination, the male/female ratio is balanced. Both species are considerably polyphagous and feed on leaves of many forest and fruit trees. Their favourites are oak, hornbeam, beech, birch, walnut, mountain ash, the genus *Prunus*; but the choice of woody species is also influenced by the stage of leaf unfolding. In the beginning of swarming, they look for birch as the leaves are already unfolding. It was also confirmed that cockchafer seek male flowers of spruce and pine (Kratochvil et al. 1953). Flerov et al. (1954) discovered that *M. hippocastani* preferred pine for mating and feeding.

In Szechwan (China), large chestnut chafer, *M. melolontha* is one of the serious pests of agriculture and forestry. As soon as the beetles crawl out from the earth, they fly

toward the forest of pitchpine, on which they feed and copulate (Tsai 1965). In Turkey, at least three species of *Polyphylla*, namely *P. fullo*, *P. olivieri* and *Polyphylla turkmenoglu* Petrovitz are recorded, however, first one is considered to be the most common (Turkmenoglu 1967).

Zivanovic (1974) reported the presence of dense population of *M. melolontha* for about 15 years in the highland regions of western Yugoslavia. The adults flew at first to wild and cultivated cherry trees, plum and various species of oak; they were observed on walnut in late May and June. In Czechoslovakia, *M. melolontha* has been reported to cause serious damage to forest and fruit trees and the frequency of outbreaks was common (Muska 1975). In United States, *M. melolontha* has been recorded on more than 60 plants as reported by Woodruff (1978). These include, fruit, ornamental, and forest trees like strawberry, grape, apple, pear, plum, cherry, pea, bean, onion, lettuce, potato, crucifers, oats, barley, corn, soybean, tobacco, alfalfa, and clover.

Gupta et al. (1977) conducted a survey to collect and identify defoliating and fruit eating beetles attacking temperate fruit trees at Chaubattia in Almora district of Uttarakhand. *M. furcicauda* was recorded on apple, apricot and cherry, whereas *M. indica* has been reported to feed only on apple. Both species occur in low numbers and were active from June to August. Joshi and Joshi (1980) also reported *Melolontha* species to defoliate preferably apple and apricot in Kumaon hills. Occasionally the walnut and cherry trees were also defoliated by *Melolontha* beetles.

Khramtsov (1979) reported that *M. hippocastani* is distributed in Europe and Asia; it is found throughout European USSR and in Siberia as far as Transbaikalia. *Melolontha melolontha* is found only in Europe confined in the west and south of the European USSR. The adult beetles have been reported to feed on the leaves of trees, predominantly birch, maple, and oak; in years of mass flight they often completely eat off the leaves, causing severe damage to plantings. The flight of the beetles begins in May, and in the south, in late April. In Romania, *M. melolontha* is the most widespread beetle and is present in the steppe regions and up to the oak and beech forest regions (Perju et al. 1983).

In Mexico, Young (1988) has described 28 species of genus *Polyphylla* in his monograph. They stated that *P. occidentalis* feeds on the foliage of pine, while *Polyphylla variolosa* (Hentz) has been reported to be injurious to fruit trees in the adult stage and as a

heavy feeder on the leaves of various forest trees such as elm, maple, and oak. *Polyphylla decemlineata* (Say) occurred in considerable numbers during July feeding on the needles of ponderosa pine. The adults begin their feeding distally and work inwards toward the needle base consuming everything except the midrib. The adults of *Polyphylla crinita* LeConte have also been recorded to feed on coniferous foliage. Under caged conditions they consumed douglas fir, Norway spruce, and western hemlock, with apparent preference for the last. Lodgepole pine was taken only under hunger stress.

Luisa and Mauro (1996) studied the presence and diffusion of the common cockchafer, *M. melolontha* L. in the areas of Mezzocorona and San Michele in Trento province. They reported that the preferred direction during this flight was towards the woods, but a part of the population remained also in the orchards feeding on cherry and apple trees. Fraval (1998) reported that the adults of *M. melolontha* fly singly during April to May in search of food and defoliate leaves of deciduous fruit and forest trees, particularly oaks, maple, sweet chestnut, beech, plum, and walnut trees.

In Switzerland, adults of *M. melolontha* swarm between end-April to mid-May and concentrate mainly at the borders of deciduous or mixed forests where they feed preferentially on oak and beech trees. The adult beetles cause defoliation mainly along the borders of the forests (Keller et al. 1999). The beetles feed from the margins without leaving the midribs or stout veins causing host plants completely denuded (Keller 2000). Sushil et al. (2006) conducted host preference studies under choice method for *M. indica* involving 31 host plants and reported *Alnus nepalensis* to be the most preferred host for adults of *M. indica* in Kumaon hills of Uttarakhand. Svestka (2006) reported that three species of *Melolontha* are found in Czech Republic and the predominant one is *M. melolontha* which is wide spread all over the country up to an altitude of 800 m especially in agricultural crops. The species, *M. hippocastani* is widespread in some isolated forests in warm locations with sandy soil. They damage the forest stands as well as the neighbourhoods of forests like new vineyards and other cultures.

Chandel et al. (2010) reported *M. indica* as most damaging in Baragraon area of Barot valley and observed its beetles feeding on wheat spikes in first week of July. *Melolontha furcicauda* occurs mainly in association with coniferous forests up to an altitude of about 3000 amsl. The adults are on wings during second half of June and feed on stone fruits and *Ficus* species. (Mehta et al. 2008). Kumar et al. (2007) recorded

observations on host plants of scarabaeid beetles in lower Kullu valley of Himachal Pradesh, and reported *koish*, *Alnus nitida* Mill. as an important host of *M. furcicauda*.

In Western Europe, *M. melolontha* has been designated as a major endemic scarab pest species and the beetles attack several fruit and forestry trees (Jackson and Klein 2006). In Nigeria, adult beetles of *M. furcicauda* defoliate *Acacia nilotica* and *Azadirachta indica*, which are especially attractive to chafer beetles (Alao et al. 2011). Sood et al. (2009) studied the population dynamics of scarabaeid beetles from 2004-2006 in Sangla valley in north-western Himalaya. The sampling of fruit trees in the valley revealed the abundance of nine species of beetles. *Melolontha furcicauda* constituted 5.99 to 8.69 per cent of total catch on different fruit trees. Ahmad and Faisal (2012) have listed as many as 133 insect species recorded by different workers in different parts of India. They have reported *M. furcicauda* as one of the serious defoliators of poplar in different parts of India.

According to Trotus et al. (2013), the adults of *M. melolontha* attack the leaves from different species of forest and fruit trees preferring oak, elm, beech, birch, hornbeam, plum, cherry and walnut; less attacked are apple, pear and grapes. The beetles also attack various shrubs like rose, hawthorn, privet, and other herbaceous plant species. At strong attacks, the leaves are completely destroyed, leaving only the main ridge; sometimes attacks the flowers and fruits in training. Reinecke et al. (2006) reported European cockchafer, *M. melolontha* and forest cockchafer, *M. hippocastani* as serious pests in many parts of central Europe. Adults from both the species emerge at the end of April or at the beginning of May, depending on the local climate. They reported that the adults feed and mate on a variety of deciduous trees like *Quercus* spp., *Fagus sylvatica* L., *Acer* spp. and *Carpinus betulus* L.

In Netherlands, Huiting et al. (2006) reported that the adults of *M. melolontha* rarely cause serious damage, but only occasionally, they are harmful in cherry or plum orchards because they feed also on the blossoms. The adults appear in April- May, fly particularly at dusk, then migrate towards the feeding trees in road-side plantings, in forest edges and in orchards. Wagenhoff et al. (2014) reported that forest cockchafer, *M. hippocastani* and field cockchafer, *M. melolontha* are among the most dreaded insect-pests in many European countries, causing economic losses in agriculture, horticulture and forestry. They reported that adult emergence occurs in good synchrony with the foliation of the main host trees which are *Quercus rubra*, *Quercus robur*, and *Quercus petraea*. The

other host trees like *Fagus sylvatica*, *Acer pseudoplatanus*, *Juglans regia* and *Juglan nigra* are readily accepted as well, whereas *Carpinus betulus* and *Betula pendula* are of minor importance. *Fraxinus excelsior*, *Alnus glutinosa* and *Tilia* sp. are nearly or completely avoided. Ordinarily, cockchafer are observed feeding on preferred deciduous trees as long as their leaves are not too much hardened.

Chandra (2005) reported that the adults of *M. furcicauda* feed on stone fruits and *Ficus* species. In Germany, *M. melolontha* and *M. hippocastani* have been reported to often cause foliage damage in deciduous forests. Some deciduous trees, oak particularly, are deeply affected in the spring by the swarming beetles, as they eat leaves and cause damage to the foliage at mating time. Consequently, the trees produce less wood in the summer returning generally to normal growth during the successive years corresponding to the larval development of the next insect generation (Billamboz 2014).

Woreta et al. (2016) studied the effects of food source quality on the adults of *M. melolontha* and *M. hippocastani* in Poland. The results showed that feeding on leaves of *Q. robur* and *Quercus petraea* had a positive impact on the development of adult beetles. Leaves of *Fagus sylvatica* and *Larix decidua* also constituted an adequate food source for the development of *M. melolontha* and *M. hippocastani*. The plant species namely *Alnus glutinosa*, *Sambucus nigra*, *Prunus serotina* and *Robinia pseudoacacia* had a negative impact on the survival and development of these two species. Martinek et al. (2018) reported that food preference of *M. hippocastani* mainly comprises of trees which have a potential to accumulate manganese in their assimilation apparatus. They contaminated food by soaking in solutions of $\text{MnCl} \cdot 4\text{H}_2\text{O}$ in concentrations from 0.5 to 10 mg/ml and showed that very high Mn content in the diet significantly influenced food consumption by *M. hippocastani* adults, and thereby their development.

Melolontha beetles have also been reported to feed on oak in Australia. When their populations become superabundant, adults may congregate on host plants and cause significant defoliation, particularly on *Eucalyptus*. Damage is more likely to occur near pastures and other environments that support large populations of scarab beetles (Manner 2017). Woreta et al. (2018) fed adults of *M. melolontha* and *M. hippocastani* on the leaves of *Betula pendula*, *Q. robur*, and *P. silvestris* during 2015. In 2016, *M. hippocastani* adults were fed on *B. pendula*, *Q. robur*, *Q. rubra*, *Tilia cordata*, and in 2017, on *B. pendula*, *Q. robur*, *T. cordata* and *Acer platanoides*. They showed that feeding on the leaves of *Q. robur* and *Q. rubra* had the greatest positive effects on the development of *M.*

hippocastani, whereas *M. melolontha* females reared on the leaves of *B. pendula* laid no eggs. *P. silvestris* inflorescences proved to be the right food only for *M. melolontha* females. *Melolontha hippocastani* adults feeding on *T. cordata* and *B. pendula* were characterized by a short life, decreasing body weight and reduced fecundity. In north Kashmir, *M. furcicauda* has been reported feeding on *Malus domestica*, *Pyrus communis* and *Prunus persica* (Altaf et al. 2019). Altaf and Ahad (2020) reported *M. furcicauda* to be a serious pest of apple in Kashmir.

2.3 Distribution and abundance of *Melolontha* grubs in different crop ecosystems

White grubs are soil inhabiting and root feeding immature stages of scarab beetles. They have become serious pests of different agricultural crops, fruits, vegetables, ornamental plants, plantation crops, pastures, turf and meadow grasses, lawns, golf courses and forest trees in different parts of the world (Potter et al. 1992). They cause wilting which is characterized by an initial yellowing of the leaves, followed by death of small plants and reduced vigour or lodging of larger ones. In tuber crops, larvae feed by making circular holes into them and thus rendering them unfit for marketing (Dhoj 2006). *Melolontha melolontha* and *M. hippocastani* are the major pests of forest, agricultural and orchard crops. Feeding of cockchafer larvae on the roots of trees and shrubs results in plant deterioration. Plant damage increases with the growth of grubs, and more older the grubs are the thicker plant roots are damaged due to their feeding (Woreta et al. 2018).

Stebbing (1914) reported that the grubs of *Melolontha* species cut through the roots of deodar seedlings and young plants, or more often gnaw away the bark all round, thus girdling them in north western Himalaya. Doane et al. (1936) stated that the *Polyphylla* larvae live mainly in sandy soils such as in seacoast areas, sand dunes, and the sandy bottom lands bordering streams. Craighead (1950) reported that under experimental conditions in south Carolina, the larvae of the south-eastern species *P. occidentalis* feed heavily on the roots of pine seedlings, whereas *P. variolosa* normally feeds on the roots of sedge grass. *Polyphylla hammondi* LeConte, commonly found in the south-central and south-western states, has been recorded as one of the few *Polyphylla* species which oviposits in rotten wood. Keen (1952) made general statement that *Polyphylla* larvae feed on the roots of many plants.

Klinger (1957) reported that the larvae of *M. melolontha* in soil are attracted to roots on which they feed and move over a distance of several inches. Ritcher (1966)

recorded *P. decemlineata* grubs in the sandy soils of western Oregon and Washington as feeding on strawberry plants, coniferous seedlings, roots of fruit trees, corn, table beets, hops, mint, potato tubers, and blueberry plants. In western Yugoslavia, Zivanovic (1974) reported that populations of *M. melolontha* have been present for some 15 years in the highland regions. Studies have shown a density of 40 third instar larvae/m² in grasslands, and 6 to 8 third instar larvae/m² in tilled soils.

Hauss (1975) reported that the larvae of *M. melolontha* are the major native below ground herbivores of *Taraxacum officinale*. Although the larvae are polyphagous, they prefer to feed on *T. officinale* (Hauss and Schutte 1976). In Germany, the dandelion, *T. officinale* is the most preferred food for the larvae of *M. melolontha*, and the roots of this plant are known to be the best source of nutrition for its grubs (Hauss and Schutte 1978). Huiting et al. (2006) reported that the larvae of *Melolontha* species attack the roots of several wild grasses and weeds. Host plants like *Rumex*, *Chenopodium*, *Stellaria*, *Achillea*, *Daucus*, *Solanum*, *Festuca* and *Cirsium*; *Taraxacum* and *Plantago* are highly preferred. In Russia, young pines suffer the greatest damage from the larvae of *M. melolontha* and *M. hippocastani*. Seedlings and slips damaged by white grubs quickly die or are retarded in development (Khramtsov (1979). In Kashmir, *M. melolontha* grubs infest poplar nurseries (Rishi 1981). Singh and Singh (1986) also reported *M. furcicauda* as pest of poplar nurseries in India.

Hurpin (1955) reported that the most economic damage by *M. melolontha* grubs in Switzerland is caused in the year after a swarming flight, when the larvae moult from the second to the third instar and gain most of their body weight. Severe economic losses have also been observed to be inflicted by grubs of *M. melolontha* in grasslands and orchards (Keller 1986).

In Netherlands, *M. melolontha* occurs predominantly in loamy, clay and calcareous soils, while *M. hippocastani* develops only in sandy soils. The grubs do more damage than the adults and they are attracted towards the roots of crops such as strawberry, lettuce, batavian endive and grass (Goffau 1990). Vlug (1996) has observed the damage of *M. melolontha* in a number of crops including potato, various legumes, fruit trees, ornamentals and grassland in Netherlands.

In Trentino province of Italy, *M. melolontha* grubs cause substantial damage to the roots of apple trees and young vines until 2-3 years of age. In case of apple trees, the root

system is superficial and limited to the upper 20-30 cm of the soil and the roots are always susceptible to attack by the white grubs (Luisa and Mauro 1996). According to Cardiff (2001), the larvae of *M. melolontha* gnaw at small roots of field plants, and are indiscriminate feeders; they eat grain, grass, tree, and beet roots, moving as much as 30 cm a day while eating large sections of crop rootlets. The larvae usually feed on fresh roots and do not eat decaying organic matter (Fraval 1998).

In Hungary, the grubs of *Melolontha* species cause serious damage in forests and have been reported to kill even older trees by feeding on the roots (Toth 1998). Strasser (2004) indicated that *M. melolontha* cause an increasing damage in cultivated grasslands all over Austria. Nuri et al. (2010) reported that in Iran (Qazvin), about 460 ha landscape has been damaged by grubs of *P. olivieri*. The larvae cause damage to the lawns and ornamental trees by feeding on the plant roots. In Czech Republic, grubs of *M. hippocastani* feed on roots and cause considerable damage to plants of forest trees, especially pine, oak, linden and others, and sometimes causing destruction up to 100 per cent (Svestka 2007).

In Solvenia, there was complete destruction of grass sod by third instar grubs of *M. melolontha* on an area of 370 ha in Idrija region during 2002. The population of grubs was reported to range from 60 to 120 grubs/m², and in the year 2003 the damage was on increase on areas where mechanical oppress was not performed (Pozenel 2005). Kumar et al. (2005) have recorded *M. furcicauda* and *M. nepalensis* to cause damage in maize and paddy crops in Kullu district of Himachal Pradesh. In Austria, the larvae of *M. melolontha* are most common in sandy soil environments with low precipitation. They feed on the roots of a wide range of economically important plants causing significant damage in grassland, arable crops and forests (Laengle 2005).

Lane et al. (2000) reported that grubs of *M. melolontha* occasionally damage sugar beet, especially in well wooded areas where the soil is light. The grubs feed entirely below the soil surface, down to the depth of 30 cm. In Baden-Wuerttemberg, there exists four populations of *M. hippocastani*, inhabiting about 25,000 ha of forests that grow on comparatively sandy and dry soils alongside the northern part of the upper Rhine valley (Delb 2004). Keller and Zimmermann (2005) reported the results of the survey conducted in 11 European countries indicating that in central Europe, *M. melolontha* and *M. hippocastani* occur on an area of 200 thousand hectares causing economic losses on 80 thousand hectares in plant production and forestry.

Buss (2006) reported that the larvae of *P. fullo* attack the root system of many crops, including young fruit trees, grapevines, turfgrass, and ornamentals. Young larvae feed on humus and the roots of herbaceous plants; older larvae gnaw through the roots of shrubs and trees, and severe feeding injuries result in wilting and often death of infested plants.

Huiting et al. (2006) reported that grubs of *M. melolontha* attack the roots of weeds and various crops like cereals, red beet, potato, lettuce, raspberry, strawberry, meadow grasses and fruit or forest trees with high preference for *Taraxacum* and *Plantago*. The larvae have been reported to be occasional pests in pastures, nurseries, gardens, and in grassy amenity areas like golf courses. The injury to grassland and lawns results in poorly growing patches that quickly turn brown in dry weather. Injury to the roots and rootstock causes small saplings and tender tap rooted plants like lettuce, which can wilt suddenly or show stunted growth or premature shedding of leaves. Plants growing in rows are usually attacked in succession as the grubs move from one plant to another.

In Black Sea region of Turkey, the common cockchafer, *M. melolontha* is one of the most important insect-pests of hazelnuts, causing 15-20 per cent damage annually (Sezen et al. 2007). Hann et al. 2008 reported that grubs of *M. melolontha* cause significant damage to Austrian cultivated grassland. They concluded that draught and elevated soil temperatures intensify the effect of grub feeding to the sward by accelerating withering. According to Svestka and Drapela (2009), the grubs of *M. hippocastani* feed on the roots and cause considerable losses to the forest tree species namely pine, oak, linden and others. In Turkey, the damage rate of *P. fullo* has been reported to vary from 50 and 80 per cent depending on crop grown and soil type, but from time to time, in the vineyards grown in sandy soil, 100 per cent damage can occur (Anonymous 2011).

Yanfang et al. (2011) studied the spatial distribution patterns of *M. hippocastani* in China. They indicated an aggregative distribution of grubs in the soil of 0-10 cm in depth, with a group of individuals as a unit. Weissteiner (2012) reported that the larvae of the cockchafer of the genus *Melolontha* cause conspicuous root damage to a range of horticulturally and silviculturally important plants, especially to young oaks on reforestation sites in Germany. Varga et al. (2014) monitored stages of *M. melolontha* in the lands of Gurghiu in Roamnia. They calculated the value of infestation analysis parameter as 1.94 larvae/m², which indicated a strong level of infestation in the forestry nursery fields.

Trotus et al. (2013) reported that grubs of *M. melolontha* attack the grassy and woody plant roots, producing great damage in some years in vineyards and orchard nurseries, agricultural land and vegetable crops, piercing galleries in potato tubers, rhizomes and bulbs in Romania. In vineyards and fruit tree nurseries, and in rare row crops like sugar beet, sunflower and corn, the low density of larvae between 0.5 - 1 larvae/m² present a hazard; instead at fodder crops and cereal crop the larval density may exceed 10 larvae/m² without any danger of crop destruction (Rosca et al. 2011).

Schmidt and Hurling (2014) reported that *M. hippocastani* is one of the most serious biotic risks especially in the forest regeneration phase in northern Upper Rhine region of Germany. An area covering 13000 ha of the total forest area of 30000 ha has been reported to be populated by this species, and about 4000 hectares are assumed to be infested by extremely high larval densities. According to Wagenhoff et al. (2014) the larvae of *Melolontha* spp. cause damage to the roots of plants which negatively affect the uptake of water and nutrients, leading to the dieback of young forest plants and at higher grub densities possibly to a chronic weakening of the older ones. In Turkey, *P. fullo* has been reported as major polyphagous agricultural pest. The larvae feed on the roots of many agricultural cultivated plants, and in severe infestations, cause considerable yield losses and plant death (Anonymous 2008). One of the main crops affected by *P. fullo* is strawberry (Demir et al. 2014).

In Romania, *M. melolontha* larvae are one of the main pests of forestry inflicting severe damage by consuming seeds or roots in forest nurseries and also in young forest plantation as observed in Lunca Bradului by Oltean et al. (2015). Erler and Ates (2015) reported that in Turkey, considerable economic damage to vineyards, almond, cherry, prune, and apple orchards can occasionally be caused by the feeding of the *Polyphylla* grubs. Niemczyk et al. (2017) observed that grubs of *Melolontha* spp. are more problematic in forest management because they damage the roots of nursery plants, seedlings, and trees. According to the Instruction of Forest Protection (2012), third instar grubs per 0.5 m² are likely to cause serious damage and economic losses in plantations and nurseries in areas with fertile soils. In areas with poorest soils, even one second instar larva per 0.5 m² is a critical number for young forest plantation.

In Poland, the grubs of *Melolontha* spp. have been causing great damage to the strawberry plantations, particularly those managed by organic farming methods. Losses of the plants and indirectly of yield have been recorded in several areas due to the feeding

grubs, with strawberry and highbush blueberry plantations being among the most vulnerable (Tartanus et al. 2017). Szmidla et al. (2019) have estimated *Melolontha* species to be a pest on 200000 ha mainly in central Europe, over the past 20 years. They have reported substantial impacts of *Melolontha* grubs on forest management in European countries.

Gorres and Chesmore (2019) have also mentioned *M. melolontha* and *M. hippocastani* to be the important insect pests in Europe with *M. melolontha* generally occurring in more open habitats like pastures, vegetable crops, orchards and vineyards. *Melolontha hippocastani* has been reported to mainly thrive in deciduous forests (Sukovata et al. 2015). In north Kashmir, *M. furcicauda* along with other species of white grubs have been recorded to damage oats during the seedling stage (Kumar et al. 2019). Tereba and Niemczyk (2018) reported that the grubs of *M. melolontha* and *M. hippocastani* are very dangerous to biological waste production system in Poland. The first instar grubs have been reported to feed and damage herbaceous plant roots in upper soil layer, whereas second and third instar grubs feed on tree and shrub roots even more voraciously making reforestation and aforestation difficult and impossible to implement.

2.4 Biology and morphometrics of *Melolontha* species

The life cycles of white grubs vary with the climate, being longest in temperate regions. The shortest life cycles are to be found in tropical areas with no climatic seasons (Gressitt 1953). Life cycles may be comparatively long in some regions having a dry, hot season since larvae may aestivate for several months until the rainy season begins (Moutia 1940). The life cycles of many scarabaeids belonging to genera *Holotrichia*, *Brahmina*, *Anomala*, *Adoretus*, *Leucopholis* and *Maladera* have been worked in details in India (Chandel et al. 1995; Shah and Shah 1990; Pathania 2014; Kumar 2019; Veeresh et al. 1982). In the Melolonthinae, most species of the tribe Melolonthini have rather long life cycles, especially in temperate regions (Ritcher 1958). According to Korschefsky (1940), *M. hippocastani* requires from three to five years to complete its life cycle in Europe; while *M. melolontha* requires three to four years depending upon latitude (Regnier 1950). Boas and Thomsen (1922) reported that the life cycle of *M. melolontha* and *M. hippocastani* lasts three year in the southern part of their range in Switzerland and France, but is lengthened to four years in *M. melolontha*, and five years in *M. hippocastani* in the north in Denmark. The adults emerge in April or May and feed on the leaves of trees. *M. melolontha* lays their eggs in cultivated fields and *M. hippocastani* more often in woods,

where the larvae feed on roots; the last winter is spent underground in the adult form, but still in the pupal cell. In regions where they have a three year life cycle, though they also appear in much smaller numbers in intermediate years (Bulmer 1977).

Thiem (1944) studied the life cycle of *Melolontha* spp. and indicated that differences in the length of life cycle of the given species depend on the development of the first and second instars. In Germany, the life cycle of *P. fullo* takes three to four years (Korschefsky 1940).

Downes and Andison (1941) published a comprehensive study of *P. crinita*, under the name *Polyphylla perversa* Casey as its synonym. They reported that newly laid eggs measured 2 mm wide and 3.4 mm long, but increase considerably in size within few days. The eggs hatched in 54 days during third week of September. Larvae are found only in sandy or gravelly soil with permeable subsoil. Larvae move deeper into the soil in December and January and have been found as deep as 30 inches. Younger stages occur at greater depths where the finer roots are present. The mature larvae are found near the surface from February until pupation in May and throughout the summer. The pupae are found about four inches deep in soil and occupy about five weeks. Adult emergence usually begins in the first week of July, but it has been recorded as early as the third week of June.

Sreedevi et al. (2015) studied the egg morphology of 12 species of Melolonthinae and Rutelinae. They reported that the shape of eggs was elliptical to cylindrical in Melolonthinae and spherical in Rutelinae. They analyzed diagnostic characters with respect to chorion microsculpturing at the subfamily and species levels, and revealed variation in the pattern exhibiting reticulations in Melolonthinae to projections in Rutelinae.

Scheerpelz (1950) stated that *M. melolontha* mainly occurs in areas with annual mean temperature of 7°C and has a three or four year life cycle in Austria. Faber (1961) discovered a transition from a four year to a three year development cycle of *M. melolontha*, particularly in some Alpine valleys. This trend is probably connected to increasing mean temperatures (Scheifinger et al. 2007). In Switzerland, adults of *M. melolontha* swarm between the end of April and the beginning of June concentrating mainly at the borders of forests. After a feeding or egg maturation period of 7-10 days, the females fly back to the breeding sites, burrow into the soil and deposit their eggs at a

depth of 10-20 cm. Some are developed into a second, or even third, egg batch (Vogel 1950). The larvae hatch after 5-7 weeks. They have three instars and their development lasts about two years. Metamorphosis takes place in the soil where the adults overwinter (Hurpin 1962).

Wille and Wildbolz (1953) reported that under natural conditions, the life cycle of *M. melolontha* takes three to four years and that of *M. hippocastani* three to five years. Under laboratory conditions and at constant temperature, this period may be much shorter. First instar larvae do not survive until their first moult if the temperature either below 12°C or above 32°C. At a constant temperature of 18°C, the total duration of larval or pupal period is 358 days, but this period is shortened to 222 days at a constant temperature of 23.5°C.

Tsai (1965) studied the life history of large chestnut chafer, *M. melolontha* in north-west of Szechwan. The complete life cycle needs about six years; the first five years being spent in the larval stages and the last winter is passed in adult stage only. The eggs hatch from late July to the beginning of October with an incubation period of 45 to 66 days. The larval stage lasts more than 58 months. At the middle of October, the larvae burrow down into a depth of more than 40 cm in the soil where they pass the winter. In the mid of April in the second year, they begin to move upward and feed on plants. After five winters, pupation takes place at a depth of 12-14 cm below the surface of the soil. Pupal stage lasts about two months from late July to the beginning of September. The beetle emerges and remains underground until the beginning of May in next year. The swarming of the beetles may be found in the middle or latter part of May. Eggs are often gathered in clusters, each containing 10-28 eggs. The adult stage lasts about 10 months.

Muska (1975) investigated the distribution and frequency of outbreaks of *M. melolontha* in Czechoslovakia and showed that populations with both three year and four year cycles were present. The speed of development was found to depend on air temperatures during April-September. The threshold of average temperature during this period above which three year cycles and below which four year cycles occur, has been calculated as 13-14 °C for Slovakia and 14-15 °C for Moravia-Silesia, respectively. Woodruff (1978) also reported that the life cycle of *M. melolontha* is extremely variable usually taking atleast three years and sometimes five years from egg to adult in various parts of its geographical range. In most areas, flight years occur during the year coinciding with average life cycle length, although a few emerge every year. In Russia, *Melolontha*

spp. begin their flight, from late April to May and mating takes place 10-15 days after the beginning of flight followed by oviposition. The females deposit at different times a total of 50-70 eggs in the soil and then die. The beetles are 22-29 mm, the body is black; the elytra are reddish brown and covered with white hairs (Khramtsov 1979). Hauss and Schutte (1978) carried out experiment in the German Federal Republic to determine the preference of *M. melolontha* to oviposit on *T. officinale*. Females were allowed to choose between plots containing various species and empty plots, and reported that plots containing *T. officinale* had 19 per cent of the oviposition holes, while those containing *Rumex acetosa* had nine per cent, and those without plants three per cent.

In Europe, early larval development of *M. melolontha* takes approximately 4 to 6 weeks. Larvae slow development over the winter and in mid April they suddenly spring back to activity and eat until October. They then hibernate until the third year, when they resume feeding in July and become fully mature in August. However, the fully mature adults do not become active until the following spring, giving the cockchafer a lengthy life cycle (Blum 1985; Fraval 1998). Keller (2000) reported that *M. melolontha* has at least three years of life cycle and many others have biannual life cycle. Female beetles lay eggs in groups of 12- 46 which after absorption of water, become elongated and spherical in shape. In central and northern Europe, *M. melolontha* has a four years life cycle. The adults emerge from the soil in April for feeding and mating. Eggs usually hatch by June, and the grub stage occupies three years. During this time, larvae overwinter below the freezing level of the soil and move into the rhizosphere as soon as soil temperature rises to above 8°C in the spring. The main feeding period during which the most damage occurs is the second year after hatching. In the summer of the subsequent year, the third larval instar pupate and, after a metamorphosis period of nine weeks, hatch as adult beetles which overwinter in the soil and emerge in the fourth year (Laengle 2005).

Svestka and Drapela (2009) studied bionomics and the abundance of grubs between two swarmings of *M. hippocastani* in Czech Republic. Results of the soil checks for the numbers of grubs confirmed the preference of *M. hippocastani* females for localities heavily shaded by the forest stand. In their choice of the locality, the *M. hippocastani* females prefer certain temperature and humidity. Billamboz (2014) observed that the development of cockchafer population is based on r-selection. Their body size is small and generation time is short, this reproductive strategy results in a greater ability to adapt to unstable environments through high fecundity and rapid change of the

reproduction rate (Krebs 1985). This fact leads to cyclic, high-density variations called gradations, which show a mass development between two phases of latency (Schwenke 1978).

Erler and Ates (2015) reported the biology of pine chafer, *P. fullo* in Turkey where it has a biennial or triennial life cycle. Adult beetles emerge during June or July, fly during evenings, feed on the leaves and needles of trees, and then lay eggs in the soil. The larvae feed during the late summer and early fall and move deep in the soil to overwinter. After the hibernation, they return to the root zone and feed throughout the following summer. The larvae cause most injury during this second season of their life cycle. During spring and early summer of the next year, the larvae complete development, cease feeding, and turn into pupae and adults that remain inactive in the soil. Adult beetles emerge next season. In cold regions of the Turkey, the larvae overwinter three times in the soil, then pupate in May of the third year; the adults appear from mid June to mid July, feed and then lay eggs (Anonymous 2008).

Sukovata et al. (2015) reported that biology of *M. melolontha* and *M. hippocastani* is very similar in Poland. The beetles usually fly in late April and in May. Their development usually lasts four years, although it may be shortened or extended by environmental conditions (Sliwa 1993). According to Niemczyk et al. (2017) cockchafers from the genus *Melolontha* have a developmental cycle of three to five years. The length of life cycle depends primarily on temperature; cockchafers in places with higher temperatures have a shorter developmental cycle (Svestka 2006).

2.5 Molecular analysis for identification of *Melolontha* species

The morphological identification keys are often effective only for a particular life stage and therefore, many individuals cannot be identified (Hebert et al. 2003). This is also true for white grubs because their identification is a challenging task due to variable morphological differences among species and delineation among the immature forms. It is difficult to link adults and larvae of the same species because of the distinct morphological differences (Dittrich et al. 2006). Proper identification of the scarabaeid species and knowledge of their bioecology is essential for developing environmentally compatible integrated pest management strategies (Murthy et al. 2018). One way of identifying field collected larvae of scarabs is to conduct molecular analysis that can link larval genetic profiles to those of identified adult specimens. Among the molecular markers used for analysing molecular variation, sequencing mitochondrial DNA (mtDNA)

and ribosomal DNA (rDNA) have been recognized as effective markers for identification of different insects (Qiu et al. 2009).

Mico et al. (2003) analysed the nucleotide sequences of a fragment of mitochondrial gene cytochrome c oxidase subunit I (COI) of six species and two sub species of genus *Hoplia* (Scarabaeidae: Hopliinae) to re-examine the species limits on the basis of results, *Hoplia freyi* Baraud and *Hoplia philanthus ramburi* Hayden were found to be junior synonyms of *Hoplia chlorophana* Erichson and *Hoplia philanthus philanthus* (Fuessly), respectively. Monaghan et al. (2005) studied the patterns of variation in both mitochondrial and nuclear genes in 71 individuals of neotropical dung beetle, *Canthon* sp. Mitochondrial DNA sequence variation proved to be highly structured, with more than 95 per cent of the observed variation existing between discrete sets of very closely related genotypes. Sequence variation in nuclear 28S rRNA among the same individuals was lower by atleast an order of magnitude, but 12 genotypes were found to be differing from each other by a minimum of two base pairs.

In South Africa, Dittrich-Schroder et al. (2009) carried out studies on identification of scarabaeids collected from sugarcane fields using mitochondrial cytochrome c oxidase subunit I (*cox I*) gene. Sequence of *cox I* from scarabaeid larvae were compared with sequence from scarabaeid adults of known species. The major groupings delimited specimens belonging to the sub families Dynastinae, Melolothinae and Rutelinae. Mitochondrial DNA (mtDNA) of larval specimens was linked with mtDNA of identified adult specimens through phylogenetic analysis.

In Nepal, comparative molecular studies were conducted to identify and describe unknown larvae of Sericini with the help of mitochondrial and nuclear DNA markers in combination with character and tree-based species delimitation methods. In the latter analysis they used a sequence of a specimen that resulted in the first analysis conspecific with the larvae of *Maladera affinis* (Blanchard) as the query sequence in GenBank, and checked in a minimum evolution tree whether larva-adult matches from the local approach were altered through interference with other taxa of the worldwide database (Sipek and Ahrens 2011).

Karimi et al. (2012) provided DNA barcodes for three important species of white grubs viz., *P. olivieri*, *P. adspersa* Motschulsky and *M. melolontha*, using mitochondrial DNA cytochrome oxidase I (COI). The relationship between theses three species of Iranian white grubs and related species has been addressed in Iran. Tereba and Niemczyk

(2018) suggested that the COI region, a standard in phylogenetic analysis and invertebrate species identification is appropriate for distinguishing between *M. melolontha* and *M. hippocastani*. In south India, Murthy et al. (2018) presented a molecular phylogenetic analysis of 23 species of scarabeid beetles collected from various geographical locations. Genomic DNA of beetles was characterized by using Cytochrome c oxidase I (COI) gene and a total of 19 barcodes were generated. The lowest genetic variation was observed between *Hybosorus illigeri* Reiche and *Oryctes rhinoceros* (L.) i.e. 0.008, whereas the highest genetic variation of 0.384 was observed between *Onthophagus auritus* Erichson with *Calcinemis obesan* and *O. rhinoceros*.

Swamy et al. (2019) sequenced mitochondrial cytochrome oxidase subunit I gene (*cox I*) for the identification and phylogenetic analysis of *Leucopholis lepidophora* Blanchard, *Leucopholis burmeisteri* Brensk, and *Leucopholis coneophora* Burmeister species by employing LCO-1490 and HCO-2198 mt COI primers. Phylogenetic analysis revealed that the *cox I* DNA barcode fragment of *Leucopholis* spp. and out-group taxa, available in genetic databases, confirms the species identity.

2.6 Management of *Melolontha* grubs using chemical insecticides

Chemical insecticides to save crops were generally applied during 1940s, especially after the Second World War. DDT (Dichlorodiphenyltrichloroethane) discovered as an insecticide in 1939 was one of them which seemed to be a cure for variety of pest problems throughout the world (Woreta 2015). In Poland, first generation highly toxic insecticides like lindane or chlordane were used to control white grubs in forest plantations. Lindane was applied as dust and its effectiveness relied on the dose used, given that too much of the chemical produced phytotoxicity in treated plants (Sierpinski 1975). Schnaider and Karlikowski (1953) studied the efficacy of lindane and showed that root dip of Scots pine seedlings in a paste consisting of humus+lindane+water significantly contain die-back due to grub feeding. Dominik (1958) demonstrated that soil treatment with organochlorine preparation Intox 8 @ 2 kg/ acre done during summer and repeated next year @ 4 kg/acre sufficiently protected tree seedlings against feeding of two year old grubs. Sierpinski (1963) investigated the effect of time of HCH application on the efficacy of grub control, and showed that treatments done during spring and fall were equally useful. Based on the findings of their results, several products based on lindane were recommended for use in reforested areas threatened by large grub populations. According to Dominik (1954), the organochlorine insecticides maintained their properties

in the soil for two to three years, and the treatments were highly effective to control first and second instar grubs of *Melolontha* species.

Szczepanska (1972) tested 17 soil insecticides against second and third instar grubs of *M. melolontha* under laboratory conditions in Poland. Parathion, thionazin and Nefusa and granular formulations of parathion, Dyfonate, diazinon, phoxim and carbofuran were the most effective against different instar grubs. In France, increasing quantities of spores of *Beauveria tenella* (Delacroix) and low doses of parathion or trichloronate were mixed with the medium in which the larvae were reared. There was considerable increase in mortality from mycosis when 40, 80 or 120 mg of a preparation containing 5 per cent parathion or 120, 150 or 180 mg of one containing 5 per cent trichloronate was incorporated into each 800 g peat treated with spores at the rate of 5×10^4 /g (Ferron 1971).

Blaisinger et al. (1978) tested alternative insecticides to chlorinated hydrocarbons in two localities to control larvae of *M. melolontha* in permanent meadowland in France. They concluded that for surface treatments, granules containing parathion or chlorpyrifos applied at 5 kg toxicant/ha were most effective, but that granules incorporated into the soil during mechanical cultivation should be applied at 2 kg/ha.

There was 100 per cent ovicidal effect when adults of *M. melolontha* were fed in laboratory on leaves of beech that had been sprayed with diflubenzuron (Dimilin WP 25) (Buchi and Jossi 1979). Woreta and Malinowski (1998) tested four pyrethroids viz., alphas-methrin, deltamethrin, lambda-cyhalothrin, zeta-cypermethrin and etofenprox against several insects that are severe pests in Polish forests. They indicated that adults of the common cockchafer are equally susceptible to the four pyrethroids, but less susceptible to etofenprox. According to Malinowski et al. (1998), basudin 10G @80-120 kg/ha, dursban 480 EC @ 2.5-3.0 litres in 450-600 litres of water and carbosulfan @ 80-120 kg/ha are mainly used in surface treatments before planting, to control *Melolontha* grubs in Polish forestry.

Froeschle and Glas (2000) organized a campaign to control the severe infestation of *M. melolontha* in crops and trees in northern Kaiserstuhl, Germany. They applied *Beauveria brongniartii* (Saccardo) and two insecticides viz., phosalone and azadirachtin at 2.5 and 3.01 kg/ha in crops and forest trees in the farmland and woodland regions using a helicopter and practically controlled the grubs. Meinert et al. (2001) applied two insecticides viz., Rubitox-Spritzpulver and NeemAzal-T/S by helicopter on 78 and 50 ha

of forest area in north Kaiserstuhl, Germany. Both insecticides showed good efficacy against the adults and the grubs as well, but Rubitox-Spritzpulver resulted slightly better than NeemAzal-T/S.

Lisova and Petroviene (2007) evaluated the effect of Actara (thiamethoxam @ 250 g/kg) against *M. melolontha* in potato plots in, Lithuania, and reported considerable reduction in tuber injury caused by *M. melolontha*. Benker and Leuprecht (2007) carried out pilot experiments with Neem Azal, Lambda-cyhalothrin, dimethoate and *Bacillus thuringiensis* in Germany. The most effective insecticide in the experiment was lambda-cyhalothrin, followed by dimethoate.

Nuri et al. (2010) evaluated thiamethoxam @ 0.0005 per cent, imidacloprid 350 SC, 0.001, 0.002 and 0.003 for preventive control and carbaryl 85% WP 0.005 , 0.002 , 0.003 and 0.005 for curative control of *P. olivieri* in ornamental plants in Iran. They reported that imidacloprid (0.003 %) had the best effect for preventive control and carbaryl (0.005 %) was most effective treatment in curative control of *P. olivieri*. Yanfang et al. (2010) compared the effects of injection 40 per cent phoxim EC, 50 per cent trichlorphon+phoxim EC, penpropathrin EC, 1 per cent matrine in the soil with the fertilization of the root device and spraying pesticides in the grass surface with watering can to control *Melolontha hippocastani* subsp. *mongolica* Menetries third instar larvae. They demonstrated that control effects of injection 40 % phoxim EC 400 times and 50 % trichlorphon+phoxim EC 300 times with the fertilization of the root device were 95.87 per cent and 97.96 per cent, significantly superior than spraying pesticides.

Malinowski (2011) tested the susceptibility of third instar grubs of *Melolontha* spp. to the contact action of neonicotinoid insecticides viz; acetamiprid, clothianidin, imidacloprid and carbosulfan under laboratory conditions. There was low sensitivity of third instar *Melolontha* white grubs to the contact action of these insecticides. Insecticides of SP and WG formulations applied to the soil at a dose of 25 mg active substance per kg soil did not cause 100 per cent mortality after 28 days of exposure. In the case of clothianidin, mortality after 28 days was 40 per cent and in the case of imidacloprid it was 80 per cent. GR forms containing clothianidin used at a dose of 50 mg/ kg soil, and imidacloprid used at a dose of 250 mg/ kg soil, caused 70-80 per cent mortality after 28 days. Carbosulfan GR used as a standard at a dose of 500 g active substance per kg soil, caused 100 per cent mortality after 28 days.

In Poland, Glowacka and Sierpinska (2012) evaluated the efficacy of insecticide treatments using aircraft and ground equipments to control *M. melolontha* with Mospilan 20 SP at a rate 0.4 kg/ha. Regardless the form of treatment application, the leaf damage on sprayed trees ranged from 0.2– 1.75 per cent, while leaf damage on unsprayed trees was 86-95 per cent. Pozenel et al. (2015) tested the efficacy of granular soil insecticides based on active substances tefluthrin and chlorpyrifos during the years 2012-2014 in Idrija. The number of *M. melolontha* grubs in the soil decreased the most after tefluthrin application. The efficacy of tefluthrin applied at the dose of 13 kg/ha was 80 per cent, and the efficacy of chlorpyrifos at the dose of 15 kg/ha was 68 per cent.

Rodstrom and Brown (2017) reported that Ecozin (3 per cent), an organic pesticide containing azadirachtin, caused larvae of *P. decemlineata* to cease feeding for several weeks, but mortality of larvae was low. A broad spectrum treatment with diazinon 50 WP eventually killed the grubs in the top six inch layer of soil (Johnson et al. 2006). Use of imidacloprid can be an effective soil drench, but additional water to reach field capacity is recommended. Imidacloprid delivered through the drip system may control grubs after one year (Rodstrom and Brown 2017). Clothianidin has been reported to have promise in California (Johnson et al. 2012).

3. MATERIALS AND METHODS

The adults of *Melolontha* beetles remain active only during a part of the year in different areas of Himachal Pradesh but the grubs can be found feeding on roots of various crops for a longer duration and thus inflicting a great damage to the crops. The field survey work relevant to the present study was initiated in 2017 and continued for about two years in Himachal Pradesh. During field surveys, beetles of *Melolontha cuprescens* Blanchard, *Melolontha furcicauda* Ancy, *Melolontha indica* Hope and *Melolontha virescens* (Brenske) [= *Polyphylla fullo* (Linnaeus)] were collected with the help of light traps and from host trees, and their culture was maintained separately in the white grub laboratory, Department of Entomology, CSK HPKV, Palampur (latitude N 32⁰,05.666' and longitude E 76⁰,32.781' at 1222 m amsl) to record observations on comparative biology and morphometrics of these species. Molecular work pertaining to these species was carried out in the Department of Agricultural Biotechnology. The grubs of *Melolontha* species collected from different areas were maintained in soil under laboratory conditions and different chemicals having new chemistry were evaluated against them by means of different methods. The materials and methods followed for this study are discussed in this chapter.

3.1 Collection of *Melolontha* beetles from different parts of Himachal Pradesh

The beetles were collected on light trap and directly from the host trees at night. Data on latitude, longitude and altitude were recorded using GPS (76 CSx, Gamin make) at each surveyed location.

3.1.1 Monitoring of *Melolontha* beetles by using light trap: The population of *Melolontha* beetles was monitored with the help of ultra violet light traps installed at various locations of six different districts in zone II (sub-temperate sub humid mid hills), zone III (wet-temperate high hills) and zone IV (dry-temperate high hills and cold deserts) of Himachal Pradesh during 2017 and 2018 (Fig. 3.1). The study areas were mostly fruit orchards, vegetable fields or farmland with different cropping patterns. The details of study sites are given in table 3.1. The light traps were set up from June-September and data on beetles were recorded daily at Palampur and fortnightly at other locations.

The body of light trap consisted of PVC plastic of red colour. The height of plastic funnel was 25 cm with diameter of 30 cm at the top and 5-6 cm at the bottom. For the

safety of ultra violet tube, a rain-shed cone was positioned approximately 17 cm above the funnel using three white metal sheets. The diameter of rain-shed cone was about 20 cm. There were three baffles placed at a uniform distance of 10 cm around the circumference of funnel. The white coloured baffles, made of M.S. sheet were 30 cm long and 11 cm wide. The baffles add characteristic feature to this trap. The baffles were fixed in such a manner so that light rays were scattered uniformly in all the directions without any interference. The beetles, when attracted to light collide with these baffles and drop into the trap. An efficient transparent poly bag was attached to the bottom of this funnel. The light source had a hard glass tube with choke of copper wire. The tube had a capacity of 120 W and with ultra violet radiation with visible spectrum producing bluish light (Plate 3.1).

The light trap was installed at a height of about 10 feet in the centre of the field to attract the beetles and the observations were recorded from 7:00 PM to 11:00 PM at all the locations. The captured beetles were regularly taken out of poly bags and *Melolontha* beetles were separated from rest of the beetles and other insects. These beetles were grouped on the basis of relative abundance and frequency values for the purpose of investigating the relative importance of different species of *Melolontha* beetles.

3.1.2 Collection of *Melolontha* beetles on host trees: In order to determine species occurrence and relative abundance of *Melolontha* beetles, surveys were conducted in various fruit growing areas of Himachal Pradesh (Table 3.1) during the month of June-September in 2017 and 2018. In 2017, beetles were collected at locations near Palampur, Chattanseri, Badagaon, Janjehli, Ahla, Mashobra and Sangla, (Table 3.1; Fig. 3.1). In 2018, the surveys were repeated at all the above mentioned locations. Scarabaeid beetles being nocturnal came out of soil at dusk. Chandel et al. (1995) reported that beetles come out of soil at around 7:50 PM at light intensity of 3 lux and settle on host trees for mating and feeding. After suitable rainfall events, representative samples of beetles were collected during June-September from trees including apple, plum, peach, persimmon, walnut, wild rose, *bathua*, and Indian siris. The trees of 3-5 m height were commonly selected. Five plants were marked at random in each orchard/ farm in all the surveyed localities and three branches of almost equal length were selected. The beetles were collected between 19:00 - 21:30 hours using a powerful flash light. The dislodged beetles were handpicked and transferred to metal cages containing shoots of host trees on which collection was done.



(a)



(b)

Plate 3.1 UV light traps used for monitoring of beetles
(a) Close-up view; (b) In operation during night

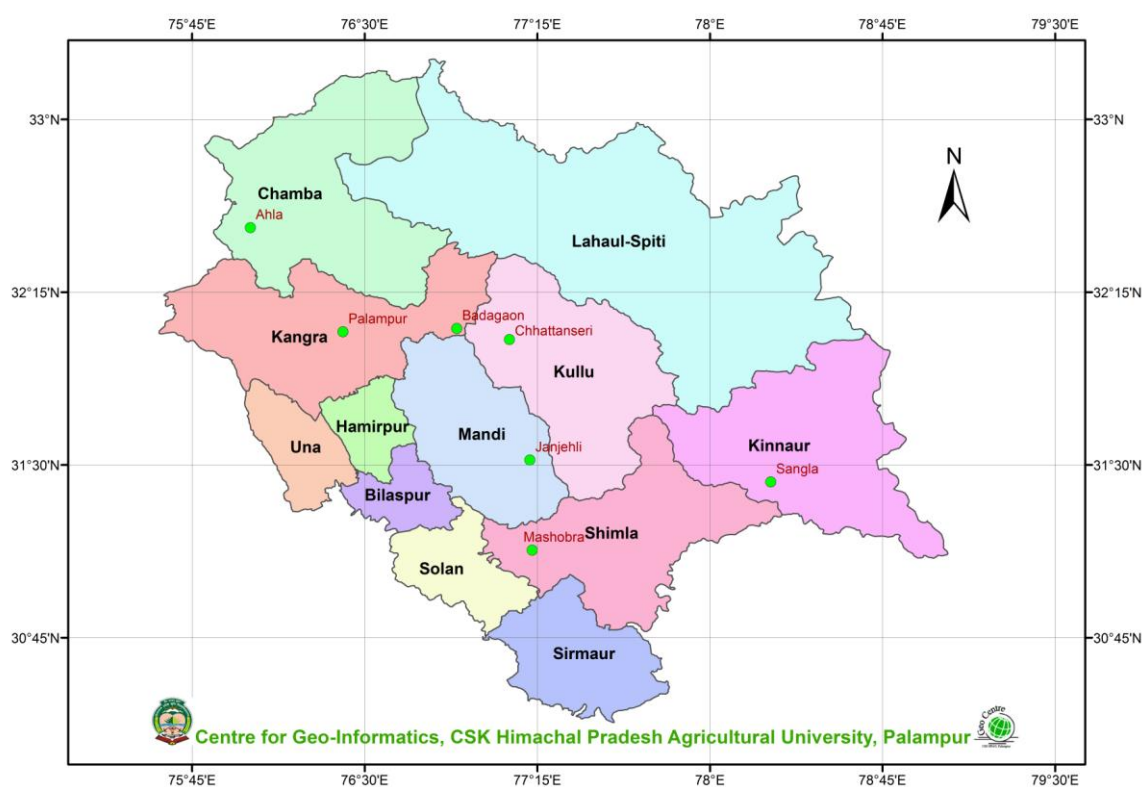


Fig. 3.1 Locations selected for beetle sampling on light trap and host trees in Himachal Pradesh

and transferred to metal cages containing shoots of host trees on which collection was done. At Palampur, the data were recorded twice in a week, whereas in other locations, collections were done fortnightly during the entire season from June-September.

All the collected beetles were brought to the laboratory and the species of beetles relevant to the study were separated from rest of the scarabaeids and their percentage was worked out species-wise and location-wise. For this survey, any tree on which *Melolontha* adults could be collected mating and/or feeding was considered to be a host tree.

Table 3.1 Sampling locations for *Melolontha* beetles on light traps and host trees

District	Location	Zone	Latitude	Longitude	Altitude (m amsl)	Source of collection
Kangra	Palampur	II	N 32 ⁰ , 05.666'	E 76 ⁰ , 32.781'	1222	Peach, apple, light trap
	Badagaon	III	N 31 ⁰ , 58.957'	E 77 ⁰ , 07.907'	2268	Apple, persimmon, walnut, wild rose, <i>bathua</i> , light trap
Kullu	Chhattanseri	II	N 32 ⁰ , 04.483'	E 77 ⁰ , 12.674'	1189	Apple, plum, light trap
Mandi	Janjehli	III	N 31 ⁰ , 52.148'	E 77 ⁰ , 21.833'	2044	Light trap
Shimla	Mashobra	III	N 31 ⁰ , 12.962'	E 77 ⁰ , 22.835'	2286	Light trap
Kinnaur	Sangla	IV	N 31 ⁰ , 25.932'	E 78 ⁰ , 15.060'	2588	Apple trees, light trap
Chamba	Ahla	III	N 32 ⁰ , 53.083'	E 76 ⁰ , 00.346'	2039	Apple, Wild rose, Indian siris., light trap

3.2 Identification of beetles

The identity of *Melolontha* beetles up to species level was confirmed at National Bureau of Agricultural Insect Resources, Karnataka, India.

3.3 Determination of diversity indices

Alpha Diversity: Alpha diversity was calculated as number of observed species per location. Species richness (number of species), relative abundance (number of individuals of each species) and three diversity measures were used in this study. The diversity indices assume that individuals are sampled randomly from a population at different locations. The Shannon index (H') explains the evenness of the abundance of species, while the Simpson's index of diversity (D) is less sensitive to species richness but more sensitive to the most abundant species, (Price 2004). Pielou's evenness index (J') explains the evenness of allotment of individuals among the species. Diversity indices were determined from the information gathered during the period of study on each site by using the following equations (Krebs 2001).

i) Shannon index (H')

$$H' = - \sum_{i=1}^S (p_i \log_2 p_i)$$

ii) Simpson's index (D)

$$D = 1 - \sum_{i=1}^S (p_i)^2$$

iii) Pielou's evenness index (J')

$$J' = H' / H_{max}$$

Where,

H'= Shannon diversity index

p_i= Proportion of total sample belonging to the ith species.

S= Number of species.

Σ= Sum from species 1 to species S

D= Simpson's index of diversity.

N= Total percentage cover or total number of organisms.

n= Percentage cover of a species or number of organisms of a species

J'= Evenness of allotment of individuals among the species.

H_{max} = Maximum species diversity (H') = Log₂S

Beta Diversity: Beta diversity was calculated to estimate species similarity between two habitats

i) Sorensen's similarity index

$$\beta = 2C / (C + S_1 + S_2)$$

Where,

C= no. of shared species between different landscapes

S₁= no. of species present in site 1

S₂= no. of species present in site 2

ii) Jaccard's similarity index was calculated according to formula given by Jaccard (1912)

$$S = a / (a + b + c)$$

Where,

a= No. of shared species between different landscapes

b= No. of species in site 1

c= No. of species present in site 2.

iii) Bray-Curtis similarity index

$$C_N = 2jN / (Na + Nb)$$

Where,

Na= the total number of individuals in site Aj

Nb= the total number of individuals in site Bj

2jN= the sum of the lower of the two abundance for species found in both sites.

The index value ranges from one (or 100) when two samples are identical, zero when there are no shared species between them. The index shows the difference in total abundance rather than relative abundance (Magurran 2004; Pathania et al. 2015).

iv) Bray-Curtis distance index

$$C_N = 1 - \{2jN / (Na + Nb)\}$$

Where,

Na= total number of individuals in site Aj

Nb= total number of individuals in site Bj

2jN= sum of the lower of the two abundance for species found in both sites.

The index value ranges from zero (or 100) when two samples are identical, one when there are no shared species between them.

3.4 Estimation of *Melolontha* grub population in soil and their extent of infestation

Larval collection was also done during March-January of 2017 and 2018 at the locations that were surveyed for beetle collection (Table 3.2; Fig. 3.2). In crops like rajmash and cabbage, the wilted plants and the plants that had died prematurely were uprooted and the soil around these plants was searched for larvae. In potato and onion, sampling sites were selected randomly by throwing a one square feet quadrant over ones shoulder, with no two sampling pits overlapping. Pit sampling was done and a sample consisted of one cubic feet of soil carefully dug by a shovel and searched for larvae. Ten samples in each crop were taken during all observations. Larvae were transferred to the laboratory and reared up to adult stage to facilitate identification. The averages of all observations were worked out and plotted to draw conclusions. At the same time, the data on white grub damage were also taken from different locations in different crops. The average of all observations was worked out and plotted to draw conclusions.

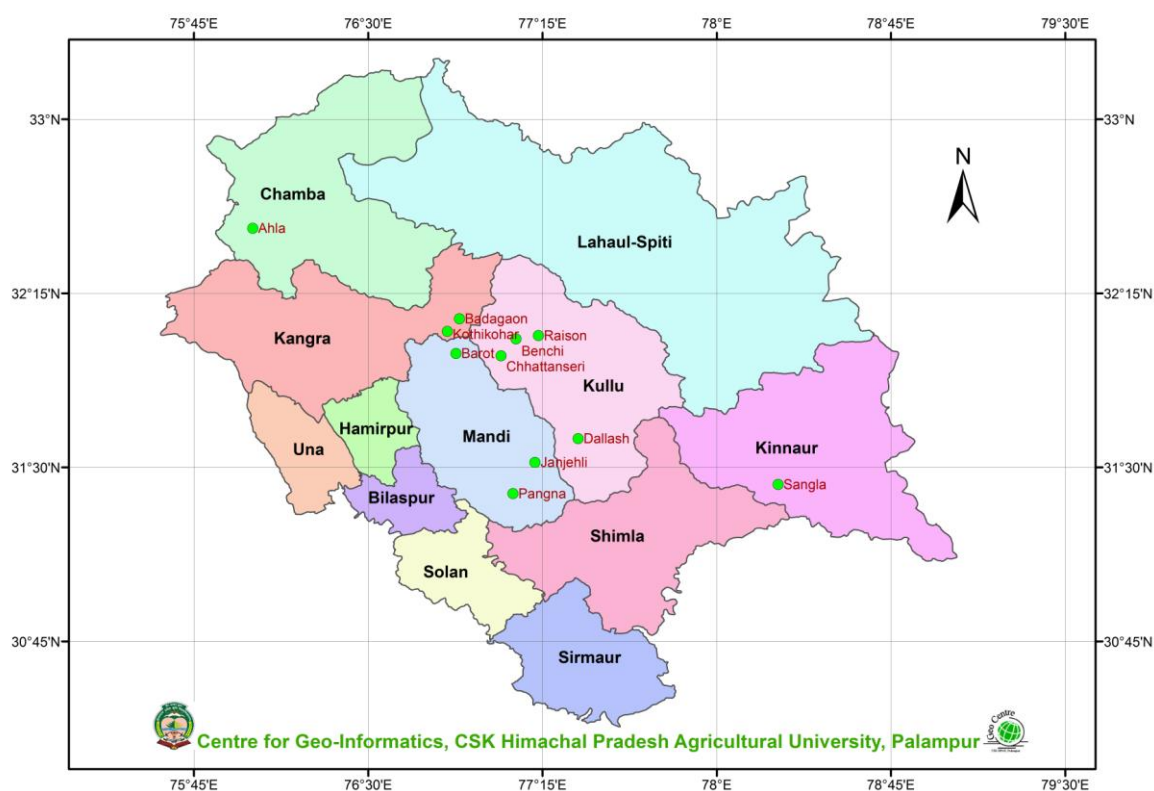


Fig. 3.2 Locations selected for *Melolontha* grubs sampling in Himachal Pradesh

Table 3.2 Description of locations surveyed for the collection of grubs in Himachal Pradesh

District	Location	Zone	Latitude	Longitude	Altitude (m amsl)	Crop habitat
Kangra	Badagaon	III	N 31 ⁰ , 58.957'	E 77 ⁰ , 07.907'	2268	Cabbage, potato, rajmash
	Kothi kohar	III	N 32 ⁰ , 07.847'	E 76 ⁰ , 80.052'	2213	Potato, Cabbage, rajmash,
Kullu	Chhattanseri	II	N 32 ⁰ , 04.483'	E 77 ⁰ , 12.674'	1189	Potato, Apple
	Benchi	II	N 32 ⁰ , 06.170'	E 77 ⁰ , 12.400'	1180	Cabbage
	Raison	II	N 32 ⁰ , 05.490'	E 77 ⁰ , 13.270'	1433	Apple
	Dallash	III	N 31 ⁰ , 23.036'	E 77 ⁰ , 6.024'	2020	Apple
Mandi	Barot	III	N 32 ⁰ , 02.647'	E 76, 50.415'	1847	Potato, cabbage rajmash
	Janjehli	III	N 31 ⁰ , 52.148'	E 77 ⁰ , 21.833'	2044	Potato, apple
	Pangna	II	N 31 ⁰ , 38.658'	E 77 ⁰ , 12.405'	1484	Apple
Chamba	Ahla	III	N 32 ⁰ , 53.083'	E 76 ⁰ , 00.346'	2039	Apple
Kinnaur	Sangla	IV	N31 ⁰ , 25.932'	E 78 ⁰ , 15.060'	2588	Cabbage, potato, rajmash, apple

3.5 Food preference studies for adult beetles of *Melolontha* species

Food preference studies of *Melolontha* beetles were carried out in multi-choice and no-choice tests under laboratory conditions. In multi-choice test, five known host plants viz., rose, plum, apple, peach and pecan nut were tested for feeding preference by adults of *M. indica*, *M. furcicauda* and *M. cuprescens* beetles. Two healthy shoots of about 15 cm long of each host tree were placed in glass jar (height: 30 cm x diameter: 22 cm) containing moist soil and covered with thick muslin cloth. The shoots were arranged in two concentric rings each containing one shoot of each host. Field collected and pre-conditioned eight beetles (starved for 24 hours) were released in each of three jars kept as replicates. Shoots were changed daily and leaf area consumed by beetles after three days of release was quantified by using leaf area meter (Systronics Model 211).

In no-choice test, five shoots of each host were placed in moist soil in the glass jar (10 cm diameter) and four preconditioned and starved beetles were released in it. Three replicates were maintained for each host. Data on leaf consumption were recorded after three days using leaf area meter.

3.6 Studies on comparative biology and morphometrics of *Melolontha* species

The beetles collected through light trap and from host trees were brought to the laboratory in metal cages (15x10 cm²) and two pairs of beetles were released in each glass jar (10 cm diameter) half filled with mixture of moist sand, soil and farm yard manure in equal proportion. Regular watering was done to keep soil moist. Fresh cut twigs of apple/

rose were provided as food and were replaced daily. The glass chimneys were used to cover jars. The upper grooved end of chimney was covered with double-layered muslin cloth with the help of rubber band to prevent escape of beetles (Plate 3.2).

The soil was taken out of glass jars every 3-4 days, and eggs were removed and counted. The same soil was used for about a month, and was examined every time it was taken out of the jars. Eggs collected from oviposition jars were placed in separate jars containing fresh moist soil and another thin layer of soil was sprinkled over the eggs to maintain optimum moisture. These jars were maintained at room temperature. The eggs were checked after every third day until eclosion. After eclosion, hatched out first instar larvae were removed from the jars very carefully and placed in soil mixture in small cups containing maize/ rajmash seedlings (Plate 3.3; 3.4). Each larva was placed in a single cup and each cup was filled with about 250 g of soil. Regular watering was done to maintain optimum soil moisture. Second and third instar grubs were reared on potato tubers. The food material was provided as often as necessary or desired. Development and survival was monitored by examining the emptied out soil every week. The general culture was also maintained in glass jars and development inside the earthen cell was followed by viewing either through a small hole or when the cell had been constructed adjacent to the glass, through the side of the jar. During the development of a batch of larvae, once high number of grubs was attained, the third instar larvae were killed and preserved in 70 per cent alcohol for further morphological studies.

Measurements on head capsule width and body length were taken from sequentially killed larvae during development from newly hatched larvae to pupation. The grubs were observed regularly and different larval instars were distinguished on the basis of head capsule width as suggested by Chandel et al. (1995). Newly formed grubs were used to measure their size in each instar ($n = 10$). Data on head capsule width were recorded using NIKON SMZ-745 T. The size of larvae, pupae and adults was measured using digital Vernier Caliper (0-150 mm, Aerospace make). The raster patterns of larvae were drawn with the help of Camera Lucida.

In Scarabaeidae, most diagnostic characteristics are found on head (antennae), mouth parts (mandible, maxillae, epipharynx, clypeus, labrum, labium and hypopharynx) and on the ventral surface of the last abdominal segment i.e. raster (Ritcher 1966). To study larval characters, third instar larvae of *Melolontha* were fixed in KAAD solution for



Plate 3.2 Glass jars used for mating and feeding of adult beetles



Plate 3.3 Maize seedlings used for rearing of first instar grubs of *Melolontha* species



Plate 3.4 Rajmash seedlings used for rearing of first instar grubs of *M. virescens* (= *P. fullo*)

12 hours and then preserved in 70 per cent ethanol. The head capsule was removed from the body and placed in 10 per cent KOH for 30 minutes.

Dissection took place under microscope and separate parts were studied in temporary slides under microscope. Drawings were made on a plain paper with the help of camera lucida at required magnifications. The photographs were taken with digital camera attached with stereo zoom microscope. The terminology of immature morphology follows Jia et al. (2020) and Ritcher (1966).

3.7 Studies on male genitalia of *Melolontha* beetles

After night collection, the beetles of *Melolontha* species were brought to laboratory in metal cages. Some of the male and female beetles were separated at the time of mating and remaining were separated on the basis of antennal characters. The beetles were preserved sex-wise in separate plastic containers. For male genitalia study, dried male specimens were placed in 70 per cent alcohol for genitalia extraction. The abdomen was removed with the help of pointing needle and forceps from thorax. Aedeagus and speculum gastrale were removed carefully from the dissected abdomen by means of forceps and placed in the cavity plates. Aedeagus was placed in 10 per cent KOH solution for about 3-4 hours until all fat bodies and tissues were macerated and aedeagus became translucent. A fluffy structure known as endophallus is present inside the aedeagus, which is of taxonomic importance. The endophallus was everted out by injecting water with little pressure from the base of the aedeagus using a syringe attached to the hollow needle (D'Hotman and D'Scholtz 1990; Sreedevi and Sakshi 2016). Aedeagus measurements were recorded by measuring the length and width of the phallobase and paramere as well as the length and width of spiculum gastrale. These studies were carried out under a stereo zoom microscope (NIKON SMZ-745T) and line diagrams drawn with the help of Camera Lucida by keeping them in 70 per cent alcohol. Aedeagus and spiculum gastrale were then transferred to 70 per cent ethanol for final preservation.

3.8 Sequencing analysis of *Melolontha* species

3.8.1 Extraction of genomic DNA: Total genomic DNA was extracted using CTAB (Cetyl Trimethyl Ammonium Bromide) method (Doyle and Doyle 1990) from entire head and legs of adult beetles or grubs. The musculature of head and legs was ground to fine powder using liquid nitrogen in sterilized pre-cooled pestles and mortars. The ground tissues were transferred to 2 ml eppendorf tubes containing 700 µl of extraction buffer (2 per cent CTAB, 100 mM Tris, 20 mM EDTA, 1.4 mM NaCl and 1 per cent PVP, pH 8.0)

maintained at 65°C in water bath and mixed vigorously. The mixture was incubated at 65°C for 1 h with occasional mixing. An equal volume of chloroform: isoamyl alcohol (24:1) was added to the tubes followed by gentle mixing. The mixture was centrifuged at 10,000 rpm for 10 minutes at 4°C. The aqueous phase was transferred to fresh tube, followed by addition of 500 µl of pre-chilled isopropanol. The contents of the tubes were mixed gently and the mixture was incubated at -20°C for 1 h. DNA was precipitated by centrifugation at 10,000 rpm for 10 minutes using centrifuge (SIGMA, Laborzentrifugen, Germany). The supernatant was drained and the resulting pellet was washed twice with 1 ml of 70 per cent chilled ethanol. The pellet was dried for 3-4 h. Dried DNA pellet was dissolved in 1 ml TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). The dissolved DNA was treated with 1 µl of RNase (10 mg/ml). The quantity and quality of DNA was estimated through electrophoresis using 1 per cent agarose gel (Himedia).

3.8.2 Electrophoresis of DNA: Agarose (1g) was added in 100 ml of TAE buffer (1 X) in a 200 ml flask to prepare 1% agarose gel. The contents were boiled in a microwave oven for 2 minutes to dissolve agarose in buffer completely. After cooling, 2µl of ethidium bromide (10 mg/ml) was added to the solution and poured into a gel tray and left for solidification. DNA samples were loaded on the gel with loading dye. The gel was run at 100V and DNA band was visualized and photographed under UV light using bioilluminator.

3.8.3 Polymerase Chain Reaction (PCR): PCR was performed for DNA amplification. Reaction mixture (25µl) containing template DNA, 10X Taq polymerase buffer (100mM Tris HCl, pH 9.1, 500 mM KCl), 0.2 mM dNTPs mix, 25 mM MgCl₂, 0.1 µl each forward and reverse primers and 0.2 µl of Taq polymerase (5U/Himedia) was prepared and the PCR reaction was carried out in 0.2 ml PCR tube. PCR mixture was denatured at 94°C for 5 minutes followed by 35-40 cycles and final extension at 72°C for 10 minutes (Table 3.3). PCR product (25µl) was then run on 1% agarose gel. Agarose gel was prepared in 1X TAE buffer and 2µl of ethidium bromide (10mg/ml) was added. The sample was mixed with loading dye prior to loading on the gel. The sizes of fragments were compared with 1kb DNA ladder.

3.8.4 Cloning of PCR Products

Ligation: Amplified PCR products were cloned in pGEM[®]-TEASY vector (Promega, USA) using standard molecular biology tools (Sambrook et al. 1989). Ligation mixture

(10µl) comprised of 1 µl PCR product, 1µl PGM-T easy vector, 5 µl ligation buffer, 1 µl DNA ligase and 2 µl dH₂O. The ligation mixture was incubated at 12-14°C overnight.

Transformation: Ligation mixture (2µl) was mixed in an eppendorf tubes containing 100 µl competent cells and incubated on ice for 20 minutes. The cells were then heat shocked at 42°C in a dry bath for 2 minutes. The cells were immediately transferred on ice for 2 min and 900 µl LB medium was added. The cells were then placed at 37°C for 1 hour with constant shaking at 150 rpm. Transformed mixture (100 µl) was plated out on LBA plates with ampicillin and X gal + IPTG and incubated for 24 h at 37°C. Thereafter, blue and white colonies were produced on LBA plates.

Table 3.3 PCR conditions for different primers

Gene (Primer)	Parameters	Initial Denaturation	Denaturation	Annealing	Extension	Final extension	Storage
<i>Cox1</i> (Jerry + Pat)	Temp (°C)	94	94	58	72	72	4
	Time (min)	3.00	0.40	0.45	1.00	7	∞
	Cycles	1	39	39		1	1
28S <i>rRNA</i> (DD + FF)	Temp (°C)	94	94	48	72	72	4
	Time (min)	3.00	0.30	0.45	1.00	7	∞
	Cycles	1	39	39		1	1

3.8.5 Isolation of plasmid DNA by alkali-lysis method (mini-prep method)

a) Reagents for plasmid isolation

I. Alkali lysis solution I (50 mM glucose, 25 mM Tris-HCl (pH 8.0).

a. 10 mM EDTA (pH 8.0)

b. Glucose = 901 mg

c. Tris (1M) = 2.5 ml

d. EDTA (0.5M) = 2 ml

e. Add dH₂O to make vol. To 100 ml. Autoclave and store at 4°C

II. Alkali lysis solution II (0.2 N NaOH, 1% SDS)

a. NaOH (5M) = 4 ml

b. SDS = 1g

c. Add dH₂O to make vol. to 1000 ml. Autoclave and store at room temperature.

III. Alkali lysis solution III

a. Potassium acetate (5M) = to ml

b. Glucose acetate acid = 11.5 ml

c. Add dH₂O to make vol. To 100 ml

d. Store at 4°C and transfer to ice bucket before use

b) Procedure

- i. LB containing appropriate antibiotic (5 ml) was inoculated with single colony of transformed bacteria and incubated at 37°C overnight on a rotary shaker.
- ii. 1.5 ml media was poured in centrifuge tube and centrifuged at maximum speed for 30 seconds.
- iii. Medium was removed by aspiration leaving bacterial pellet as dry as possible.
- iv. The bacterial pellet resuspended in 100 µl of ice cold alkali lysis solution I by vigorous vortexing.
- v. Freshly prepared alkali lysis solution II (200 µl) + 4 µl of RNase (10 mg/ml) was added to each bacterial suspension and incubated at room temperature for 10 minutes.
- vi. Ice-cold alkali lysis solution III (150 µl) was added and alkali lysis solution III was dispersed by inverting tubes several times. This mixture was stored on ice for 3-5 minutes. Then lysate centrifuged at maximum speed at 4°C for 5 min.
- vii. An equal volume of phenol: chloroform mix was added and centrifuged at maximum speed for 2 min at 4°C.
- viii. The aqueous phase was then transferred to fresh tubes.
- ix. Nucleic acid precipitated from supernatant by adding 2 volume of ethanol at room temperature. The solution was mixed by vortexing and then allowed to stand for 20 min at room temperature.
- x. Centrifuged at maximum speed for 5 min at 4°C.
- xi. Supernatant was removed and tubes were stored in an inverted position on paper towel to drain away the fluid.
- xii. 1 ml of 70 % ethanol was added and tubes were inverted several times. Then the solution was centrifuged at maximum speed for 2 min at 4°C.
- xiii. Removed all the supernatant by aspiration. Open tubes were stored at room temperature until ethanol has evaporated.
- xiv. The nucleic acid was dissolved in 50 µl of TE and tubes were stored at -20°C.

3.8.6. Molecular analysis for DNA sequencing

The sequencing of cloned genes (*Cox1* and *28S rRNA*) was done by automated sequencer (Eurofins Genomics, Bengaluru). The plasmid (pGEM-T Easy Vector; Promega) containing respective cloned genes were used for the sequencing. The

sequencing was done using T7 (TAATACGACTCACTATAGGG) and SP6 (ATTTAGGTGACACTATAG) primers followed by standard operating protocols. Sequences on the 5' and 3' ends were trimmed to remove unwanted vector sequences using the GATT and AATC sequences. Forward and reverse primers were highlighted. Nucleotide-Basic Local Alignment Search Tool (BLASTn) of NCBI database was used to perform a similarity search. The highly similar gene sequences were retrieved and these sequences along with our genes sequences were used for multiple sequence alignment using Clustal Omega tool (Thompson et al. 1997) with default settings in BioEdit 7.2.5 (Hall 1999). Phylogenetic analysis was done by using MEGA X program. In MEGA X, data sets were compiled using sequences generated in this study and sequences from previous studies obtained from the NCBI GenBank databases. Using all sequences generated in the study and the reference sequences, a maximum likelihood (ML) analysis based on the K-2 parameter model (Kimura 1980) with complete gap deletion and resampling with 1000 bootstrap replications was done.

3.9 Bioefficacy studies of insecticides against white grubs

3.9.1 Evaluation of insecticides against grubs of *M. virescens* (= *P. fullo*)

Six insecticides, as given in table 3.4 were evaluated against second and third instar grubs of *M. virescens* (= *P. fullo*) under laboratory conditions at Palampur during 2018. These insecticides (Plate 3.5) were used at doses ranging from 0.02 – 0.64 g a.i./kg soil. For all insecticides, preliminary trials were run to determine the range of concentration, which could give mortality from 10 - 90 per cent. The serial dilutions were further prepared using the following equation:

$$C_1V_1 = C_2V_2$$

Where, C_1 = Initial concentration of stock solution; V_1 = Volume of initial concentration
 C_2 = Final concentration; V_2 = Final volume

Table 3.4 List of insecticides tested against grubs of *M. virescens* (= *P. fullo*)

S.No.	Name of chemical	Group	Trade name	Source
1.	Chlorantraniliprole	Diamide groups	Coragen 18.5 SC	Dupont India Pvt. Ltd.
2.	Bifenthrin	Synthetic pyrethroids	Bicep 10 EC	Crop Chemicals
3.	Clothianidin	Neonicotinoids	Dantotsu 50 WDG	Sumitomo Chemicals
4.	Thiamethoxam	Neonicotinoids	Suckgan 25 WDG	Adama India Pvt. Ltd.
5.	Imidacloprid	Neonicotinoids	Victor 17.8 SL	Bayer Crop Science
6.	Chlorpyrifos	Organophosphate	Lethal 20 EC	Insecticides (India) Ltd.



Plate 3.5 Insecticides tested against *Melolontha* grubs

The calculated doses of insecticides were thoroughly mixed in one kg of moist soil, and the treated soil was filled in plastic pots. Each treatment was replicated thrice, and total 10 grubs were released in each replication. Fresh potato tubers were provided as food for the grubs. The data on grub mortality were recorded after 48 hours of treatment. To record observations, the soil was tipped out of pot on a paper, and the grubs were observed carefully for mortality. A grub was considered dead if it failed to respond when probed. The mortality data were converted to per cent mortality and per cent mortality was corrected by using Abbott's formula (Abbott 1925).

$$\text{Per cent corrected mortality} = \frac{\% \text{ mortality in treatment} - \% \text{ mortality in control}}{100 - \% \text{ mortality in control}} \times 100$$

The corrected per cent mortality was subjected to probit analysis (Finney 1971) to calculate LC_{50} and LC_{90} values. Relative toxicity was calculated by dividing LC_{50} value of an insecticide to a population with lowest LC_{50} value of the insecticide among the population.

3.9.2 Evaluation of insecticides as seed treatment against grubs *M. indica*

The third instar grubs of *M. indica* were collected from apple orchards in Pangna area of Mandi district. These grubs were brought to the laboratory and were maintained for about one week before treatment. Six insecticides viz., chlorpyrifos 20 EC, imidacloprid 17.8 SL, clothianidin 50 WDG, thiamethoxam 25 WG, bifenthrin 10 EC and chlorantraniliprole 18.5 SC were used for seed treatment at different doses. There were total six treatments, and each treatment was replicated three times. For seed dressing, the required quantity of each insecticide along with rajmash seeds was placed inside polythene bag. To facilitate uniform coating, few drops of water were sprinkled over the mixture. The mixture was swirled gently to ensure uniform coating of insecticide over seeds. The treated seeds were then shade dried in Petri plates. The experiment was conducted in plastic trays (45 cm x 35 cm x 7 cm) filled with the mixture of soil + FYM. In each tray, 10 grubs were released, and 20 treated rajmash seeds were sown at a depth of about 5 cm. The grub mortality was observed after 20 days of sowing by taking out the soil and counting the number of healthy and dead grubs in both treated and control trays. The data on plant mortality were recorded 10 and 20 days after sowing, and per cent mortality data on plants and grubs were subjected to arcsin transformations.

4. RESULTS AND DISCUSSION

The present work on bioecology and management of *Melolontha* species in Himachal Pradesh was undertaken during 2017-2019. The abundance and diversity of *Melolontha* beetles in different agro climatic zones of Himachal Pradesh was investigated using light traps and by sampling beetles directly from their host trees. To examine the diversity and density of grubs in soil, pit sampling was done and the collected white grubs were reared in laboratory for further identification. The observations on bionomics of four species were recorded under laboratory conditions. The data recorded on these aspects are presented and discussed under this chapter in the light of existing literature.

4.1 Species diversity and population abundance of *Melolontha* beetles on light trap

The light trap studies were carried out during May-September in six districts covering seven locations during 2017 and 2018 (Table 4.1). The altitude of these locations varied from 1189 to 2588 m amsl, and the latitude of the study sites ranged from 31°12.962' to 32°53.082' N. The longitude of study sites ranged from 76°00.346' to 78°15.060' E. The rainfall was recorded in the range of 254.2 to 2293 mm during 2017, whereas in 2018, it was slightly higher varying from 251.7 to 2584.7 mm. During the survey, total five species of *Melolontha* beetles were collected from Kangra, Kullu, Mandi, Chamba, Shimla and Kinnaur districts of Himachal Pradesh (Table 4.1). Chandra (2005) has reported four species belonging to the genus *Melolontha* from Chamba, Kinnaur, Kullu, Mandi, Shimla, Sirmour and Solan districts of Himachal Pradesh. In the present study, *Melolontha furcicauda* Ancy was recorded at all the locations. *Melolontha cuprescens* Blanchard was absent in Kinnaur and *Melolontha indica* Hope in Shimla district among surveyed locations. *Melolontha* sp.1 was collected in very low numbers during May-September in 2017 and 2018 in Kangra and Mandi districts. *Melolontha virescens* (Brenske) [= *Polyphylla fullo* (Linnaeus)] was recorded in Mandi, Kangra, Chamba and Kinnaur districts of Himachal Pradesh. The data recorded on number of *Melolontha* beetles on light traps are presented separately species-wise.

4.1.1 Abundance and distribution of *M. cuprescens* on light trap in Himachal Pradesh during 2017 and 2018

The data on abundance of *M. cuprescens* recorded on light traps at different locations during 2017 and 2018 are given in table 4.2. This species was collected at

Palampur, Chhatanseri, Badagaon, Janjehli, Ahla and Mashobra. It is said by Chandra (2005) to occur in Chamba, Kinnaur and Kullu districts of Himachal Pradesh. Pathania (2014) found it to be common species in Chamba, Sirmaur, Kinnaur and Kullu districts of Himachal Pradesh. Among selected sites, the areas like Chhatanseri, Badagaon, Janjehli and Ahla showed higher abundance of *M. cuprescens*. Maximum beetles of *M. cuprescens* (92 and 88 beetles) were collected in Chhatanseri where this species comprised 69.17 and 73.33 per cent of total *Melolontha* beetle catch during 2017 and 2018, respectively. According to Pathania et al. (2015), *M. cuprescens* comprised 2.63 to 3.70 per cent of total scarab fauna in Kullu district of Himachal Pradesh, but the contribution of *M. cuprescens* to total *Melolontha* beetle catch has been reported as 33.13 to 36.36 per cent by these workers. In Badagaon area, 50 and 65 beetles were collected between June to September during 2017 and 2018 with relative abundance of 27.47 and 35.14 per cent, respectively. At Janjehli and Ahla, the total catch of *M. cuprescens* consisted of 57 and 41 beetles during 2017, and 54 and 51 beetles during 2018, respectively. At Janjehli, *M. cuprescens* comprised 62.07 to 62.63 per cent of total *Melolontha* beetle catch, whereas at Ahla, the relative abundance of *M. cuprescens* varied from 43.62 to 48.11 per cent during 2017-18. According to earlier reports, the relative abundance of *M. cuprescens* has been recorded to be ranging from 44.89 to 46.32 per cent in Chamba district of Himachal Pradesh (Pathania et al. 2015).

Table 4.1 Checklist of *Melolontha* beetles collected on light trap during 2017-18 in HP

S. No.	Species	Palampur	Chhatanseri	Badagaon	Janjehli	Ahla	Mashobra	Sangla
1.	<i>M. cuprescens</i>	+	+	+	+	+	+	-
2.	<i>M. furcicauda</i>	+	+	+	+	+	+	+
3.	<i>M. indica</i>	+	+	+	+	+	-	+
4.	<i>Melolontha</i> sp.1	+	-	+	+	-	-	-
5.	<i>M. virescens</i> (= <i>P. fullo</i>)	-	-	+	+	+	-	+

Present (+), Absent (-)

The frequency of occurrence was recorded to be 100 per cent at Chhatanseri, Badagaon and Ahla, whereas at Janjehli, the frequency of occurrence was found to be 75.0 per cent during both the years. In Mashobra and Palampur areas, very low population of *M. cuprescens* was recorded during July to September. At Mashobra, *M. cuprescens* beetles constituted 33.33 to 40.0 per cent of total *Melolontha* beetle catch, whereas at Palampur, *M. cuprescens* constituted only 1.07 to 1.13 per cent of total *Melolontha* beetle

catch during 2017 and 2018. Sreedevi et al. (2017) reported that *M. cuprescens* accounts for 4.40 per cent of total scarab fauna in Himachal Pradesh.

Table 4.2 Abundance of *M. cuprescens* beetles on light trap in HP during 2017 and 2018

Location	Year	No. of beetles collected				Total catch	Dominance (%)	Frequency (%)
		June	July	Aug	Sept			
Palampur	2017	0	3	0	0	3	1.09	25.0
	2018	0	0	2	1	3	1.14	50.0
Chhatanseri	2017	15	55	20	2	92	69.17	100.0
	2018	11	60	16	1	88	73.33	100.0
Badagaon	2017	3	34	11	2	50	27.93	100.0
	2018	5	43	16	1	65	36.11	100.0
Janjehli	2017	2	46	9	0	57	65.51	75.0
	2018	1	43	10	0	54	63.53	75.0
Ahla	2017	2	19	15	5	41	43.62	100.0
	2018	1	27	16	7	51	48.11	100.0
Mashobra	2017	0	6	3	0	9	33.33	50.0
	2018	0	4	4	0	8	40.0	50.0

The beetles of *M. cuprescens* started appearing on light trap in third week of June at Chhatanseri, and during last week of June at Badagaon, Janjehli and Ahla. At Mashobra, the activity of *M. cuprescens* began during first to third week of July, whereas at Palampur, its first appearance on light trap was noticed between second week of July to third week of August during 2017 and 2018, respectively. The peak activity of *M. cuprescens* beetles was recorded in the month of July at all the locations during both the years (Fig. 4.1; 4.2). At Chhatanseri, 59.78 to 68.18 per cent of total *M. cuprescens* beetles were collected during July, followed by 18.18 to 21.74 per cent in August during 2017 and 2018, respectively. At Badagaon, 66.15 to 68.0 per cent, at Janjehli, 79.63 to

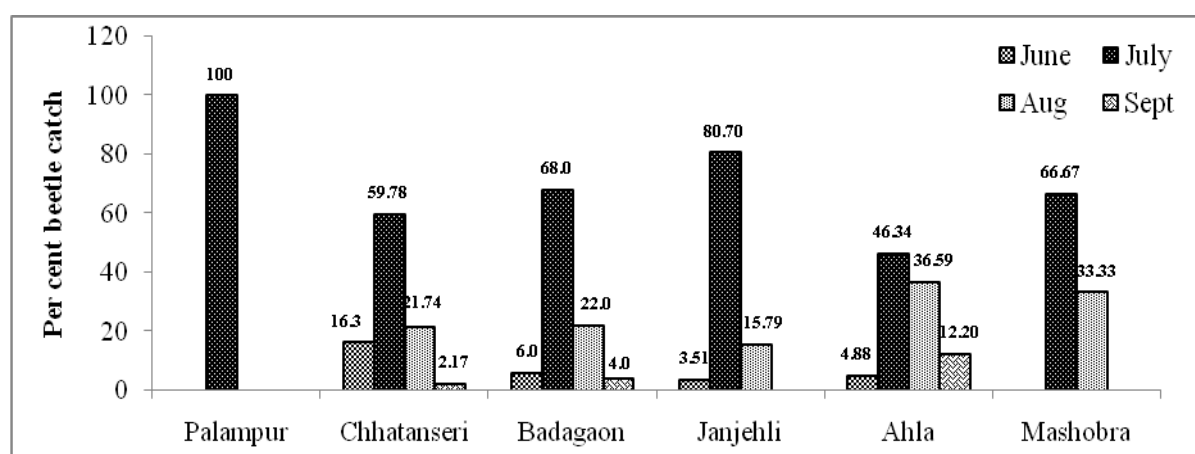


Fig. 4.1 Month-wise occurrence of *M. cuprescens* beetles on light trap during 2017

80.70 per cent, and at Ahla, 46.34 to 52.94 per cent of total *M. cuprescens* beetles were taken in the month of July, whereas in August, the respective trap catch varied from 22.0 to 24.62 per cent, 15.79 to 18.52 per cent and 31.37 to 36.59 per cent during 2017 and 2018. Pathania (2014) also reported that beetles of *M. cuprescens* appeared late in June in Sirmaur and Kullu districts, and during July in Kinnaur and Chamba districts which corroborates our findings.

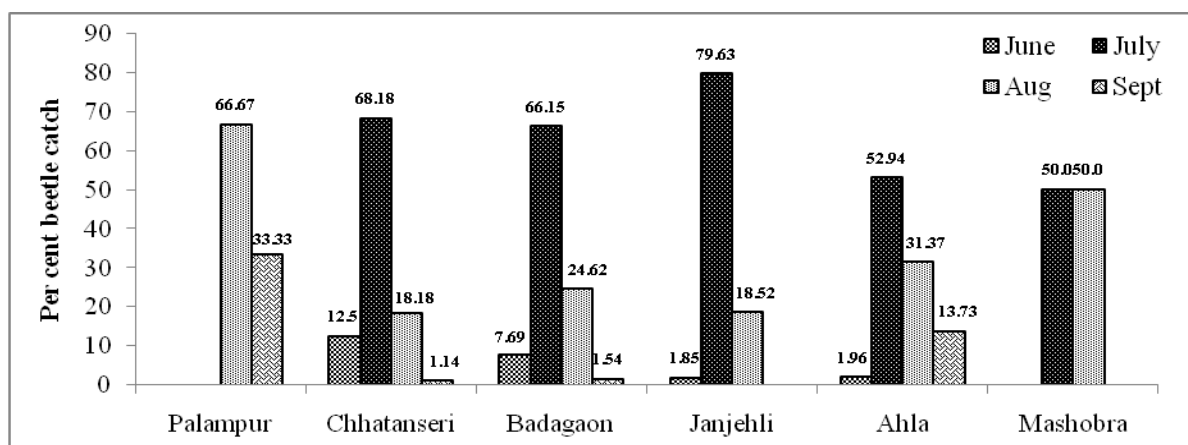


Fig 4.2 Month-wise occurrence of *M. cuprescens* beetles on light trap during 2018

The analysis of data from six different locations during 2017 and 2018 indicated that 64.68 and 65.80 per cent of the *M. cuprescens* beetles appeared on light trap only during July. The mean catch of *M. cuprescens* beetles in

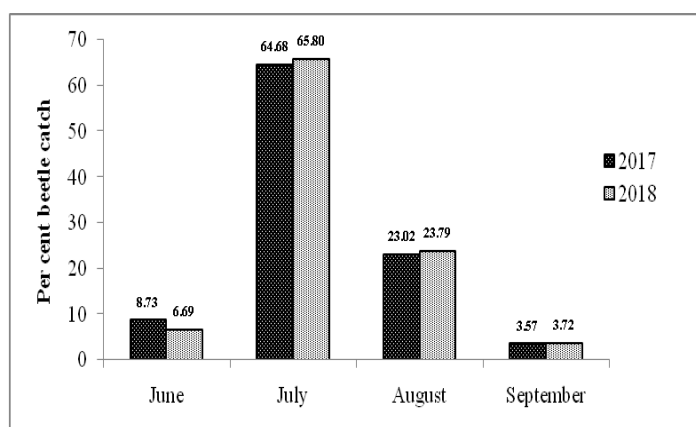


Fig. 4.3 Cumulative month-wise catch of *M. cuprescens* beetles during 2017 and 2018

June, August and September was computed to be 8.73 and 6.69 per cent, 23.02 and 23.79 per cent, and 3.57 and 3.72 per cent during 2017 and 2018, respectively, as shown in fig. 4.3.

4.1.2 Abundance and distribution of *M. furcicauda* on light trap in Himachal Pradesh during 2017 and 2018

The data in table 4.3 showed that *M. furcicauda* was predominantly collected in Palampur and Badagaon in Kangra district, and at Ahla in Chamba district. According to Chandra (2005), *M. furcicauda* is widely distributed in Himachal Pradesh, and has been

reported to occur in Chamba, Kinnaur, Kullu, Mandi, Sirmaur and Solan districts of Himachal Pradesh. Pathania et al. (2015) reported that *M. furcicauda* occur in Kangra, Kullu, Sirmaur and Chamba districts of Himachal Pradesh. In Kashmir (Arif et al. 2019) and Uttarakhand (Shah and Garg 1988; Sreedevi et al. 2017), *M. furcicauda* has been reported as a minor pest.

A cursory look on the data indicated that the total beetle catch of *M. furcicauda* was marginally higher during 2017 as compared to 2018 at all the locations except Ahla. At Palampur during 2017, 135 beetles, and in 2018, 132 beetles were collected during a period of four months between June to September. Perusal of data in table 4.3 indicated that *M. furcicauda* comprised 48.21 per cent of total *Melolontha* beetle catch during 2017, and 49.62 per cent of total *Melolontha* beetle catch during 2018 at Palampur. Earlier workers have reported that *M. furcicauda* contributed to 43.32 to 43.42 per cent of total *Melolontha* beetle catch on light trap in different parts of Himachal Pradesh (Pathania et al. 2015). At Badagaon, the total trap catch for *M. furcicauda* beetles comprised of 35 beetles during both the years contributing to 19.23 and 18.91 per cent of total *Melolontha* beetle catch during 2017 and 2018, respectively. At Ahla in Chamba district, 36 and 41 beetles of *M. furcicauda* were captured on light trap during the period of study (June-September) in 2017 and 2018, respectively. The relative abundance of *M. furcicauda* at Ahla was calculated to be 38.29 per cent during 2017, and 38.68 per cent during 2018.

At Chhatanseri in Kullu valley, 32 and 24 beetles of *M. furcicauda* were taken on light trap during 2017 and 2018, respectively, and the contribution of this species to *Melolontha* assemblage has been worked out to be 24.06 and 20.0 per cent during 2017 and 2018, respectively. At Janjehli in Mandi district, *M. furcicauda* comprised 18.68 and 17.24 per cent of total *Melolontha* beetle catch with total count of 17 and 15 beetles during July and August in both the years. Almost similar trend was noticed at Mashobra where 18 and 12 beetles of *M. furcicauda* were observed on light trap between June to August. This species accounted for 66.67 and 60.0 per cent of total *Melolontha* beetle catch at Mashobra during 2017 and 2018, respectively. Mishra and Singh (1999) collected 37 beetles of *M. furcicauda* in Uttarakhand, and reported that *M. furcicauda* accounts for 68.52 per cent of total *Melolontha* beetle catch at Ranichauri. According to Sreedevi et al. (2017), *M. furcicauda* contributes up to 3.72 per cent of total beetle catch in Uttarakhand. Similarly, in Kumaon hills, *M. furcicauda* comprised 74.07 per cent of total *Melolontha* beetle catch at Saraikhet (Mishra and Singh 1997).

The total catch of *M. furcicauda* beetles was lowest at Sangla in Kinnaur district with a total catch of seven and five beetles during the entire season. The relative abundance of *M. furcicauda* at Sangla was worked out to be 15.91 and 11.11 per cent during 2017 and 2018, respectively. Raina and Khan (2007) have also reported low population of *M. furcicauda* from Kashmir accounting for 0.75 per cent of total scarab beetle catch on light trap at Pahalgam. The frequency of occurrence was recorded to be 100 per cent at Palampur, Chhatanseri, Badagaon and Ahla. At Mashobra, the frequency of occurrence was 75.0 per cent, whereas at Janjehli and Sangla, the frequency of occurrence of *M. furcicauda* beetles on light trap was 50.0 per cent during both the years, and at both the locations.

Table 4.3 Abundance of *M. furcicauda* beetles on light trap in HP during 2017 and 2018

Location	Year	No. of beetles collected				Total catch	Dominance (%)	Frequency (%)
		June	July	Aug	Sept			
Palampur	2017	36	86	11	2	135	48.91	100.0
	2018	31	78	19	4	132	50.19	100.0
Chhatanseri	2017	6	14	11	1	32	24.06	100.0
	2018	3	11	9	1	24	20.00	100.0
Badagaon	2017	2	27	5	1	35	19.55	100.0
	2018	4	22	8	1	35	19.44	100.0
Janjehli	2017	0	5	12	0	17	19.54	50.0
	2018	0	7	8	0	15	17.65	50.0
Ahla	2017	2	19	12	3	36	38.29	100.0
	2018	2	23	15	1	41	38.68	100.0
Mashobra	2017	5	8	5	0	18	66.67	75.0
	2018	2	7	3	0	12	60.00	75.0
Sangla	2017	2	5	0	0	7	15.91	50.0
	2018	0	3	2	0	5	11.11	50.0

At Palampur during 2017, and at Badagaon and Ahla during 2017 and 2018, the beetles of *M. furcicauda* made their first appearance on light trap during third week of June, and their activity continued up to first week of September. At Palampur during 2018, the period of beetle activity was found to be extended by about two weeks. The beetles started emerging during second week of June, and their activity continued up to second week of September. At Chhatanseri, the activity of *M. furcicauda* began in third week of June and continued up to second week of September as evident from the light trap data given in table 4.3. Altaf and Ahad (2020) also reported that beetles of *M. furcicauda* appeared on light trap between May to September in Kashmir Himalaya with peak activity in first fortnight of June. Mishra and Singh (1997) recorded this species occurring on light trap at Saraikhet from June to September with peak catch of 12 beetles per month in July.

At Mashobra, the beetle emergence began in the third week of June during both the years, but there was no activity of beetles on light trap after second week of August during 2017. In 2018, the beetle activity ceased in first week of August. At Sangla, the beetle emergence was found to be inconsistent during the study period. In 2017, the beetles emergence started in the last week of June and it continued up to second week of July, whereas in 2018, the beetles started appearing on light trap in second week of July and their activity continued up to first week of August. At Janjehli, there was delayed emergence of *M. furcicauda* beetles in 2017. The beetle emergence commenced during third week of July and continued up to second week of August, whereas in 2018, the beetle emergence commenced one week in advance but there was no activity of *M. furcicauda* beetles after second week of August. The peak activity of *M. furcicauda* beetles was recorded in the month of July at Palampur, Chhatanseri, Badagaon, Ahla, Mashobra and Sangla, whereas at Janjehli, *M. furcicauda* exhibited peak activity in the month of August during both the years (Fig. 4.4; 4.5). Raina and Khan (2007) recorded *M. furcicauda* appearing on light trap at Pahalgam from June to August with peak emergence in July.

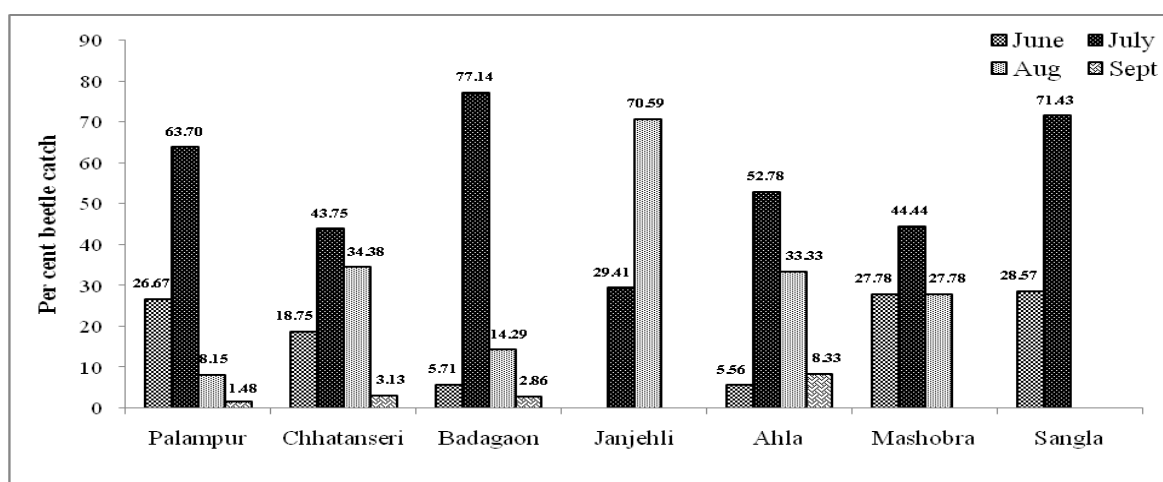


Fig 4.4 Month-wise occurrence of *M. furcicauda* beetles on light trap during 2017

At Palampur, 63.70 and 59.09 per cent of total *M. furcicauda* beetles were collected in July, followed by June (26.67 % in 2017 and 23.48 % in 2018), and August (8.15 % in 2017 and 14.39 % in 2018). Similarly, at Badagaon, the total beetle catch for *M. furcicauda* was maximum in July (77.14 and 62.86 %), followed by August (14.29 and 22.86 %), and June (5.71 and 11.43 %) during 2017 and 2018, respectively. Similar trend was noticed at Chhatanseri as shown in fig. 4.4 and fig. 4.5. At Ahla, the total beetle catch of *M. furcicauda* in July was calculated to be 52.78 and 56.10 per cent, followed by 33.33

and 36.59 per cent in August, and 8.33 and 2.44 per cent during September in 2017 and 2018, respectively. At Mashobra, the per cent beetle catch for *M. furcicauda* during July was recorded to be 44.44 and 58.33 per cent during 2017 and 2018, respectively, but the beetle catch was 27.78 per cent both in June and August during 2017. In 2018, the beetle catch was higher in August (25.0 %) as compared to June (16.67 %). In Janjehli area, 70.59 and 53.33 per cent beetles of *M. furcicauda* were collected in August, whereas in July, the respective trap catch was found to be 29.41 and 46.67 per cent during 2017 and 2018, respectively.

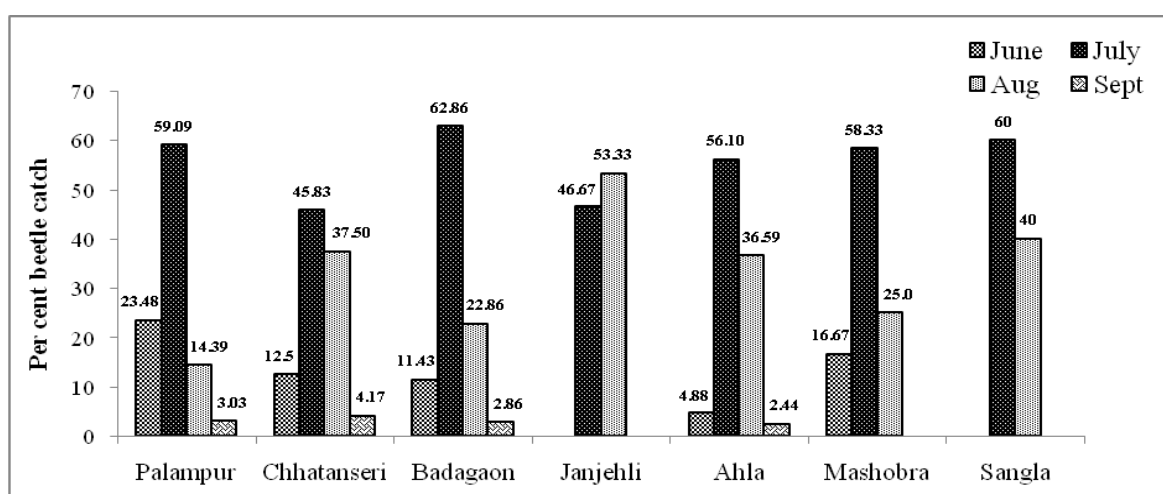


Fig 4.5 Month-wise occurrence of *M. furcicauda* beetles on light trap during 2018

The overall pooling of data from all seven locations indicated that 58.57 and 57.20 per cent beetles of *M. furcicauda* appeared on light trap in the month of July. The present results are in conformity with those of Raina and Khan (2007). In

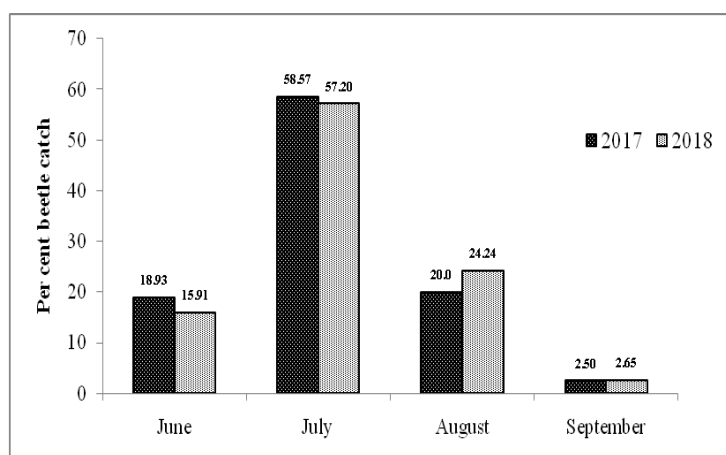


Fig. 4.6 Cumulative month-wise catch of *M. furcicauda* beetles during 2017 and 2018

August, the cumulative catch of *M. furcicauda* was recorded to be 20.0 and 24.24 per cent during 2017 and 2018, respectively. In June, the mean beetle catch for all seven locations during 2017 and 2018 has been worked out to be 18.93 and 15.91 per cent, respectively, as shown in fig. 4.6. Similarly, in Uttarakhand, the trap catch for *M. furcicauda* has been

reported to be 51.35 per cent in July, followed by 45.95 per cent in June and 2.70 per cent in August (Mishra and Singh 1999).

4.1.3 Abundance and distribution of *M. indica* on light trap in Himachal Pradesh during 2017 and 2018

Out of seven locations surveyed in the present study, *M. indica* was recorded at six sites viz. Palampur, Chhatanseri, Badagaon, Janjehli, Ahla and Sangla. Chandra (2005) has reported *M. indica* to occur in Kullu, Shimla and Solan districts of Himachal Pradesh. The data on number of *M. indica* beetles captured on light trap during 2017 and 2018 are presented in table 4.4. This species was recorded in higher numbers at Palampur, whereas at Badagaon, the beetles of *M. indica* appeared in moderate numbers. At Chhatanseri, Janjehli, Ahla and Sangla, very low population of *M. indica* beetles was recorded on light trap during both the years. At Palampur, total 138 beetles of *M. indica* were captured on light trap during July to September in 2017. The corresponding catch during 2018 consisted of 128 beetles. According to Pathania et al. (2015), *M. indica* is fairly common species at Palampur and have collected 33-38 beetles of this species between May to August during 2011 and 2012. In Doon valley of Uttarakhand, Lal and Sharma (2017) had collected 20 beetles of *M. indica* from four different sites, and these reports partially substantiate our results.

The total catch for *M. indica* beetles at Badagaon varied from 36 to 37 beetles during 2017-18, whereas at Janjehli, 6 to 11 beetles of *M. indica* were caught on light trap in July and August during 2017-18. At Chhatanseri, Ahla and Sangla, 9, 15 and 5 beetles of *M. indica* were recorded on light trap during 2017. Similarly, during 2018, 8, 11 and 4 beetles of *M. indica* were caught on light trap at Chhatanseri, Ahla and Sangla, respectively. Among different species of *Melolontha*, *M. indica* was found to be leading species at Palampur comprising 49.29 and 48.12 per cent during 2017 and 2018, respectively. In Badagaon area, *M. indica* constituted 19.78 and 20.0 per cent of total *Melolontha* beetle catch during 2017 and 2018, respectively. At Ahla, the relative abundance of *M. indica* beetles has been computed to be 15.96 and 10.38 per cent during 2017 and 2018, respectively. Contrary to our results, Pathania et al. (2015) have reported higher abundance of *M. indica* in Kullu district as compared to Palampur. At Sangla, the contribution of *M. indica* to the total catch of *Melolontha* beetles has been worked out to be 11.36 per cent during 2017, and 8.89 per cent during 2018. At Chhatanseri, *M. indica* beetles accounted for 6.77 and 6.67 per cent during 2017 and 2018, respectively.

According to Sreedevi et al. (2017), *M. indica* occurs in low numbers in north western Himalaya and accounts for 1.09 and 0.22 per cent of total scarab beetle catch in Himachal Pradesh and Uttarakhand, respectively. The beetles of *M. indica* appeared more regularly on light trap at Palampur, Badagaon and Ahla with 75.0 per cent frequency of occurrence during both the years. Similarly, at Sangla, the frequency of occurrence was recorded to be 75.0 per cent during 2017. At Chhatanseri and Janjehli, the beetles of *M. indica* appeared less regularly on light trap, and their frequency of occurrence at both the locations has been worked out to be 50.0 per cent during 2017 and 2018.

Table 4.4 Abundance of *M. indica* beetles on light trap in HP during 2017 and 2018

Location	Year	No. of beetles collected				Total catch	Dominance (%)	Frequency (%)
		June	July	Aug	Sept			
Palampur	2017	0	14	84	40	138	50.0	75.0
	2018	0	20	71	37	128	48.67	75.0
Chhatanseri	2017	0	7	2	0	9	6.77	50.0
	2018	0	5	3	0	8	6.67	50.0
Badagaon	2017	0	29	5	2	36	20.11	75.0
	2018	2	32	3	0	37	20.56	75.0
Janjehli	2017	0	2	4	0	6	6.90	50.0
	2018	0	4	7	0	11	12.94	50.0
Ahla	2017	0	2	8	5	15	15.96	75.0
	2018	0	3	6	2	11	10.38	75.0
Sangla	2017	1	3	1	0	5	11.36	75.0
	2018	0	3	1	0	4	8.89	50.0

The emergence of *M. indica* beetles started in first week of July at Ahla, and continued up to first week of September during both the years. At Palampur, the emergence of *M. indica* beetles began in second week of July, and continued up to last week of September during both the years. At Chhatanseri, during 2018 the beetle emergence began in second week of July and the activity ceased in second week of August. There was delayed emergence in 2017 as the beetles of *M. indica* were first noticed on light trap in last week of July. No beetle activity was observed at Chhatanseri after second week of August during 2017. At Janjehli, the beetle emergence started in last week of July and the emergence continued up to second week of August in low numbers during both the years. At Badagaon, lot of variation existed in beetle emergence during 2017 and 2018. In 2018, the *M. indica* beetles started appearing on light trap in last week of June, and their activity continued up to third week of August, whereas in 2017, the beetle activity commenced in the last week of July, and continued up to first week of August. In Sangla area, the emergence initiated during last week of June to first week of

July, but beetle activity terminated in first week of August during both the years. Shah and Garg (1988) reported that emergence of *M. indica* begins in last week of June and its activity continues up to third week of September with peak activity during third week of August in Almora district of Uttarakhand. The peak activity of *M. indica* beetles was recorded in the month of August at Palampur, Janjehli and Ahla, whereas at other locations, the beetle catch was comparatively higher in July than in August (Fig. 4.7; 4.8).

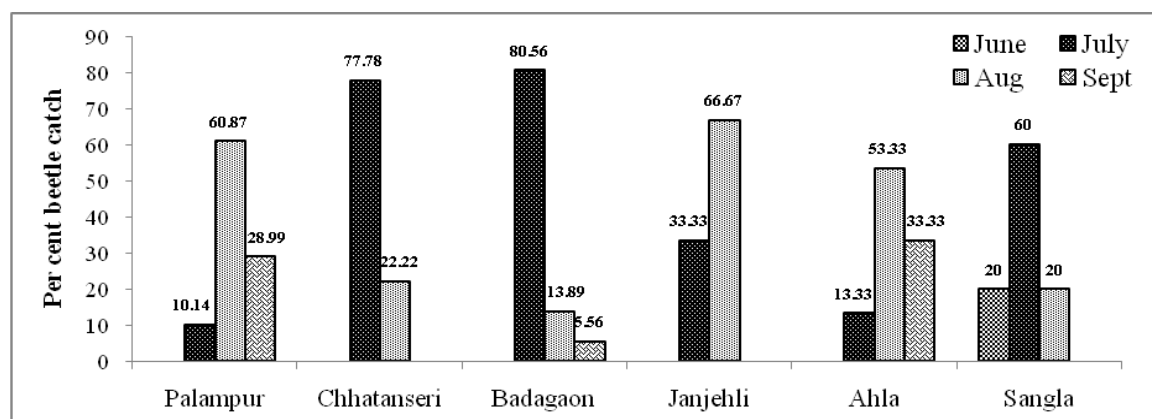


Fig 4.7 Month-wise occurrence of *M. indica* beetles on light trap during 2017

Out of the total 128-138 beetles collected during July to September, 60.87 and 54.47 per cent of the beetles were captured on light trap in the month of July at Palampur. In August, 28.99 and 28.91 per cent beetles of *M. indica* were collected on light trap during 2017 and 2018, respectively. Similarly, at Ahla, 53.33 and 54.55 per cent of *M. indica* beetles were taken on light trap in the month of June, whereas during August, 33.33 and 18.18 per cent of *M. indica* beetles appeared on the light trap during 2017 and 2018, respectively. Contrary to Ahla and Palampur, *M. indica* beetles at Badagaon exhibited maximum emergence in July (80.56 % in 2017 and 86.49 % in 2018), followed by a catch of 13.89 and 8.11 per cent during August in 2017 and 2018, respectively (Fig. 4.7; 4.8).

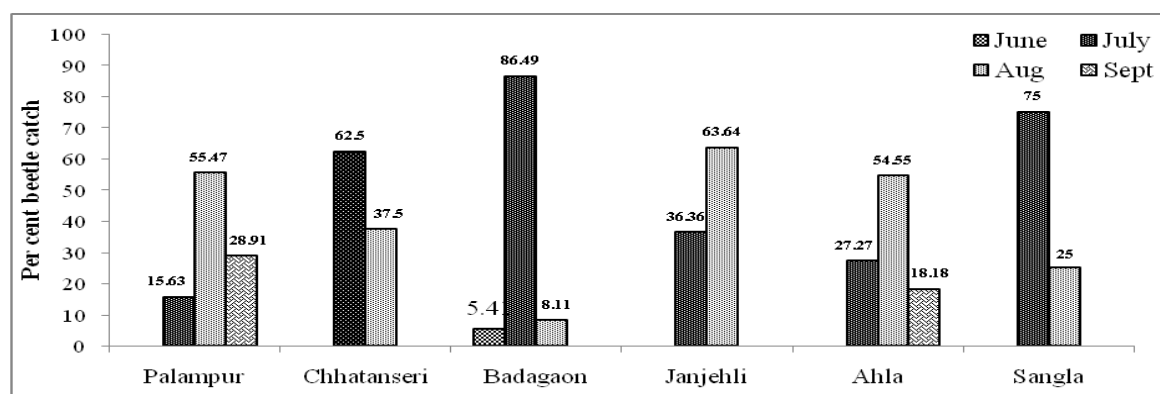


Fig 4.8 Month-wise occurrence of *M. indica* beetles on light trap during 2018

Irrespective of locations, the overall catch of *M. indica* beetles was maximum in the month of August (49.76 % in 2017 and 45.73 % in 2018), followed by July (27.27 % in 2017 and 33.67 % in 2018) and September (22.49 % in 2017 and 19.60 % in 2018) as shown in fig. 4.9.

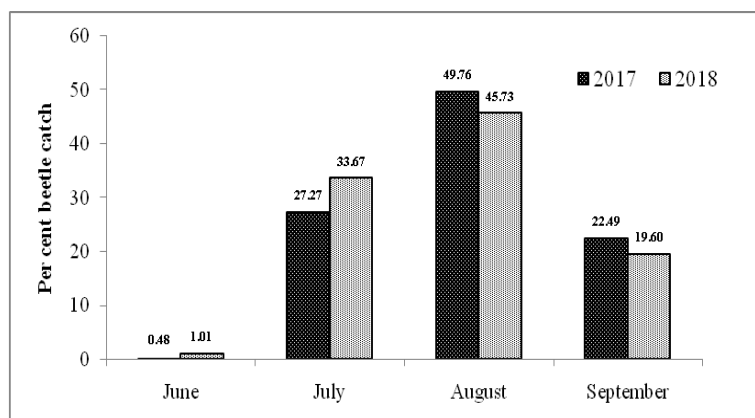


Fig 4.9 Cumulative month-wise catch of *M. indica* beetles during 2017 and 2018

4.1.4 Abundance and distribution of *M. virescens* (= *P. fullo*) on light trap in Himachal Pradesh during 2017 and 2018

The data on the seasonal abundance of *M. virescens* (= *P. fullo*) recorded on light trap during June to September in 2017 and 2018 are given in table 4.5. *Melolontha virescens* (= *P. fullo*) was not recorded on light trap at Palampur, Chhatanseri and Mashobra. Maximum activity of this species was recorded in high altitude areas at Badagaon (2268 m amsl) and Sangla (2588 m amsl). Earlier, *M. virescens* has been reported to occur in low numbers in Himalaya by Beeson (1941). Jarfas and Toth (1977) made comparisons of trap catches taken on different lights in Hungary. They reported that *P. fullo* flew to the mercury vapour lamps in the highest numbers. At Badagaon, total 58 beetles were collected during 2017, and comprehended 31.87 per cent of total *Melolontha* beetle catch during 2017. In 2018, the trap catch for *M. virescens* (= *P. fullo*) comprised of 43 beetles and the relative abundance has been calculated to be 23.24 per cent. Pathania et al. (2015) have reported *M. virescens* to be a predominant species in Badagaon area of Kangra district at an altitude of 2268 m amsl. At Sangla, 32 and 36 beetles of *M. virescens* (= *P. fullo*) were observed on light trap during 2017 and 2018, and accounted for 72.73 to 80.0 per cent of total *Melolontha* beetle catch during 2017-18. At Ahla and Janjehli, *M. virescens* (= *P. fullo*) beetles were collected in very low numbers (2-7 beetles) during the study period. The relative proportion of *M. virescens* (= *P. fullo*) at Janjehli and Ahla was calculated to be 7.69 and 5.75 per cent, and 2.13 and 2.83 per cent during 2017 and 2018, respectively. The frequency of occurrence has been recorded to be 100 per cent at Sangla, and 75.0 per cent at Badagaon during both the years. However, at Janjehli and Ahla, the

beetles were collected less frequently with 50.0 per cent frequency of occurrence during both the years at both the locations.

Table 4.5 Abundance of *P. fullo* beetles on light trap in HP during 2017 and 2018

Location	Year	No. of beetles collected				Total catch	Dominance (%)	Frequency (%)
		June	July	Aug	Sept			
Badagaon	2017	0	21	25	12	58	32.40	75.0
	2018	0	16	18	9	43	23.89	75.0
Janjehli	2017	0	0	4	3	7	8.05	50.0
	2018	0	0	3	2	5	5.88	50.0
Ahla	2017	0	1	1	0	2	2.13	50.0
	2018	0	1	2	0	3	2.83	50.0
Sangla	2017	4	12	14	2	32	72.73	100.0
	2018	5	13	17	1	36	80.0	100.0

At Badagaon, the activity of *M. virescens* (= *P. fullo*) beetles commenced in third week of July and their activity continued up to first week of September during both the years. At Sangla, there was early emergence of beetles as is evident from the light trap catches indicating onset of emergence in last week of June. The activity of *M. virescens* (= *P. fullo*) beetles ceased in the first week of September at Sangla. At Janjehli, the beetles started appearing on light trap in first week of August, and their activity continued for about one month. At Ahla, the beetle emergence began in second week of July and their emergence was recorded up to second week of August in 2017, and up to third week of August in 2018. The peak activity was recorded in the month of August at all the locations (Fig. 4.10; Fig. 4.11). At Badagaon, more than 40.0 per cent of total beetle catch for entire season was taken in the month of August (43.10 % in 2017 and 41.86 % in 2018). Almost similar trend was evident at Sangla showing 43.75 per cent of total beetle catch in August during 2017, and 47.22 per cent in 2018.

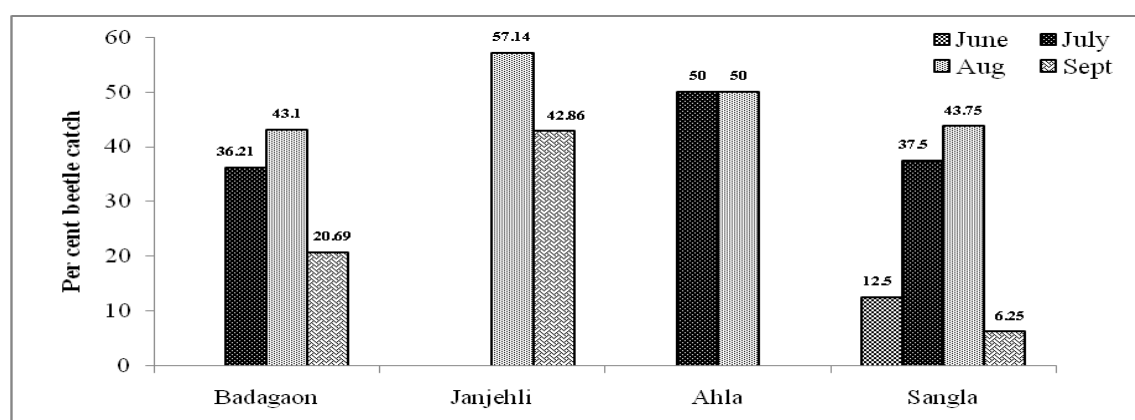


Fig 4.10 Month-wise occurrence of *M. virescens* (= *P. fullo*) beetles on light trap during 2017

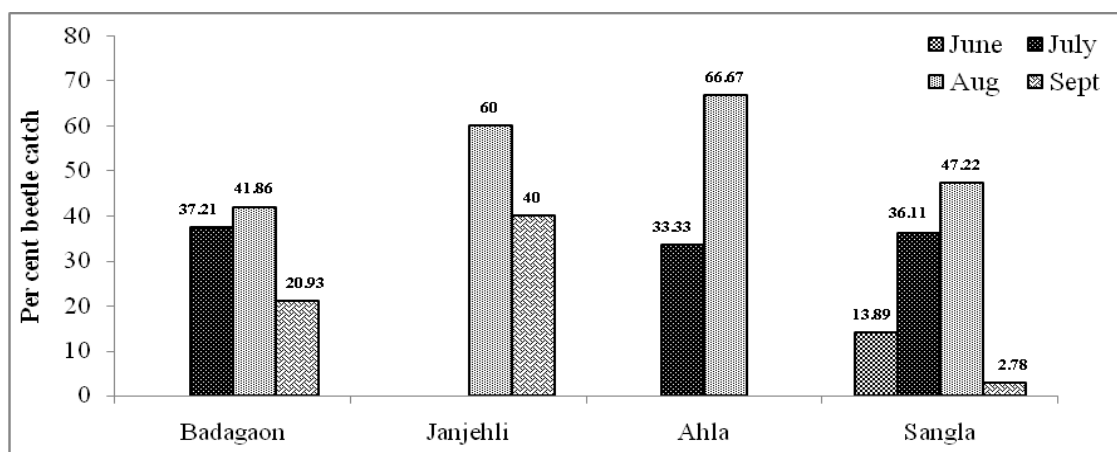
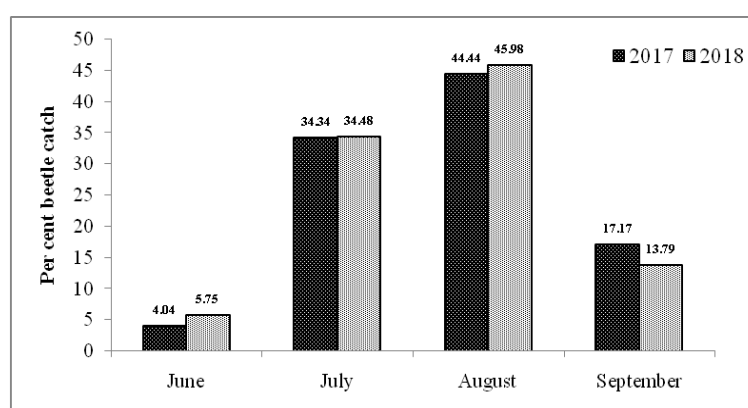


Fig 4.11 Month-wise occurrence of *M. virescens* (=P. *fullo*) beetles on light trap during 2018

The analysis of data for Badagaon, Janjehli, Ahla and Sangla during 2017 and 2018 revealed that 44.44 and 45.98 per cent of *M. virescens* (=P. *fullo*) beetles were collected only during August. The average catch of *M.*



4.12 Cumulative month-wise catch of *M. virescens* (=P. *fullo*) beetles during 2017 and 2018

virescens (=P. *fullo*) beetles was calculated to be 34.34 and 34.48 per cent in July, and 17.17 and 13.79 per cent in September during 2017 and 2018, respectively, as shown in fig. 4.12.

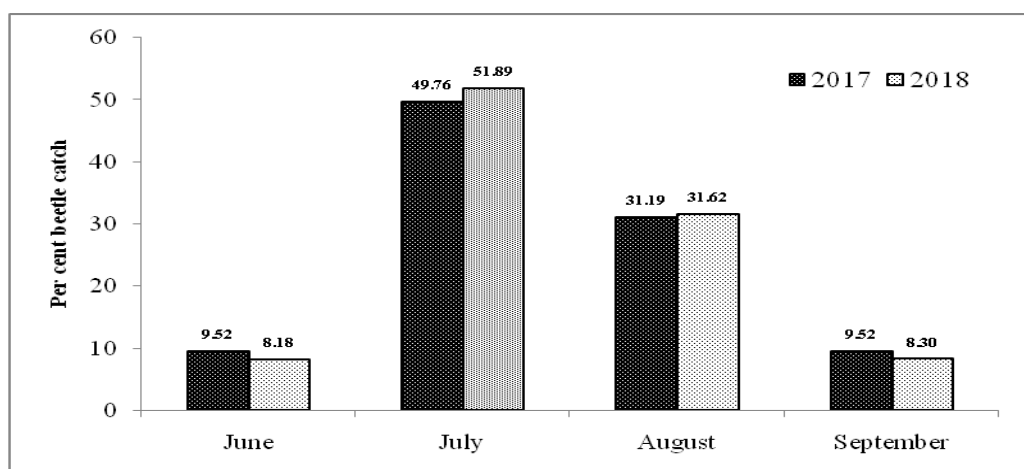


Fig. 4.13 Overall diversity of *Melolontha* beetles in different locations of HP

4.1.5 Alpha diversity indices in relation to abundance of *Melolontha* beetles in different locations of Himachal Pradesh

Richness (number of species), abundance (number of individuals) and three alpha diversity indices were used in the present study (Table 4.6). The Shannon index (H') which explains the evenness of abundance of species was calculated to be maximum at Badagaon ($H' = 1.36$) during 2017. During 2018, the value of Shannon index (H') was calculated to be 1.35 at Badagaon. This indicates evenness in abundance at Badagaon during both the years. At Badagaon, there was no dominance of a single species, and four species out of total five collected species viz. *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*) comprised 27.47 to 35.14, 18.91 to 19.23, 19.78 to 20.0 and 23.24 to 31.87 per cent, respectively. These species contributed to 97.29 to 98.35 per cent of total beetle catch which explains the maximum value of Shannon index (H') at Badagaon in the present study. This shared dominance of several species amounts to lesser variation in scarab community between the species. At Ahla and Janjehli, the value of Shannon index (H') ranged from 0.98 to 1.11 during 2017, and 1.03 to 1.06 during 2018. The value of Shannon index (H') was found to be low at Palampur ($H' = 0.75$ in 2017 and 2018), Chhatanseri ($H' = 0.78$ in 2017 and $H' = 0.73$ in 2018), Mashobra ($H' = 0.64$ in 2017 and $H' = 0.67$ in 2018) and Sangla ($H' = 0.77$ in 2017 and 0.64 in 2018). This low value of Shannon index (H') is mainly due to poor species to specimen ratio. Out of the four *Melolontha* species, *M. furcicauda* and *M. indica* contributed 97.5 to 97.74 per cent of total *Melolontha* beetle catch validating the low value of Shannon index (H') at Palampur. Similarly, at Chhatanseri, *M. cuprescens* and *M. furcicauda* comprised 93.23 per cent, whereas at Sangla *P. fullo* alone contributed 80.0 per cent of total *Melolontha* beetle catch. The low value of Shannon index (H') at Palampur, Chhatanseri, Mashobra and Sangla indicated more unevenness in abundance of *Melolontha* species at these locations during both the years.

The Pielou's evenness index (J') was calculated to be maximum at Badagaon ($J' = 0.98$ in 2017 and $J' = 0.97$ in 2018), followed by Mashobra ($J' = 0.92$ in 2017 and $J' = 0.97$ in 2018). At Ahla, the value of Pielou's evenness index (J') was calculated to be 0.79 and 0.76 during 2017 and 2018, respectively. At Janjehli, the value of Pielou's evenness index (J') ranged from 0.71 to 0.74 during 2017-18. The lowest value of Pielou's evenness index (J') was calculated to be at Sangla during 2018 ($J' = 0.58$), whereas during 2017, the value of Pielou's evenness index (J') at Sangla was worked out to be 0.70. At Palampur, the Pielou's evenness index (J') was calculated to be 0.68 during both the years. This showed

that relative abundance of different *Melolontha* species was more even in Badagaon and Mashobra areas as compared to Palampur, Chhatanseri, Janjehli and Sangla (Table 4.6). This unevenness of *Melolontha* beetle community is mainly due to the dominance of particular species at a particular location.

The overall general diversity of *Melolontha* beetles in Himachal Pradesh as indicated by light trap catches is depicted in fig. 4.13. The value of Simpson's index of diversity (D) was calculated to be maximum (D= 0.74 in 2017 and D= 0.73 in 2018) for *Melolontha* species at Badagaon. At Ahla, the value of Simpson's index of diversity (D) was computed to be 0.60 and 0.58 during 2017 and 2018, respectively. At Janjehli during 2017 and at Palampur during both the years, the value of Simpson's index of diversity (D) was calculated to be 0.52. At Chhatanseri, Mashobra and Sangla, the value of Simpson's index of diversity (D) varied from 0.34 to 0.48 during 2017-18. On the basis of Simpson's index of diversity (D), it can be inferred that lesser diversity among *Melolontha* species exists at Chhatanseri, Mashobra and Sangla.

Since the value of Shannon index (H') varied between 0.64 to 1.36 throughout Himachal Pradesh which indicated that there exists dominance of a particular species at selected sites. Dhoj et al. (2006) reported that a community dominated by few species is considered to be less diverse than one with high species richness and evenness. It was observed that there exists less variation in diversity in genus *Melolontha* at different selected locations in Himachal Pradesh. Several workers have observed that in Himachal Pradesh the beetle diversity and abundance varied highly with locations (Chandel et al. 1994; Pathania et al. 2015; Chandel 2020). The variation in beetle diversity might be due to variation of hosts, vegetation, crops grown and soil type.

Table 4.6 Diversity indices calculated for light trap catches of *Melolontha* beetles during 2017 and 2018

Diversity indices		Palampur	Chhatanseri	Badagaon	Janjehli	Ahla	Mashobra	Sangla
Shannon index (H')	2017	0.75	0.78	1.36	0.98	1.11	0.64	0.77
	2018	0.75	0.73	1.35	1.03	1.06	0.67	0.64
Simpson's index (D)	2017	0.52	0.46	0.74	0.52	0.60	0.44	0.43
	2018	0.52	0.42	0.73	0.55	0.58	0.48	0.34
Pielou's evenness index (J')	2017	0.68	0.71	0.98	0.71	0.79	0.92	0.70
	2018	0.68	0.66	0.97	0.74	0.76	0.97	0.58

4.1.6 Beta diversity analysis in relation to abundance of *Melolontha* beetles in different locations of Himachal Pradesh

The Sorensen's similarity index (SI), Jaccard similarity index (JI) and Bray-Curtis index (BCI) were computed to compare similarity among *Melolontha* species at seven different locations covering six districts of Himachal Pradesh (Table 4.7). The species richness and abundance showed total similarity among *Melolontha* species between Badagaon and Janjehli as indicated by the values of Sorensen's similarity index (SI) and Jaccard similarity index (JI) which were calculated to be 1.0 during both the years. The comparison of *Melolontha* assemblages revealed high similarity for Palampur with Chhatanseri, Badagaon and Janjehli, for Chhatanseri with Ahla and Mashobra, for Badagaon with Ahla, for Janjehli with Ahla and for Ahla with Sangla. The value of Sorensen's similarity index (SI) was calculated to be 0.89 for Palampur with Badagaon and Janjehli, for Badagaon with Ahla, and for Janjehli with Ahla during both the years. The value of Sorensen's similarity index (SI), was worked out to be 0.86 between Palampur and Chhatanseri, Chhatanseri and Ahla, and Ahla and Sangla, whereas between Chhatanseri and Mashobra, the value of Sorensen's similarity index (SI) was worked out to be 0.80. The lowest value of Sorensen's similarity index (SI) (0.57) was calculated among *Melolontha* assemblages from Palampur x Sangla, Badagaon x Mashobra, and Janjehli x Mashobra.

Similarly, the value of Jaccard similarity index (JI) was calculated to be 1.0 for Badagaon x Janjehli, followed by 0.80 for Palampur x Badagaon and Janjehli, Badagaon x Ahla, and Janjehli x Ahla. The value of Jaccard similarity index (JI) was calculated to be 0.75 for Palampur x Chhatanseri and Ahla, Chhatanseri x Ahla, and Ahla x Sangla during both the years. The lowest value of Jaccard similarity index (JI) was calculated to be 0.40. This value was calculated for *Melolontha* assemblages from Palampur x Sangla, Badagaon x Mashobra, and Janjehli x Mashobra during both the years. The values of Sorensen's similarity index (SI) and Jaccard similarity index (JI) were computed to be uniform during both the years because these values are based on number of shared species among locations, and at all the locations, the species richness was uniform during both the years.

Unlike Sorensen's similarity index (SI) and Jaccard similarity index (JI), the value of Bray-Curtis index (BCI) was found to be different during 2017 and 2018, because both richness and abundance values were used to calculate the value of Bray-Curtis index (BCI). In the present study, the maximum value of Bray-Curtis index (BCI) was calculated

to be 0.77 and 0.83 between Janjehli and Ahla during 2017 and 2018, respectively. The value of Bray-Curtis index (BCI) for Chhatanseri x Janjehli ranged from 0.71 to 0.74, and for Chhatanseri x Ahla, the value of Bray-Curtis index (BCI) varied from 0.72 to 0.73 during 2017-18. The value of Bray-Curtis index (BCI) for Badagaon x Janjehli, and Badagaon x Ahla was calculated to be 0.61 and 0.67 during 2017, and 0.64 and 0.69 during 2018, respectively. Comparison of Sangla with rest of the locations revealed very low value of Bray-Curtis index (BCI) ranging from 0.61 to 0.94. Similarly, comparison of Palampur with all other locations exhibited low values of Bray-Curtis index (BCI= 0.65 to 0.94).

Maximum dissimilarity was recorded among *Melolontha* assemblages from Sangla and Palampur as indicated by highest value of Bray-Curtis distance index (BCDI= 0.93 to 0.94). Chhatanseri, Janjehli and Mashobra also exhibited high dissimilarity with Palampur. Sangla also showed high dissimilarity with Chhatanseri, Ahla and Mashobra (Table 4.8). The *Melolontha* beetle communities at most of the locations were largely stable having a balance between common species and rare species. Colwell and Coddington (1994) reported that most individuals belong to a few common species in a typical community but this fact does not hold true in the present study. Perhaps poor species richness in genus *Melolontha* contributes to balance between common and rare species among *Melolontha* beetle communities. The white grub endemic areas surveyed in six districts of Himachal Pradesh viz. Kangra, Kullu, Mandi, Chamba, Shimla and Kinnaur, revealed variations in species richness and abundance. Both these observations are important in representing changes in diversity. The species like *M. cuprescens*, *M. furcicauda* and *M. indica* were abundant in mid-hills (1189 to 1222 m amsl), whereas *M. virescens* (= *P. fullo*) was abundant in high hills (2268 to 2588 m amsl). The species diversity exhibited some differences among the surveyed locations with considerable variations in their composition. Comparatively high similarity between Palampur x Badagaon and Janjehli can be attributed to similarity in topography, weather and vegetation in mid and high hills. Similarly, the high dissimilarity between Palampur and Sangla could be attributed to differences in topography and crops grown.

Table 4.7 Beta diversity showing similarity among *Melolontha* species in different locations of HP

Locations → Locations ↓	Chhatanseri				Badagaon				Janjehli				Ahla				Mashobra				Sangla			
	SI	JI	BCI		SI	JI	BCI		SI	JI	BCI		SI	JI	BCI		SI	JI	BCI		SI	JI	BCI	
	2017 & 2018	2017 & 2018	2017	2018	2017 & 2018	2017 & 2018	2017	2018	2017 & 2018	2017 & 2018	2017	2018	2017 & 2018	2017 & 2018	2017	2018	2017 & 2018	2017 & 2018	2017	2018	2017 & 2018	2017 & 2018	2017	2018
Palampur	0.86	0.75	0.21	0.18	0.89	0.80	0.33	0.35	0.89	0.80	0.16	0.18	0.75	0.60	0.29	0.30	0.67	0.50	0.14	0.10	0.57	0.40	0.07	0.06
Chhatanseri					0.75	0.60	0.58	0.64	0.75	0.60	0.71	0.74	0.86	0.75	0.72	0.73	0.80	0.67	0.34	0.29	0.67	0.50	0.14	0.11
Badagaon									1.00	1.00	0.61	0.64	0.89	0.80	0.67	0.69	0.57	0.40	0.26	0.20	0.75	0.60	0.39	0.39
Janjehli													0.89	0.80	0.77	0.83	0.57	0.40	0.43	0.37	0.75	0.60	0.28	0.21
Ahla																	0.67	0.50	0.45	0.32	0.86	0.75	0.20	0.16
Mashobra																					0.67	0.50	0.20	0.15

SI=Sorensen's Index; JI=Jaccard's Index; BCI=Bray Curtis Index

Table 4.8 Bray Curtis distance index of *Melolontha* species in different locations of HP

Sites → ↓	Chhatanseri		Badagaon		Janjehli		Ahla		Mashobra		Sangla	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Palampur	0.79	0.82	0.67	0.65	0.84	0.82	0.71	0.70	0.86	0.90	0.93	0.94
Chhatanseri			0.42	0.36	0.29	0.26	0.28	0.27	0.66	0.71	0.86	0.89
Badagaon					0.39	0.36	0.33	0.31	0.74	0.80	0.61	0.61
Janjehli							0.23	0.17	0.57	0.63	0.72	0.79
Ahla									0.55	0.68	0.80	0.84
Mashobra											0.80	0.85

4.2 Species diversity and population abundance of *Melolontha* beetles on host trees

Scarab beetles are highly polyphagous and flights of *Melolontha* beetles were largely noticed on foliage of different pome and stone fruits during June to September in different parts of Himachal Pradesh. Chandel and Kashyap (1997) reported that scarab beetles usually remain unnoticed throughout the year and their emergence in large numbers occurs during May-June just after break in monsoon. To collect *Melolontha* beetles from host trees, surveys were conducted from June to September at five locations in mid and high hills of Himachal Pradesh. The beetle diversity has been plotted month-wise as the mean of different sampling dates. The data of *Melolontha* beetles in Himachal Pradesh on different host trees during 2017 and 2018 are given in table 4.9 and 4.10. A total of four *Melolontha* species were collected on apple, peach, plum, persimmon, walnut, wild rose, *bathua* and Indian siris.

Table 4.9 Abundance of *Melolontha* beetles on different hosts in HP during 2017

Locations	Species	Number of beetles collected				Total	Host (s)
		June	July	August	September		
Palampur	<i>M. cuprescens</i>	0	0	2	1	3	Apple
	<i>M. furcicauda</i>	5	6	2	0	13	Apple
	<i>M. indica</i>	0	8	10	6	24	Apple, peach
Chhatanseri	<i>M. cuprescens</i>	6	13	11	1	31	Apple, plum
	<i>M. furcicauda</i>	2	3	2	0	7	Apple, plum
	<i>M. indica</i>	0	3	1	0	4	Apple
Badagaon	<i>M. cuprescens</i>	0	9	6	1	16	Apple, persimmon, walnut, wild rose
	<i>M. furcicauda</i>	1	5	2	1	9	Apple
	<i>M. indica</i>	0	7	1	0	8	Persimmon, walnut
	<i>P. fullo</i>	0	8	12	0	20	Apple, walnut, persimmon, <i>bathua</i>
Ahla	<i>M. cuprescens</i>	0	2	8	3	13	Apple, wild rose
	<i>M. furcicauda</i>	0	6	2	1	9	Wild rose
	<i>M. indica</i>	0	1	4	3	8	Wild rose, Indian siris
	<i>P. fullo</i>	0	0	1	0	1	Indian siris
Sangla	<i>M. indica</i>	0	1	1	0	2	Apple
	<i>P. fullo</i>	0	1	2	2	5	Apple

At Palampur and Chhatanseri, three *Melolontha* species viz. *M. cuprescens*, *M. furcicauda* and *M. indica* were recorded during 2017 and 2018. At Palampur, *M. indica* and *M. furcicauda* were predominant, whereas at Chhatanseri, *M. cuprescens* was found to be the leading species. At Palampur, more activity of *M. furcicauda* was recorded during June to August, whereas *M. indica* was recorded between July to September with peak activity in the month of August. *M. cuprescens* was recorded in low numbers only from August to September at Palampur during 2017, and during July to September in 2018. Pathania (2014) has collected two species viz. *M. furcicauda* on pear and peach, and *M. virescens*

Table 4.10 Abundance of *Melolontha* beetles on different hosts in HP during 2018

Locations	Species	Number of beetles collected				Total	Host (s)
		June	July	August	September		
Palampur	<i>M. cuprescens</i>	0	1	3	2	6	Apple
	<i>M. furcicauda</i>	2	4	4	1	11	Apple, peach
	<i>M. indica</i>	0	6	9	4	19	Apple, peach
Chhatanseri	<i>M. cuprescens</i>	4	10	5	0	19	Apple, plum
	<i>M. furcicauda</i>	0	4	1	1	6	Apple
	<i>M. indica</i>	1	2	1	0	4	Apple
Badagaon	<i>M. cuprescens</i>	0	7	3	0	10	Persimmon, walnut
	<i>M. furcicauda</i>	1	4	1	1	7	Apple
	<i>M. indica</i>	0	3	1	0	4	Persimmon, walnut
	<i>P. fullo</i>	0	7	8	2	17	Apple, persimmon, bathua
Ahla	<i>M. cuprescens</i>	0	3	6	2	11	Wild rose
	<i>M. furcicauda</i>	0	3	3	1	7	Wild rose
	<i>M. indica</i>	0	1	2	0	3	Wild rose, Indian siris
Sangla	<i>M. indica</i>	0	1	1	0	2	Apple
	<i>P. fullo</i>	0	1	2	2	5	Apple

on walnut at Palampur. Both *M. furcicauda* and *M. indica* showed slightly higher preference to feed on apple as compared to peach at Palampur (Fig.

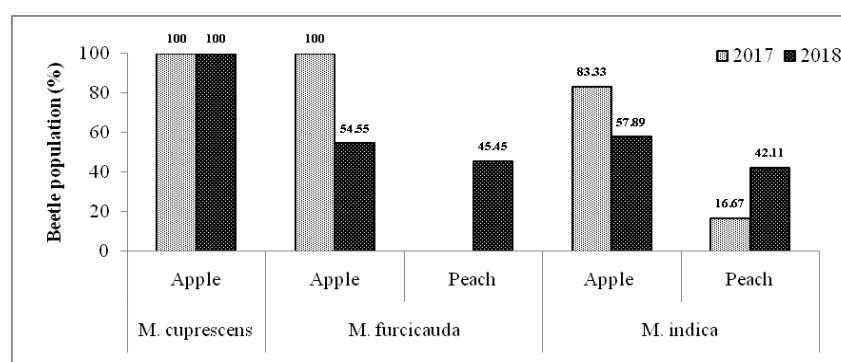


Fig. 4.14 Occurrence of *Melolontha* beetles on host trees during 2017 and 2018 at Palampur

4.14). Earlier reports indicate higher preference of *M. furcicauda* beetles to feed on pear, followed by peach (Pathania 2014).

At Chhatanseri, the emergence of *M. cuprescens* was recorded during last week of June, and peak emergence was recorded in the month of July during both the years. *M.*

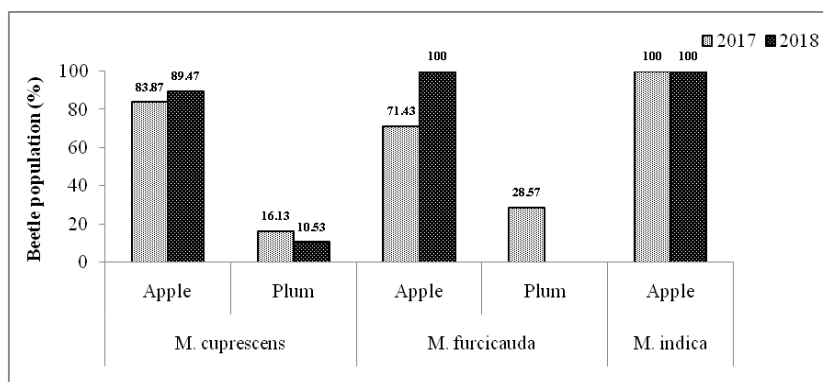


Fig. 4.15 Occurrence of *Melolontha* beetles on host trees during 2017 and 2018 at Chhatanseri

furcicauda beetles were collected on apple between June to August in 2017, and from July to September in 2018, whereas *M. indica* beetles occurred on apple during July to August in 2017, and during July to September in 2018. *Melolontha furcicauda* beetles were also observed to feed on plum during 2017 in low numbers (Fig. 4.15). The beetles of *M. cuprescens* displayed distinct preference to feed on apple as compared to plum at Chhatanseri. Our observations on species diversity and host preference find support with the survey work from Kullu as reported by Pathania (2014).

At Badagaon during 2017 and 2018, and at Ahla during 2017, four species viz. *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*) were observed to feed on different plants. At Ahla during 2018, only three species except *P. fullo* were recorded. At Badagaon, the predominant species were *M. virescens* (= *P. fullo*) and *M. cuprescens*, whereas *M. furcicauda* and *M. indica* were observed to appear in moderate numbers during both the years. Beeson (1941) reported that *M. virescens* occurs at low levels in Himalaya, breeding in moist sandy soil permeated by roots of grasses and herbs. At Badagaon, the emergence of *M. cuprescens* started in first week of July and continued up to first week of September during 2017, and up to third week of August during 2018. The emergence of *M. furcicauda* and *M. indica* was recorded to be comparatively more in the month of July. Mehta et al. (2008) reported occurrence of *M. furcicauda* up to an altitude of 3000 m amsl in Himachal Pradesh with adults feeding on stone fruits during second fortnight of June. Unlike other species, the emergence of *M. virescens* (= *P. fullo*) commenced in first week of July and its peak population was recorded in the month of August. There was no activity of *M. virescens* (= *P. fullo*) in September during 2017, but in 2018, the activity of *M. virescens* (= *P. fullo*) continued up to second week of August. *M.*

cuprescens displayed higher preference to feed on persimmon as compared to apple, walnut and wild rose during 2017, whereas *M. furcicauda* was recorded exclusively on apple during both the years. Like *M. cuprescens*, *M. indica* also exhibited higher preference for persimmon as compared to walnut during both the years. *M. virescens* (= *P. fullo*) was recorded to feed on apple, walnut, persimmon and *Chenopodium album* during 2017 and 2018, but 60 per cent of the beetles in 2017, and 58.82 per cent of the population in 2018 was recorded to feed on persimmon (Fig. 4.16). Chandel et al. (2010) reported *M. virescens* as most damaging in Badagaon area of Chuhar valley, and observed its beetles feeding on wheat spikes in the first week of July.

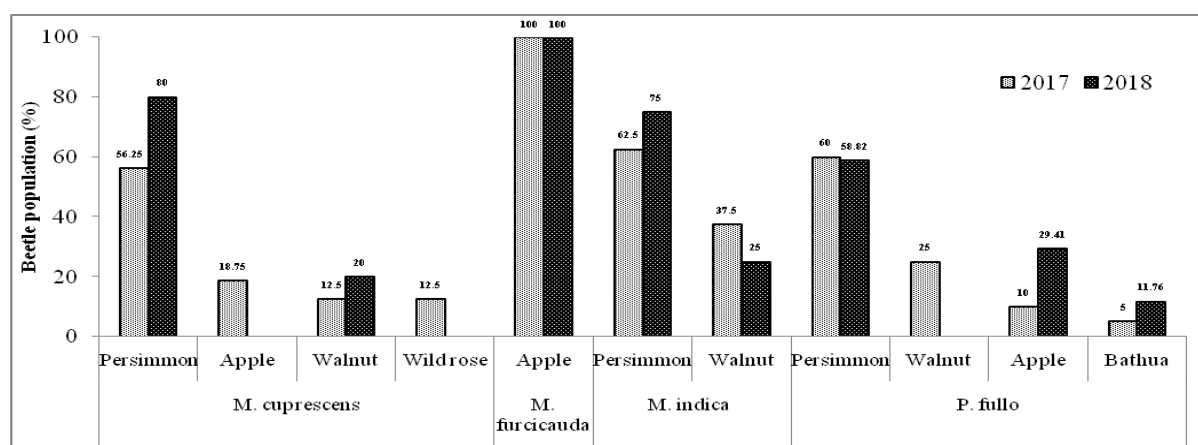


Fig. 4.16 Occurrence of *Melolontha* beetles on host trees during 2017 and 2018 at Badagaon

At Ahla, *M. cuprescens* was found to be the predominant species, and the beetles displayed maximum activity in the month of August. During 2017, 61.53 and 38.46 per cent of beetles were recorded to feed and mate on wild rose and apple, respectively, whereas during 2018, the beetles of *M. cuprescens* were recorded feeding exclusively on wild rose. *M. furcicauda* beetles showed more activity in the month of July, whereas *M. indica* appeared in more numbers in the month of August during both the years.

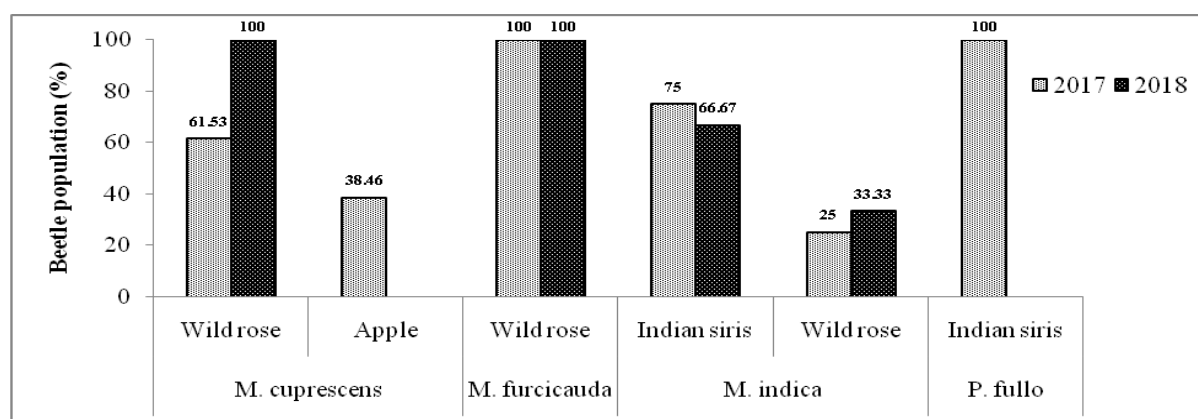


Fig. 4.17 Occurrence of *Melolontha* beetles on host trees during 2017 and 2018 at Ahla

M. furcicauda was caught exclusively on wild rose in both the years, whereas *M. indica* beetles were recorded in more numbers (75 % in 2017 and 66.67% in 2018) on Indian siris. At Ahla, 25.0 and 33.33 per cent beetles of *M. indica* beetles were recorded feeding on wild rose during 2017 and 2018, respectively (Fig. 4.17). Pathania (2014) has reported apple as major host of *M. cuprescens* and *M. furcicauda* with peak activity during July in Bharmour area of Chamba district. Our findings are partially in agreement with these reports.

At Sangla, only two species viz. *M. indica* and *M. virescens* (= *P. fullo*) were recorded to feed on apple during 2017 and 2018. Both these species were caught in very low numbers as shown in table 4.9 and 4.10. *Melolontha indica* beetles were recorded during July-August, whereas *M. virescens* (= *P. fullo*) beetles were observed during September. Earlier, *M. furcicauda* has been reported to feed on walnut, apple and apricot, and its relative abundance on different hosts varied from 6.51 to 6.71 per cent at Sangla (Sood et al. 2009). In north Kashmir, *M. furcicauda* is active from May to August with peak activity occurring in first fortnight of June. The adults have been reported to feed on apple, pear and peach (Altaf et al. 2019). Ahmad and Faisal (2012) have reported *M. furcicauda* as serious defoliator of poplar from Dehradun in Uttarakhand. Pathania (2014) has reported *M. cuprescens* feeding on apple, pear, peach, apricot, and wild rose during August at Reckong Peo in Kinnaur district of Himachal Pradesh.

4.2.1 Host preference behaviour of *Melolontha* beetles

The beetles of *Melolontha* species are polyphagous and under field conditions, these beetles were observed to feed on different fruit/forest trees in varying proportion at different locations. Therefore, five plants were tested for their food preference under confinement in multi-choice and no-choice tests (Table 4.11; 4.12). In multi-choice test, rose was found to be the most preferred host for *M. indica* and *M. furcicauda* with mean leaf area consumption of 33.73 cm² and 29.55 cm², after three days of their release, respectively. According to Kumar et al. (2006), scarab beetles are most important and economical pests of rose which cause considerable damage by feeding both on leaves and flowers. Field studies on choice of host for feeding and mating indicated decided preference of *M. cuprescens*, *M. furcicauda* and *M. indica* for wild rose in Ahla. At Palampur, Chhatanseri and Mashobra, apple is largely preferred by *Melolontha* beetles for feeding and mating. The preference of *M. cuprescens*, *M. furcicauda* and *M. indica* beetles for rose and apple in the field finds support from the present multi and no-choice feeding

tests. Contrary to our results, pecan nut is highly favoured host of *Holotrichia longipennis* Blanchard and *Holotrichia sikkimensis* Brenske, whereas wild rose is an unsuitable host (Kalpana 2015). This indicates that despite polyphagous nature, different species of scarabaeid beetles have definite choice for one host over the other, and certain plants might be having chemicals, which act as antifeedant to these beetles. In case of *M. indica*, the next best preferred host was plum showing leaf area consumption of 26.80 cm², followed by apple where leaf area consumption was recorded to be 13.71 cm². Peach and pecan nut were almost rejected by *M. indica* in multi-choice tests. The leaf area consumption on rose and plum was statistically at par with each other, but leaf area consumption differed significantly on apple.

Table 4.11 Leaf area consumption by *Melolontha* beetles in multi-choice test

Plant species	Leaf area consumption (cm ²) after 3 days		
	<i>M. indica</i>	<i>M. fuscicauda</i>	<i>M. cuprescens</i>
Rose	33.73 (5.88)	29.55 (5.52)	18.21 (4.34)
Plum	26.80 (5.26)	14.58 (3.95)	29.35 (5.51)
Apple	13.71 (3.82)	21.84 (4.77)	38.77 (6.31)
Peach	0.41 (1.16)	0.07 (1.03)	2.41 (1.84)
Pecan nut	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
CD (P=0.05)	(0.61)	(0.26)	(0.67)

Figures in parentheses are $\sqrt{n+1}$ transformed values

The beetles of *M. fuscicauda* showed higher preference to feed on apple (21.84 cm²) as compared to plum (14.58 cm²). The leaf area consumption by *M. fuscicauda* beetles after three days was found to be statistically different in rose, plum and apple. Contrary to *M. indica* and *M. fuscicauda*, *M. cuprescens* beetles showed higher preference to feed on apple leaves, followed by plum and rose. The mean leaf area consumption on apple, plum and rose was calculated to be 38.77 cm², 29.35 cm² and 18.21 cm², respectively. There was no feeding on pecan nut leaves by either of the species in multi-choice test in captivity. Sushil et al. (2006) also conducted host preference studies using choice method and reported walnut as most preferred host for *Anomala dimidiata* Hope, *Rosa* sp. for *Holotrichia seticollis* Moser, and *Alnus nepalensis* for *M. indica* at Almora. Similar host preference to guava has also been reported for *Holotrichia consanguinea* Blanchard under laboratory conditions (Bhadauria and Nigam 1982). Chandel et al. (1995) studied food preference of related melolonthid beetle (*B. coriacea*) in multi-choice test on seven fruit trees, and maximum intake was recorded in apricot, followed by plum, peach and almond.

When these plants were subjected to beetle feeding in no-choice test, similar results were obtained as shown in table 4.12. Maximum consumption by *M. indica* beetles was recorded on rose leaves (31.24 cm²), followed by plum (24.06 cm²) and apple (21.52 cm²). The mean leaf area consumption in peach and pecan nut was found to be 5.14 cm² and 0.45 cm², respectively. In case of *M. furcicauda*, maximum leaf area consumption was recorded on rose (30.16 cm²), followed apple (22.76 cm²) and plum (12.56 cm²). *M. cuprescens* beetles registered maximum consumption on apple leaves (45.23 cm²) and this species was found to be more voracious feeder as compared to *M. indica* and *M. furcicauda*. After three days of exposure, the leaf area consumption was 18.21 and 22.45 per cent more as compared to *M. indica* and *M. furcicauda* on rose, which was their most preferred host, respectively. In case of rose and plum, the mean leaf area consumption was recorded to be 36.93 cm² and 33.11 cm², respectively. The leaf area consumption differed significantly among all tested hosts. Peach and pecan nut seems to be the unsuitable host plants for feeding among studied species with mean leaf consumption varying from 1.93 to 6.92 cm² in peach, and 0.30 to 2.16 cm² in pecan nut leaves even in the absence of any choice. According to Chandel et al. (1995), feeding capacity of scarab beetles vary significantly on different hosts in no-choice feeding test under laboratory conditions. Field studies on choice of host for feeding and mating by *Melolontha* beetles showed considerable variation.

Table 4.12 Leaf area consumption by *Melolontha* beetles in no-choice test

Plant species	Leaf area consumption (cm ²) after 3 days		
	<i>M. indica</i>	<i>M. furcicauda</i>	<i>M. cuprescens</i>
Rose	31.24 (5.68)	30.16 (5.58)	36.93 (6.16)
Plum	24.06 (5.01)	12.56 (3.68)	33.11 (5.84)
Apple	21.52 (4.75)	22.76 (4.89)	45.23 (6.79)
Peach	5.14 (2.48)	1.93 (1.70)	6.92 (2.81)
Pecan nut	0.45 (1.20)	0.30 (1.13)	2.16 (1.76)
CD (P=0.05)	(0.23)	(0.30)	(0.45)

Figures in parentheses are $\sqrt{n+1}$ transformed values

The overall survey for *Melolontha* beetles on light traps and host trees revealed a complex of five species in Himachal Pradesh. In addition to these five species, two more species are known to occur in Himachal Pradesh. *Melolontha nepalensis* (Blanchard) has been reported from Kullu (Barwal 1998; Kumar et al. 2005) and *Melolontha melolontha* Linnaeus from Palampur (Devi et al. 1994). There seems to be some confusion in identification of these species. The picture of *M. nepalensis* given by Kumar et al. (2019)

looks like *M. furcicauda* as recorded in the present study. Similarly, *M. melolontha* reported from Palampur by Devi et al. (1994) seems to be like *M. indica*. The *M. virescens* reported by Pathania (2014) has been identified and confirmed as *P. fullo* from National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru in the present study. According to Sehna and Bezdek (2011), the subgenus *Polyphylla* is synonym to *Granida*. Beeson (1941) has reported *Granida albosparsa* Moser a large Melolonthini beetle commonly occurring in Himalaya up to 9000 feet and causing maximum damage during May-June and his description fits appropriately into the description of *M. virescens* (= *P. fullo*).

There was lesser collection of *Melolontha* beetles both on light traps and host trees at all locations as compared to other species of scarabaeid beetles even in areas like Badagaon, Janjehli, Chhatanseri and Reckong Peo where the grubs of *Melolontha* cause economic damage in several crops like pulses, potato and vegetables. Despite of having large population of their grubs in the soil, the beetle catch was comparatively poor. It seems that the beetles of *Melolontha* species are less phototactic in nature, which could be the reason for low beetle catch on light traps. In the present study, the light trap used consisted of ultra violet tube, but Jarfas and Toth (1977) in Hungary made comparisons of trap catches recorded on different light sources, and reported that *P. fullo* flew to mercury vapour lamps in the highest numbers. Similarly, during adult sampling on host trees, very less population of beetles for all the species was recorded at almost all the locations. The beetles in genus *Melolontha* are large in size as compare to other species of scarabaeid beetles and these species may be flying high during swarming and settle on tall trees for feeding and mating where adult sampling is not possible. Rodstrom and Brown (2017) also reported that *Polyphylla decemlineata* (Say) beetles settle at a height of 8-18 feet high in the trees for mating and feeding. Their feeding and mating behaviour also differed considerably from other scarabaeid beetles which generally return to soil at dawn, whereas in *Melolontha* beetles, it was interesting to observe that several beetles were observed to feed on host trees even during day time. Similar behaviour has also been reported in *Lepidiota stigma* (Fabricius) by Pathania (2014).

4.3 Population abundance and infestation of *Melolontha* grubs in different crop habitats

The white grubs feed on roots of a wide variety of cultivated as well as uncultivated plants. In general, underground parts of all plants are subjected to grub

feeding and show different types of damage (Chandel et al. 2015). Chandel and Kashyap (1997) reported that white grubs inflict heavy damage on various vegetables, fruit/ forest nurseries, lawns and field crops in Himachal Pradesh. In the present survey, data on *Melolontha* grub damage were recorded on potato, rajmash, cole crops, onion and apple at seven locations of Himachal Pradesh.

4.3.1 Abundance and infestation of *Melolontha* grubs in potato

Total seven locations were surveyed from Kangra, Mandi, Chamba, Kullu and Kinnaur districts of Himachal Pradesh during 2017 and 2018 to ascertain the population of *Melolontha* grubs and magnitude of losses inflicted by them in potato. The maximum tuber damage was recorded at Badagaon (41.25-47.5 %). Very high population of *Melolontha* grubs was observed at Badagaon, ranging from 16-26 grubs/ ft³ and 0-23 grubs/ ft³ in 2017 and 2018, respectively (Table 4.13). At Kothi kohar and Barot, the tuber

Table 4.13 Abundance and infestation of *Melolontha* grubs in potato fields during 2017 and 2018

District	Location	Grub population /ft ³ in 2017		Tuber infestation (%)	Grub population / ft ³ in 2018		Tuber infestation (%)
		Range (Mean±SE)	Frequency (%)		Range (Mean±SE)	Frequency (%)	
Kangra	Badagaon	16-26 (20.1±1.04)	100.0	47.5 (43.5) ^a	0-23 (17.5 ±2.05)	90.0	42.5 (40.60) ^a
	Kothi Kohar	0-18 (11.5 ±1.67)	90.0	41.25 (39.78) ^a	0-23 (9.40 ±2.54)	70.0	37.5 (37.14) ^a
Mandi	Barot	0-14 (10.8 ±1.30)	90.0	36.25 (36.57) ^{ab}	0-18 (7.70 ±2.49)	60.0	32.5 (33.73) ^{ab}
	Janjehli	0-9 (2.90 ±1.02)	70.0	27.5 (31.25) ^{bc}	0-5 (1.80 ±0.53)	70.0	23.75 (28.76) ^{bc}
Chamba	Ahla	0-5 (1.50 ±0.60)	50.0	23.75 (28.61) ^c	0-3 (1.10 ±0.46)	40.0	18.75 (25.20) ^c
Kullu	Chhattanseri	0-12 (4.10 ±1.44)	80.0	12.5 (17.55) ^c	0-8 (3.90 ±1.03)	70.0	15.0 (21.51) ^c
Kinnaur	Sangla	0-9 (2.50 ±1.08)	60.0	22.5 (27.99) ^d	0-6 (2.20 ±0.81)	70.0	23.75 (28.91) ^{bc}
CD (p=0.05)				(7.71)			(7.96)

Figures in parentheses are arcsine transformed values

infestation recorded to be 41.25 and 36.25 per cent during 2017, and in 2018, the per cent tuber damage was 37.5 and 32.5 per cent. At these two locations, the mean grub population was found to be 11.5 ± 1.67 grubs/ ft³ and 10.8 ± 1.30 grubs/ ft³ in 2017 and in 2018; the grub population averaged 9.40 ± 2.54 grubs/ ft³ and 7.70 ± 2.49 grubs/ ft³. The average grub population at Janjehli, Sangla, Ahla and Chhattanseri was recorded to be 1.80



(a)



(b)



Plate 4.1 Infestation by *M. cuprescens* grubs in potato tubers (a) Tuber damaged by second instar grubs; (b) Tubers damaged by third instar grubs

± 0.53 - 2.90 ± 1.02 grubs/ ft^3 , 2.20 ± 0.81 - 2.50 ± 1.08 grubs/ ft^3 , 1.10 ± 0.46 - 1.50 ± 0.60 grubs/ ft^3 and 3.90 ± 1.03 - 4.10 ± 1.44 grubs/ ft^3 with corresponding tuber infestation of 23.75-27.5 per cent, 22.5-23.75 per cent, 18.75-23.75 per cent and 12.5-15.0 per cent during 2017-18. The frequency of occurrence varied between 90-100 per cent during 2017-18 at Badagaon. Pathania (2014) has reported that, *Melolontha* sp. grubs predominated in potato fields in Badagaon and Barot areas and inflicted tuber damage by forming single large cavity in the tubers (Plate 4.1), which were filled with the soil. Chandel et al. (2010) also mentioned similar nature of damage of *Melolontha* spp. on potato in Himachal Pradesh.

4.3.2 Abundance and infestation of *Melolontha* grubs in cole crops

In cole crops, especially cabbage, field observations on abundance and infestation of grubs were recorded during May-June in Badagaon, Kothi Kohar, Barot, Benchi and Sangla areas. In these areas, cole crops are grown as 'off season' crops from May-September. Perusal of the data in table 4.14 indicated considerable plant mortality as a result of *Melolontha* grub infestation at all the locations. The grub damage was noticed in

Table 4.14 Abundance and infestation of *Melolontha* grubs in cole crops during 2017 and 2018

District	Location	Grub population / ft^3 in 2017		Plant infestation (%)	Grub population / ft^3 in 2018		Plant infestation (%)
		Range (Mean $\pm$ SE)	Frequency (%)		Range (Mean $\pm$ SE)	Frequency (%)	
Kangra	Baragran	0-35 (14.6 $\pm$ 3.86)	80.0	26.25 (5.05) ^a	0-23 (13.4 $\pm$ 2.40)	90.0	28.75 (5.35) ^a
	Kothi Kohar	0-24 (10.5 $\pm$ 2.41)	80.0	22.5 (4.55) ^a	0-19 (5.80 $\pm$ 2.14)	50.0	21.25 (4.39) ^{ab}
Mandi	Barot	0-9 (4.70 $\pm$ 1.33)	60.0	17.5 (4.01) ^{ab}	0-8 (2.80 $\pm$ 1.15)	40.0	12.5 (2.99) ^b
Kullu	Benchi	0-8 (2.60 $\pm$ 1.09)	40.0	11.25 (3.17) ^b	0-9 (1.60 $\pm$ 0.97)	30.0	11.25 (2.89) ^b
Kinnaur	Sangla	0-5 (1.30 $\pm$ 0.47)	70.0	16.25 (4.04) ^{ab}	0-3 (0.80 $\pm$ 0.39)	40.0	12.5 (3.32) ^b
CD (p=0.05)				(1.18)			(1.51)

Figures in parentheses are square root transformed values

25-30 days old plants. Maximum plant mortality as a result of *Melolontha* grub attack was recorded at Badagaon (26.25-28.75 %), followed by Kothikohar (21.25-22.5%), Barot (12.5-17.5 %), Sangla (12.5-16.25 %) and Benchi (11.25 %). The mean population of



(a)



(b)

Plate 4.2 Infestation by *M. virescens* (= *P. fullo*) grubs in cabbage
(a) Wilted plant; (b) Roots pruned by grubs

grubs was estimated to be 13.4 ± 2.40 - 14.6 ± 3.86 , 5.80 ± 2.14 - 10.5 ± 2.41 , 2.80 ± 1.15 - 4.70 ± 1.33 , 0.80 ± 0.39 - 1.30 ± 0.47 and 1.60 ± 0.97 - 2.60 ± 1.09 grubs/ ft³ during 2017-18, respectively. The *Melolontha* grubs were present near the root zone of the attacked plants. The infested plants showed stunted growth, which could be easily pulled out, and their roots were found to be totally pruned by the grubs (Plate 4.2). According to Pathania (2014), the leading species responsible for causing plant mortality in cabbage/cauliflower were *Melolontha* spp. in different parts of Kinnaur and Chamba districts. Mehta et al. (2008) observed total failure of cabbage due to attack of whitegrubs in Chotta Bhangal area of Kangra district during July-August.

4.3.3 Abundance and infestation of *Melolontha* grubs in rajmash

Rajmash is being cultivated in hilly areas of Himachal Pradesh during May-September. In rajmash, field survey was carried out at four locations in Kangra, Mandi and Kinnaur districts. At all the locations, the predominant species attacking rajmash was identified as *M. virescens* (= *P. fullo*). At Badagaon, the population of *M. virescens* (= *P. fullo*) grubs averaged 3.50 ± 1.38 grubs/ ft³ (range: 0-14 grubs/ ft³) and 2.60 ± 0.99 grubs/ ft³ (range: 0-9 grubs/ ft³), and the plant infestation as a consequence to attack of grub ranged from 18.75-20.0 per cent during 2017-18 (Table 4.15). At Kothi Kohar, Barot and Sangla, the mean grub population was found to range from 1.50 ± 0.50 - 1.90 ± 0.62 , 1.0 ± 0.49 - 1.60 ± 0.76 and 0.40 ± 0.27 - 0.50 ± 0.34 grubs/ ft³ during 2017-18. The corresponding plant infestation was recorded to be 8.75, 2.5-7.5 and 6.25-7.5 per cent, respectively. The grubs attacked rajmash plants at 4-5 leaf stage in Chuhar valley. The grubs inflicted damage by feeding on the root system at a depth of about 10 cm, causing sudden wilting and death of rajmash plants (Plate 4.3). According to Pathania (2014), the grubs of *M. virescens* completely destroyed root system leading to death of plants.

Table 4.15 Abundance and infestation of *Melolontha* grubs in Rajmash during 2017 and 2018

District	Location	Grub population /ft ³ in 2017		Plant infestation (%)	Grub population / ft ³ in 2018		Plant infestation (%)
		Range (Mean $\pm$ SE)	Frequency (%)		Range (Mean $\pm$ SE)	Frequency (%)	
Kangra	Baragaon	0-14 (3.50 $\pm$ 1.38)	70.0	20.0 (4.33) ^a	0-9 (2.60 $\pm$ 0.99)	60.0	18.7 (4.15) ^a
	Kothi Kohar	0-6 (1.90 $\pm$ 0.62)	60.0	8.75 (2.74) ^b	0-5 (1.50 $\pm$ 0.50)	70.0	8.75 (2.74) ^b
Mandi	Barot	0-6 (1.60 $\pm$ 0.76)	50.0	7.5 (2.45) ^b	0-6 (1.0 $\pm$ 0.49)	50.0	2.5 (1.29) ^c
Kinnaur	Sangla	0-3 (0.50 $\pm$ 0.34)	20.0	6.25 (2.16) ^b	0-2 (0.40 $\pm$ 0.27)	20.0	7.5 (2.45) ^{bc}
CD (p=0.05)				(1.32)			(1.28)

Figures in parentheses are square root transformed values



(a)



(b)

Plate 4.3 Infestation by *M. virescens* (= *P. fullo*) grubs in rajmash
(a) Wilted plant; (b) Grubs feeding on roots

4.3.4 Abundance and infestation of *Melolontha* grubs in onion

The attack was noticed in the month of February. The mean grub population was recorded to be 3.70 ± 1.14 grubs/ feet³ (range: 0-9 grubs/ feet³) and 2.10 ± 0.75 grubs/ feet³ (range: 0-6 grubs/ feet³) during 2017 and 2018, respectively (Table 4.16). The bulb damage as a result of grub attack was recorded to be 8.75 and 6.25 per cent during 2017 and 2018. Kaundal (2018) has reported bulb infestation in onion by grubs of *M. furcicauda* in Kullu valley.

Table 4.16 Abundance and infestation of *Melolontha* grubs in onion at Chhatanseri

Year	Number of grubs /ft ³			Plant infestation (%)
	Range	Mean $\pm$ SE	Frequency (%)	
2017	0-9	3.70 ± 1.14	60.0	8.75
2018	0-6	2.10 ± 0.75	50.0	6.25

4.3.5 Abundance of *Melolontha* grubs in apple

The data on abundance of *Melolontha* grubs in apple plants were recorded at six locations, viz., Chhatanseri, Raison, Dallash, Janjehli, Pangna and Sangla in three districts of Himachal Pradesh. The sampling was done in the basins of apple trees at each location. Very high population of grubs, ranging from 0-51 grubs/ feet³ (mean: 30.5 ± 5.57 grubs/ feet³) and 0-41 grubs/ feet³ (mean: 22.4 ± 5.15 grubs/ feet³) was recorded at Sangla during 2017 and 2018, respectively. At Chhatanseri and Pangna, the *Melolontha* grub population ranged from 0-49 grubs/ feet³ (average: 22.4 ± 3.22 - 31.2 ± 4.24 grubs/ feet³) and 0-42 grubs/ feet³ (average: 17.4 ± 2.28 - 24.70 ± 3.63 grubs/ feet³) during 2017-18. At Raison, Dallash and Janjehli, the mean population of *Melolontha* grubs was recorded to be 1.50 ± 0.56 - 3.9 ± 1.54 grubs/ ft³ in 2017, and 1.30 ± 0.52 - 3.30 ± 1.0 grubs/ ft³ in 2018. The frequency of occurrence at Pangna has been recorded to be 100 per cent in 2017, and 90 per cent in 2018. At Chhatanseri, the frequency of occurrence was found to be 90 per cent during both the years, whereas at other locations, the frequency of occurrence ranged between 50-80 per cent (Table 4.17). The grubs were observed feeding on small apple rootlets (Plate 4.4) and roots of other grasses or weed plants growing in the basin of the trees. The severally attacked plants showed partial drying of leaves and the fruits remained undersized (Plate 4.4). Altaf et al. (2019) reported *M. furcicauda* as a minor root feeding pest in apple nurseries in north Kashmir. According to Weissteiner et al. (2012), the *Melolontha* grubs show a pronounced preference for a variety of tree roots and are able to migrate as far as several meters through the soil towards their host plants. White grubs

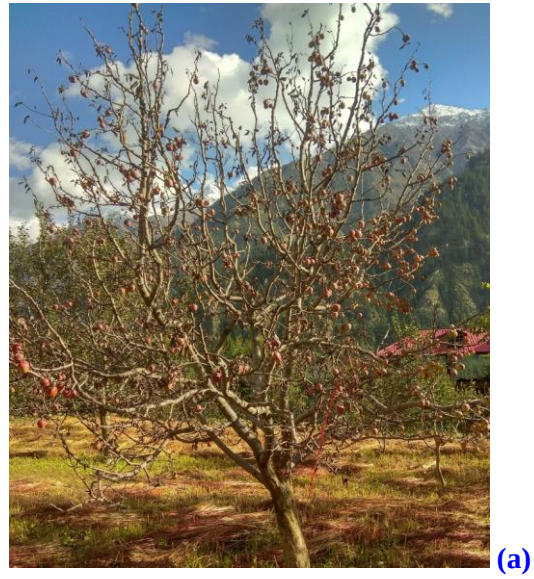


Plate 4.4 (a) Apple plant damaged by *P. fullo*; (b) Apple plant damaged by *M. indica*; (c) *M. cuprescens* grubs feeding on apple roots

generally feed on the roots of field crops and attack fruit/ forest trees, their nurseries, vegetables, lawns and field crops (Chandel and Kashyap 1997). The damage by grubs in unruly large numbers leading to wilting and drying of leaves is uncommon and rarely seen in Himachal Pradesh as observed in the present study.

Table 4.17 Distribution and abundance of *Melolontha* grubs in apple during 2017 and 2018

District	Location	Grub population /ft ³ in 2017			Grub population / ft ³ in 2018		
		Range	Mean±SE	Frequency (%)	Range	Mean±SE	Frequency (%)
Kullu	Chhatenseri	0-49	31.2 ± 4.24	90.0	0-42	24.70 ± 3.63	90.0
	Raison	0-5	1.50 ± 0.56	60.0	0-5	1.30 ± 0.52	60.0
	Dallash	0-14	3.9 ± 1.54	50.0	0-8	2.10 ± 1.02	50.0
Mandi	Janjehli	0-7	3.4 ± 0.82	80.0	0-9	3.30 ± 1.0	90.0
	Pangna	1-36	22.4 ± 3.22	100.0	0-24	17.4 ± 2.28	90.0
Kinnaur	Sangla	0-51	30.5 ± 5.57	80.0	0-41	22.4 ± 5.15	70.0

4.4 Biology and morphometrics of *M. cuprescens*, *M. furcicauda* and *M. indica*

4.4.1 Eggs: Adult emergence of *M. cuprescens* and *M. furcicauda* from soil started between second-third week of June, remained abundant throughout July-August months, and then perished in second week of September. The emergence of *M. indica* beetles commenced in first week of July, peak activity occurred during July-August and their activity continued up to last week of September in Himachal Pradesh. Pathania (2014) indicated emergence of *M. cuprescens* beetles in June at Kheradhar, Dallash and Kullu, and in July at Sangla and Holi. Svestka (2006) reported that swarming in *Melolontha hippocastani* Fab. begins during mid April to early May, and culminates 8-14 days after the flight of first beetle in Czech Republic. According to reports from Austria, *M. melolontha* and *M. hippocastani* begin to swarm together during April-May, but swarming of *M. hippocastani* culminates and ends earlier. Males prevail in the beginning of swarming and at the time of culmination, the male: female ratio gets more or less balanced in all *Melolontha* species (Kratochvil et al. 1953).

Copulation was observed under laboratory and field conditions. The male and female beetles mated in end to end position (Plate 4.5) and the duration of the coital phase lasted for 16-25 minutes in *M. cuprescens*, *M. furcicauda* and *M. indica*. In the present study, heavy intensive swarming was seen between July-September, and during this time interval intensive egg laying into soil was recorded. Field collected beetles of *M. cuprescens*, *M. furcicauda* and *M. indica* started laying eggs after 5-11 days of captivity



(a)



(b)



(c)

Plate 4.5 Mating pairs (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

under laboratory conditions at Palampur. Pathania (2014) also reported pre-oviposition period of 7-10 days for *M. cuprescens* in Himachal Pradesh. Vogel (1950) has reported egg maturation period of 7-10 days for *M. melolontha* in Switzerland, and these reports back up our results. Contrary to our results, Huiting et al. (2006) reported oviposition period of about 10-15 days for *M. melolontha* in Netherlands. Similarly, in case of *M. hippocastani*, the period of the first maturation feeding has been reported to last for 8-14 days, and the females fly off to lay eggs (Svestka 2006). In *M. cuprescens* and *M. furcicauda*, the egg laying was observed during July-August, whereas in *M. indica*, the egg laying was recorded between September to October in the present study. Svestka and Drapela (2009) reported egg laying by *M. hippocastani* during mid-May in Czech Republic, and in Germany, peak egg laying by *M. hippocastani* occurs during second week of May (Wagenhoff et al. 2014). In case of *M. melolontha*, the egg laying has been reported towards the end of May in China (Tsai 1965), and during mid-June in Switzerland and Netherlands (Keller et al. 1997; Huiting et al. 2006). All these reports are contradicting to our observations.

Female beetles deposited eggs inside the soil up to a depth of 16 cm. *Melolontha cuprescens* and *M. furcicauda* deposited eggs at a depth of 8-11 and 10-11 cm, respectively, whereas *M. indica* laid eggs more deep at a depth of 13-16 cm in soil. According to Keller et al. (1997), the females of *M. melolontha* burrow into the soil and deposit their eggs at a depth of 10-20 cm. In Florida, the females of *M. melolontha* have been reported to burrow into the soil up to 15 inches for oviposition (Woodruff 1978). Svestka (2007) reported that *M. hippocastani* females frequently lay eggs in clusters of 10-36 eggs in a depth of about 10-30 cm. How deeply they lay eggs perhaps depends on the looseness and type of soil.

The eggs were found without any earthen cavities in all *Melolontha* species, whereas most other species of white grubs like *Brahmina coriacea* (Hope), *H. longipennis*, *H. sikkimensis*, (Chandel et al. 1995; Kalpana 2015; Pathania 2014), *Holotrichia serrata* (Fab.) (Veeresh 1977) and *H. consanguinea* (Vishwanath 1979) lay their eggs in small earthen cavities which provide extra protection to the eggs. *Melolontha* beetles are quite large in size and deposit their eggs deeper in soil, whereas white grub species which are smaller in size generally deposit their eggs in upper soil layer where chances of desiccation and abruption are maximum. In deep soil layers, the eggs become less prone to desiccation and abruption, and thereby eliminate the need for earthen

cavities. The chorion of the eggs was very thin and delicate, and it was found to be damaged even at slight disturbance while removing them from the soil. They have a preference for soft soil where the female beetles quickly dug up to a depth of 15-20 cm. The soft soil was closed after the female has dug, and this place becomes hard to find. Svestka (2007) also reported that under same conditions, *M. melolontha* females lay their eggs deeper in the soil than those of *M. hippocastani* females which corroborates our findings.

The egg laying occurred in batches, and 3-19 eggs per batch were observed in different *Melolontha* species in the present study. There was sufficient space between the two eggs inside the soil. The eggs were oval in all the species, but the colour of eggs was found to be variable in different species. In *M. cuprescens*, the eggs were shiny milky white, and in *M. furcicauda*, the colour was slightly dirty white. However, in case of *M. indica*, there was more tinge of yellow, and the eggs appeared creamish to slightly yellowish in colour (Plate 4.6). The eggs of *M. melolontha* have also been reported to be dirty white in colour (Anonymous 2009). According to Sreedevi et al. (2015), egg size, shape and colour vary among species, and the chorion microsculpturing exhibits variation among and within genus. The length/ width ratio has been reported to be comparatively higher in *Lepidiota mansueta* Burn. (1.43), and this value is close to length/ width ratio (1.36-1.40) computed in the present study for *M. cuprescens*, *M. furcicauda* and *M. indica*.

The eggs of *M. indica* were smaller in size. The average length x width of newly laid eggs of *M. indica* was measured to be $3.63 \pm 0.02 \times 2.60 \pm 0.09$ mm. The newly laid eggs of *M. furcicauda* measured from 3.70-4.24 mm in length and 2.63-3.16 mm in width. The eggs of *M. cuprescens* were significantly larger in size as compared to *M. furcicauda* and *M. indica*. The average length x width of newly laid eggs of *M. cuprescens* was recorded to be $4.58 \pm 0.06 \times 3.33 \pm 0.04$ mm. The observations recorded on length x width of *M. cuprescens* are in agreement with the findings of Pathania (2014), where the newly laid eggs of *M. cuprescens* were 4.62 ± 0.04 mm x 3.5 ± 0.06 mm in length x width, and near to hatching, the length x width of eggs was measured to be $5.31 \pm 0.06 \times 3.80 \pm 0.03$ mm. Huiting et al. (2006) have reported dimensions of oval eggs of *M. melolontha* as 2 x 3 mm in Netherlands. With advancement in embryonic development, the shape of eggs changed from elongate to spherical prior to hatching in about 15-20 days old eggs. The length x width of 20 days old eggs of *M. cuprescens* ranged from 5.11-5.50 x 3.61-3.80



Newly laid eggs



Prior to hatching

(a)



Newly laid eggs



Prior to hatching

(b)



Newly laid eggs



Prior to hatching

(c)

Plate 4.6 Eggs (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

mm. In *M. furcicauda* and *M. indica*, the average length x width prior to hatching (20 days for *M. furcicauda*; 25 days for *M. indica*) was recorded to be $5.23 \pm 0.06 \times 3.81 \pm 0.02$ mm and $4.49 \pm 0.05 \times 3.20 \pm 0.05$ mm, respectively (Table 4.18). This increase in egg size could be due to absorption of water from the surrounding soil or entrapping of the air in the egg shell through thin elastic chorion during embryonic development. Such an increase in size with the age of eggs has been noticed in *M. melolontha* by Huiting et al. (2006) who reported an enlargement of eggs by water absorption. Keller (2000) and Pathania (2014) also reported that after absorption of water, the elongated oval eggs of *M. melolontha* and *M. cuprescens* attained spherical shape. In *M. cuprescens*, the duration of egg stage ranged from 19-22 days with an average of 20.3 ± 0.52 days. Pathania (2014) recorded incubation period ranging from 16-20 days for *M. cuprescens* in Himachal Pradesh. There was an increase in incubation period with decrease in egg size among the studied species. The mean incubation period for *M. furcicauda* was recorded to be 23.5 ± 0.48 days (range = 22-25 days). In case of *M. indica*, the incubation period was found to be longest ranging from 24-30 days with an average of 26.5 ± 0.92 days (Table 4.19).

Table 4.18 Size of eggs of *M. cuprescens*, *M. furcicauda* and *M. indica*

Size	Stage	<i>M. cuprescens</i>		<i>M. furcicauda</i>		Stage	<i>M. indica</i>	
		Range	Mean $\pm$ SE	Range	Mean $\pm$ SE		Range	Mean $\pm$ SE
Length (mm)	Newly laid	4.31-4.89	4.58 $\pm$ 0.06	3.70-4.24	3.94 $\pm$ 0.06	Newly laid	3.55-3.68	3.63 $\pm$ 0.02
	20 days old	5.11-5.50	5.29 $\pm$ 0.05	4.99-5.46	5.23 $\pm$ 0.06	25 days old	4.25-4.71	4.49 $\pm$ 0.05
Width (mm)	Newly laid	3.18-3.51	3.33 $\pm$ 0.04	2.63-3.16	2.90 $\pm$ 0.06	Newly laid	2.22-3.01	2.60 $\pm$ 0.09
	20 days old	3.61-3.80	3.70 $\pm$ 0.02	3.73-3.88	3.81 $\pm$ 0.02	25 days old	2.98-3.40	3.20 $\pm$ 0.05

In closely related species like *M. melolontha*, the incubation period has been observed to be of 4-6 weeks in Netherlands (Huiting et al. 2006), 5-7 weeks in Switzerland (Keller et al. 1997), 40-50 days in Russia (Anonymous 2009) and 6-7 weeks in Florida. Svestka (2007) also recorded incubation period of 40-50 days for *M. hippocastani* in Czech Republic. Svestka (2006) reported that the development of eggs from the time of their laying until the larval hatch depends on the temperature and humidity of the soil.

Table 4.19 Incubation period of *M. cuprescens*, *M. furcicauda* and *M. indica*

Species	Duration of egg stage in days		Period of oviposition
	Range	Mean $\pm$ SE	
<i>M. cuprescens</i>	19-22	20.3 $\pm$ 0.52	July-August
<i>M. furcicauda</i>	22-25	23.5 $\pm$ 0.48	July-August
<i>M. indica</i>	24-30	26.5 $\pm$ 0.92	August-September

4.4.2 Larva: Body movement of grubs inside the eggs was clearly visible under the microscope after 20-25 days of egg laying. The egg membrane appeared comparatively thin and papery at this stage. The young larvae ruptured the papery egg shell by vigorous body movement of its appendages, and gradually crawled out of it. The newly hatched grubs were milky-white in colour with shiny tips of mandibles and translucent head. The head turned reddish brown after 3-5 days. The grubs became active in about 2-4 hours, and entered in the soil. In the present study, the first instar grubs were reared on tender maize and rajmash seedlings in small cups. They seldom moved away from the root zone.

To complete post eclosion development, the *Melolontha* grubs moulted two times and thus has three distinct instars as shown by the width of head capsule. Ritcher (1958) reported that all scarabaeids without exception undergo three larval stages. Emden (1941) states that the larvae of lamellicornia moult thrice, the third ecdysis releasing the pupa.

First instar: The first instar grubs were milky-white in colour with light yellowish head (Plate 4.7). After feeding for 3-4 days, all segments of the body became distinct from each other among themselves. The size prominence of head was also finished. The first instar grubs of *M. cuprescens* were observed to be comparatively larger in size as compared to *M. furcicauda* and *M. indica*. The fully fed first instar larvae of *M. cuprescens* measured 13.11-16.87 mm in length. The head capsule width ranged from 3.01-4.02 mm. The average body length of first instar grubs of *M. furcicauda* and *M. indica* was recorded to be 11.15 ± 0.37 and 13.49 ± 0.70 mm, respectively. The width of head capsule for first instar grubs of *M. furcicauda* and *M. indica* was observed to be 2.99-3.95 mm and 2.23-2.52 mm, respectively (Table 4.20). Pathania (2014) reported body length of fully fed first instar grubs of *M. cuprescens* as 15.98 ± 0.13 mm, and 2.63 ± 0.01 mm as head capsule width. Sukovata et al. (2015) gave the head capsule width of about 2.6-2.7 mm for first instar grubs of *Melolontha* species. Similarly, in case of *M. melolontha*, the width of head capsule in first instar grubs has been reported to be 2.7 mm (Huiting et al. 2006). In *M. hippocastani*, the width of head capsule of first instar has been recorded as 2.5 mm (Anonymous 2009). The present data on body length and head capsule width of *M. cuprescens*, *M. furcicauda* and *M. indica* are in confirmity with the earlier reports. After hatching, the young grubs started to gnaw the small roots of maize after about two days. Huiting et al. (2006) also reported that young larvae of *M. melolontha* start to gnaw the small roots directly after hatching and moves about horizontally distances of up to 30 cm/day. The larvae of *M. cuprescens*, *M. furcicauda* and *M. indica* became more active after about one week, and showed considerable horizontal movement in the soil. Huiting et al.



(a)



(b)



(c)

Plate 4.7 First instar grubs (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

(2006) reported that first instar grubs of *M. hippocastani* and *M. melolontha* are attracted to the plants by the CO₂ released from the roots, and olfactory and taste stimuli are used for orientation to short distances. After about one month, the first instar grubs started downward movement in the soil, and became inactive.

Under laboratory conditions, the duration of development for first instar grubs was found to be overlapping in all three *Melolontha* species in the present study (Table 4.21). The first instar grubs of *M. cuprescens* occupied 56-64 days (mean: 59.60 ± 1.29 days). In *M. furcicauda*, first instar grubs occupied 54-56 days with a mean of 54.80 ± 0.32 days. The duration of first instar grubs of *M. indica* has been recorded to range from 53-58 days, and average duration was calculated to be of 55.90 ± 0.83 days. Pathania (2014) has reported somewhat lesser duration of first instar grubs of *M. cuprescens* (24.8 ± 0.55 days) in Himachal Pradesh. Wille and Wildbolz (1953) observed variation in the duration of first instar grubs of *M. melolontha* from 33 to 41 days by changing temperature from 23.3°C to 18°C. Hurpin (1955) reared grubs of *M. melolontha* individually in soil in the laboratory, and observed that first instar averaged nine weeks at 20°C, and longer at 15°C which vindicates our findings. According to Wille and Wildbolz (1953), the first instar larvae of *M. melolontha* do not survive until their first moult if the temperature either below 12°C or above 32°C. In the present study, the ambient temperature during the period of observations varied between 13.8°C and 29°C. The first instar grubs of *M. cuprescens* and *M. furcicauda* occurred from August-October, whereas, in *M. indica*, the first instar lasted from October-December. The fully fed first instar grubs stopped feeding after about 1½ month, and became inactive. In laboratory at Palampur, the first instar grubs of *M. cuprescens* and *M. furcicauda* moulted into second instar during the month of October, whereas in case of *M. indica*, moulting of first instar was observed during December.

Second instar: The second instar grubs resembled to that of first instar to some extent except size. The last abdominal segment was more enlarged in size to some extent. The body colour of *M. cuprescens* and *M. indica* grubs during second instar was observed to be dirty white, whereas in *M. furcicauda*, the second instar grubs appeared slightly yellowish in colour. The head capsule was dark brown in all the species (Plate 4.8). The body length during second instar was almost in the same range for all three studied species. In *M. cuprescens*, the body length was observed to range from 22.29-24.18 mm for full fed second instar grubs. The head capsule width varied from 4.28-5.19 mm. Pathania (2014) reported body length of second instar grubs of *M. cuprescens* ranging from 34.13-36.54 mm with average head capsule width of 4.74 ± 0.01 mm. The present



(a)



(b)



(c)

Plate 4.8 Second instar grubs (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

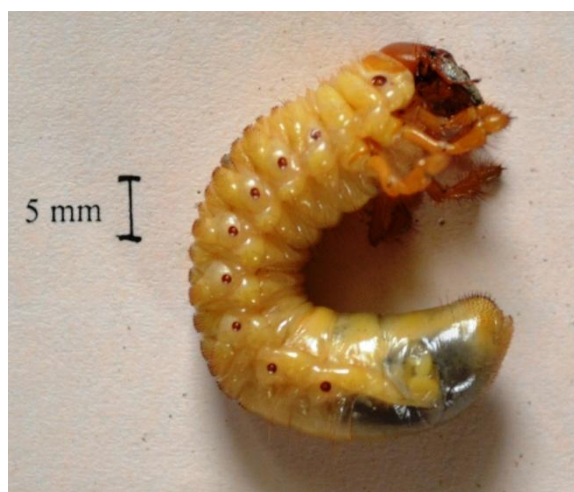
data on body length are on lesser side. It seems that this data on body length pertains to about two months old grubs, whereas in the present study, the data were recorded after about one month of first moult. The data on head capsule width show total agreement with our results. The second instar grubs of *M. furcicauda* were slightly larger in size as compared to *M. cuprescens* and *M. indica*. The average length of second instar grubs of *M. furcicauda* was recorded to be 23.20 ± 0.36 mm with a range of 23.38-24.08 mm. The average head capsule width was recorded to be 5.04 ± 0.09 mm. In case of *M. indica*, the body length varied from 21.87-23.57 mm with an average of 22.61 ± 0.35 mm. The width of head capsule averaged 4.63 ± 0.20 mm (range: 4.18-5.25 mm) as shown in table 4.20. In closely related species like *M. hippocastani*, the width of head capsule of second instar grubs has been reported to be 4.0 mm, which corroborates our findings (Anonymous 2009). Sukovata et al. (2015) reported head capsule width of about 4.2-4.5 mm for second instar grubs of *Melolontha* species, which finds distinct overlap with our data.

In *M. cuprescens*, the growth ratio (GR) for head capsule width was calculated to be 1.34 for second instar as compared with the first instar. The corresponding values of GR in *M. furcicauda* and *M. indica* have been computed to be 1.57 and 1.95, thus indicating that there exist considerable variations in the growth ratio in different *Melolontha* species. Pathania (2014) reported this value to be 1.80 for *M. cuprescens*. However, in *M. melolontha*, the head capsule width as reported by Huiting et al (2006) is 2.7 and 4.5 mm for first and second instar grubs and the growth ratio (GR) for second to first instar comes out to be 1.67. Similarly, in case of *M. hippocastani*, the head capsule width has been reported to be 2.6 and 4.2 for first and second instars grubs, respectively, and the growth ratio (GR) for second to first instar was 1.62. However, in *H. serrata*, this value has been reported to be 2.7 by Veeresh (1997). The ratio of body length to head capsule width has been worked out to be 4.71, 4.60 and 4.88 in *M. cuprescens*, *M. furcicauda* and *M. indica*, respectively. Yakir et al. (1996) reported that in second instar grubs of *Maladera matrida* Argaman, the ratio of body length to head capsule width was below 3.0 in Israel, whereas in case of *M. insanabilis*, the ratio of body length to head capsule width has been worked out to be 4.68 in Himachal Pradesh (Gupta 2019) which validates our findings.

During the course of our observations, the first moult occurred in the same year (2017) that the egg hatched, while second moult occurred in the following year (2018). In case of *M. cuprescens* and *M. furcicauda*, the second instar grubs occurred between October, 2017 to May, 2018, while in *M. indica*, the second instar grubs were recorded

between December, 2017 to July, 2018 under laboratory conditions at Palampur. The duration of second instar lasted for 226-230 days and 218-224 days in *M. cuprescens* and *M. furcicauda*, respectively. In *M. indica*, it ranged from 234-237 days with an average of 235.30 ± 0.39 days (Table 4.21). The duration of second instar in the present study was relatively shorter as compared to that of 274.7 ± 1.05 days recorded for *M. cuprescens* by Pathania (2014) in Himachal Pradesh. In other related species, *Leucopholis lepidophora* Blanchard, second instar was completed in 60-110 days (Patil and Hapase 1991). The second instar grubs in all studied *Melolontha* species were quite active, feeding voraciously on roots of maize/rajmash, and potato tubers. In potato, the second instar grubs inflicted large deep holes in the tubers rendering them unfit for marketing. Second instar grubs constructed earthen cells and moulted inside these cells. According to Pathania (2014), the second instar larvae of *M. virescens* overwintered in earthen cells. Earlier workers have also reported the formation of earthen cells by second instar larvae in other related species of white grubs (Chandel et al. 1995).

Third instar: There was considerable increase in size in third instar. The second and third instar larvae did not show much variation in morphological characters. During this final instar, there was slight change in body colour, but the colour of head capsule remained dark brown. In *M. furcicauda*, the body colour was creamish-white to slightly yellowish, whereas third instar grubs of *M. cuprescens* were more yellowish in colour as compared to *M. furcicauda*. In case of *M. indica*, the third instar grubs appeared greyish-white in colour (Plate 4.9). The third instar grubs appeared bulky, robust and highly active. The body length of *M. cuprescens* and *M. furcicauda* showed distinct overlap, but the grubs of *M. indica* were significantly smaller in size as compared to these two species. The third instar grubs of *M. cuprescens* measured 51.12-58.20 mm in body length, and 7.68-8.99 mm in head capsule width. The body length of third instar grubs of *M. furcicauda* ranged from 50.11-53.39 mm with an average of 51.85 ± 0.63 mm. The head capsule width for third instar grubs of *M. furcicauda* was recorded to be 7.0 ± 0.05 mm (range: 6.89-7.13 mm). In case of *M. indica*, the third instar grubs measured 44.81 ± 1.82 mm (range: 40.13-50.03 mm), and width of head capsule was recorded to be 6.98-7.58 mm (mean: 7.34 ± 0.14) as indicated in table 4.20. According to Pathania (2014), the body length of third instar grubs of *M. cuprescens* ranged from 58.76-64.01 mm and the width of head capsule has been reported to be varying between 7.67 to 8.11 mm in Himachal Pradesh. In *M. hippocastani*, the width of head capsule of third instar grub has been reported to be 6.0 mm (Anonymous



(a)



(b)



(c)

Plate 4.9 Third instar grubs (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

2009). The present data are in total agreement with these reports. Stebbing (1914) has reported *Melolontha* ? sp. attacking deodar in western Himalaya having body length in the range of 38.1-50.8 mm, and the colour of these grubs has been reported to be yellowish white. These observations on body length and colour are similar to our observations recorded for *M. indica* and *M. cuprescens* in the present study. In *M. melolontha*, the body length and head capsule width of third instar grubs have been reported to be 40-46 mm and 6.9 mm in Netherlands (Huiting et al. 2006).

The growth ratio (GR) for head capsule width of *M. cuprescens* and *M. indica* was on higher side as compared to *M. furcicauda*. The GR ratio for head capsule width of *M. cuprescens* and *M. indica* was calculated to be 1.67 and 1.59 for the third instar as compared with the second instar, respectively. The corresponding value of GR for head capsule width of *M. furcicauda* has been calculated to be 1.39. The overall growth ratio

Table 4.20 Body length and head capsule width for grubs of different *Melolontha* spp.

Instar		Body length and head capsule width (mm)					
		<i>M. cuprescens</i>		<i>M. furcicauda</i>		<i>M. indica</i>	
		Length	Width	Length	Width	Length	Width
I	Range	13.11-6.87	3.01-4.02	10.01-12.33	2.99-3.95	11.94-15.75	2.23-2.52
	Mean $\pm$ SE	15.33 $\pm$ 0.66	3.65 $\pm$ 0.22	11.15 $\pm$ 0.37	3.21 $\pm$ 0.19	13.49 $\pm$ 0.70	2.37 $\pm$ 0.05
II	Range	22.29-24.18	4.28-5.19	22.38-24.08	4.85-5.35	21.87-23.57	4.18-5.25
	Mean $\pm$ SE	22.99 $\pm$ 0.32	4.88 $\pm$ 0.17	23.20 $\pm$ 0.36	5.04 $\pm$ 0.09	22.61 $\pm$ 0.35	4.63 $\pm$ 0.20
III	Range	51.12-58.20	7.68-8.99	50.11-53.39	6.89-7.13	40.13-50.03	6.98-7.58
	Mean $\pm$ SE	53.80 $\pm$ 1.24	8.18 $\pm$ 0.23	51.85 $\pm$ 0.63	7.0 $\pm$ 0.05	44.81 $\pm$ 1.82	7.34 $\pm$ 0.14

(GR) has been worked out to be 1.51, 1.48 and 1.77 for *M. cuprescens*, *M. furcicauda*, and *M. indica*, respectively. According to Brooks Dyar rule (Daly 1985), the increment in size of stable character is 1.4-1.6. The values obtained in the present study are on higher side for *M. indica*, but the values obtained for *M. cuprescens* and *M. furcicauda* falls within the normal range of 1.4-1.6. Chandel et al. (1995) also reported overall growth ratio (GR) of 1.74 for grubs of *B. coriacea* at Solan in Himachal Pradesh. Kalpana (2015) has calculated overall growth ratio (GR) for *H. longipennis* and *H. sikkimensis* as 1.72 and 1.73, respectively. In *H. serrata*, the overall growth ratio (GR) comes out to be 2.05 on the basis of head capsule width reported for instars I-III (Veeresh 1977). In case of *M. melolontha* and *M. hippocastani*, the overall growth (GR) ratio of 1.60 and 1.59 on the basis of head capsule width was reported for I-III instars by Huiting et al. (2006) in Netherlands. Thus there exists considerable variation in growth ratio (GR).

The duration of third instar grubs ranged from 325-329 days in *M. cuprescens*, 310-312 days in *M. indica* and 299-311 days in *M. furcicauda* in the present study. Average duration of third instar grubs was found to be 326.7 ± 0.58 , 310.60 ± 0.29 and 305.60 ± 1.88 days in *M. cuprescens*, *M. indica* and *M. furcicauda*, respectively. In *M. cuprescens*, third instar grubs occurred from May, 2018 to April, 2019, and in *M. furcicauda*, the third instar grubs occurred from May, 2018 to March, 2019. In *M. indica*, the third stage grubs were recorded between July, 2018 to June, 2019 in the present study (Table 4.21). The average longevity of third instar of *M. cuprescens* at Palampur has been reported to be of 325.1 ± 1.53 days by Pathania (2014). According to Burakowski (1993), the third instar grubs of *M. hippocastani* occupied 234 days at 18°C , and at 23.3°C , the duration of third instar was reduced to 121 days in Poland. Svestka and Drapela (2009) reported that third instar grubs of *M. hippocastani* occupy about 12 months from June to May. These reports clearly corroborated our findings.

The fully fed third instar grubs in all three species moved downward up to a depth of 15 cm, stopped feeding and constructed earthen cells near to the bottom of glass jars. The formation of earthen cells by third instar grubs was noticed in the month of December in *M. cuprescens* and *M. furcicauda*, and during January in *M. indica*. These cells provided protection against biotic and abiotic factors throughout winter months in north western Himalaya. Thus, it was the overwintering stage in these species. Third instar grubs of *M. cuprescens* were found to be infected with *Beauveria brongniartii* (Saccardo) Petch at Chhatanseri in Kullu valley. The diseased grubs showed white mycelium growth on their body, and the ellipsoid conidia were $2-3 \times 1.5-2.5 \mu\text{m}$ long.

The economic importance of white grubs is primarily due to the feeding activity of third instar grubs. The white grubs damage is extensive in a variety of crops (Chandel et al. 2015). Third instar grubs produced large irregular cavities deep into the potato tubers and such tubers were rendered unfit for marketing. Although the duration of third instar was about 11 months, the duration of active feeding period was about seven months during May-November in *M. cuprescens* and *M. furcicauda*. In Russia the duration of active feeding by third instar grubs of *M. melolontha* has been reported from May-September (Anonymous 2009). The grubs remained inactive inside earthen cells for about two months from December-February. Third instar grubs of *M. indica* were found to feed actively for about five months from August-December and then hibernated for about $2\frac{1}{2}$ months. The overwintering grubs of *M. furcicauda* and *M. cuprescens* resumed feeding



(a)



(b)



(c)

**Plate 4.10 (a) *M. cuprescens* grub infected with *B. brongniartii*;
(b) *B. brongniartii* isolated on PDA; (c) Conidia of *B. brongniartii***

activity during second and last week of February, whereas in case of *M. indica*, the overwintering grubs resumed their activity during second week of March. The larval stage in the present study was recorded from August, 2017 to April, 2019 (20.63 months) for *M. cuprescens*, August, 2017 to March, 2019 (19.67 months) for *M. furcicauda*, and October, 2017 to June, 2019 (20.17 months) for *M. indica*. According to Ritcher (1958), the larval stage is longest in white grubs and larvae are encountered more often in the soil. The total larval period ranged from 608-619 days in *M. cuprescens*, and 598-605 days in *M. indica*. In case of *M. furcicauda*, the total larval period varied from 572-590 days. Average total larval duration was little more in *M. cuprescens* (mean: 614.40 ± 1.51 days) as compared to *M. furcicauda* (mean: 581.30 ± 2.60 days) and *M. indica* (mean: 601.80 ± 0.94 days). Pathania (2014) reported total larval period of 610-631 days for *M. cuprescens* in Himachal Pradesh. These reports are well within the range for *M. cuprescens* obtained in the present study. Contrary to our findings, in *M. melolontha*, the total larval duration has been reported to be of 58 months in China (Tsai 1965), four to five years in Europe and USSR (Khramtsov 1979), and three years in United States (Cardiff 2001) and Austria (Laengle 2005). *Melolontha hippocastani* also required three to five years to complete its total larval period in Russia (Anonymous 2009).

Table 4.21 Larval period of *M. cuprescens*, *M. furcicauda* and *M. indica*

Stage	<i>M. cuprescens</i> (Duration in days)		<i>M. furcicauda</i> (Duration in days)		<i>M. indica</i> (Duration in days)	
	Mean $\pm$ SE (Range)	Period of Year	Mean $\pm$ SE (Range)	Period of Year	Mean $\pm$ SE (Range)	Period of Year
Instar I	59.60 $\pm$ 1.29 (54-64)	Aug 2017- Oct 2017	54.80 $\pm$ 0.32 (54-56)	Aug 2017- Oct 2017	55.90 $\pm$ 0.83 (53-58)	Oct 2017- Dec 2017
Instar II	228.10 $\pm$ 0.59 (226-230)	Oct 2017- May 2018	220.9 $\pm$ 0.85 (218-224)	Oct 2017- May 2018	235.3 $\pm$ 0.39 (234-237)	Dec 2017- July 2018
Instar III	326.7 $\pm$ 0.58 (325-329)	May 2018- April 2019	305.6 $\pm$ 1.88 (299-311)	May 2018- March 2019	310.6 $\pm$ 0.29 (310-312)	July 2018- June 2019
Total larval period	614.40 $\pm$ 1.51 (608-619)	Aug 2017- April 2019	581.30 $\pm$ 2.6 (572-590)	Aug 2017- March 2019	601.8 $\pm$ 0.94 (598-605)	Oct 2017- June 2019

Larval morphology

The larvae of all these species are of typically scarabaeiform, bearing a pair of thoracic legs on the C-shaped body. Certain structures of the larvae are of taxonomic importance and several workers have classified the Melolonthine beetles on the basis of larval characters (Hayes 1925; Boving and Craighead 1931; Gardner 1935; Emden 1941 and Ritcher 1966). Sreedevi et al. (2014) reported that raster and anal lobes of last abdominal

segments possess definite taxonomic significance that delineates the species of white grubs. The larval head is heavily sclerotized, bearing a pair of antennae and a set of chewing mouth parts directed ventrally.

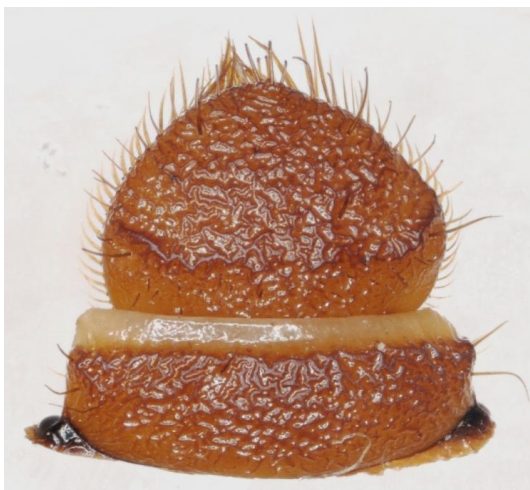
A detailed description of the mouth parts (clypeus, labrum, mandibles, maxilla, epipharynx, labium, hypopharynx), raster, spiracles, thoracic legs of the third instar larva and last abdominal segment of pupa is given below with the help of suitable diagrams.

Mouth parts

The mouth parts are of typical mandibulate type consisting of clypeus, labrum, epipharynx, a pair of mandibles, a pair of maxillae, labium and hypopharynx complex.

Clypeus and labrum: The clypeus is nearly trapezoidal in shape, bearing an anterior membranous band and posterior sclerotised area in all three *Melolontha* species. The posterior part of post clypeus is comparatively flatter in *M. indica*, whereas in *M. cuprescens* and *M. furcicauda*, it is more circular in outline (Plate 4.11; Fig. 4.18). The width of ante clypeus between postclypeus and labrum was more in *M. furcicauda* among three *Melolontha* species. The clypeus is furnished with two pairs of setae laterally and one pair centrally in *M. furcicauda* and *M. indica*. In case of *M. cuprescens*, the central pair of setae was absent. Jia et al. (2020) observed two pairs of setae on lateral sides and one pair in the centre of clypeus in *Melolontha incana* Motschulsky. The labrum is heart shaped, articulated distally to the clypeus, equipped symmetrically with three pairs of longer setae on dorsal surface, and two groups of shorter setae on proximal part in *M. indica*. In case of *M. cuprescens*, only two pairs of longer setae are present on dorsal surface alongwith two groups of shorter setae on proximal part. In *M. furcicauda*, three pairs of longer setae are present, but groups of shorter setae are missing.

Antennae: The antennae were four segmented, cylindrical with second and third segments comparatively longer in all three *Melolontha* species (Plate 4.12; Fig. 4.19). The basal segment possesses 11 prominent setae in *M. cuprescens*, seven in *M. indica* and five in *M. furcicauda*. The second segment is longest in all three species possessing one setae in *M. cuprescens* and *M. indica*, and five in *M. furcicauda*. The third segment bears an apical protrusion on the ventral surface. The protrusion is more prominent in *M. cuprescens*. This process tapers gradually, and is more curved in *M. cuprescens* as compared to *M. furcicauda*. In *M. indica*, it is slightly swollen in the middle and the tip is less pointed as compared to *M. cuprescens* and *M. furcicauda*. The distal antennal segment is cambiform



(a)



(b)



(c)

Plate 4.11 Clypeus and labrum (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

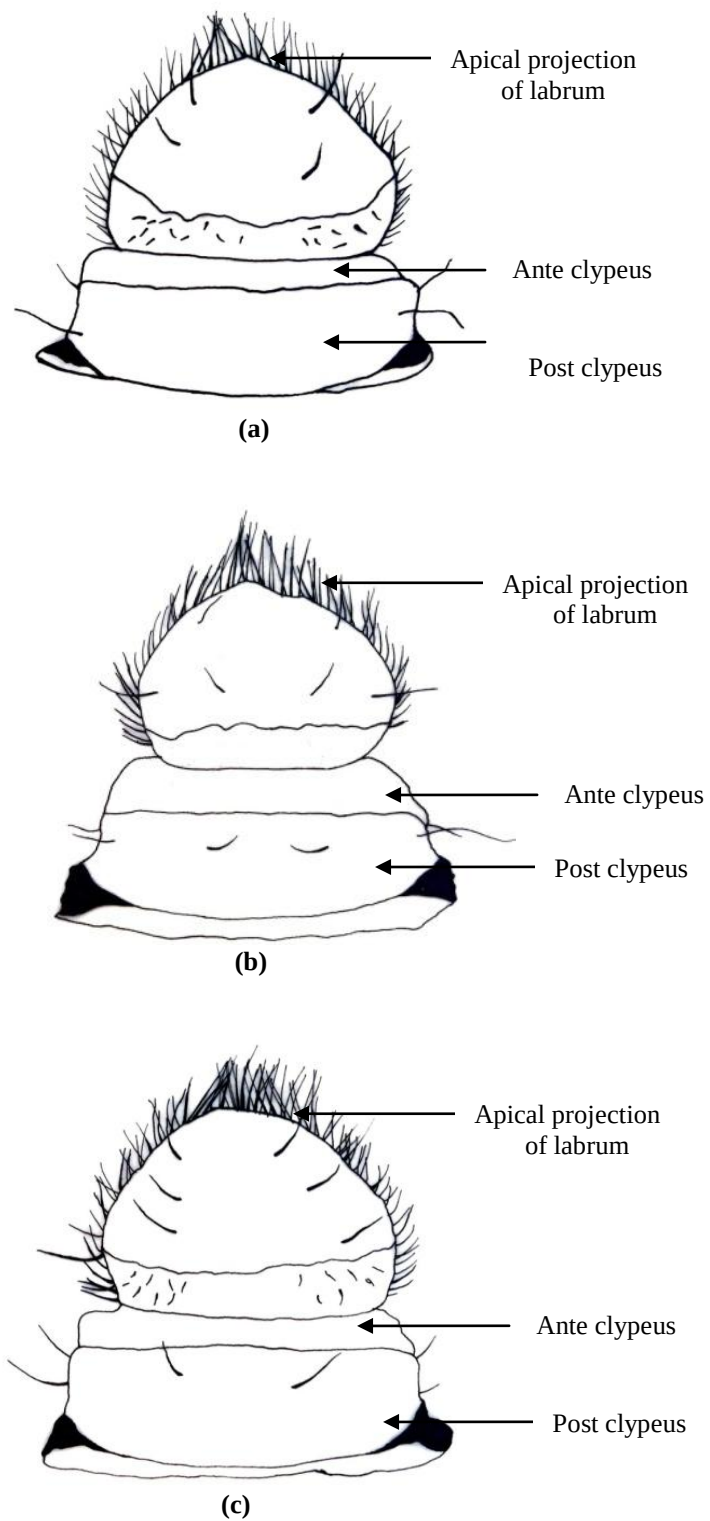
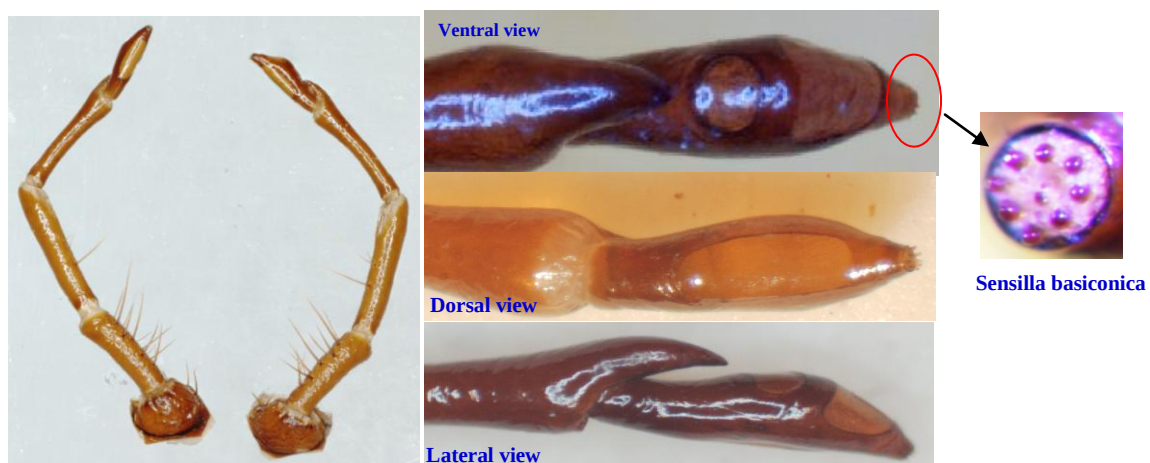
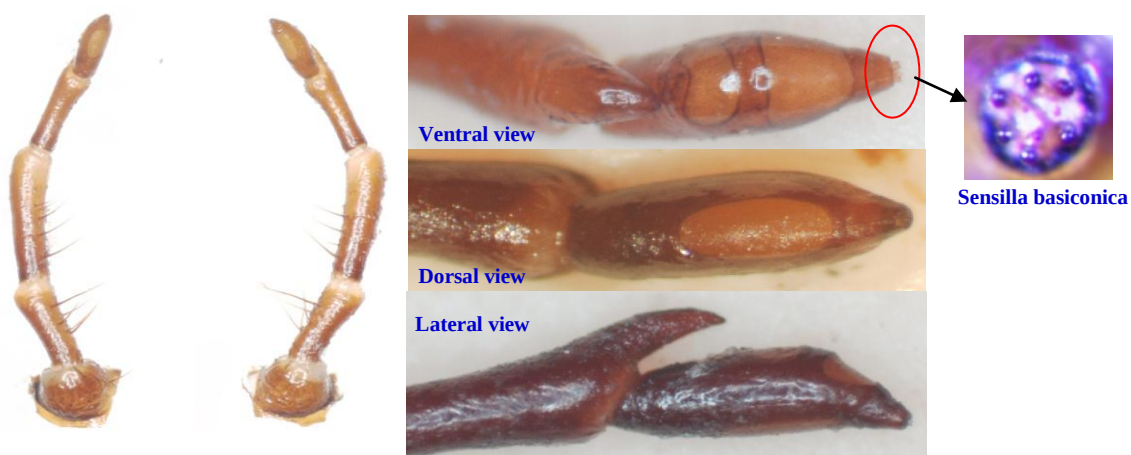


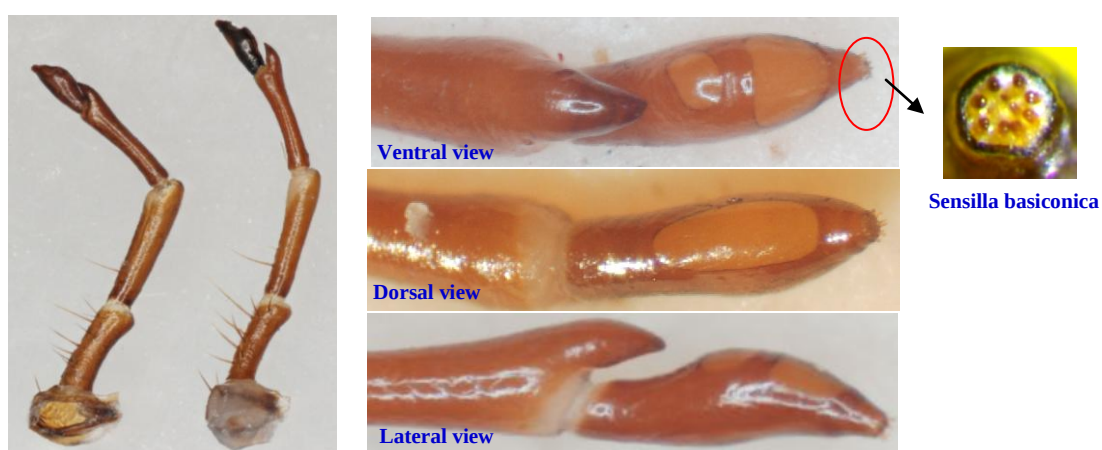
Fig 4.18 Clypeus and labrum (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*



(a)



(b)



(c)

Plate 4.12 Antennae of grub (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

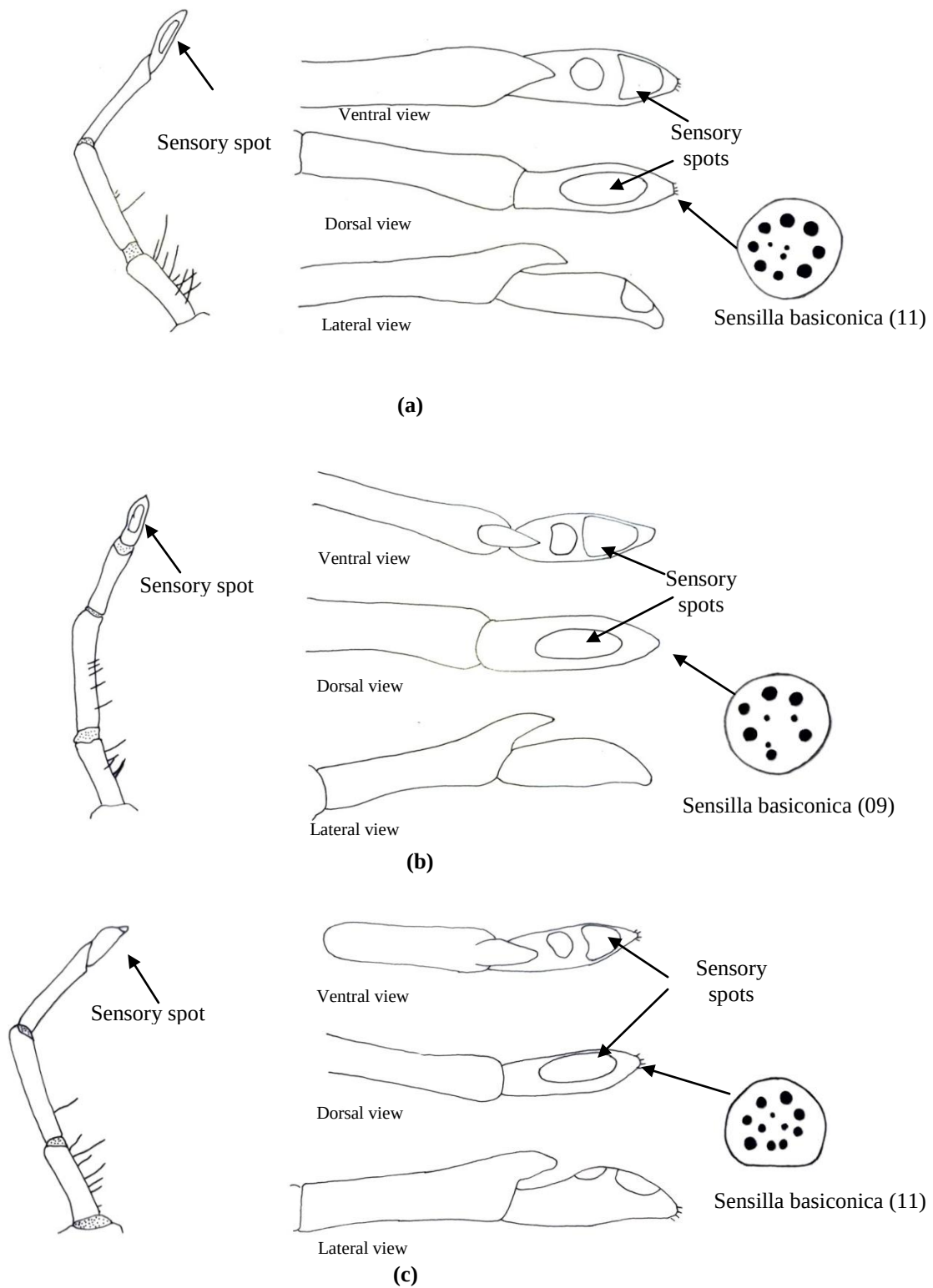


Fig. 4.19 Grub antennae (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

bearing a sensory plate on dorsal surface and a crown shaped sensory area at the apex. In *M. cuprescens*, the dorsal sensory plate is elliptical covering about $3/4^{\text{th}}$ of the area. On ventral surface, two sensory spots are present. The posterior one is circular in outline, and the anterior one is cone shaped. In case of *M. furcicauda*, the dorsal sensory plate is elliptical and covers about 60 per cent of the length of the segment. On ventral surface, two sensory areas were seen as shown in fig. 4.19. The dorsal sensory spot and sensory areas on ventral surface on terminal segment in *M. indica* are almost similar to that of *M. furcicauda* except posterior sensory spot on ventral surface which appears more oval in shape. The apical sensory area is furnished with sensilla basiconica, which are arranged in a circle. In case of *M. cuprescens* and *M. indica*, there were total 11 sensilla basiconica, whereas in *M. furcicauda*, only nine sensilla basiconica were present. The arrangement of sensilla basiconica on apical sensory area of last antennal segment also varies among these three species. In *M. cuprescens* and *M. indica*, eight distinct sensilla basiconica were present in a circle, and three discrete inside the circle. In case of *M. furcicauda*, only six prominent sensilla basiconica were present in a circle, and three discrete inside the circle. In *Melolontha incana* (Motschulsky), the apical sensory area is furnished with 11 sensilla basiconica, eight of which are comparatively thicker and are arranged in circle (Jia et al. 2020). The apical sensory area is circular in *M. cuprescens* and *M. furcicauda*, and it is flattened at one end in *M. indica*.

Epipharynx: The epipharynx appears more symmetrical in *M. indica* as compared to *M. cuprescens* and *M. furcicauda* (Plate 4.13). Epipharynx possesses a haptomerum on the apex, paired plegmatia on the lateral margins, paired chaetoparia on the central region, a haptolachus and a paired tormae on the basal part. The haptomerum is protuberant, having 18 heli, pointing posteriorly and six minute sensilla basiconica in *M. cuprescens*. In *M. indica*, the haptomerum bears 15 heli and eight sensilla basiconica (Fig. 4.20). In closely related species, *M. incana*, 14 heli (Jia et al. 2020), whereas in *M. melolontha*, 12 heli have been reported (Boving 1942). There is reduction in number of heli (7) and sensilla basiconica (3) in *M. furcicauda*. In *M. cuprescens*, each plegmatium comprises 18 parallel plegmata with inner end line up. The plegmata are of maximum length in the centre, and gradually the length decreases to the both sides. The plegmata are intertwined with same number of acanthopariae (18) pointed ventrally. In case of *M. indica*, 16 plegmata were present on each plegmatium, and accordingly 16 acanthopariae pointing ventrally were present. In *M. furcicauda*, the number of plegmata and acanthopariae were recorded to be



(a)

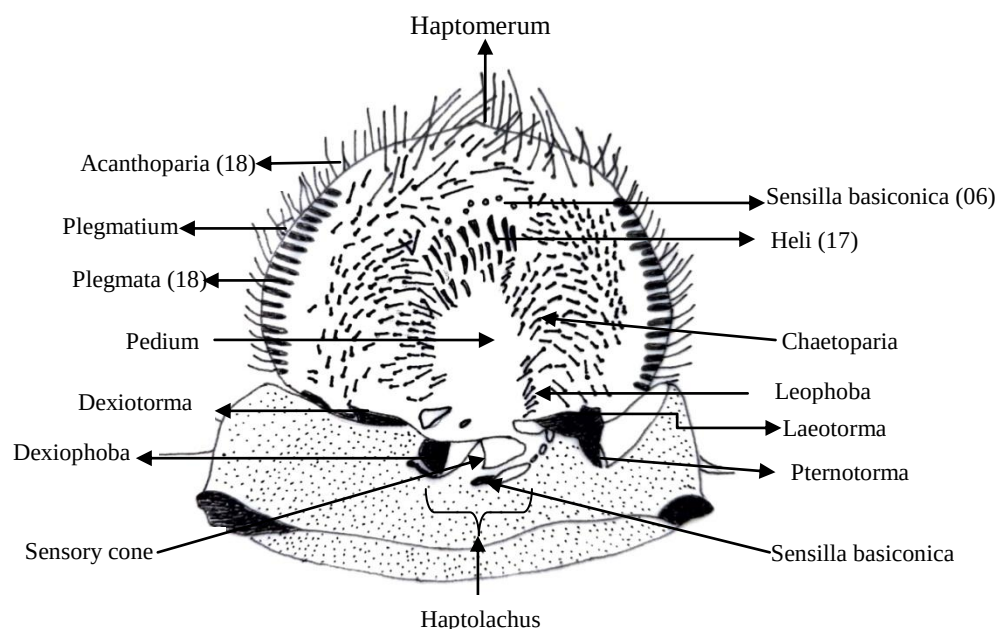


(b)

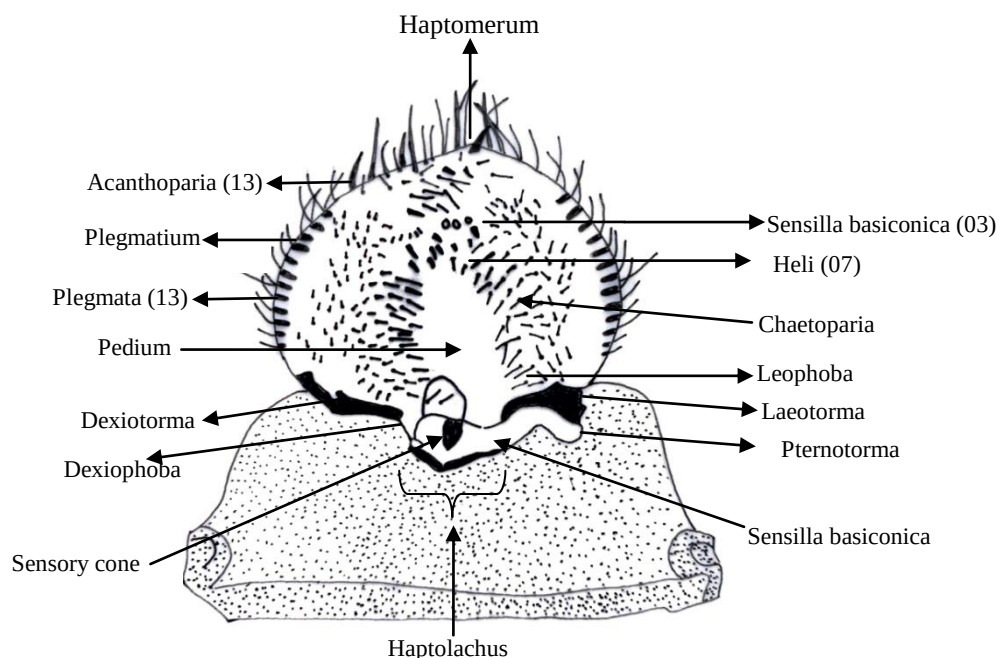


(c)

Plate 4.13 Epipharynx (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*



(a)



(b)

Fig 4.20. Epipharynx (a) *M. cuprescens*; (b) *M. furcicauda*

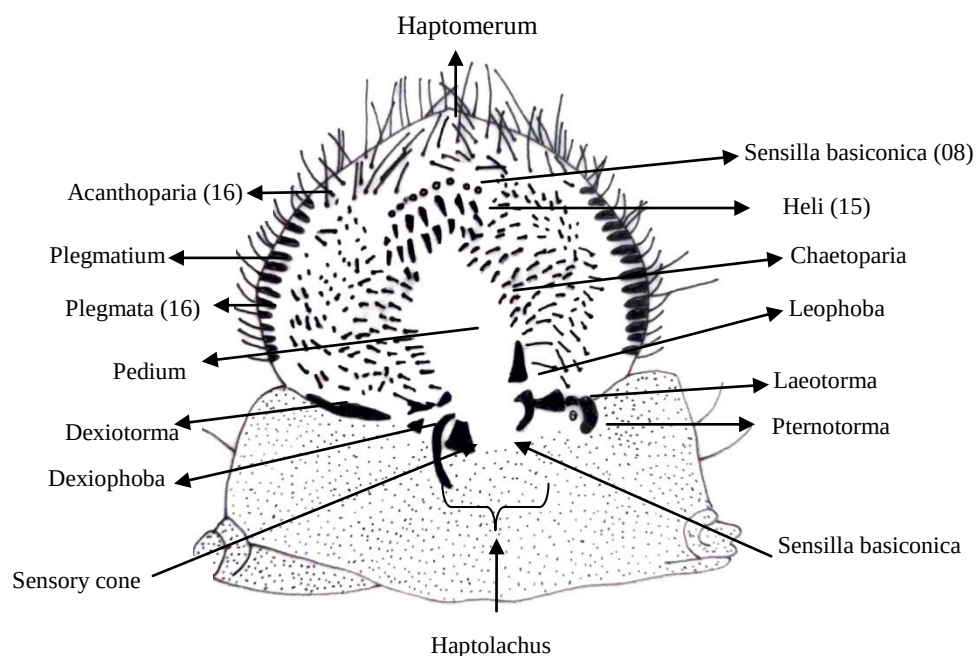


Fig. 4.21 Epipharynx of *M. indica*

13 (Plate 4.13; Fig. 4.21). Several workers have reported that epipharynx provides numerous valuable characters for the larval taxonomy in melolonthinae, mainly based on the number of heli on haptomerum, shape of plegmatia, and absence or presence of proplegmatia (Boving 1931; Ritcher 1966). The cryptic functions of these structures still need further anatomical studies. The pedium was narrow and oblong in *M. furcicauda*, whereas in *M. cuprescens* and *M. indica*, the pedium is broader and shape was more regular in *M. indica*. The haptolachus composed of a sclerotised plate, a sensory cone, several sensilla basiconica and a cluster of dexiophoba between the sclerotised plate and sensory cone. The dexiotorma is more or less straight in all the species and the leotorma was equipped with pternotorma. The pternotorma appears to be peg shaped and is directed posteriorly in *M. cuprescens*. In *M. furcicauda*, the pternotorma appears to be somewhat oval in shape, whereas in *M. indica*, the pternotorma is oval to oblong in shape.

Mandibles: The mandibles were distinct, and usually the left mandibles are considered for species description (Sreedevi and Tyagi 2013). The paired mandibles were darkish laterally and yellowish in the middle region. The yellowish patch is more prominent in case of *M. indica* (Plate 4.14). The mandibles are heavily sclerotized, strong, sub-triangular in outline, curved inward, each with a strong blade like scissorial area and a rather strong molar area. The scissorial area is equipped with a larger blade-like apical tooth and a smaller, rounded tooth following the scissorial notch. The scissorial notch was more distinct in *M. cuprescens* and *M. furcicauda*. The scissorial area is blackish with a nearly straight cutting edge in *M. cuprescens* and *M. furcicauda*. In *M. indica*, the cutting edge was slightly slanting inward. The width of scissorial area was recorded to be maximum in *M. indica* (1.58 mm), and minimum in *M. cuprescens* (1.04 mm). In case of *M. furcicauda*, the width of scissorial area was recorded to be 1.26 mm (Plate 4.14; Fig 4.22). The molar region is asymmetrical between the two mandibles, each bearing a group of large molar teeth that seems to be chimeric with each other. The length of molar area was recorded to be maximum in *M. furcicauda* (2.32 mm), followed by *M. cuprescens* (2.24 mm) and *M. indica* (1.89 mm). The inner edge of the smaller scissorial area was concave in shape and there was a gap of 1.71 mm in between scissorial and molar structure in *M. cuprescens*. The corresponding values in *M. furcicauda* and *M. indica* have been recorded to be 1.83 mm and 1.72 mm, respectively. The base of the mandible was of minimum width in *M. cuprescens* (3.08 mm). In *M. furcicauda*, the width of mandible base measured to be 3.12 mm. In case of *M. indica*, the base of mandible was of maximum

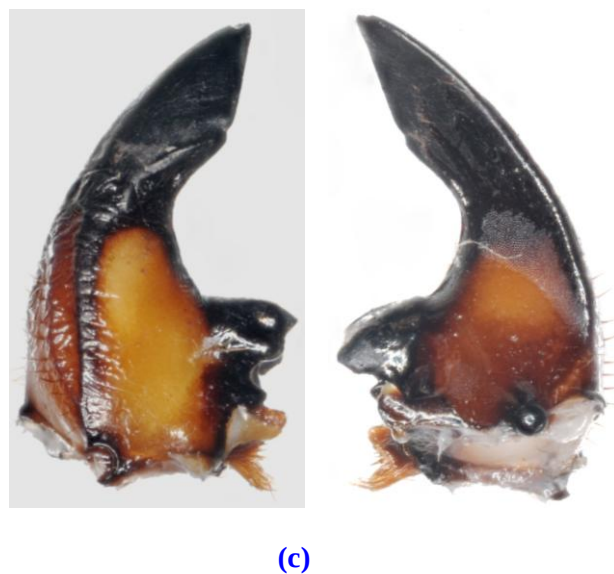
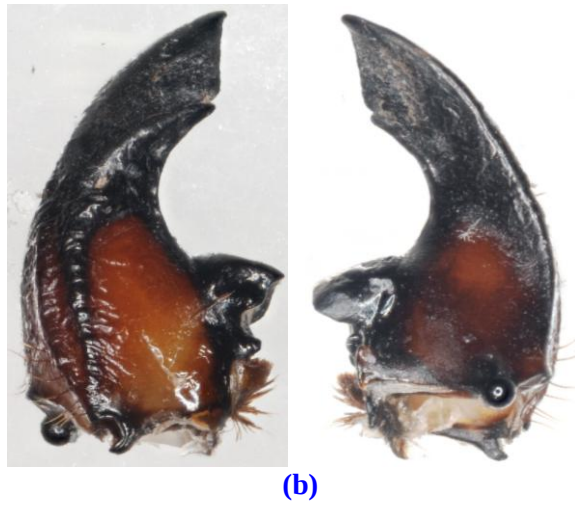
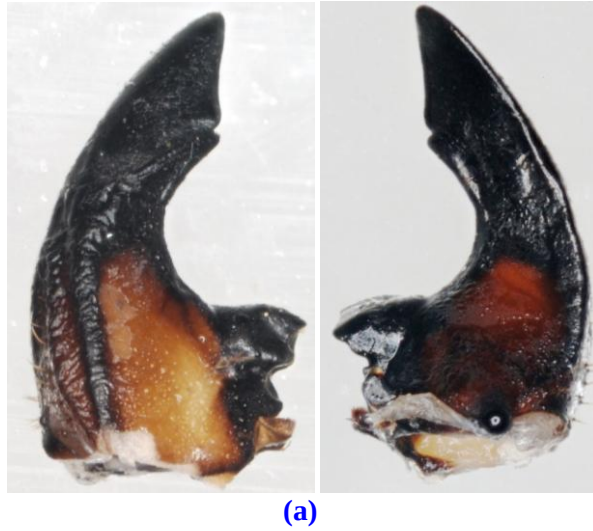


Plate 4.14 Mandibles (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

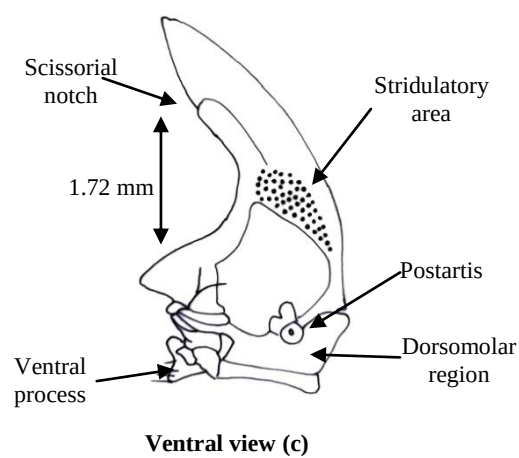
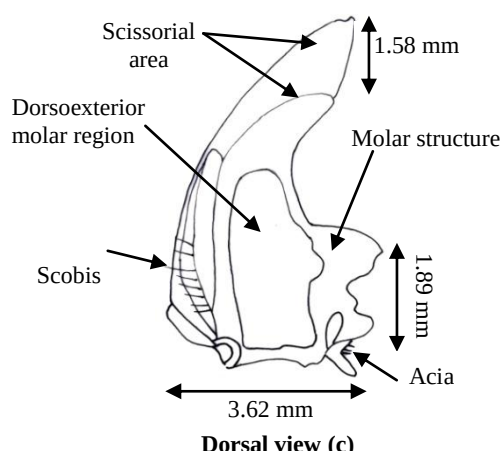
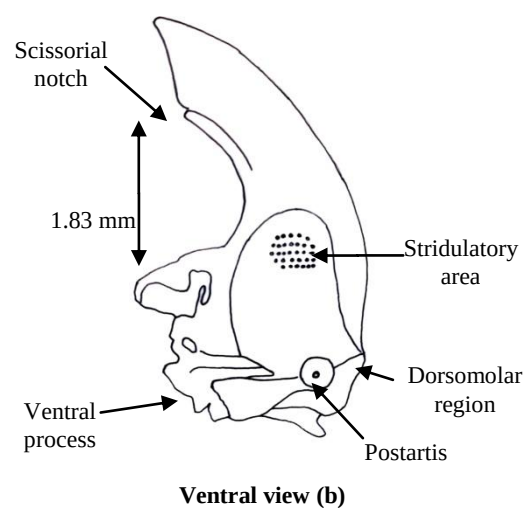
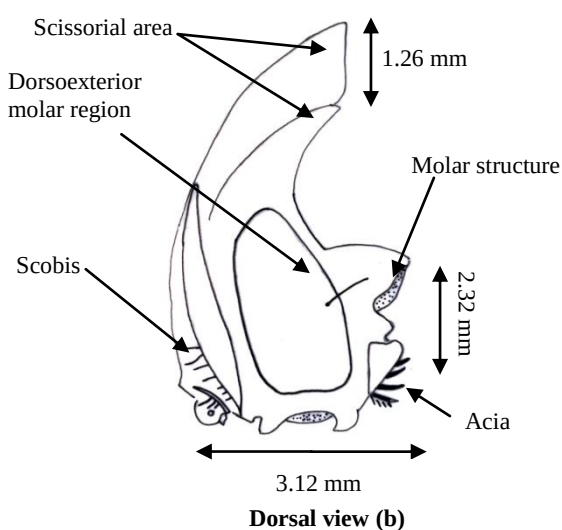
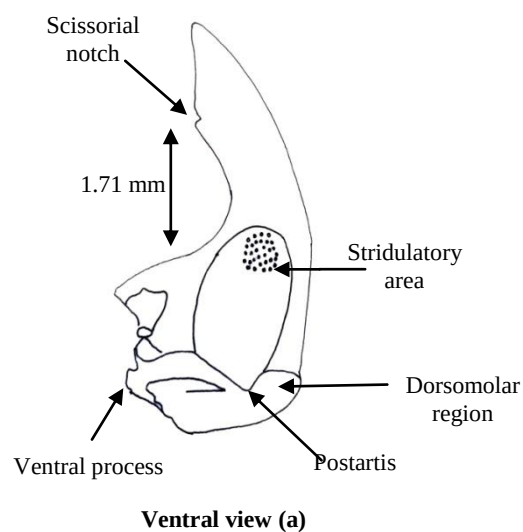
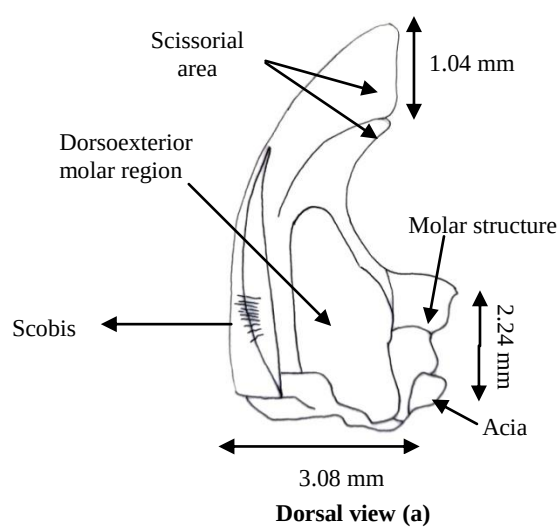


Fig. 4.22 Mandibles (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

width (3.62 mm). In scobis area, 10, seven and nine setae were present in *M. cuprescens*, *M. furcicauda* and *M. indica*, respectively. The left mandible possesses an acia and ventral process in all three *Melolontha* species. Each mandible is equipped with a stridulatory area, which is characterized by the presence of, granulate patch as shown in fig. 4.22

Maxillae: Each maxilla is subdivided into a cardo, stipes, a maxillary palp and a mala lobe fused from the galea and the lacinia (Plate 4.15; Fig. 4.23). The cardo possesses numerous minute setae on the lateral surface and several tiny setae on the ventral surface. The stipe is furnished with 16 stridulatory teeth in *M. cuprescens*, 12 stridulatory teeth in *M. furcicauda* and 13 stridulatory teeth in *M. indica*. These stridulatory teeth are arranged in a line on the dorsal surface of stipe (Plate 4.15). Jia et al. (2020) also reported the presence of a single row of 18 teeth arranged on the dorsal surface of stipe in *M. incana*. The maxillary palp is four segmented and the last segment is columnar in shape and longest in all the species. The distal segment bears a digitiform organ on the lateral surface and several sensilla basiconica are present at the apex. These sensilla basiconica are present in circular fashion protruding outward. The central sensilla basiconica were comparatively thicker and long. In *M. furcicauda* and *M. indica*, 10 sensilla basiconica were present, whereas in case of *M. cuprescens*, the number of sensilla basiconica goes up to 13-14. In case of *M. indica*, all sensilla basiconica were confined to the mid-posterior side of the circular sensory area. The third segment is furnished with single seta on the lateral and ventral surface. The basal segment is also furnished with three setae. The galea is cuculiform, bearing a robust unci directed anteriorly on the apex, and single sensillum basiconicum on the basal part. The lacinia possesses three unci in all three species. In *M. cuprescens* and *M. indica*, four large setae are present on dorsal surface of galea. The basal seta is more sclerotised, stout and prominent. In case of *M. furcicauda*, the basal seta was inconspicuous. On the dorsal surface of lacinia, three sclerotised setae are present which are adjacent to unci in series. The cardo was found to be longest in *M. cuprescens* (2.64 mm), followed by *M. indica* (2.40 mm) and *M. furcicauda* (1.88 mm). Unlike cardo, the maxillary palp was found to be shortest in *M. cuprescens* (1.66 mm). In *M. furcicauda* and *M. indica*, the length of maxillary palp was recorded to be 1.68 mm and 1.79 mm, respectively.

Labium: Labium consists of a sub-mentum, mentum, pre-mentum and a pair of labial palps (Plate 4.16; Fig. 4.24). The sub-mentum is asymmetrical in all species. Width of sub-mentum at proximal end was recorded to be maximum in *M. furcicauda* (3.20 mm),

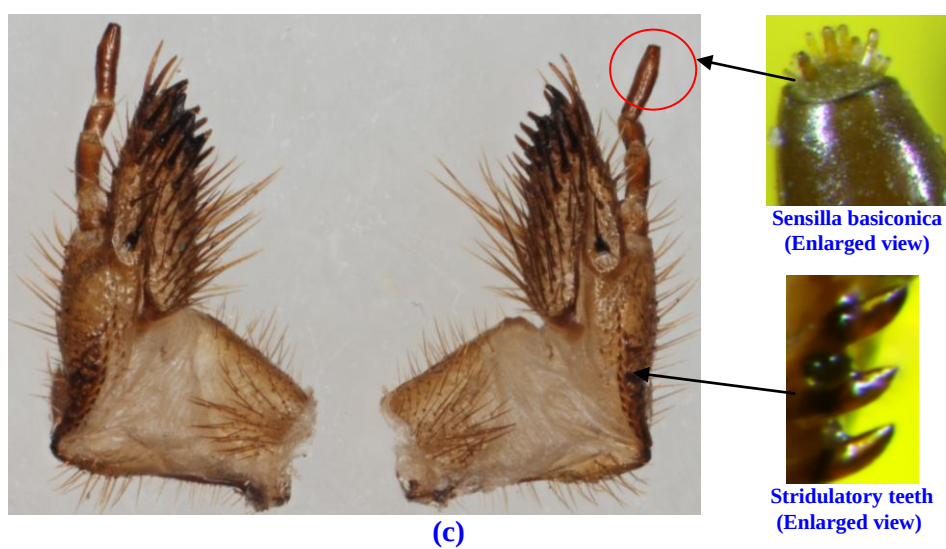
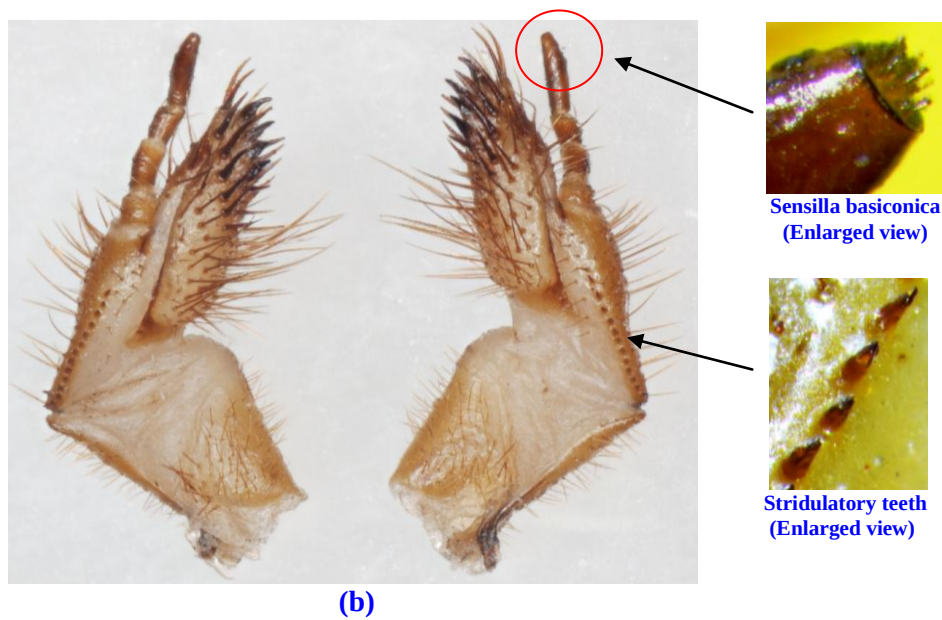
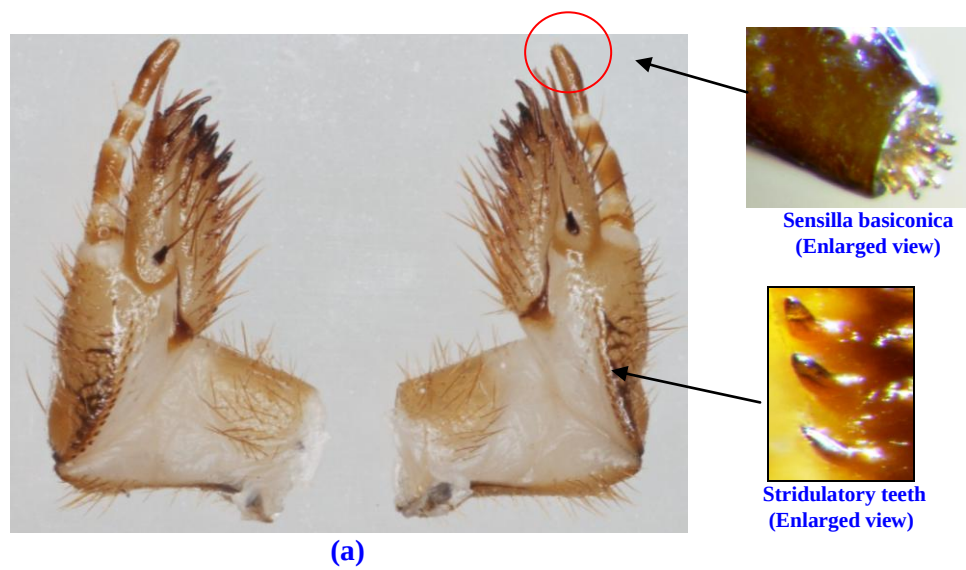


Plate 4.15 Maxillae (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

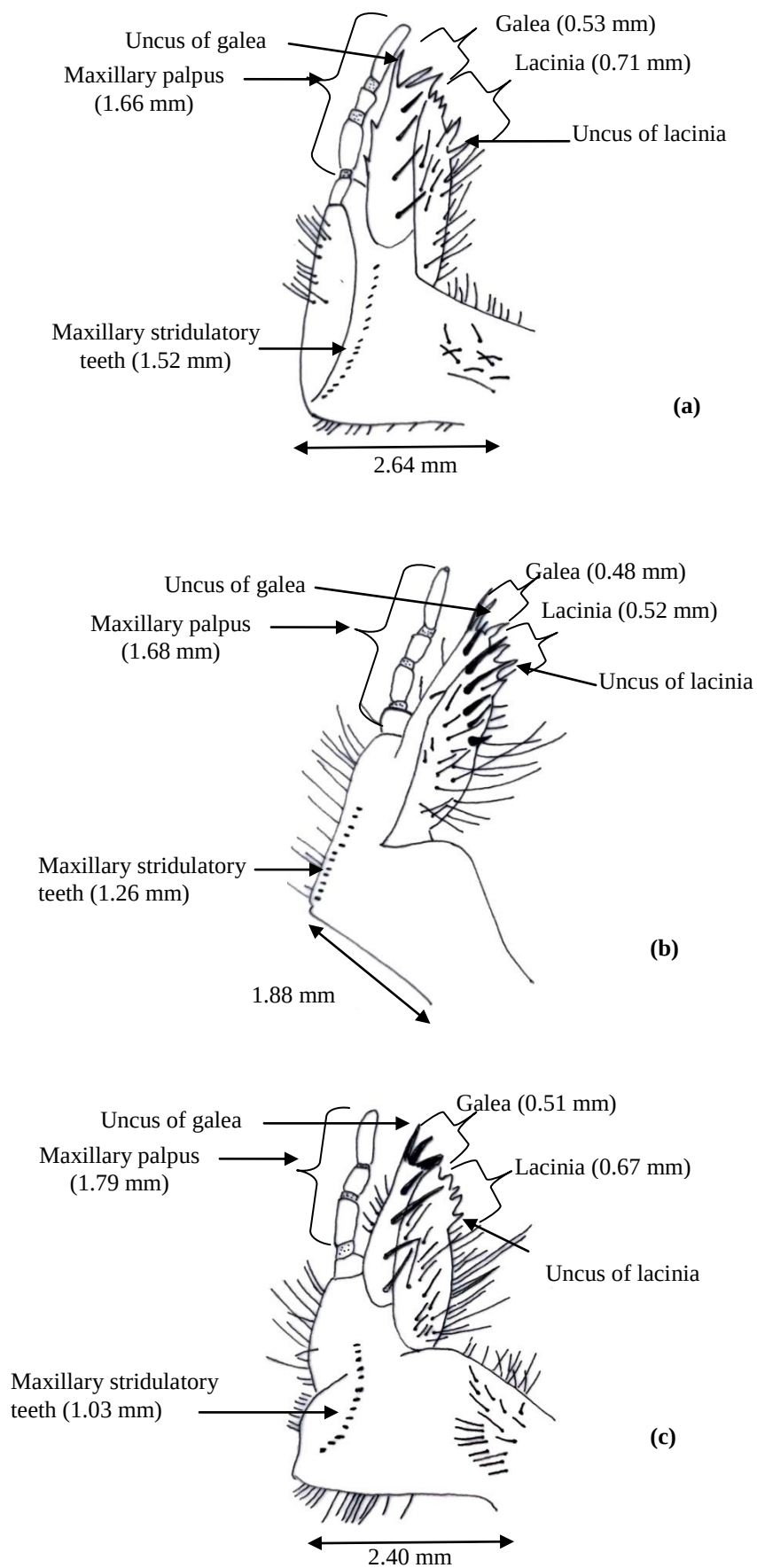


Fig. 4.23 Maxillae (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*



(a)



(b)



(c)

Plate 4.16 Labium (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

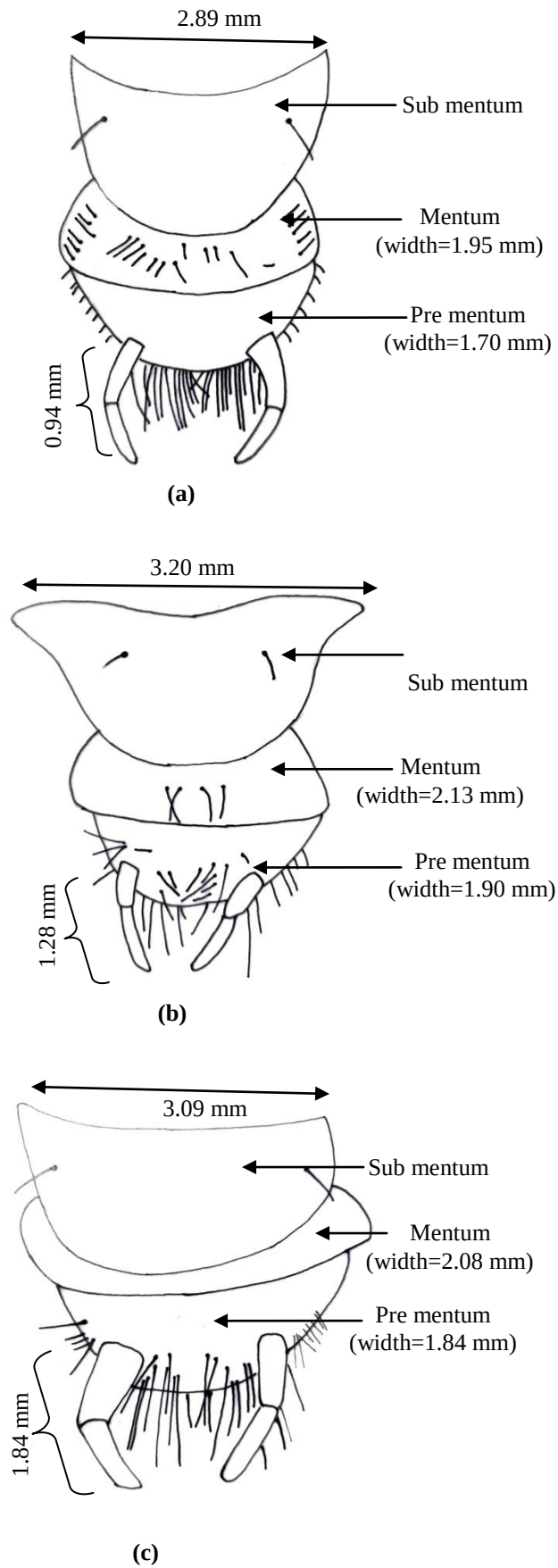
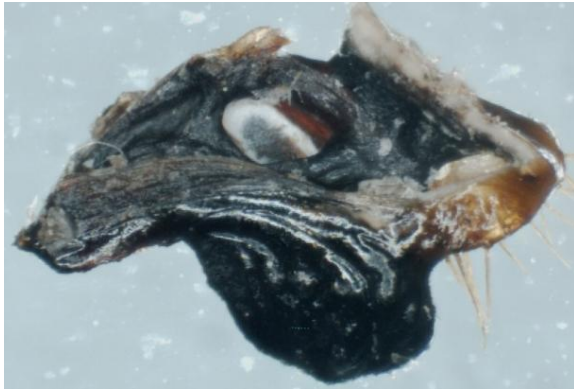


Fig. 4.24 Labium (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

followed by *M. indica* (3.09 mm) and *M. cuprescens* (2.89 mm). A pair of setae was present in upper half of the sub-mentum in all *Melolontha* species. These setae were larger in *M. indica* and *M. cuprescens* as compared to *M. furcicauda*. The mentum is more or less symmetrical in *M. cuprescens* and *M. furcicauda*, but symmetry is lost in *M. indica*. The maximum width of mentum was recorded in *M. furcicauda* (2.13 mm), followed by *M. indica* (2.08 mm) and *M. cuprescens* (1.95 mm). The pre-mentum was symmetrical in *M. cuprescens*, and it appears asymmetrical in *M. furcicauda* and *M. indica*. The maximum width of pre-mentum was recorded in *M. furcicauda* (1.90 mm), followed by *M. indica* (1.84 mm) and *M. cuprescens* (1.70 mm). Several paired setae were present on the distal part of the pre-mentum. The paired setae were directed straight foreward in *M. indica*, whereas in *M. cuprescens* and *M. furcicauda*, these setae pointed inward crossing each other. The overlap of setae is more distinct in *M. furcicauda*. The labial palp is two segmented bearing sensilla basiconica on the apex of the distal segment. The glossa was furnished with numerous sensilla basiconica on the central part, and the tuft of microtrichia on the distal and lateral margins. The length of the maxillary palp has been recorded to be maximum in *M. indica* (1.84 mm), followed by *M. furcicauda* (1.28 mm) and *M. cuprescens* (0.94 mm).

Hypopharynx: The hypopharynx is specialized in the hypopharyngeal sclerome, which is furnished with truncate process and paired lobes on the lateral margin (Plate 4.17; Fig. 4.25). The left lateral lobe is pointed in *M. cuprescens*, and more blunt in *M. indica*. In case of *M. furcicauda*, the left lateral lobe is more or less pointed and it is curved upward. A tuft of hairs was present on hypopharyngeal sclerome in all the species. The truncate process in *M. cuprescens* was sub circular in outline, whereas in *M. furcicauda* and *M. indica*, outer edge of truncate process was more or less flat in shape. On the dorsal surface of hypopharynx, a hole was observed which is known as hypopharyngeal opening. This hole was elliptical in shape in *M. indica*, whereas in *M. cuprescens* and *M. furcicauda*, the hole tends to be irregularly oval in shape.

Thoracic legs: Thoracic legs are five segmented, each consisting of a coxa, a trochanter, a femur, a tibiotarsus, and a single claw on the apex. In prothoracic legs, the trochanter is somewhat triangular in shape (Plate 4.18; Fig. 4.26). The femur is furnished with numerous fuscous setae on the ventral surface. Similar setae have also been observed in other multivoltine species such as *Melolontha japonica* Burmeister (Sawada 1991) and *M. incana* (Jia et al. 2020). The tibiotarsus is fused from tibia and tarsus, and furnished with numerous fuscous setae on the ventral surface either. The claw is sclerotized and almost



Dorsal view



Ventral view

(a)



Dorsal view



Ventral view

(b)



Dorsal view



Ventral view

(c)

Plate 4.17 Hypopharynx (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

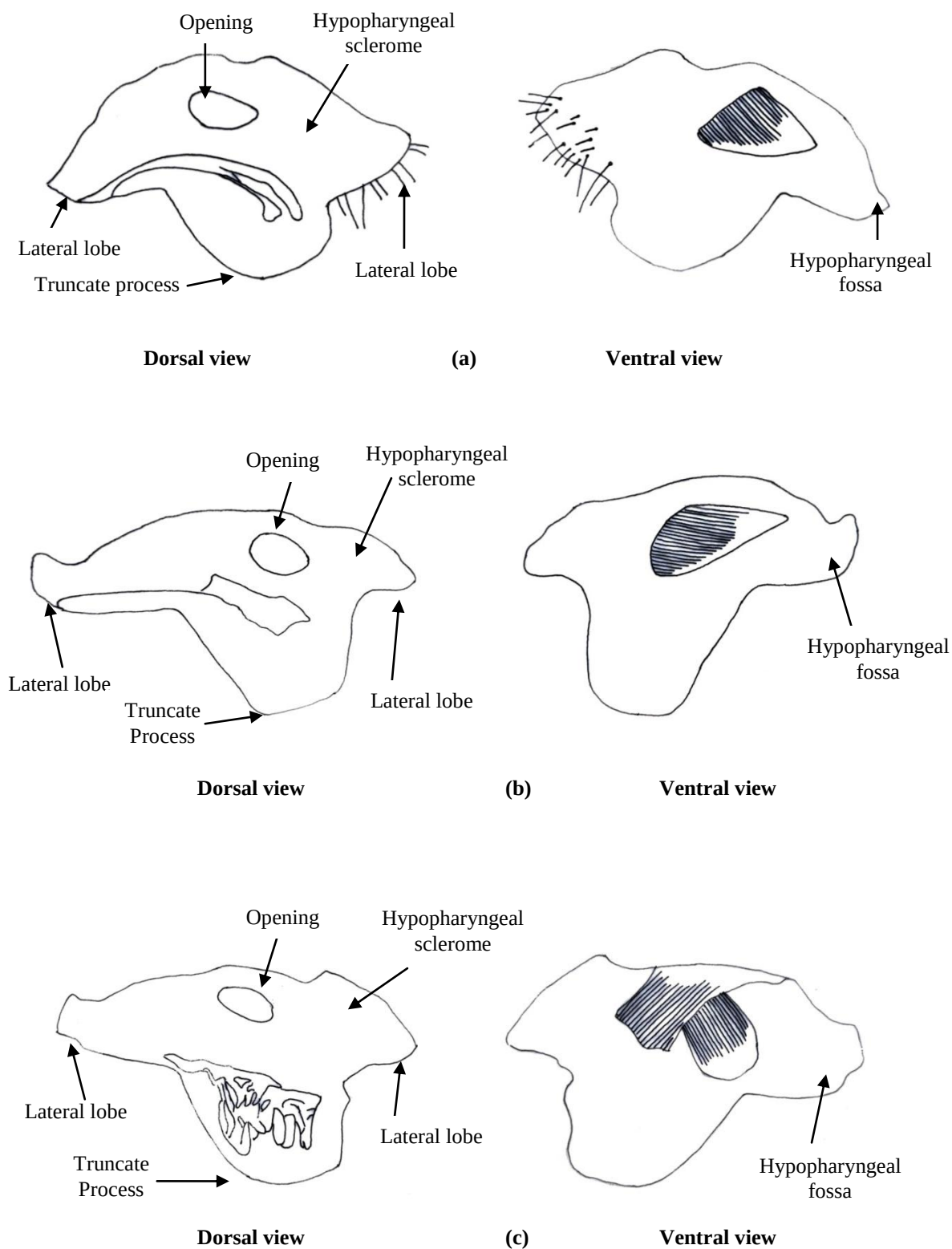


Fig. 4.25 Hypopharynx (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

straight in *M. cuprescens* and *M. furcicauda*. The length of claw has been recorded to be 0.87 mm in *M. cuprescens* and 0.94 mm in *M. furcicauda*. In *M. indica*, the claw is slightly curved, and its length has been recorded to be 0.86 mm. In *M. furcicauda*, the claws are directed forward, whereas in *M. indica* and *M. cuprescens*, the claws are directed laterally. In mesothoracic legs, the shape of trochanter showed little variation among species (Plate 4.19; Fig. 4.27). In all three *Melolontha* species, the line joining trochanter with femur was almost flat in *M. cuprescens*, whereas the line is slightly pressed inward towards the end in *M. furcicauda* and *M. indica*. Distal part of the trochanter was notched, and the notch was more prominent in *M. indica* as compared to *M. furcicauda* and *M. cuprescens*. The length of the claw was maximum in *M. indica* (0.76 mm), followed by 0.68 mm in *M. furcicauda*, and 0.37 mm in *M. cuprescens*. The fuscorial setae were also larger in *M. indica* as compared to *M. furcicauda* and *M. cuprescens*. In metathoracic legs, the shape of trochanter was more or less symmetrical in *M. furcicauda* and *M. cuprescens*, but in *M. indica*, the shape tends to be asymmetrical (Plate 4.20; Fig. 4.28). The femur was short and slightly rounded on outer side in *M. furcicauda* and *M. indica*, whereas in *M. cuprescens*, it was almost flat for most part of its length. The length of the claw was recorded to be maximum in *M. indica* (0.41 mm), followed by *M. cuprescens* (0.32 mm) and *M. furcicauda* (0.26 mm).

Spiracles: The respiratory system is of peripneustic type, bearing a pair of spiracles on the prothorax and eight pairs of spiracles on the first eight abdominal segments. The spiracles were 'C' shaped with respiratory plate completely surrounding the bulla. The thoracic spiracles were slightly larger and opened towards the caudal end. The abdominal spiracles are comparatively smaller and opened anteriorly (Plate 4.21; Fig. 4.29). The overall size of thoracic spiracles was larger in *M. cuprescens*, than *M. indica* and *M. furcicauda*. In *M. cuprescens*, the length x width of thoracic spiracles has been recorded to be 1.27 x 0.86 mm, whereas for *M. indica* and *M. furcicauda*, the length x width of thoracic spiracles measured to be 1.02 x 0.81 mm and 0.43 x 0.28 mm, respectively. Similar trend was observed for the first pair of abdominal spiracles among different *Melolontha* species. The maximum length x width was recorded in *M. cuprescens* (0.89 x 0.72 mm), followed by *M. indica* (0.85 x 0.61 mm) and *M. furcicauda* (0.37 x 0.26 mm), respectively. The distance between the two lobes of respiratory plates differed considerably among three studied *Melolontha* species. In thoracic spiracles, it was recorded to be maximum in *M. furcicauda* (0.20 mm) and minimum in *M. cuprescens* (0.06 mm). In *M. indica*, the distance between two respiratory lobes was 0.18 mm. For abdominal spiracles, the



(a)



(b)



(c)

Plate 4.18 Prothoracic legs (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

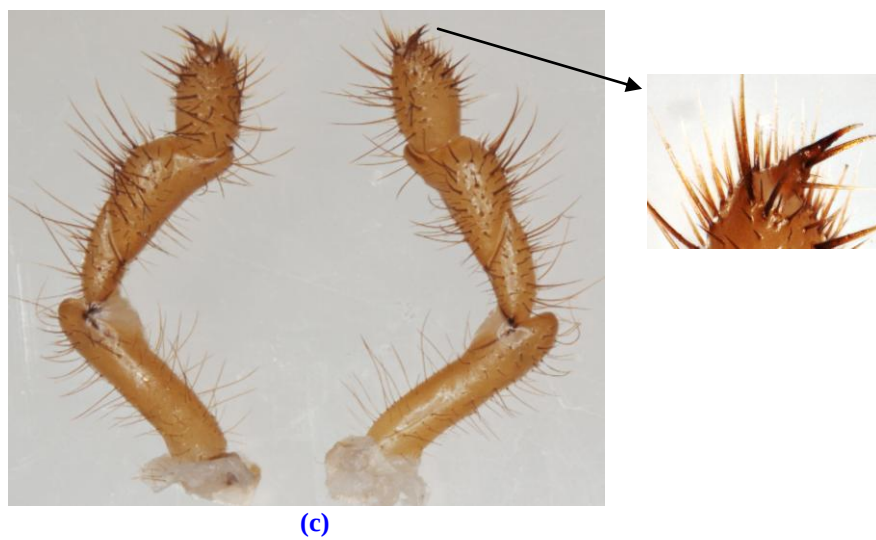
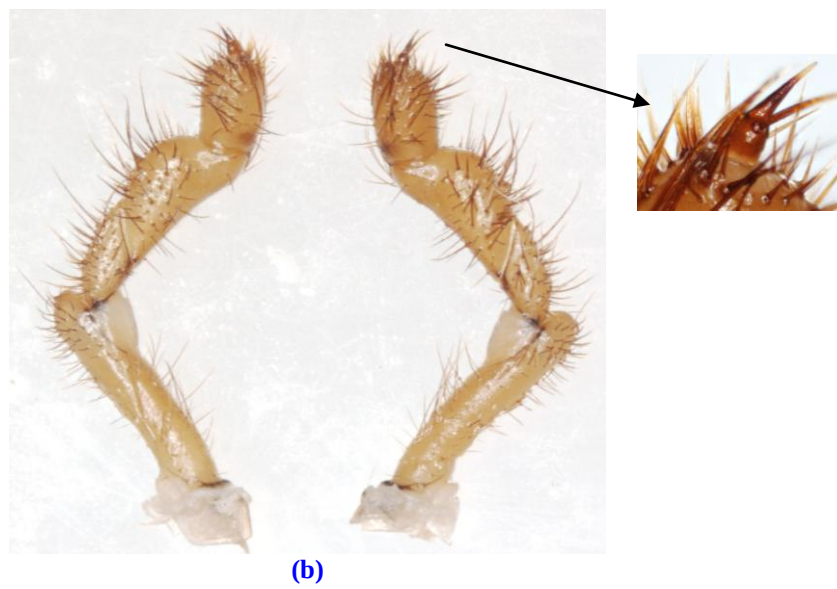
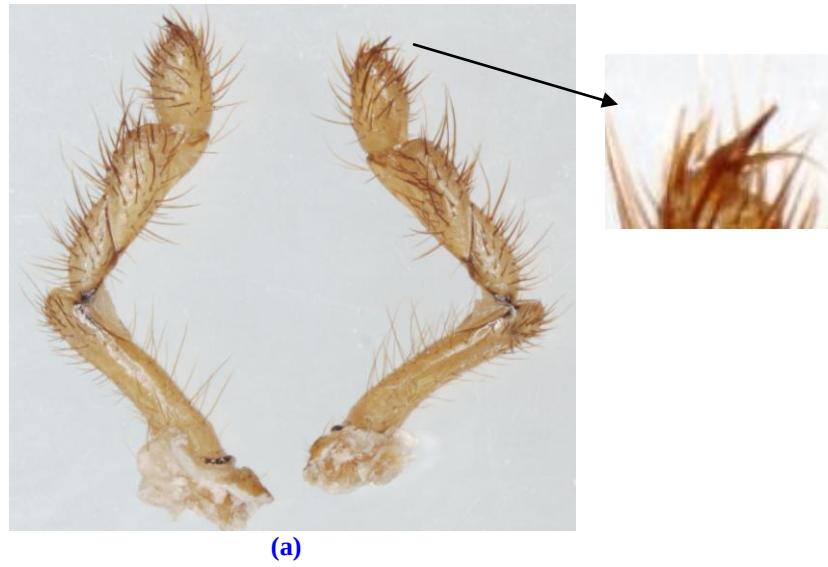


Plate 4.19 Mesothoracic legs (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

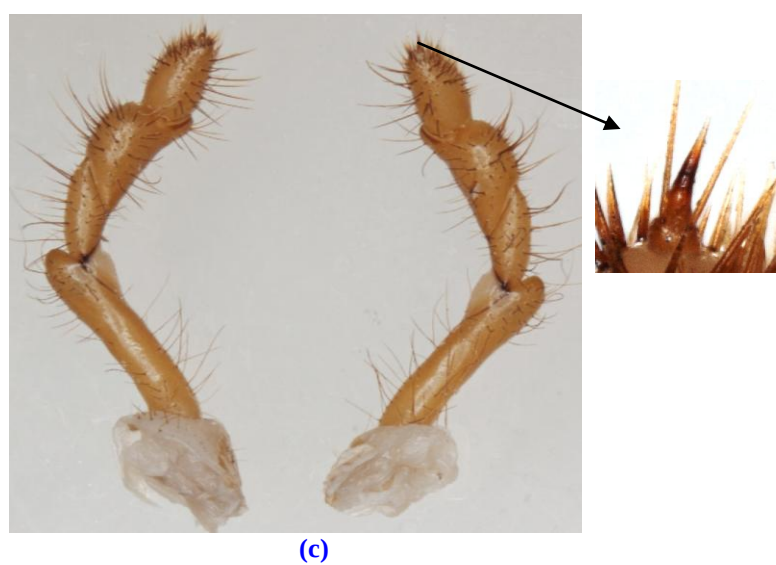
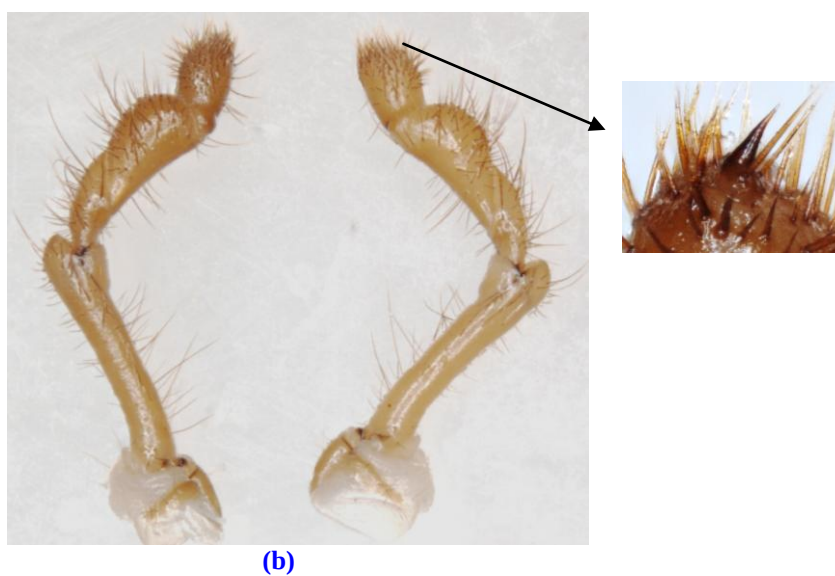
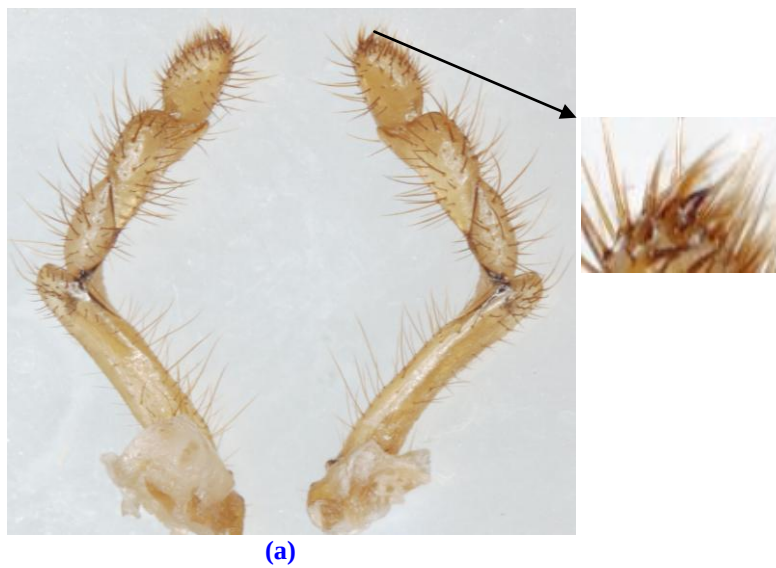


Plate 4.20 Metathoracic legs (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

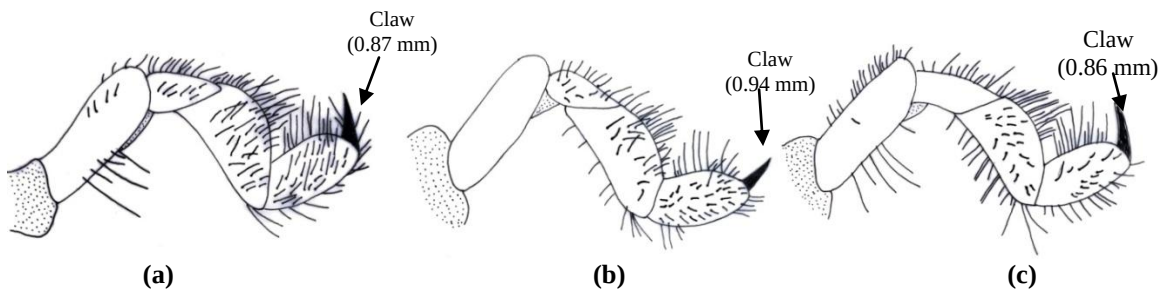


Fig. 4.26 Pro thoracic legs (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

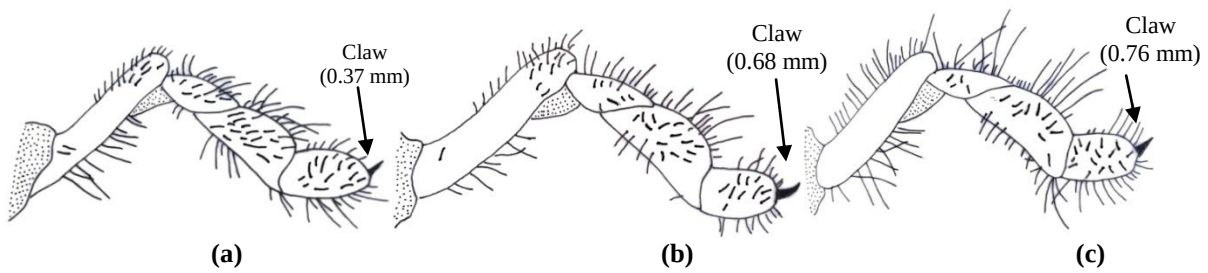


Fig. 4.27 Mesothoracic legs (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

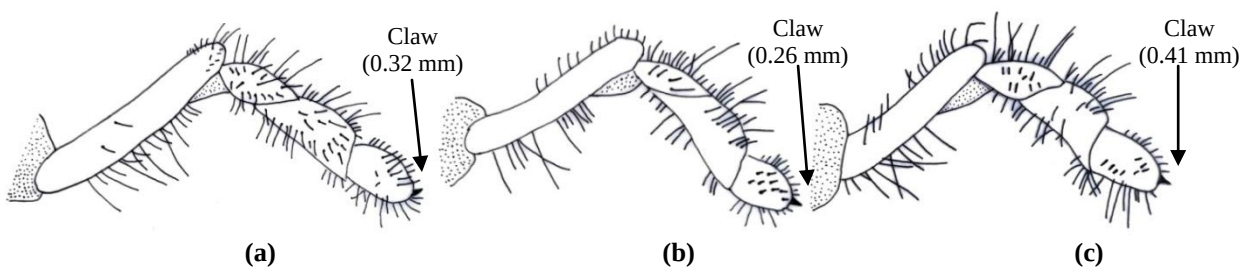
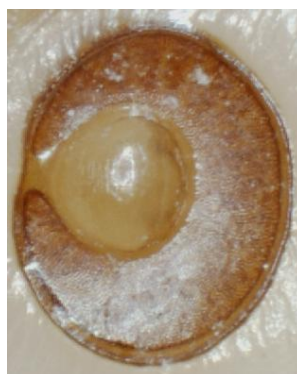


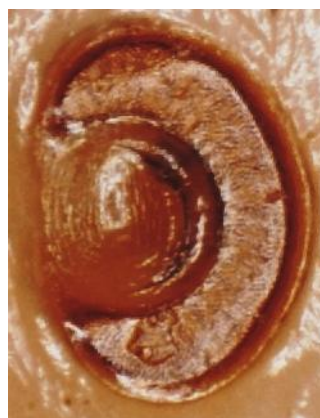
Fig. 4.28 Metathoracic legs (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*



(a)

Prothoracic spiracle

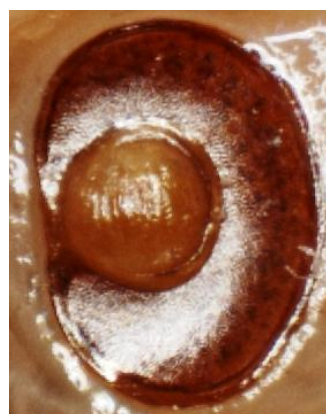
Abdominal spiracle



(b)

Prothoracic spiracle

Abdominal spiracle



(c)

Prothoracic spiracle

Abdominal spiracle

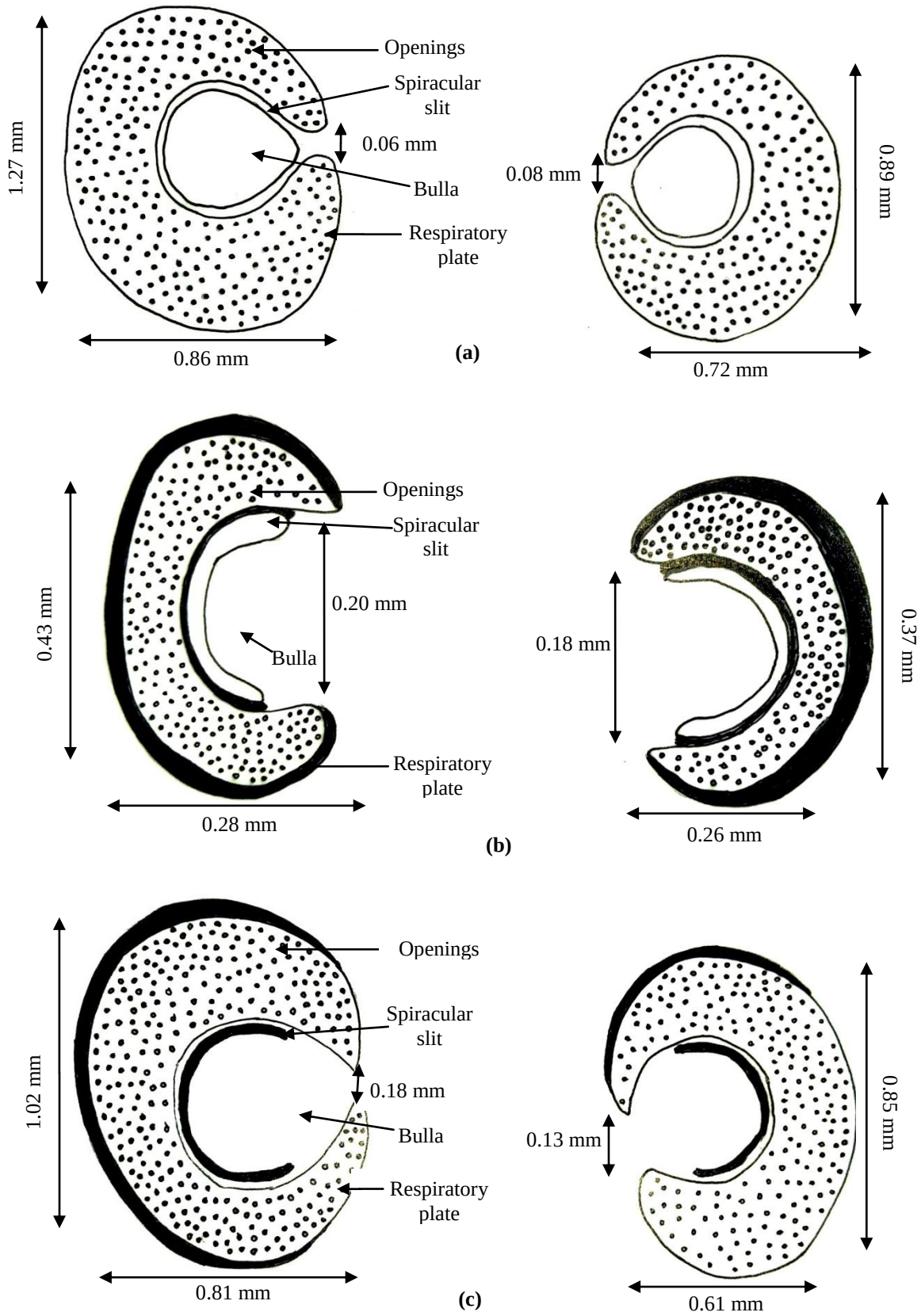
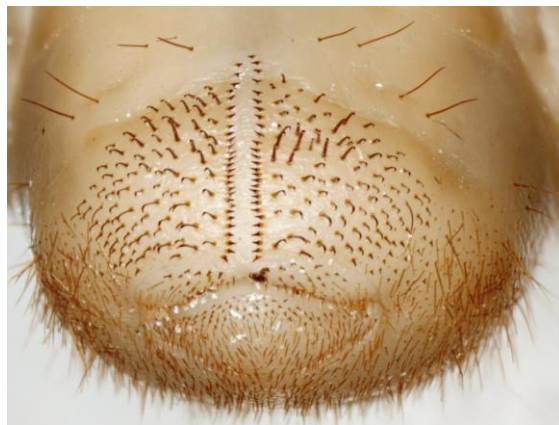


Fig. 4.29 Prothoracic and abdominal spiracles (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

maximum distance between two lobes of respiratory plates was discernible in *M. furcicauda* (0.18 mm), followed by *M. indica* (0.13 mm) and *M. cuprescens* (0.08 mm). In *M. cuprescens*, the length of abdominal spiracles gradually decreased from I-VIII abdominal segments; whereas the width of abdominal spiracles was stable from II-V segments, and gradually increases from VI-VIII abdominal segments. In *M. furcicauda*, the length of abdominal spiracles gradually increased from I-III abdominal segments, and then decreased from IV-VIII segments. The width of abdominal spiracles gradually increased from I-VIII abdominal segments. In subfamily melolonthinae, the larval spiracles have been reported to be significantly smaller on abdominal segment VI-VIII in *Phyllophaga cuicateca* Moron and Aragon (Aragon and Moron 2000), gradually larger from abdominal segment I-VII, but distinctly smaller on abdominal segment VI-VIII in *Phyllophaga ilhuicaminai* Moron (Aragon and Moron 2000). The distance between lobes of spiracular plates gradually decreased from I-VIII abdominal segments. In *M. indica*, the length of abdominal spiracles gradually decreased from I-VII abdominal segments, but larger in eighth abdominal segment. The width of abdominal spiracles and distance between lobes of respiratory plate in *M. indica* was found to be inconsistent. The diverse spiracle size arrangement can provide more information in larval identification among different *Melolontha* species.

Raster pattern: Rasters are generally furnished with hemate setae and palidial setae, which varied greatly in shape, number and arrangement. Jia et al. (2020) reported that diverse morphological features of the raster imply their peculiar functions. The palidial setae were considered to be the site of chemical emission that attract male adults (Haynes et al. 1992), and also known to be associated with defensive behaviour against nematode attack (Gaugler et al. 1994). The raster is well developed with two widely separated and prominent palidia in the central region. The palidia are diverging posteriorly in all the species. The paired palidia are nearly parallel, each consisting of 28 or 29 pali, pointing inward in *M. cuprescens*. Leaving 8-9 basal pali, all other pali in the present studied species were distinctly directed in upward direction. In *M. furcicauda*, each palidium consisted of 26 pali. The number of pali were recorded to be 31 or 33 in each palidium of *M. indica* (Plate 4.22; Fig. 4.30). Jia et al. (2020) observed 25 or 26 pali in palidium of *M. incana*. The septula was more prominent in *M. indica* as compared to *M. cuprescens* and *M. furcicauda*. The tegilla comprised of numerous hamate setae, which are slightly compressed and apically curved. The shape of pali and hemate setae also varied among species as shown in fig. 4.30. The presence or absence of these setae could not only



Palidium
Enlarged view

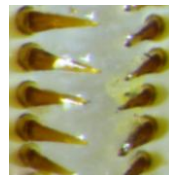


Hemate seta
Enlarged view



Anal opening

(a)



Palidium
Enlarged view

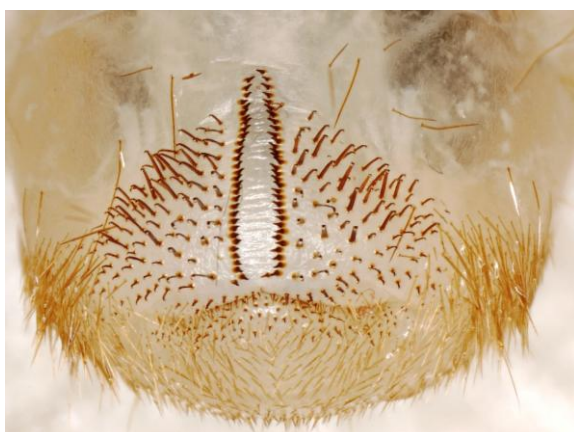


Hemate seta
Enlarged view



Anal opening

(b)



Palidium
Enlarged view



Hemate seta
Enlarged view



Anal opening

(c)

Plate 4.22 Raster pattern (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

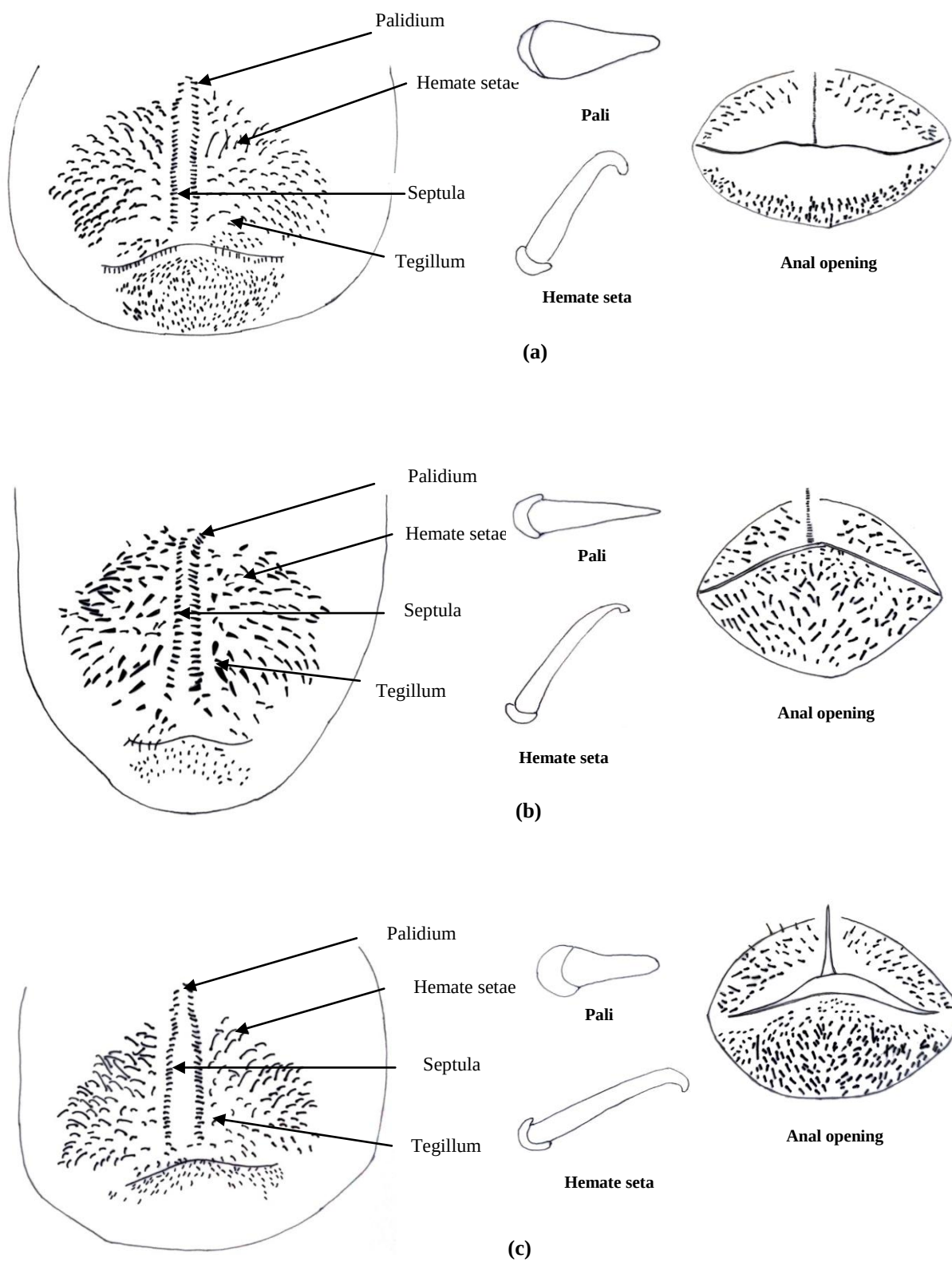


Fig. 4.30 Raster pattern (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

valuable for taxonomic purposes, but may also provide valuable information for diverse living habits in melolonthinae (Jia et al. 2020). In *M. cuprescens*, the anal slit is Y shaped, slightly depressed and hooked in the centre, slightly raised on both sides and gradually tapering laterally in a semicircular fashion. The anal cleft is straight, touching the base of septula. In case of *M. furcicauda*, the anal slit is raised in the centre and gradually tapers to both sides uniformly. The anal cleft is somewhat broadened at the base, tapering upward and its upper end remains far behind the septula. The anal slit of *M. indica* was raised in the centre, gradually tapers to the sides and again curves upward forming distinct grooves on both sides. The anal cleft is straight and goes up to the base of septula as in case of *M. cuprescens*. The lower anal lobe is sub-circular in outline in all the species (Fig. 4.30). According to Sreedevi et al. (2014), the anal slit forms an important and stable character to distinguish between subfamilies especially melolonthinae and rutelinae. It is 'V' or 'Y' shaped in melolonthinae and transverse or angulate in rutelinae (Ritcher 1966) The setae are more uniformly groomed and arranged in *M. cuprescens* and *M. indica*, but in *M. furcicauda*, the setae covered more area on the lower anal lobe, which are arranged in triangular fashion in the centre.

4.4.3 Pre-pupa: Towards the end of third instar, the normal C-shape of the body becomes somewhat straight and slightly curved upward at its abdominal end (Plate 4.23). This stage is known as pre-pupal stage. Pre-pupae were formed in the month of April in *M. cuprescens*, March in *M. furcicauda*, and during June in *M. indica* in third summer at Palampur. The ventral surface of the last abdominal segment became wrinkled and they assumed yellowish colouration. First to sixth segments of dorsal surface became brownish in colour. The spiracles appeared depressed in this stage. The size of pre-pupae is given in the table 4.22. In *M. cuprescens*, the body length of pre-pupae varied from 34.16-36.86 mm, and in *M. furcicauda*, the body length was in the range of 34.97-35.17 mm. The average body length of pre-pupae in *M. cuprescens* and *M. furcicauda* has been recorded to be 35.24 ± 0.31 and 35.03 ± 0.02 mm, respectively. In *M. indica*, the average body length was observed to be 36.01 ± 0.29 mm (range: 34.80-37.11 mm).

The duration of terminal portion of the third instar or pre-pupal period was relatively short. In *M. cuprescens*, the pre-pupal period ranged from 22-25 days with an average of 23.4 ± 0.4 days. The pre-pupal period was relatively short in *M. furcicauda* and *M. indica* where the duration of pre-pupal stage has been recorded to be 12-14 days (mean: 13.0 ± 0.30 days) and 11-12 days (mean: 11.5 ± 0.17 days), respectively. Tsai



(a)



(b)



(c)

Plate 4.23 Pre-pupae (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

(1965) and Huiting et al. (2006) did not mention pre-pupal stage in *M. melolontha*. Probably they have included the duration of this stage in the larval stage. In *H. consanguinea*, the pre-pupal period has been reported to be of three to five days (Vishwanath 1979), and in *H. serrata*, the duration of pre-pupa has been reported to be of seven days (Veeresh 1977).

4.4.4 Pupa: Pupae were without any cocoon inside the hard earthen cells. Ritcher (1958) also reported that mature scarabaeid larvae construct an earthen cell and pupate inside these cells. The cuticle of the last larval instar was pushed to its posterior end. The pupae were light yellow with orange tinge when freshly formed (Plate 4.24). The colour gradually darkened to tan yellow during the course of development. In *M. cuprescens*, *M. furcicauda* and *M. indica*, the pupae were of exarate aedeicious type. The pupae of *M. hippocastani* have also been reported to be of light-yellow colour in Russia (Anonymous 2009). According to Haq (1962), the freshly formed pupae of *H. longipennis* are creamy white, but within 24-48 hours assume tawny tinge, which get darker as transformation to the adult stage. Shah and Shah (1990) also reported that pupae of *H. longipennis* are of exarate type and possesses a pair of short pointed caudal appendages. The size of the pupa was almost same in all three *Melolontha* species. In *M. cuprescens*, the average body length x width of pupae has been recorded to be 32.22 ± 0.37 mm x 14.56 ± 0.29 mm, whereas in *M. indica*, the body length x width of pupae ranged from 31.19-32.43 mm x 11.94-13.61 mm. In *M. furcicauda*, the body length x width of pupae averaged 32.47 ± 0.11 mm x 14.55 ± 0.13 mm with a range of 32.01-32.91 mm x 14.19-15.11 mm (Table 4.22). According to Pathania (2014), the body length x width of pupae of *M. cuprescens* was 32.53 ± 0.35 mm x 13.45 ± 0.08 mm, and these values find distinct overlap with our data.

Under laboratory conditions, the pupation was observed in earthen cells at the bottom of glass jars at a depth of about 13-16 cm in the soil indicating their tendency to pupate deep into the soil. These laboratory observations were also supported by field observations where pupae were observed at a depth of about 25-35 and 20-25 cm in *M. cuprescens* and *M. indica*, respectively. Pathania (2014) also noticed pupal formation in large earthen chambers near to the bottom of the glass jars in *M. cuprescens* from Himachal Pradesh. In China, the pupation takes place at a depth of 12-14 cm below the surface of soil (Tsai 1965), but in Netherlands, the pupation in *M. melolontha* has been reported to take place in June in small cells mostly at a depth of 30-40 cm. The depth at which pupation takes place is probably influenced by the type of soil and climate. Svestka



(a)



(b)



(c)

Plate 4.24 Pupae (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

and Drapela (2009) reported that fully fed grubs of *M. hippocastani* after third stage pupates in July to August at a depth of 30-40 cm or even more.

Table 4.22 Morphometric measurements of pre-pupae and pupae

Species	Pre-pupa	Pupa	
	Length (mm)	Length (mm)	Body width (mm)
<i>M. cuprescens</i> : Range	34.16-36.86	30.50-33.78	13.42-15.98
Mean $\pm$ SE	35.24 $\pm$ 0.31	32.22 $\pm$ 0.37	14.56 $\pm$ 0.29
<i>M. furcicauda</i> : Range	34.97-35.17	32.01-32.91	14.19-15.11
Mean $\pm$ SE	35.03 $\pm$ 0.02	32.47 $\pm$ 0.11	14.55 $\pm$ 0.13
<i>M. indica</i> : Range	34.80-37.11	31.19-32.43	11.94-13.61
Mean $\pm$ SE	36.01 $\pm$ 0.29	31.97 $\pm$ 0.15	12.71 $\pm$ 0.22

The pupation in the laboratory was observed as early as 30.04.2019, 16.05.2019 and 14.06.2019 for *M. furcicauda*, *M. cuprescens* and *M. indica*, respectively. The pupal period was observed to be shortest in *M. cuprescens*, followed by *M. indica* and *M. furcicauda*. The average pupal period under laboratory conditions was 20.3 ± 0.7 , 30.0 ± 0.47 and 25.1 ± 0.67 days in *M. cuprescens*, *M. furcicauda* and *M. indica*, respectively. The minimum and maximum being 17 and 23 days for *M. cuprescens*, 28 and 32 days for *M. furcicauda* and 22 and 28 days for *M. indica* (Table 4.23). Pathania (2014) reported a pupal period of 13-25 days at Palampur for *M. cuprescens*. According to Tsai (1965), the pupal period for *M. melolontha* lasts for about two months in China. Similarly, in Florida, the pupal stage of *M. melolontha* occupies about two months (Woodruff 1978). In Austria, the third instar larvae of *M. melolontha* pupate in summer, and after a metamorphosis period of nine weeks hatch as adult beetles (Laengle et al. 2005). Huiting et al. (2006) reported that duration of pupal period in *M. melolontha* varies from 25 days at 25°C and 100 days at 12°C. This variation in pupal period in the present study seems to be an effect of variable temperature in different study sites.

The sexual diamorphism is clearly evident in the pupal stage. The male and female pupae differed from each other in many morphological characteristics. In males of all three *Melolontha* species, the antennae are long, extending beyond the apex of maxillary palps and reaching the base of meso-coxa, whereas in females, the antennae are shorter, extended up to maxillary palps only and reaching the base of pro-coxa (Plate 4.25).

In *M. cuprescens* males, the caudal appendages are widely separated, thick at the base and gradually tapering to the apical end. The tips of caudal appendages are black, sclerotised and pointed. Both caudal appendages are directed inward in a semi-circular fashion. In females, the caudal tips are directed outward in V shape. Although, tapering is



(a)



(b)



(c)

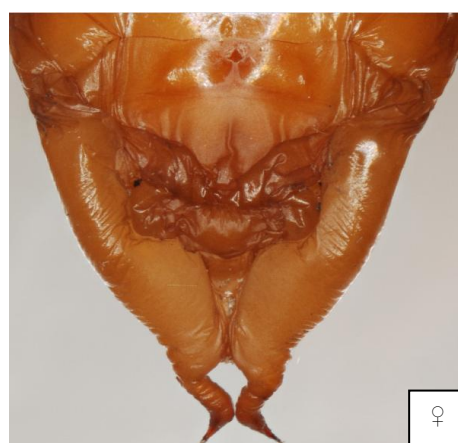
Plate 4.25 Pupal antennae (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*



(a)



(b)



(c)

Plate 4.26 Pupal tips (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

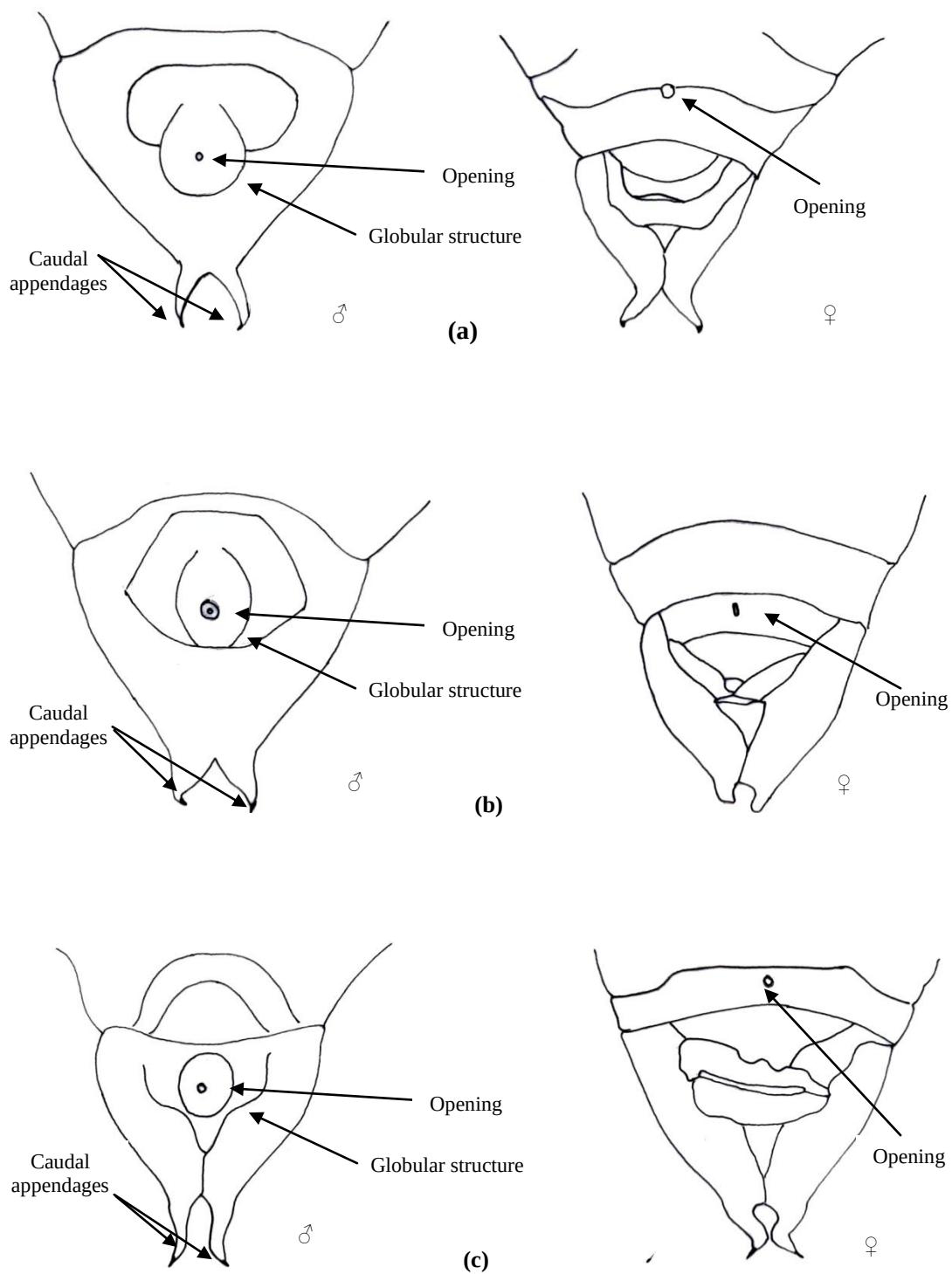


Fig. 4.31 Pupal tips (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

gradual from base to apex, but the tips are slightly hooked and directed downward. In males of *M. furcicauda*, the caudal appendages are swollen at the base and directed outward. The caudal tips are pointed and directed inward. In females, the caudal appendages are short, less prominent and caudal tips appear rounded. In *M. indica*, the caudal appendages are gradually tapers from base to apex in males and their shape is characteristically differs from that of *M. cuprescens* and *M. furcicauda*. The caudal appendages are directed outward at the base, runs straight and then again turns outward in opposite direction. The caudal tips are sharply pointed and are directed outward. In females of *M. indica*, the caudal appendages are swollen at the base and appears to be of uniform width in the mid-posterior region. The caudal appendages are directed inward and almost touches each other at the apex end. The caudal tips are pointed and are directed outward in a characteristic manner (Plate 4.26; Fig. 4.31). At the end of last sternum, a median glubose projection with a median key hole shape structure is present in males of all three *Melolontha* species. In females, an anal slit is present on last sternum, which is elliptical in shape.

Table 4.23 Duration of pre-pupa, pupa and total life cycle

Stage	Duration in days					
	<i>M. cuprescens</i>		<i>M. furcicauda</i>		<i>M. indica</i>	
	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE
Pre-pupa	22-25	23.4 $\pm$ 0.4	12-14	13.0 $\pm$ 0.30	11-12	11.5 $\pm$ 0.17
Pupa	17-23	20.3 $\pm$ 0.7	28-32	30.0 $\pm$ 0.47	22-28	25.1 $\pm$ 0.67
Total (egg - adult emergence)	672-685	678.4 $\pm$ 1.49	640-658	647.8 $\pm$ 1.91	660-674	664.9 $\pm$ 1.65

Total life cycle: Total life cycle from egg to adult emergence was determined to be of 672-685 days for *M. cuprescens*, 640-658 days for *M. furcicauda* and 660-674 days for *M. indica* indicating two years life cycle from oviposition to oviposition for these three species in Himachal Pradesh. The average duration of life cycle has been recorded to be of 678.4 $\pm$ 1.49, 647.8 $\pm$ 1.91, 664.9 $\pm$ 1.65 days for *M. cuprescens*, *M. furcicauda* and *M. indica*, respectively (Table 4.23). According to Pathania (2014), the total life cycle of *M. cuprescens* from egg to adult was calculated to 649-690 days in Himachal Pradesh. Ritcher (1958) reported that scarabaeids' life cycles vary with the climate, being longest in more temperate regions. The shortest life cycles are to be found in tropical areas with no climatic seasons (Gressitt 1953). In Europe, *M. hippocastani* requires from three to five years to complete its life cycle (Korschefsky 1940; Schwerdtfeger 1944), while *M.*

melolontha requires from three to four years depending upon latitude (Regnier 1950). Work by Thiem (1944) with *Melolontha* spp. indicates that differences in the length of life cycle of a given species depend on the rate of development of the first and second instars. In China, the life cycle of *M. melolontha* takes about six years in north west Szechwan (Tsai 1965). In Florida and Austria, *M. melolontha* has three to five years of life cycle (Woodruff 1978; Laengle et al. 2005). Muska (1975) showed that in Czechoslovakia different populations of *M. melolontha* with both three and four year life cycle are present. The threshold of average temperatures during this period above which three year cycles and below which four year cycles occur, has been calculated as 13-14°C for Slovakia and 14-15°C for Moravia-Silesia (Muska 1975).

4.4.5 Adult: In laboratory, the adult formation was noticed during first week of June in *M. cuprescens* and *M. furcicauda*, whereas in *M. indica*, the adult formation occurred during second week of July. Pathania (2014) noticed adult formation of *M. cuprescens* in third week of May at Palampur. In China, the beetles of *M. melolontha* emerge from July-end to beginning of September, and they remain underground until the beginning of May in next year (Tsai 1965). Huiting et al. (2006) reported that adults of *M. melolontha* appear during April-May in Netherlands. Similarly, in Europe, the adults of *M. melolontha* and *M. hippocastani* emerge in April or May, feed on the leaves of trees and lay their eggs on ground in cultivated fields (Bulmer 1977).

In *M. cuprescens*, the body is elongate, convex, blackish in colour with brownish tinge, and covered all over with dirty white scales. In *M. furcicauda* and *M. indica*, the general body shape was similar to that of *M. cuprescens*, but the body colour is distinctly variable. In *M. furcicauda*, the adult beetles were reddish brown with creamish white scales, whereas in *M. indica*, the body colour was yellowish brown with pale scales (Plate 4.27). According to Chandra and Uniyal (2007), the adults of *M. furcicauda* are reddish brown, while those of *M. indica* yellowish brown in colour, covered all over with pale white scales. Kumar and Vishvendra (2018) reported that beetles of *M. cuprescens* are dull brown in colour. Elytra with flat and densely scaly intervals, intervals raised, narrowed with denuded costae. Elytra does not cover whole of the abdomen. The five longitudinal ribs on each elytra were present in all three *Melolontha* species. According to Woodruff (1978), the *M. melolontha* beetles are easily distinguished from other scarabaeid beetles by the costate ridges on the elytra. The body of freshly emerged beetles was coated with whitish scales. Initially, the density of scales was very less, but after 2-3 days, the scales became more dense which could be easily rubbed off. The scales were more dense in case



(a)



(b)



(c)

Plate 4.27 Adults (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

of *M. indica* as compared to *M. cuprescens* and *M. furcicauda* as a result the ribs on elytra were less clearly visible in *M. indica*.

The head and scutellum were black in colour in *M. cuprescens*, whereas in *M. indica* and *M. furcicauda*, the head and scutellum were reddish brown in colour. In *M. cuprescens*, *M. furcicauda* and *M. indica*, less pubescence was observed on head in males, whereas females were more pubescent having dirty white hairs on head. In *M. cuprescens* and *M. furcicauda*, the punctation on head was more dense and even in males, whereas in females, the punctation was coarse and uneven (Plate 4.28). According to Feroz and Tara (2015), the head of *M. furcicauda* is closely setose, densely and unevenly punctate. In *M. indica*, the punctation on head was dense in both sexes. The clypeus was broader in females than males, rectangular, with margins strongly reflexed in front in all three *Melolontha* species (Plate 4.28). Chandra and Uniyal (2007) have also reported rectangular clypeus in *M. furcicauda* and *M. indica* beetles. The maxillary palps in male beetles are clearly visible from above clypeus, whereas in females, only a small portion of maxillary palp is visible. The scutellum is small and more round in shape in males, however, in females; the scutellum is broad and oval in shape in all three *Melolontha* species. The tip of scutellum was somewhat pointed in males, and it was rounded in females. There existed little differences in punctuation of scutellum in male and female beetles in all three species. In *M. cuprescens*, the punctation was more even in males and exhibited more crowding on lateral sides of the scutellum, and the middle portion was free from punctation. In females, the scutellum is unevenly punctate throughout. In *M. furcicauda*, the punctuation becomes sparse and shallow in both male and female beetles. In *M. indica*, the scutellum is evenly punctate with dirty white pubescence in both male and female beetles (Plate 4.29; Fig. 4.32).

In *M. cuprescens*, the anterior margin of pronotum of male beetles is deeply notched, and the posterior margin is sub-triangular in outline. On lateral sides, the pronotum tapers gradually outward for about half of its length, and then runs straight. There was a large depression in the mid-anterior region, and a small depression in post mid-region of pronotum. In females of *M. cuprescens*, the anterior margin is almost flat in the centre, and slightly raised on the sides. A small depression is present in the middle region of pronotum. The punctations sparse in the middle and dense at the sides in females, whereas in males, the punctuation is even throughout the pronotum. In *M. furcicauda* males, the anterior margin is almost flat, and it is slightly raised upward on the



(a)

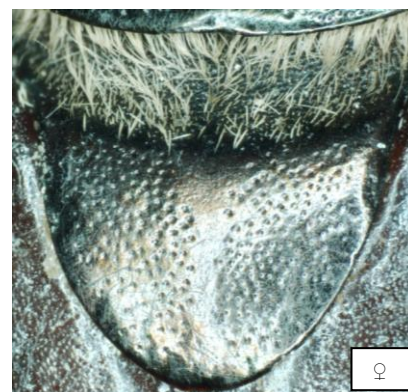
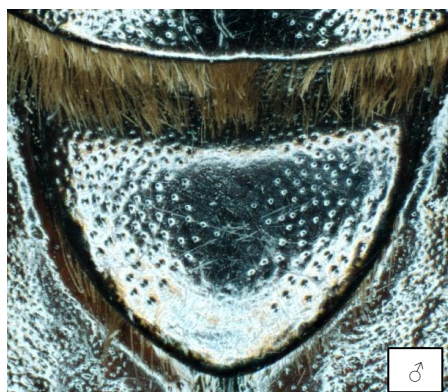


(b)

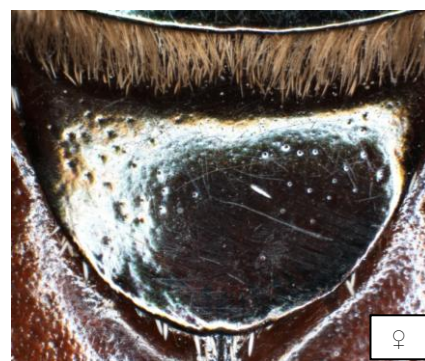


(c)

Plate 4.28 Head capsule (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*



(a)



(b)



(c)

Plate 4.29 Scutellum (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

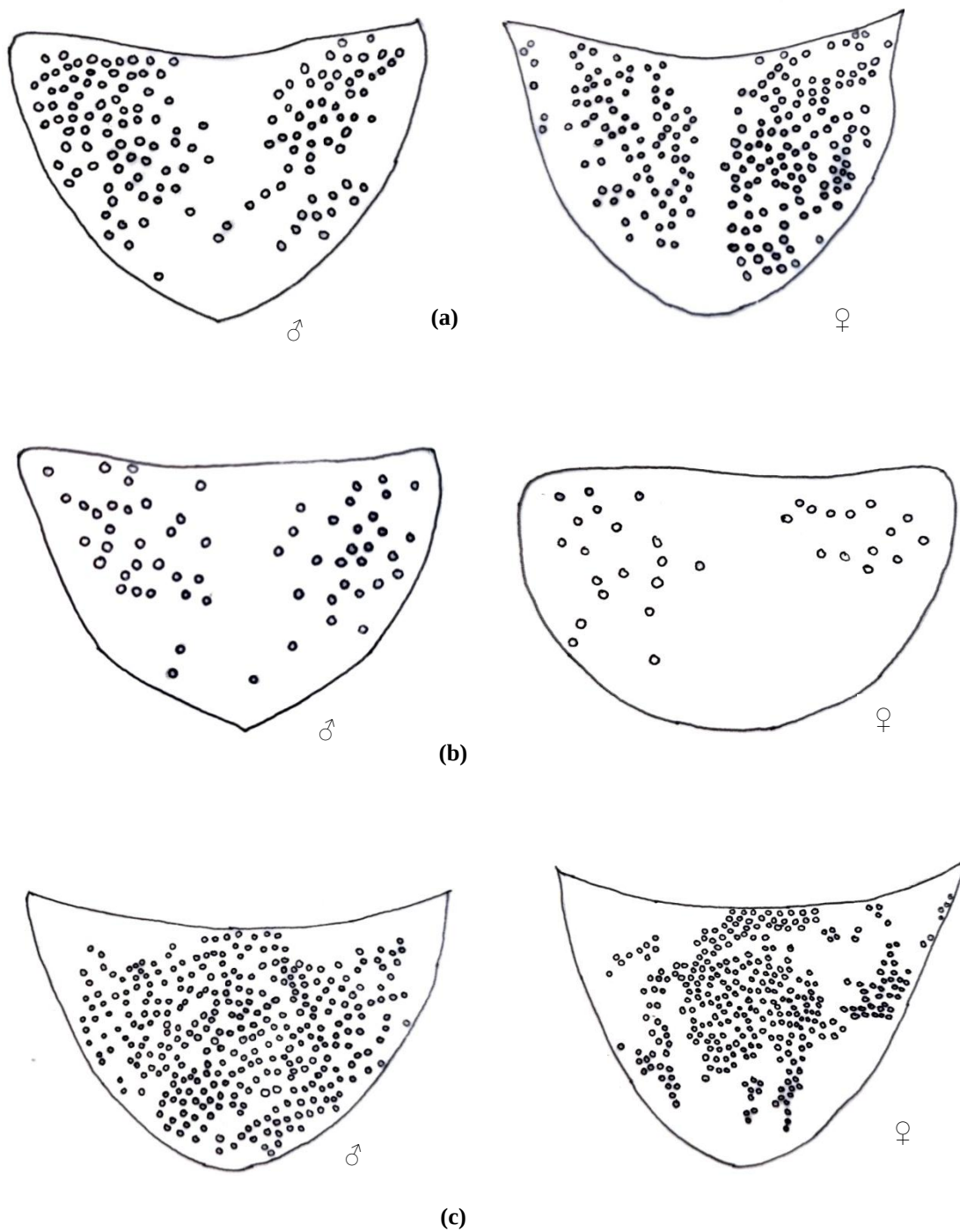
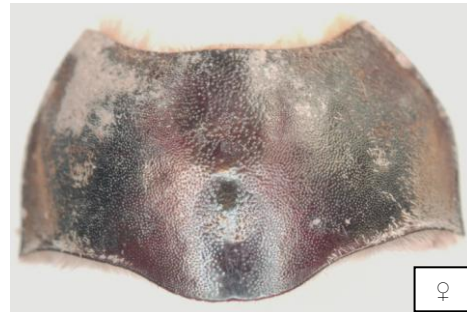


Fig. 4.32 Scutellum (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

sides. The lateral margins taper gradually upward throughout their length, and the posterior lateral margins are distinctly pointed. The posterior margin is concave, but the curvature is more gentle in the middle region. In females, the anterior notch is comparatively more deep, and curvature on the sides is more pronounced. In case of male beetles, the punctations sparse in the middle, dense at the side, whereas in female beetles, the punctuation is even throughout the pronotum (Plate 4.30; Fig. 4.33). According to Chandra and Uniyal (2007), the pronotum of *M. furcicauda* is comparatively more punctate in the middle region. In *M. indica*, the anterior margin appears sub-circular in outline in males, whereas in females, the anterior notch not so prominent and becomes slightly wavy in the centre. In males, a prominent depression is present in the central part of pronotum, whereas in females of *M. indica*, a small depression exists in the mid-posterior region. The punctuation is even throughout the pronotum in both sexes. In closely related species like *M. guttigera*, *Melolontha javanica* Keith & Li, *M. maculata* (Chang) and *M. setifera* Li, the pronotum surface is densely to confluent punctate in narrow bands along the lateral margins (Li et al. 2010).

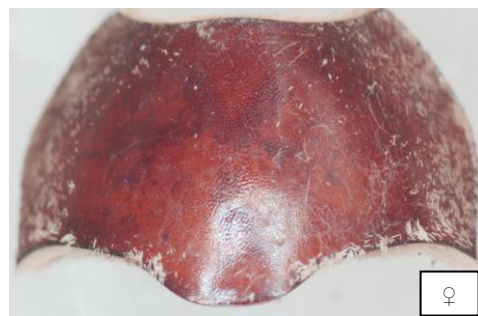
In thoracic segments, the under surface has a light brownish pubescence in all three *Melolontha* species. In males, the density of hairs was more on meta-sternum as compared to females, where a smooth area with few hairs was present (Plate 4.31). In *M. cuprescens*, the meso-metasternal process on ventral surface was observed to be extending from the base of metathorax to posterior part of the mesothorax. It was more broad and slightly more elongated in males as compared to females. In *M. indica*, this process extends up to half of the mesothorax. It was more pointed and slightly more elongated in males as compared to females. Similarly, in *M. furcicauda* males, the meso-metasternal process extends up to anterior margin of prothorax, whereas in females, this process is produced up to mesothorax (Plate 4.32). Chandra and Gupta (2012) reported that meso-metasternal process is weakly developed in *Melolontha guttigera* Sharp, produced weakly beyond meso-coxa to the posterior margin of procoxa. In other closely related species like *Melolontha setifera* Li and *Melolontha maculata* (Chang), the meso-metasternal process is glabrous, well developed, and its apex reaches posterior margin of procoxae (Li et al. 2010). The abdomen is light brown and there are white triangular well-distinguished spots on the side of abdomen in all studied species as indicated in plate 4.33. Chandra and Uniyal (2007) have also reported that sides of andomen in *M. furcicauda* are large with white patches of dense fine scales. In *M. cuprescens* males, the pygidium lobe is triangular



(a)



(b)



(c)

Plate 4.30 Pronotum (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

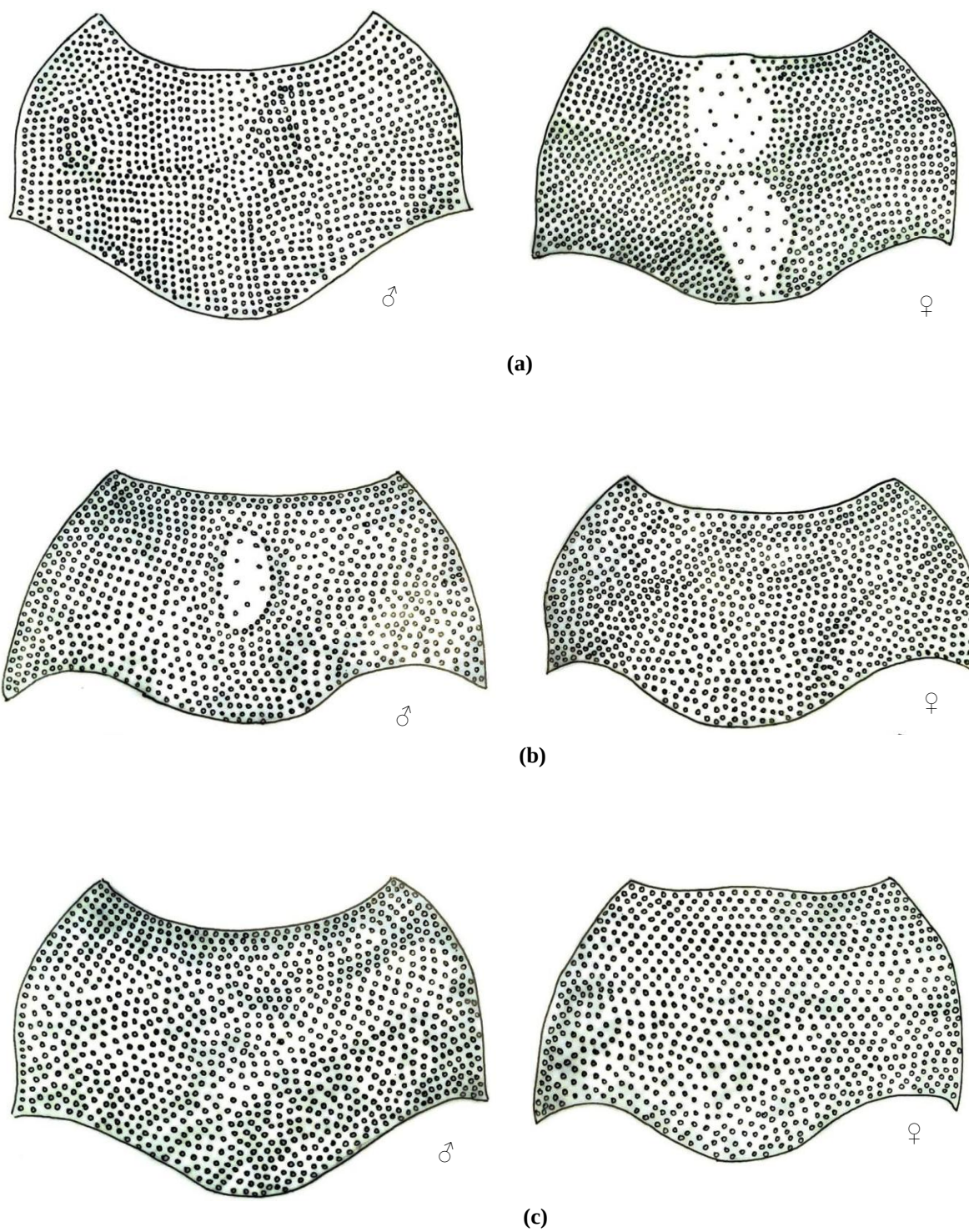


Fig. 4.33 Pronotum (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*



Plate 4.31 Pubescens on meta-sternum (a) *M. cuprescens* (b) *M. furcicauda* (c) *M. indica*



(a)



(b)



(c)

Plate 4.32 Meso-metasternal process (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*



(a)



(b)



(c)

Plate 4.33 Triangular patches on abdomen (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

and bifurcated at the tip. The caudal processes are tubular in shape. The wings almost touching the pygidium in males, whereas in females, more gap observed between wing margins and the pygidium lobe. The caudal processes are short, pointed and widely separated. In *M. furcicauda* males, the pygidium is triangular, but margins are slightly wavy near to the base. The caudal process is bifurcated, tubular and hairy. In females, the caudal processes are pointed and small (Plate 4.34; Fig. 4.34). Chandra and Uniyal (2007) also reported that the pygidium in *M. furcicauda* is long and projected behind into a bifurcated tail. In *M. indica* males, the caudal processes are less projected. The mid line of the pygidium is black. In females of *M. indica*, the mid-line of pygidium runs throughout the lobe and the caudal extremity is slightly bifid without any further extension. A slightly bifid process in caudal extremity of pygidium has been reported in *M. indica* by Chandra and Uniyal (2007) which supports our findings. According to Tereba and Niemczyk (2017), the pygidium is long and slender in *M. melolontha*, whereas in *M. hippocastani*, the pygidium is short and knob shaped. The female beetles were slightly larger in size than the male beetles in all three studied *Melolontha* species. Irrespective of sex, the overall size of beetles in *M. cuprescens* was on higher side than *M. furcicauda* and *M. indica*. Body length x width of male beetles of *M. cuprescens* ranged from 30.0-35.20 mm x 19.2-20.30 mm, and for female beetles, length x width varied from 32.0-35.10 mm x 19.10-22.10 mm. According to Pathania (2014), the body length x width of males of *M. cuprescens* was $26.23 \pm 0.30 \times 12.31 \pm 0.10$ mm, and it was recorded to be $30.53 \pm 0.18 \times 13.64 \pm 0.11$ mm in females, and these values are in close range with our data. Kumar and Vishvendra (2018) reported that the body length x width of *M. cuprescens* beetles is 23 mm x 13 mm. In *M. furcicauda*, the body length x width of male and female beetles ranged from 27.81-30.72 mm x 12.78-14.35 mm and 28.90-30.80 mm x 13.70-14.56 mm, respectively. According to Chandra and Uniyal (2007), the body length x width of *M. furcicauda* beetles measured 32 mm x 15 mm, whereas Feroz et al. (2015) recorded 34 mm x 16 mm as length x width of *M. furcicauda* beetles in Jammu and Kashmir. These values are on higher side as compared to our data. In case of *M. indica*, the body length x width has been recorded to be 25.98 ± 0.68 mm x 13.22 ± 0.21 mm in males, and 26.80 ± 0.72 mm x 14.52 ± 0.31 mm in female beetles (Table 4.24). Chandra and Uniyal (2007) reported body length x width of *M. indica* adults as 28 mm x 13 mm, and these observations are in conformity with our results. In *M. melolontha* and *M. hippocastani*, the body length of beetles range from 22-29 mm and 20-29 mm, respectively (Khramtsov



(a)



(b)



(c)

Plate 4.34 Pygidium (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

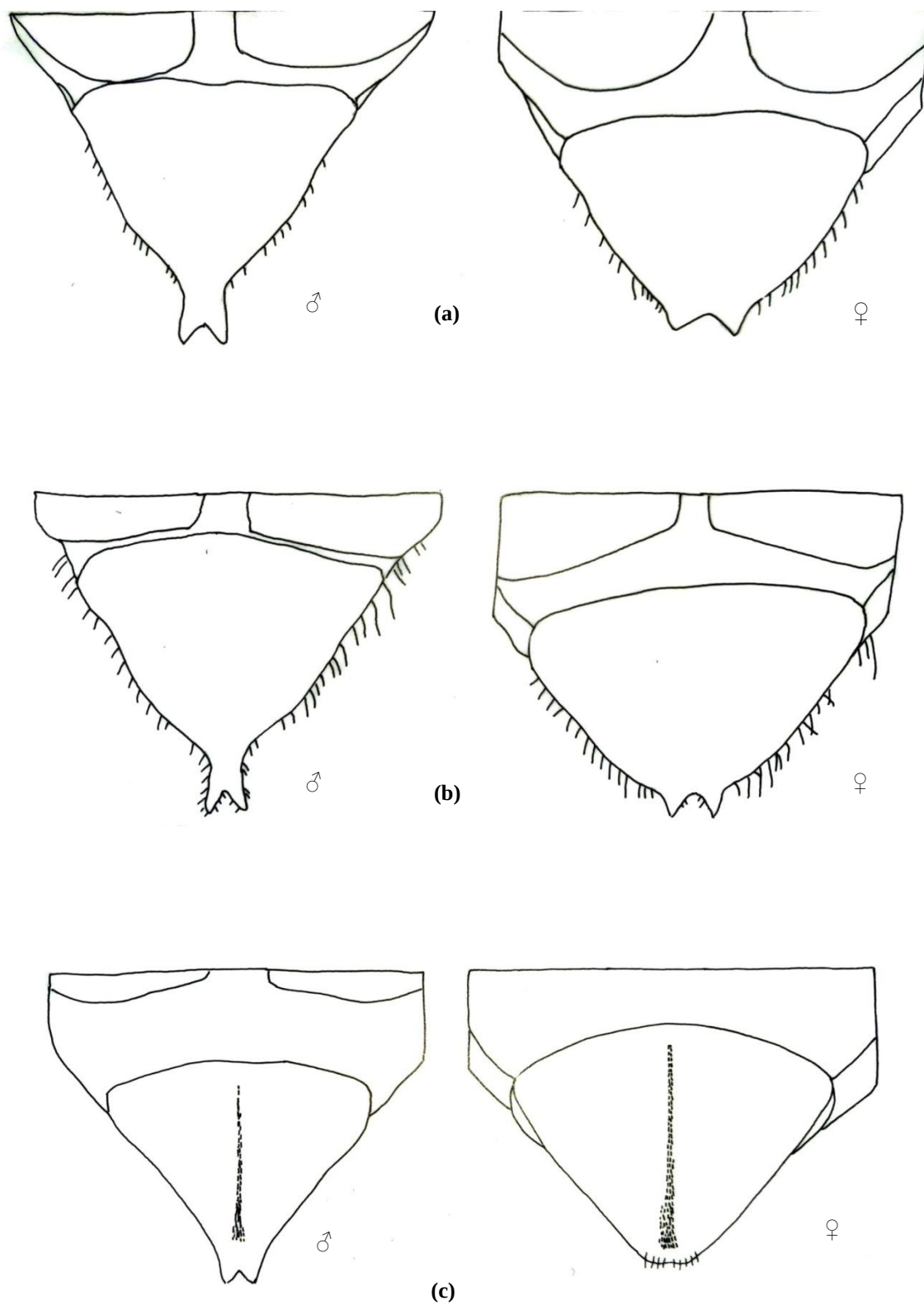


Fig. 4.34 Pygidium (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

1979; Anonymous 2009). Similarly, Woodruff (1978) also reported body length of *M. melolontha* as 25 mm, and width having range from 12-13 mm.

Table 4.24 Morphometric measurements of *Melolontha* adults

Sex	<i>M. cuprescens</i>		<i>M. furcicauda</i>		<i>M. indica</i>	
	Range	Mean±SE	Range	Mean±SE	Range	Mean±SE
Male						
Length (mm)	30.0-35.20	32.68±0.62	27.81-30.72	29.21±0.34	23.30-29.21	25.98±0.68
Width (mm)	19.2-20.30	19.70±0.13	12.78-14.35	13.56±0.20	12.30-14.0	13.22±0.21
Female						
Length (mm)	32.0-35.10	33.37±0.39	28.90-30.80	29.74±0.24	24.40-30.10	26.80±0.72
Width (mm)	19.10-22.10	20.74±0.39	13.70-14.56	14.01±0.08	13.30-15.90	14.52±0.31

In adult beetles, antennae, and legs were also studied in detail to find differences in these three species, and in different sexes within the same species.

Antennae: The lamellate antennae were dark brown, and 10 segmented in all three species. In both sexes, the scape was narrow at the base with a tuft of dense bristles on the posterior margin. Pedicel is small and without bristles. Antennal club of terminal segments was larger in males than in females, and the first segment of flagellum had a narrow base with broader apex in both sexes. In females, second segment of flagellum was reduced to plate-like structure. The antennal club was 7-segmented in males, whereas in females, it was 6-segmented (Plate 4.35; Fig. 4.35). On anterior margin of flagellum, small setae were present. The maximum width of antennal club of *M. cuprescens* has been recorded to be 6.34 mm in males, whereas in females, it measured 1.05 mm. In *M. furcicauda* and *M. indica*, the width of antennal club was recorded to be 5.79 mm and 5.13 mm in males, and 1.41 mm and 1.75 mm in female beetles, respectively. Chandra and Uniyal (2007) reported that in *M. furcicauda* and *M. indica*, the antennae are 10 segmented, long in males and with seven segmented antennal club. In *M. hippocastani*, the antennal plates are heptamerous in males and two times longer than females with antennal club consisting of six plates (Anonymous 2009). All these reports corroborated our findings.

Legs: Legs are well developed, adapted for walking and running. It is also supporting function for feeding and mating. In fore legs of *M. cuprescens*, the femur width (2.31 mm) is more and length (4.89 mm) is comparatively less in females. In males, the femur width and length is 1.74 mm and 6.73 mm, respectively. Tibia is flattened, notched and leaf-like in females and measured 1.29 mm in width and 4.25 mm in length, whereas in males, the tibia is thin with a width of 0.99 mm, and length as 5.91 mm. Earlier workers have also



(a)



(b)



(c)

Plate 4.35 Antennae (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

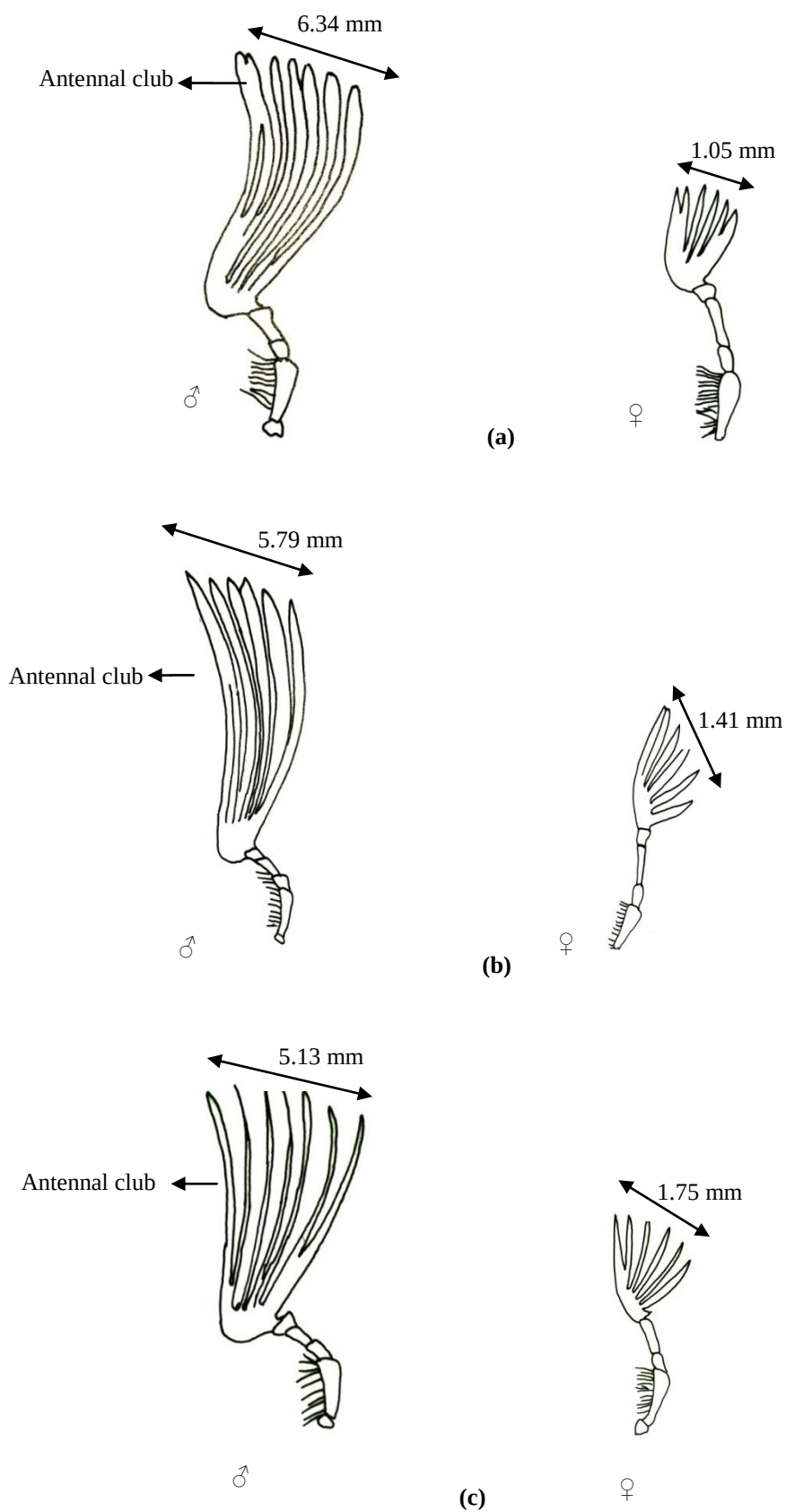
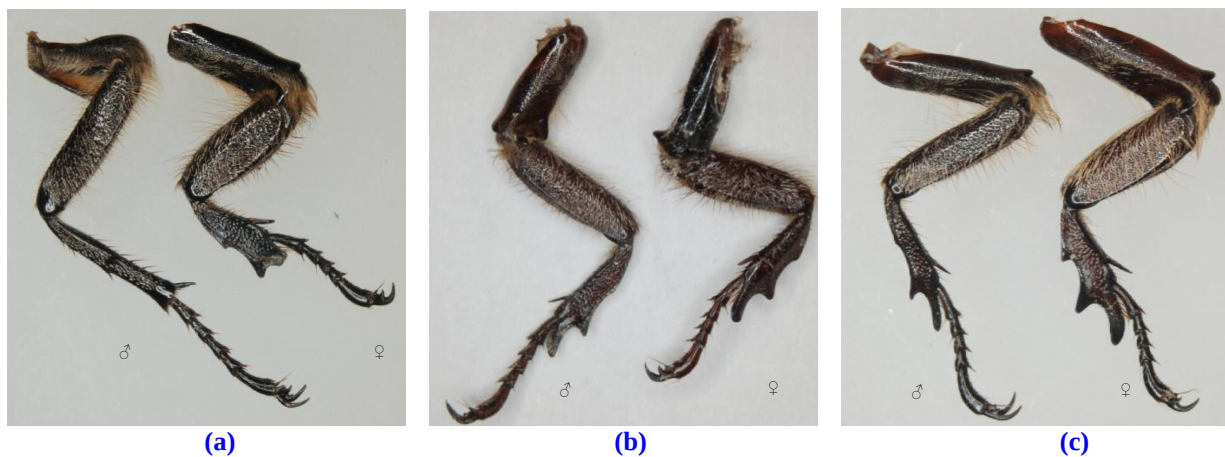


Fig. 4.35 Antennae (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

reported sexual diamorphism on the basis of notched tibia of female forelegs in *M. hippocastani* (Anonymous 2009). The tibial spines are pointed and 0.82 mm in males, whereas in females, tibial spines are less pointed, and measured 0.87 mm in length. Tarsi are short in females (4.68 mm) than in males (5.39 mm). The claws in females are single, toothed and 1.31 mm in length. In males, the claws are bifurcated measuring 1.57 mm. In *M. furcicauda*, the tibial spine was 0.70 mm (pointed) in males, and in females, it was 0.96 mm (blunt). The claws are simple, toothed and measured 1.46 mm in females; however, in males, the claws are bifurcated, widely separated and measured 1.88 mm in length. In *M. indica*, the femur width is comparatively more in females (2.07 mm) than in males (1.56 mm). Tibia is leaf-like in both sexes, but more flattened in females as compared to males. The length of tibial spine has been recorded to be 0.59 mm in females and 0.87 mm in males. The length of claw was 1.73 mm in males and 1.16 mm in females (Plate 4.36; Fig. 4.36).

In mid legs of *M. cuprescens*, the tibia is more flat, and posterior end is bifurcated in males as indicated in fig. 4.37. The tibial spines are pointed and 0.72 mm long in females, whereas in males, the tibial spines are less pointed, and 0.74 mm long. The claws are single, short and toothed in females, measuring 1.09 mm in length. In males, claws are bifurcated, long and toothed with an average length of 1.78 mm. In *M. furcicauda* and *M. indica*, tibial spines are well pointed both in males and females, but the tibial spines in females were larger than in male beetles. In *M. furcicauda* and *M. indica*, the tibial spines of female beetles measured to be 0.73 mm and 0.87 mm, and in male beetles, the tibial length has been recorded to be 0.55 mm and 0.67 mm, respectively. In *M. furcicauda*, the claws were simple, toothed and long in males (1.76 mm), but in females, the claws were bifurcated and comparatively short in length (1.48 mm). In *M. indica*, claws measured 1.29 mm in males and 1.24 mm in females (Plate 4.37; Fig.4.37).

In hind legs, the femur width is more in females than in males in all three *Melolontha* species (Fig. 4.38). In *M. cuprescens*, the femur width in female and male beetles has been recorded to be 3.05 mm and 2.29 mm, respectively. Similarly, in *M. furcicauda*, the femur width was comparatively more in females (2.81 mm) than in males (1.68 mm). In *M. indica*, the femur width in females was 2.83 mm and in males, it was 2.05 mm. The tibial spines are conspicuously thick, small in size and more pointed in females of *M. cuprescens* (1.58 mm) and *M. furcicauda* (1.07 mm) than in males (*M. cuprescens*: 1.63 mm; *M. furcicauda*: 1.44 mm). In *M. indica*, the tibial spines of females measured 1.56 mm in females, 1.14 mm in males on the present study. Claws were simple

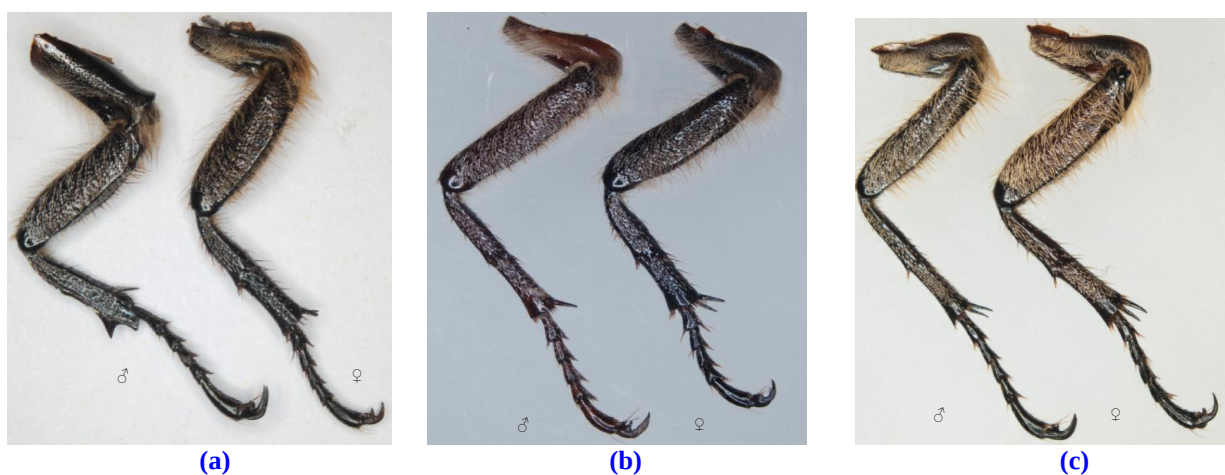


(a)

(b)

(c)

Plate 4.36 Forelegs (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

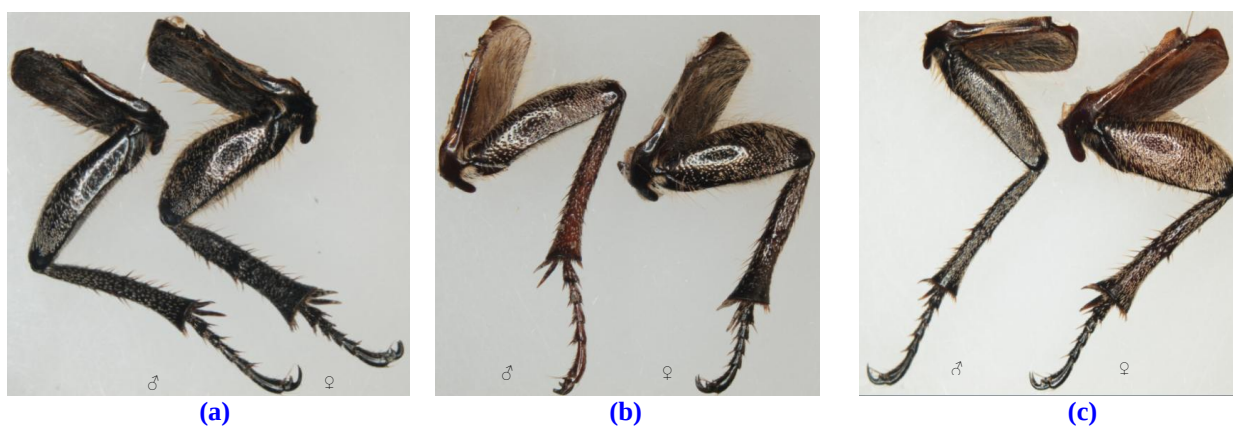


(a)

(b)

(c)

Plate 4.37 Midlegs (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*



(a)

(b)

(c)

Plate 4.38 Hindlegs (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

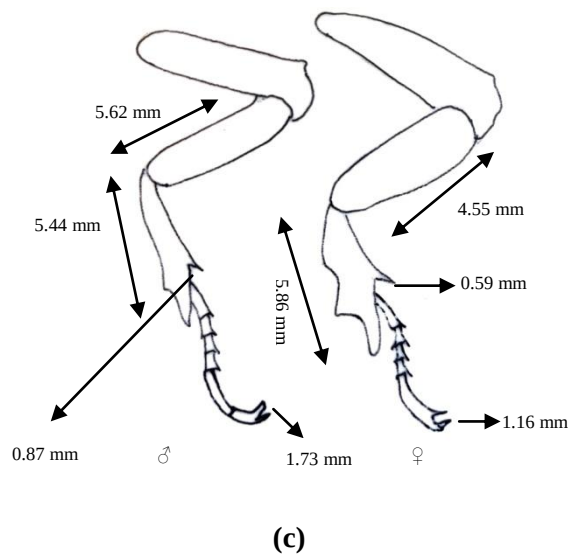
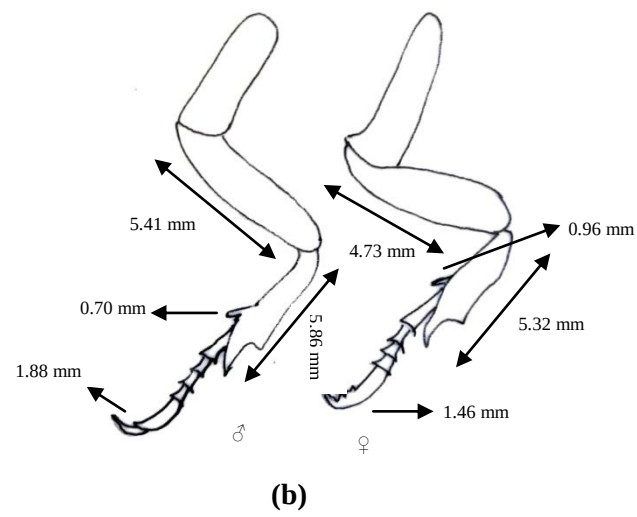
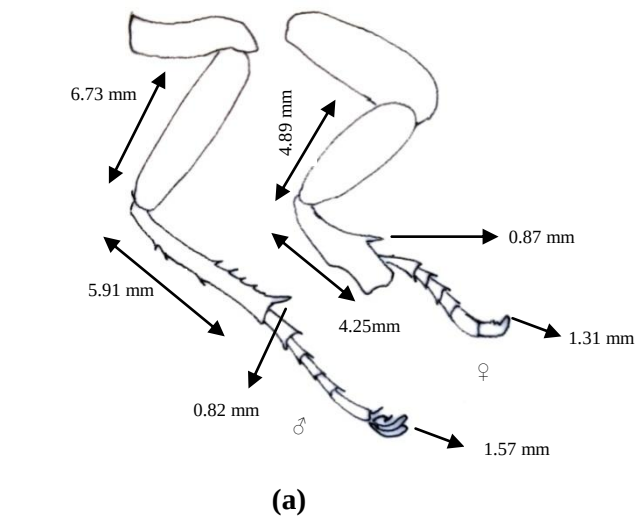
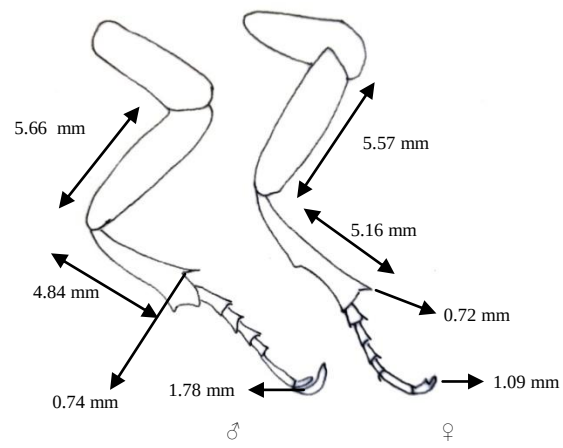
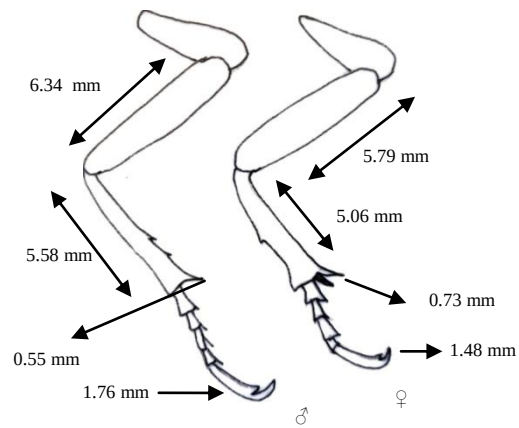


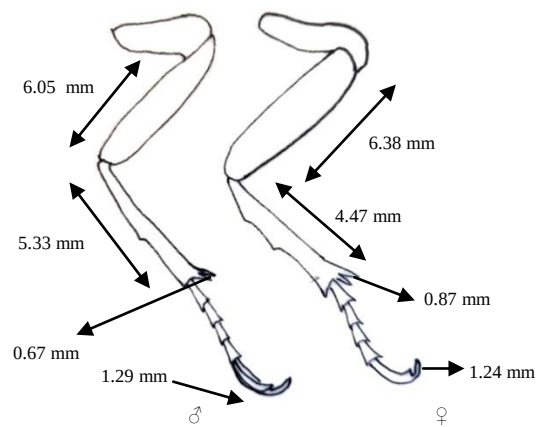
Fig. 4.36 Forelegs (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*



(a)

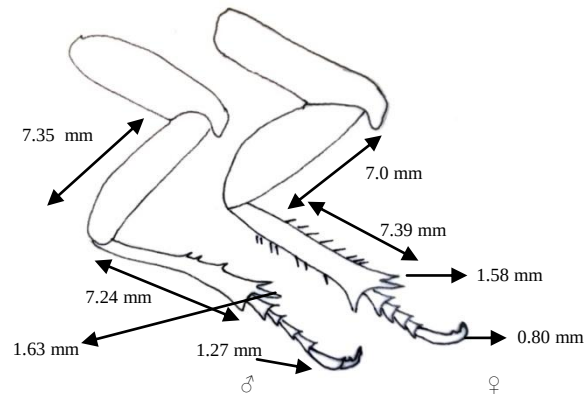


(b)

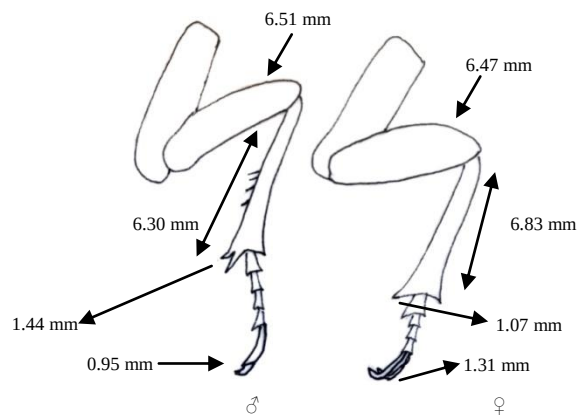


(c)

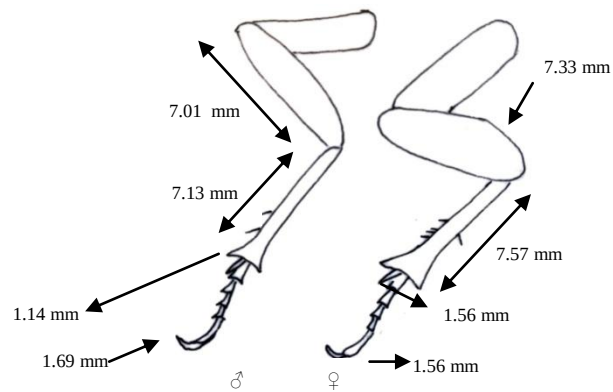
Fig. 4.37 Midlegs (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*



(a)



(b)



(c)

Fig. 4.38 Hindlegs (a) *M. cuprescens*; (b) *M. furcicauda*; (c) *M. indica*

in males and bifurcated in females in all three *Melolontha* species. The shape and size of claws differed considerably among both sexes in *M. cuprescens* and *M. furcicauda*. In *M. cuprescens*, the claws were more pointed, curved and long in males (1.27 mm) than in females (0.80 mm). Contrary to this, in *M. furcicauda*, the claws were more pointed, curved and long in females (1.31 mm) as compared to males (0.95 mm) where claws exhibited blunt apex. In *M. indica*, the claws were pointed, curved and long in both female (1.56 mm) and male beetles (1.69 mm) (Plate 4.38; Fig. 4.38).

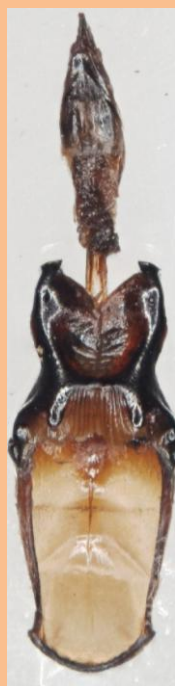
Male genitalia

***M. cuprescens*:** Phallobase is longer than paramere, sub-circle, measuring a length of 5.99 mm and width of 4.26 mm, broader anteriorly and slightly narrow at the base. Phallobase light brown in colour, whereas parameres are dark brown in colour having contrast with phallobase. Both parameres are symmetrical, separated widely and lobe-like (Plate 4.39; Fig. 4.39). Li et al. (2010) also reported that male genitalia is moderately symmetrical in *M. guttigera*. Parameres measuring length of 4.53 mm and width of 2.72 mm, broader at the base and narrower at the apical margin. Dorsal posterior margin of paramere is 'Y' shaped and connected by membranous integument. The parameres are deeply grooved and constricted in the mid-region. The endophallous long, sclerotised, enlarged in the centre and pointed at the tip. There is gradually tapering to both sides. Temones long and connected to the base by uniform width. Spiculum gastrale 'Y' shaped sclerite, stem short, measuring 2.23 mm in length and 0.47 mm in width. Arms are lengthy having width of 4.21 mm, and connected by membranous structure. The arms are pointed upward.

***M. furcicauda*:** Phallobase light brown in colour, whereas parameres are dark brown in colour. Phallobase tapers gradually from anterior region to the base, which is more narrower. The length of phallobase measured 5.69 mm, and the width was 4.55 mm. The ventral inner mid posterior margin of parameres is connected by sclerotised pointed arms. Both parameres are symmetrical, separated widely and lobe-like. In ventral view, the parameres look elongated and sickle shaped (Plate 4.40; Fig. 4.40). Parameres measuring length of 3.92 mm and width of 2.01 mm, broader at the base, and constricted in the middle. In dorsal view, the mid-posterior region is bell-shaped. Both sides of the parameres bounded by an elevated ridge. Endophallus short, membranous and asymmetrical. Temones long and connected to the base. Spiculum gastrale 'Y' shaped, sclerite, stem medium, measuring length of 3.09 mm and width of 0.41 mm. Arms are connected by a membranous structure, having width of 4.68 mm. The arms are pointed outward and upward.



Dorsal view



Ventral view



Lateral view



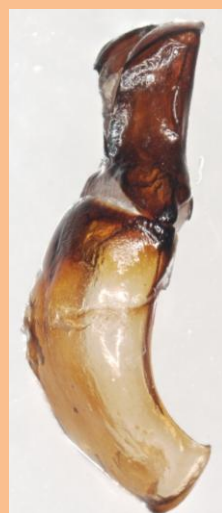
Spiculum gastrale

Plate 4.39 Genitalia of *M. cuprescens* male

Dorsal view



Ventral view



Lateral view



Spiculum gastrale

Plate 4.40 Genitalia of *M. furcicauda* male

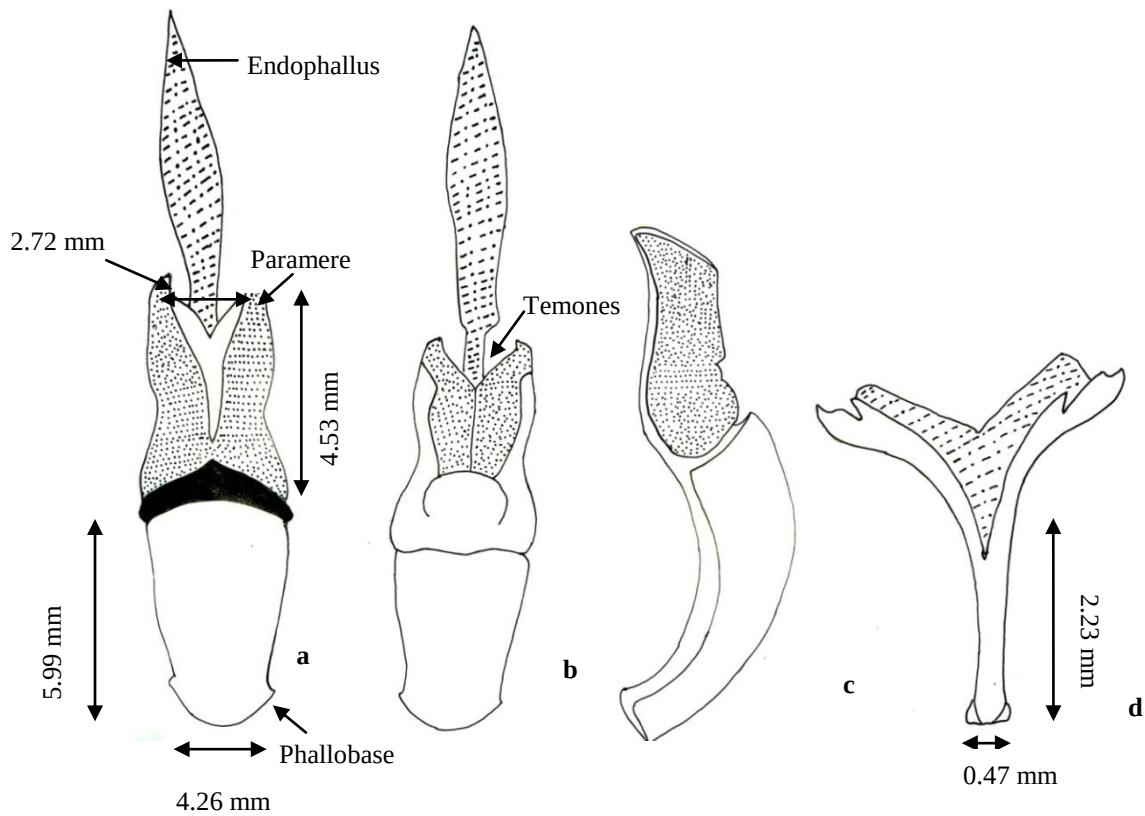


Fig. 4.39 Male genitalia of *M. cuprescens* (a) Dorsal view; (b) Ventral view; (c) Lateral view; (d) Spiculum gastrale

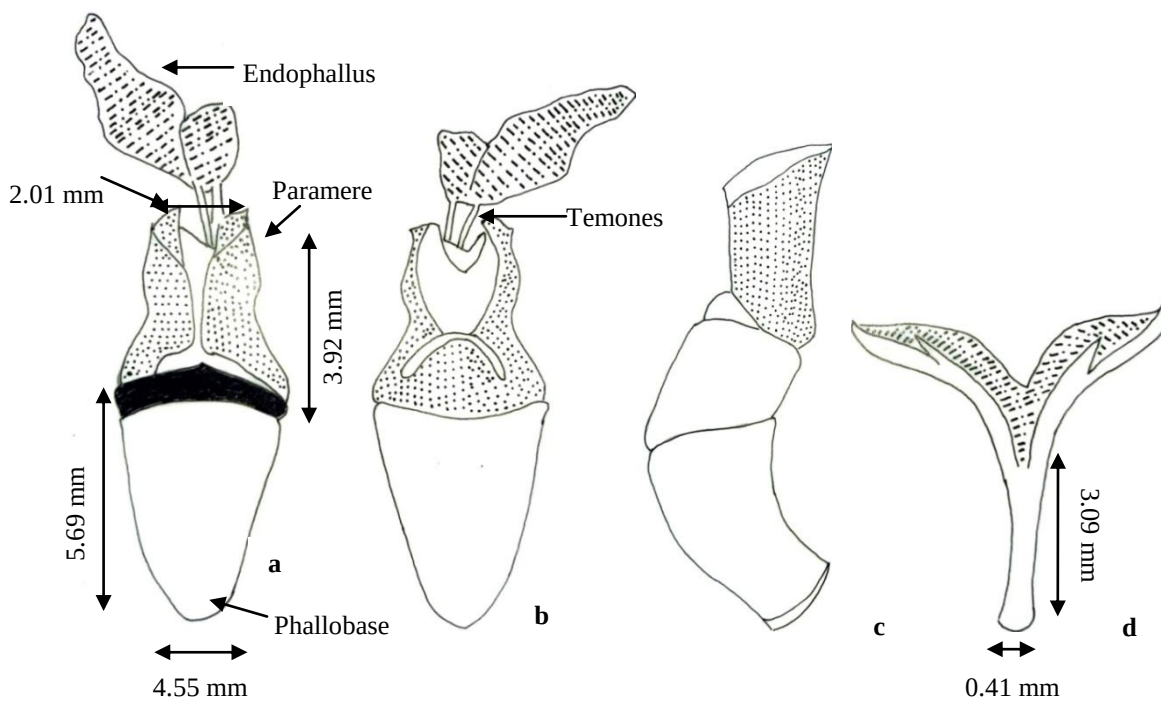


Fig. 4.40 Male genitalia of *M. furcicauda* (a) Dorsal view; (b) Ventral view; (c) Lateral view; (d) Spiculum gastrale

***M. indica*:** Phallobase is yellowish brown in colour, larger than paramere, having broader base. It is evenly sclerotised measuring the length of 5.59 mm and width of 3.46 mm. Dorsal anterior margin of phallobase is connected to parameres with membranous tissue. Parameres typically join in the centre in the form of two sub-triangular plates in dorsal mid-posterior region. Lateral processes of parameres are typically swollen at the base, slightly narrower in the middle, which further broadens, and finally becomes pointed in shape (Plate 4.41; Fig. 4.41). The parameres measured 3.75 mm in length and 2.29 mm in width. Endophallus elongated and oblong in shape. Temone long, narrower at the base, and broadens at its posterior end. Spiculum gastrale 'Y' shaped sclerite, stem short, measuring length of 2.04 mm and width of 0.39 mm. Arms are connected by a membranous structure, having width of 3.47 mm. The arms are further bifurcated into two parts in the mid-region; shorter arms are internal in position and larger arms are pointed outward and upward. The posterior end of the stem is lobe-like.

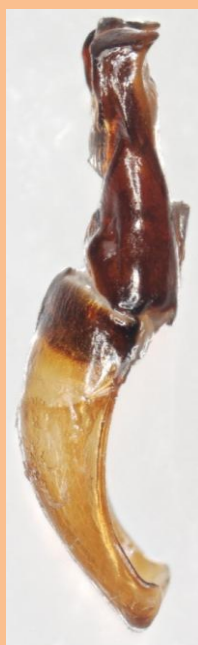
Adult longevity: There existed considerable variation in the longevity of female beetles in different *Melolontha* species in the present study. The pre-oviposition period ranged from 5-11 days in different species of *Melolontha*, maximum being in *M. indica* (mean: 8.3 ± 0.63 days) and minimum in *M. furcicauda* (mean: 5.8 ± 0.33 days). The oviposition period was very short in all three studied *Melolontha* species. The oviposition continued for a period of 2-4 days (mean: 3.0 ± 0.26 days) in *M. cuprescens*. In *M. furcicauda*, the duration of oviposition varied from 1-3 days with a mean of 2.1 ± 0.28 days. Similarly, in *M. indica*, the females deposited eggs for a period of 2-6 days, and the average oviposition period was found to be of 4.1 ± 0.50 days (Table 4.25). The mean fecundity has been recorded to be 19, 26 and 23 eggs per female in *M. cuprescens*, *M. furcicauda* and *M. indica*, respectively. According to Pathania (2014), a single female of *M. cuprescens* laid on an average of 13 eggs in Himachal Pradesh. Keller (2000) observed fecundity of 12-46 eggs/ female in *M. melolontha* from Switzerland. Huiting et al. (2006) reported that each female of *M. melolontha* deposits a batch of about 24 eggs, and many egg laying females die, but about a third return to feed and lay for a second time; some lay eggs for a third time. The post-oviposition period showed considerable variation in *M. cuprescens*, *M. furcicauda* and *M. indica*, and it was of longer duration as compared to pre-oviposition and oviposition periods. The average duration of post-oviposition period was observed to be of 8.9 ± 0.41 days in *M. furcicauda*, and 13.7 ± 0.37 days in *M. cuprescens*. In *M.*



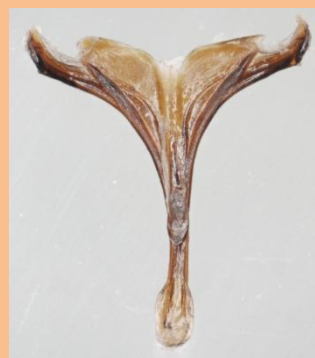
Dorsal view



Ventral view



Lateral view



Spiculum gastrale

Plate 4.41 Genitalia of *M. indica* male

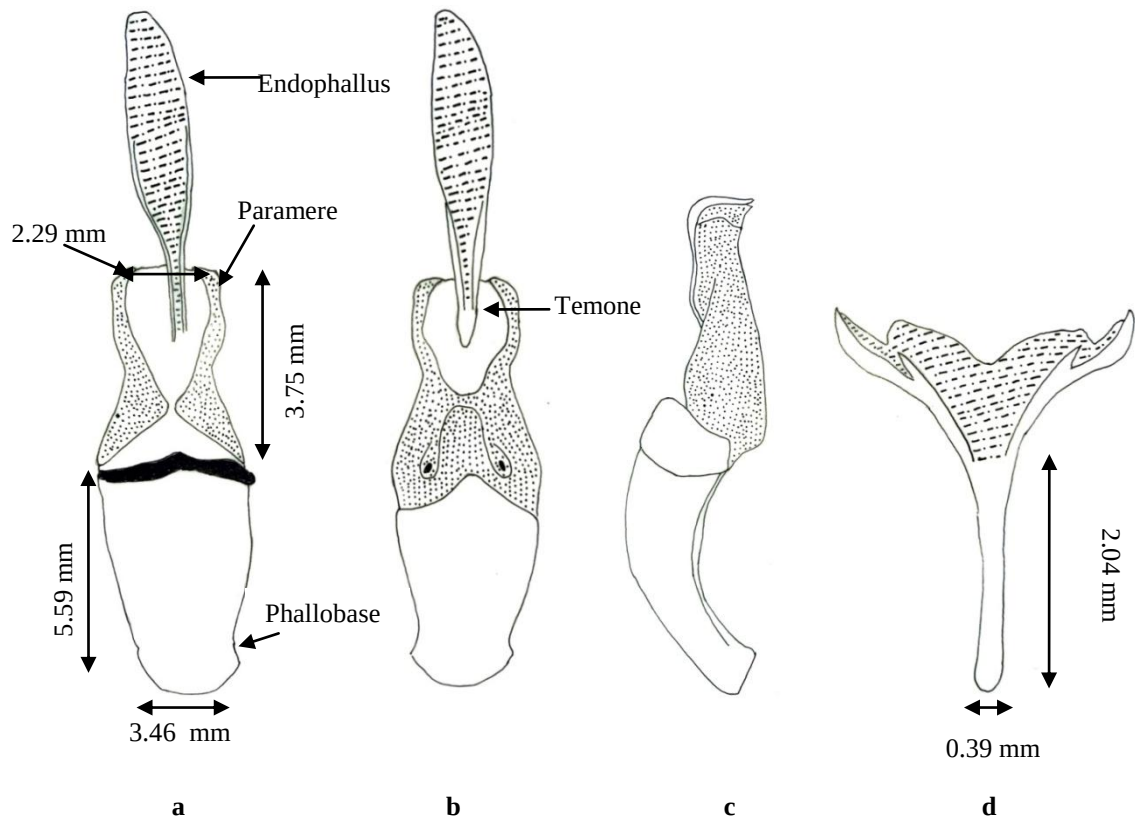


Fig. 4.41 Male genitalia of *M. indica* (a) Dorsal view; (b) Ventral view; (c) Lateral view; (d) Spiculum gastrale

indica, the post-oviposition period was longest, and it varied from 21-26 days with a mean of 23.3 ± 0.52 days (Table 4.25).

The overall survival of female beetles was considerably more as compared to males in *M. indica* and *M. furcicauda*. In *M. cuprescens*, the longevity of female and male beetles did not show any difference. It was recorded to be 25.0 ± 0.71 days for male beetles and 25.1 ± 0.71 days for female beetles. In *M. furcicauda* and *M. indica*, the average survival of male beetles was recorded to be 17.4 ± 0.40 and 27.4 ± 0.34 days,

Table 4.25 Pre-oviposition, oviposition and post oviposition periods of *Melolontha* species

Stage	Duration in days					
	<i>M. cuprescens</i>		<i>M. furcicauda</i>		<i>M. indica</i>	
	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE
Pre oviposition period	6-8	7.10 ± 0.28	5-7	5.80 ± 0.33	6-11	8.30 ± 0.63
Oviposition period	2-4	3.0 ± 0.26	1-3	2.10 ± 0.28	2-6	4.10 ± 0.50
Post oviposition period	12-15	13.70 ± 0.37	7-11	8.90 ± 0.41	21-26	23.30 ± 0.52

whereas the female beetles of *M. furcicauda* and *M. indica* survived for 23.0 ± 0.49 and 36.6 ± 1.18 days, respectively (Table 4.26). Pathania (2014) reported longevity of female *M. cuprescens* beetles varying between 15-23 days, and 19-31 days for male beetles in Himachal Pradesh. In *H. consanguinea*, it is 13-17 days as reported by Yadava and Saxena (1977).

Table 4.26 Adult longevity of *M. cuprescens*, *M. furcicauda* and *M. indica*

Species	Male (Duration in days)			Female (Duration in days)		
	Range	Mean $\pm$ SE	Period of year	Range	Mean $\pm$ SE	Period of year
<i>M. cuprescens</i>	21-28	25.0 ± 0.71	June-August	23-30	25.10 ± 0.71	July-August
<i>M. furcicauda</i>	16-19	17.40 ± 0.40	June- August	21-25	23.0 ± 0.49	July-August
<i>M. indica</i>	25-29	27.40 ± 0.34	July-August	31-44	36.60 ± 1.18	July-September

4.5 Biology and morphometrics of *M. virescens* [= *Polyphylla fullo* (Linnaeus)]

P. fullo has been described earlier as *M. virescens* by Pathania (2014) and *M. indica* by Chandel et al. (2008). According to Sehnaal and Bezdek (2011), the subgenus *Polyphylla* is synonym to *Granida*. Beeson (1941) has reported *Granida albosparsa* Moser a large Melolonthine beetle commonly occurring in Himalaya up to 9000 feet and causing maximum damage during May-June. Its larva is 45 mm as described by Gardner (1935) and the life cycle lasts for two years above 6000 feet. It seems possible that *G. albosparsa* can be an incorrect citation for *P. fullo*. *P. fullo* is mainly distributed in high hill areas of Himachal Pradesh at altitude ranging between 2039 to 2588 mm. It was recorded in

moderate numbers from Badagaon (Kangra district), Sangla (Kinnaur district) and Ahla (Chamba district) areas. The collection of beetles from forest/fruit trees have been average, they represent total five different host plants in the present study. The species has a wide distribution in Chuhar valley, becoming most numerous in Barot, Multhan, Kothikohar and Badagaon areas. In Chuhar valley, it constituted 40.66 per cent of total beetle catch and ranked first in total number of beetles collected on different hosts during 2017-18. Erler and Ates (2015) have reported *P. fullo* as the most common species of economic importance in many districts of Turkey. The beetles are not strongly attracted to light, although, it has frequently been collected on light trap in Badagaon, Sangla, Janjehli and Ahla in the present study during 2017-18. This species constituted 11.89-13.36 per cent of total *Melolontha* beetle catch on light traps in Himachal Pradesh during 2017-18.

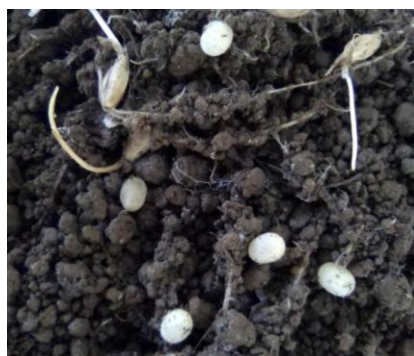
4.5.1 Eggs: The emergence of *M. virescens* (= *P. fullo*) from soil started in second week of July, peak activity occurred during August, and then perished in first week of September. Erler and Ates (2015) have reported beetle emergence of *P. fullo* from mid-June to mid-July in Turkey. Mating (Plate 4.42) was observed at dusk under laboratory conditions and it lasted for 16-20 minutes. The beetles were also observed on host plants during day time both under field and laboratory conditions. Young (1988) has also indicated diurnal searching behaviour in the males of *Polyphylla crinita* LeConte thereby confirming our observations. As in other species of white grubs, oviposition occurred in soil. The eggs were deposited at a depth of 10-12 cm. The eggs were pearly white and when freshly laid were elongated in shape (Plate 4.43). The field-collected beetles observed a pre-oviposition period of 7-9 days under laboratory conditions in captivity. Oviposition occurred in soil during third to fourth week of July. The period of oviposition was brief varying from 2-3 days. A single female laid 13-21 eggs during her life time under laboratory conditions. Newly laid eggs measured from 3.62-4.21 mm in length, and 2.57-3.09 mm in width. As the development progressed, they enlarged and assumed nearly spherical shape, as do the eggs of other scarab species. Prior to hatching, the length of eggs was recorded to be 4.73-4.98 mm with an average of 4.86 ± 0.03 mm (Table 4.27). Rodstrom and Brown (2017) made similar observations for the eggs of *P. decemlineata* in Washington. In other related species *P. crinita*, the newly deposited eggs measure about 2 mm wide and 3.4 mm long, but increase considerably in size within few days (Young 1988). These reports back up our results. Duration of egg stage ranged from 16-18 days with an average of 17.2 ± 0.35 days. The length of egg stage as determined by Young (1988) was about 54 days in closely related species, *P. crinita* in Nebraska. Under



Plate 4.42 Mating pair of *M. virescens* (= *P. fullo*)



Newly laid eggs



Eggs prior to hatching

Plate 4.43 Eggs of *M. virescens* (= *P. fullo*)

laboratory conditions, the eggs hatched during second week of August. Contrary to our findings, Young (1988) reported that the egg hatching in *P. crinita* occurs during third week of September in Nebraska.

4.5.2 Larva

First instar: The newly hatched grubs were creamy-white in colour (Plate 4.44). The width of head capsule ranged from 2.60-2.84 mm. The fully fed first instar grubs of *M. virescens* (= *P. fullo*) measured 10.16-13.97 mm in body length. The duration of first instar averaged 47.9 ± 0.57 days with a range of 46-51 days. The young grubs after 1-2 days, started gnawing roots of rajmash/oats, and became more active within 4-5 days. The fully fed first instar larvae stopped feeding and started downward movement before 10-15 days of moulting. Under field conditions, the larvae were found only in sandy soil with permeable subsoil. The upper 6-8 inches generally hosts only mature larvae with younger stages occurring at greater depths as they fed upon finer roots. The duration of first instar larvae averaged 47.9 ± 0.57 days with a range of 46-51 days. In laboratory, the first instar moulted into second instar during the month of October (Table 4.27). Contrary to our results, the first instar grubs of *P. decemlineata* have been reported to hibernate in California (Rodstrom and Brown 2017).

Second instar: The first instar grubs moulted to second instar in the month of October. On moulting, the head was whitish, and it soon turned brown (Plate 4.44). The average width of head capsule was recorded to be 4.68 ± 0.17 mm (range: 4.33-5.13 mm). The body length of fully grown second instar grubs ranged from 23.65-27.70 mm with an average of 25.89 ± 0.71 mm. According to Pathania (2014), the second instar grubs on an average measured 41.26 mm in length and the mean head capsule width has been calculated to be 4.68 mm. The growth ratio (GR) for head capsule width was calculated to be 1.72 for second instar as compared with first instar. The second instar grubs inflicted large holes in potato tubers rendering them unfit for marketing. Second instar grubs constructed earthen cells during second week of May, and moulted inside these cells. Erler and Ates (2015) reported that larvae of *P. fullo* feed during the late summer and early fall and move deep in the soil to overwinter. After the hibernation, they return to the root zone and feed throughout the following summer. These behavioural differences are attributed to the fact that *P. fullo* has triennial life cycle in Turkey. However, in *P. decemlineata*, the grubs complete their second instar in the following growing season as observed in the present study. The duration of the second instar varied from 214-229 days (mean: 221.7 ± 1.71 days) as shown in table 4.27. Rodstrom and Brown (2017) reported that in *P.*

decemlineata, the second instar is completed during second year under Washington conditions in United States.

Third instar: The grubs in third instar were robust and slightly yellowish to creamish-white in colour (Plate 4.44). The length of third instar grubs of *M. virescens* (= *P. fullo*) ranged from 48.33-54.17 mm with an average of 50.17 ± 1.03 mm. The width of head capsule in third instar varied from 7.92-8.48 mm with an average of 8.19 ± 0.11 mm. According to Pathania (2014), the third instar grubs averaged 55.38 mm in length and the head capsule width was 8.45 mm. The ratio (GR) for head capsule width of *P. fullo* was calculated to be 1.75 for third instar as compared with the second instar. Pathania (2014) has calculated the growth ratio (GR) of 1.81 for third instar grubs as compared with the second instar grubs. The overall growth ratio (GR) has been worked out to be 1.74 for *P. fullo*. The majority of the damage is done by second and third instar during second year of their development. Third instar grubs produced large irregular cavities into the potato tubers and the infested tubers were rendered totally useless. Duration of third instar averaged 314.1 ± 1.19 days, ranging from 309-320 days (Table 4.27). Fully fed third instar grubs stopped feeding, constructed hard earthen cells and remained inactive inside these cells till adult emergence in next summer. Erler and Ates (2015) have reported in Turkey that larvae of *P. fullo* complete their development during spring and early summer of next year, and turn into pupae and adults that remain inactive in the soil. According to Steenwyk and Rough (1989), the third instar grubs of *P. decemlineata* again overwinters during the second year, and pupation and adult eclosion occurs in the third growing season. In the present study, the first earthen cell was formed as early as second week of December during 2018. Grubs moved deeper into the soil in December and have been found as deep as 30 inches under field conditions. A few mature grubs may be found at such depths at any time of the year. Young (1988) believed it to be a function of nutrient searching rather than climatic stress. The third instar grubs were found near the surface from February until pupation in May. In the present study, the larval stage was recorded to occur from August, 2017 to March, 2019. The total larval period ranged from 574-594 days with an average of 583.7 ± 2.10 days. Rodstrom and Brown (2017) reported that larval development in *P. decemlineata* requires multiple years depending upon latitude and site. In California, two or three seasons are required, while in some northern sites, the life span can be of four years.



First instar



Second instar



Third instar

Plate 4.44 Different larval stages of *M. virescens* (= *P. fullo*)

Larval morphology

Clypeus and labrum: The clypeus is nearly trapezoidal in shape, bearing an internal membranous band and exterior sclerotized area (Plate 4.45; Fig. 4.42). The clypeus is furnished with two pairs of setae laterally and one pair centrally. The labrum is rounded, articulated distally to the clypeus. It is equipped symmetrically with three pairs of longer setae on its dorsal surface, but groups of shorter setae were absent. There is one pair of black spots present near to the base of labrum as indicated in fig 4.42.

Antennae: The antennae were four segmented, cylindrical with second and third segments comparatively longer. The basal segment and second segment possess four and three prominent setae, respectively (Fig. 4.43). The protrusion on the ventral surface of the third segment is straight and blunt at apex. The fourth segment is not uniformly tapered; it is swollen in the middle. The sensory plate on the dorsal surface of the fourth segment is elongated covering about 60 percent of the area. On ventral surface, two sensory spots are present. The posterior one is oval in shape and interior one is trapezoidal in shape. Both sensory spots are somewhat depressed in centre. The apical sensory area is furnished with nine sensilla basiconica which appears to be arranged in circle (Plate 4.46; Fig. 4.43).

Epipharynx: In *M. virescens* (= *P. fullo*), the haptomerum is composed of 12 heli, pointing posteriorly, and five minute sensilla basiconica (Plate 4.47; Fig. 4.44). In contrast to this, Hayes (1928) reported 20 heli in epipharynx of *Polyphylla arguta* Casey. The number of plegmata and acanthopariae were recorded to be 13. Similarly, Young (1988) has observed 10-12 very short plegmata in *Polyphylla variolosa* (Hentz). The pedium is broader at apex and narrower towards the basal part. The dextrotorma is more or less straight. The pternotorma appears to be peg shaped.

Mandibles: The scissorial area is blackish with a nearly straight cutting edge having small serrations (Plate 4.48; Fig. 4.45). The width of scissorial area and length of molar area was recorded to be 1.06 mm and 2.40 mm, respectively. The inner edge of the scissorial area was concave in shape and there was a gap of 1.59 mm in between scissorial and molar structure. The base of mandible was 3.25 mm in width. In scobis area, eight setae were present. The left mandible possessed an acia and ventral process. Stridulatory area is present in each mandible in the form of small granules (Plate 4.48; Fig. 4.45).

Maxillae: The stipe is furnished with 17 long stridulatory teeth with blunt apex in *P. fullo* (Fig. 4.46). In closely related species, *P. decemlineata*, the stipe is furnished with a row of 16 to 18 conical, sharp-pointed stridulatory teeth (Young 1988). In *M. virescens* (= *P.*



Plate 4.45 Clypeus and labrum of *M. virescens* (= *P. fullo*)

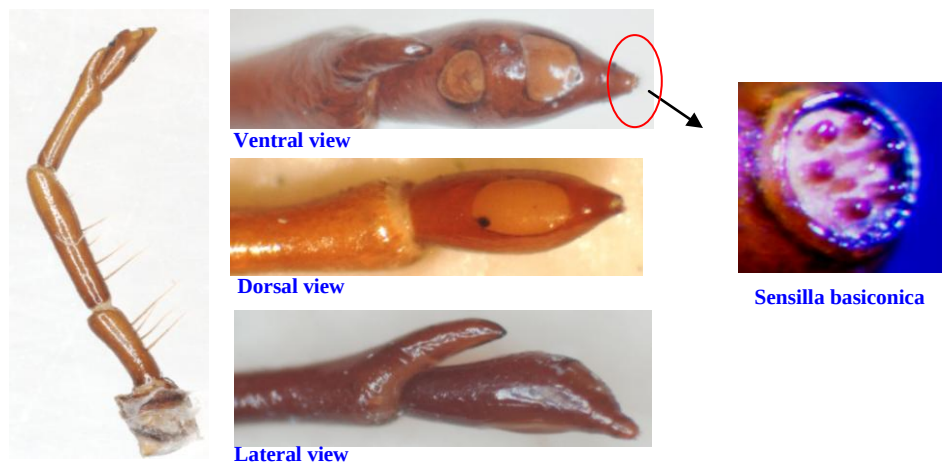


Plate 4.46 Antenna of *M. virescens* (= *P. fullo*)



Plate 4.47 Epipharynx of *M. virescens* (= *P. fullo*)

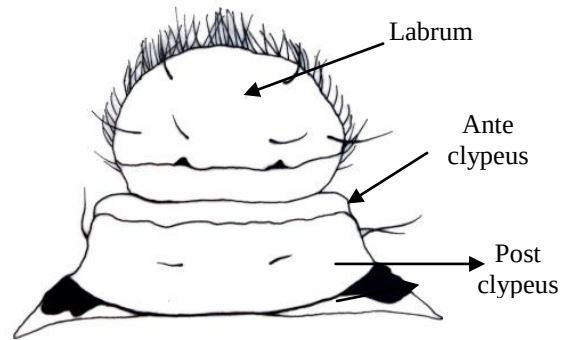


Fig. 4.42 Clypeus and labrum of *M. virescens* (= *P. fullo*)

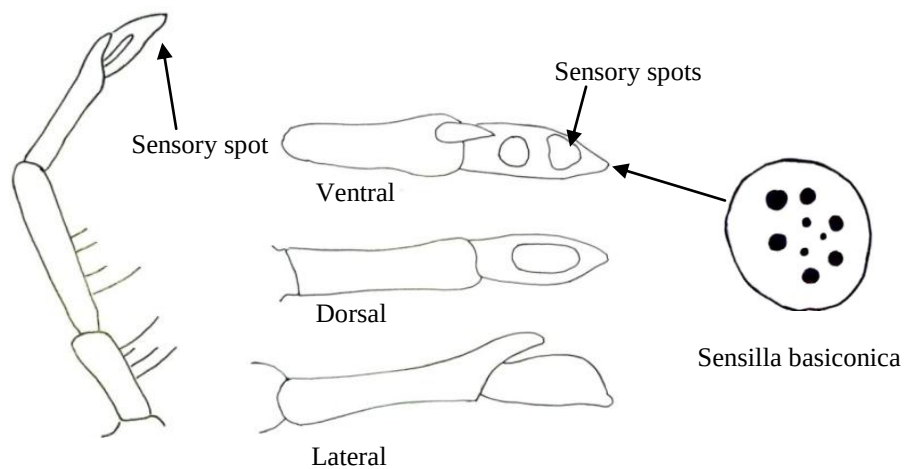


Fig. 4.43 Antenna *M. virescens* (= *P. fullo*) grub

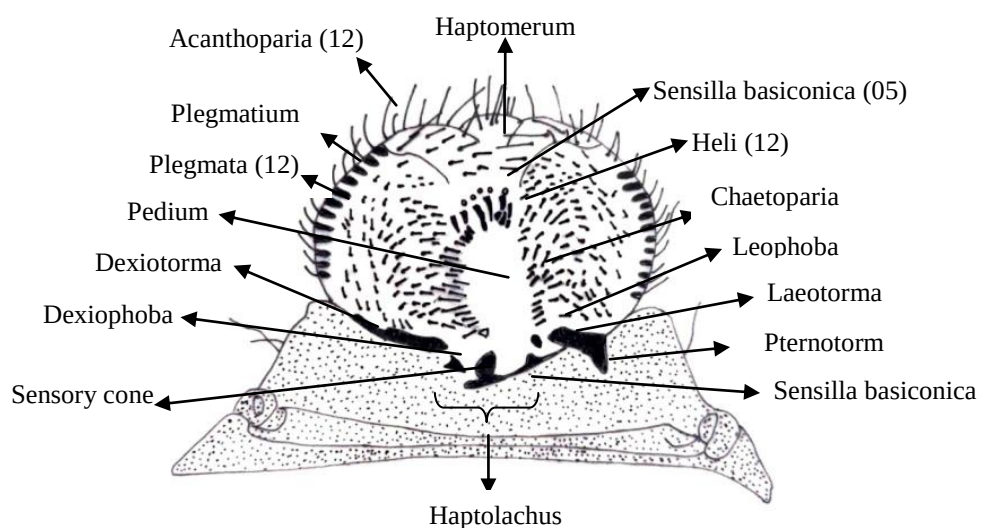


Fig. 4.44 Epipharynx *M. virescens* (= *P. fullo*)

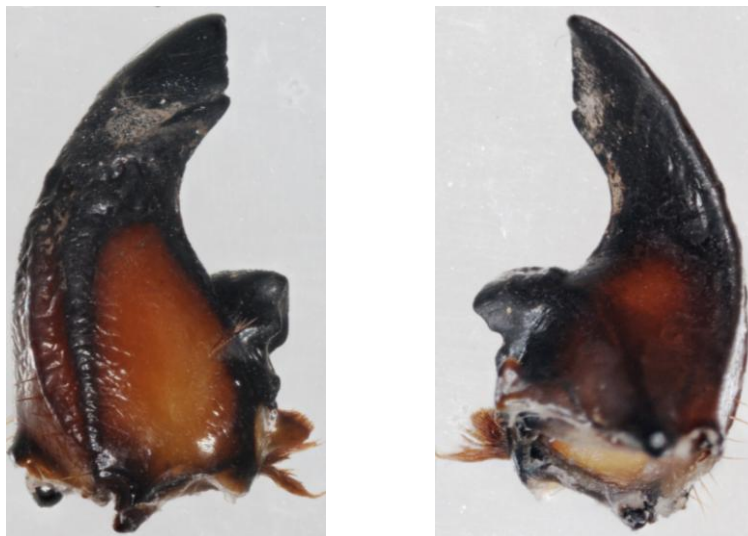


Plate 4.48 Mandibles of *M. virescens* (= *P. fullo*)

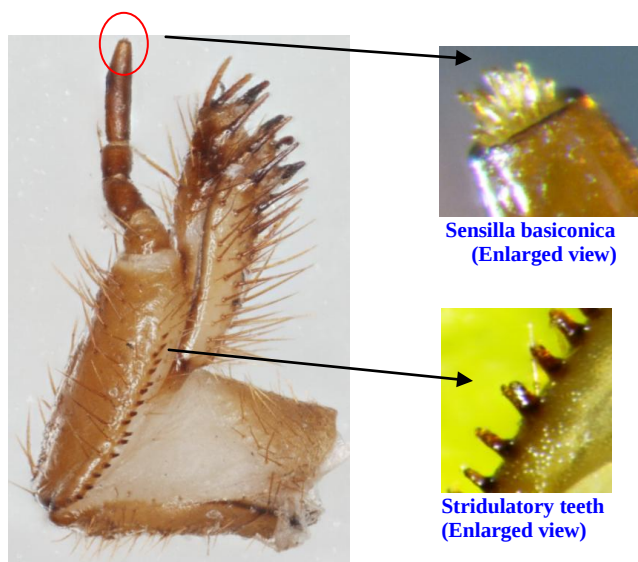


Plate 4.49 Maxilla of *M. virescens* (= *P. fullo*)

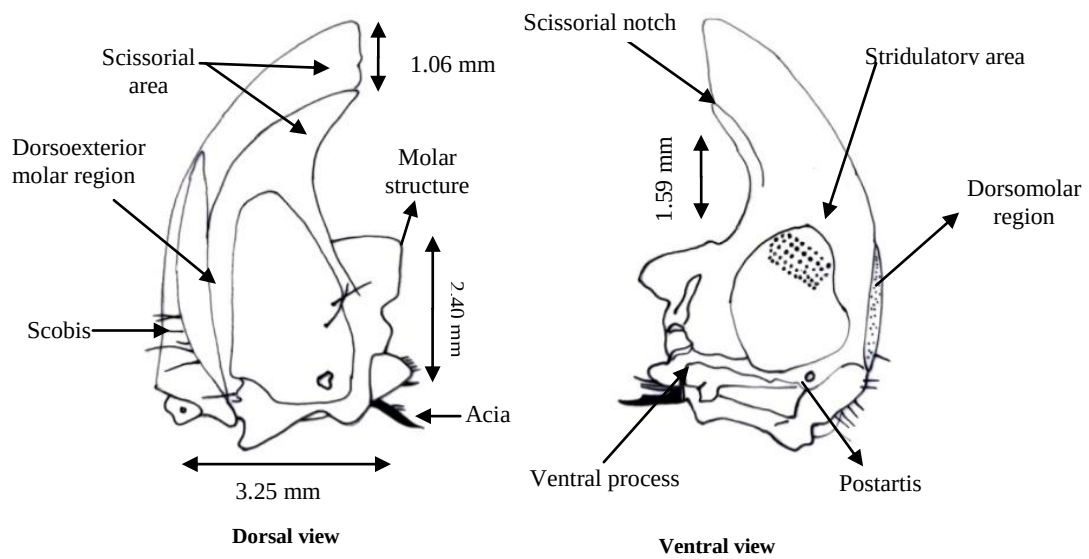


Fig. 4.45 Mandibles *M. virescens* (= *P. fullo*)

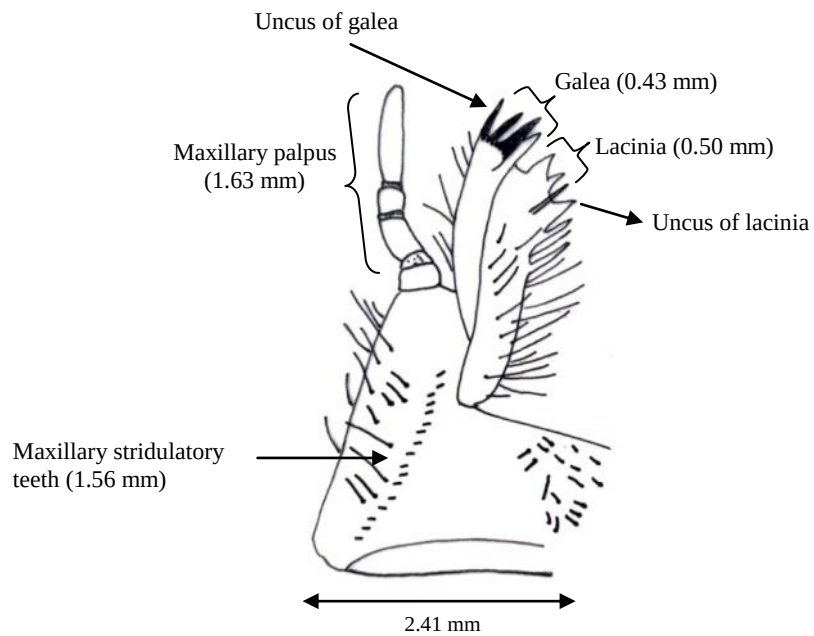


Fig. 4.46 Maxilla *M. virescens* (= *P. fullo*)

fullo), these stridulatory teeth are arranged in series on the dorsal surface of stipe. The maxillary palp is four segmented and the last segment is comparatively longest (Plate 4.49; Fig. 4.46). The distal segment bears 11-12 sensilla basiconica at the apex. The second segment bears single seta on lateral side. The basal segment is also furnished with two setae. The galea has three unci at the apex and one sclerotized seta on its dorsal surface. The lacinia possesses three unci. The length of cardo and maxillary palp measured to be 2.41 mm and 1.63 mm, respectively.

Labium: The sub-mentum was more or less symmetrical, and the width of sub-mentum at proximal end has been recorded to be 3.10 mm. A pair of setae was present in upper half of the sub-mentum (Plate 4.50; Fig. 4.47). The mentum was asymmetrical having width of 1.72 mm. The pre-mentum was symmetrical, and its width averaged 1.67 mm. There were several paired setae observed to be present on the lower part of the pre-mentum. These paired setae were straight at the distal end, and directed inward, crossing each other in the central region (Plate 4.50; Fig. 4.47). The labial palp is two segmented bearing sensilla basiconica on the apex of the distal segment. The glossa was found to be furnished with numerous sensilla basiconica on the central part, and tufts of microtrichia on the distal and lateral margins. The length of the maxillary palp has been recorded to be 0.90 mm as indicated in fig. 4.47.

Hypopharynx: The hypopharyngeal sclerome was more or less pointed. The lateral lobes were flat, and the truncate process was pointed at apex. The hole on the dorsal surface of hypopharynx was oval in shape (Plate 4.51; Fig. 4.48).

Thoracic legs: Thoracic legs are five segmented, each consisting of a coxa, a trochanter, a femur, a tibiotarsus, and a single claw on the apex. In prothoracic legs, the trochanter is triangular in shape. The femur and tibiotarsus are furnished with numerous fuscous setae on the ventral surface (Plate 4.52; Fig. 4.49). The length of claw has been recorded to be 0.67 mm. The claws are directed laterally. In mesothoracic and metathoracic legs, the distal part of the trochanter was notched, and the notch was more prominent. The length of the claw has been recorded to be 0.47 mm and 0.29 mm in mesothoracic and metathoracic legs, respectively (Fig. 4.49). The fuscous setae were sparsely distributed in mesothoracic and metathoracic legs. The femur was short and slightly rounded on outer side in mesothoracic and metathoracic legs.

Spiracles: The thoracic spiracles are slightly larger and open towards the caudal end. The abdominal spiracles are comparatively smaller and opened anteriorly (Plate 4.53; Fig. 4.50). In *P. occidentalis* (Linnaeus) (Ritcher 1966) and *P. decemlineata* (Young 1988),

the abdominal spiracles were found to be progressively smaller in size as compared to thoracic spiracles. The length x width of thoracic spiracle was recorded to be 0.42 x 0.30 mm, whereas length x width of first abdominal spiracle was found to be 0.35 x 0.22 mm. The distance between two respiratory lobes was 0.13 mm in thoracic spiracles, and 0.10 mm for abdominal spiracles (Fig. 4.50). The length of all abdominal spiracles was more or less equal, but width was found to vary. The width of abdominal spiracles gradually decreased from I-VI abdominal segments, and then increased from VII-VIII abdominal segments.

Raster pattern: In *P. fullo*, the palidia is short and each palidium consisted of 15 pali. The pali pointing inward with septula indistinct. The V shaped anal slit, more or less flat in the centre, tapers gradually on both sides and becomes almost flat for a considerable length on both sides. Anal cleft is absent. The lower anal lobe is broadly oval in shape (Plate 4.54; Fig. 4.51). In *P. decemlineata*, the anal opening is broadly V-shaped and raster with two short, nearly parallel, longitudinal palidia each sparsely set with 7 to 12 long, sharp, cylindrical pali whose tips frequently touching those of the opposite palidium (Ritcher 1966).

4.5.3 Pre-pupa: The fully fed third instar grubs stopped feeding, formed earthen cells and contracted in size (Plate 4.55). It was termed as pre-pupa. The body length of pre-pupa ranged from 33.33-38.32 mm (mean = 35.99 ± 0.59 mm). Duration of this stage was short, and this stage lasted for 15-23 days with an average of 19.3 ± 0.83 days (Table 4.27).

4.5.4 Pupa: Pupa was without any cocoon inside the earthen cells formed by third instar grubs. The last larval skin was shed off before the formation of pupae which was found at its posterior end. Sexual diamorphism was evident in pupal stage and newly formed pupae of both sexes were light yellow and gradually became yellowish brown (Plate 4.55). The average body length x width of male pupae has been recorded to be 30.92-34.10 mm x 14.20-14.27 mm with an average of 32.72 ± 0.41 mm x 14.23 ± 0.01 mm. The female pupa was larger than male pupa. The length x width of female pupa ranged from 33.67-34.10 mm x 14.21-14.27. In the present study, the first record of pupation under laboratory conditions was on 22.04.2019. Pupal duration was estimated as 14-22 days (mean = 18.6 ± 0.82 days) as given in table 4.27. Most pupal cells formed were about four inches deep, and oblong in shape. In *P. crinita*, the pupal duration has been reported as five weeks (Young 1988). Ritcher (1958) mentions that pupation lasts for about two months in 5-8 inches deep soil. In males, the antennae are long, extending beyond the apex



Plate 4.50 Labium of *M. virescens* (= *P. fullo*)



Plate 4.51 Hypopharynx of *M. virescens* (= *P. fullo*)

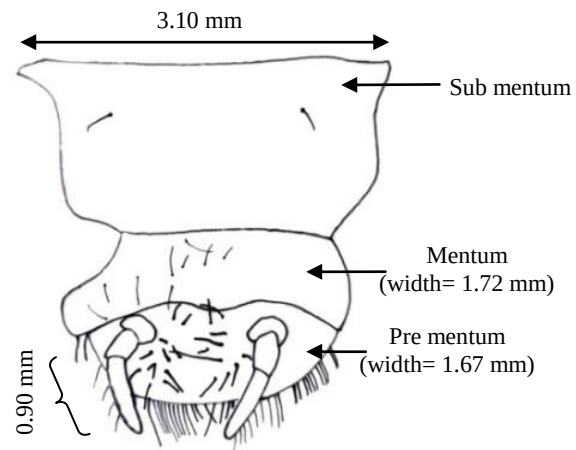


Fig. 4.47 Labium *M. virescens* (= *P. fullo*)

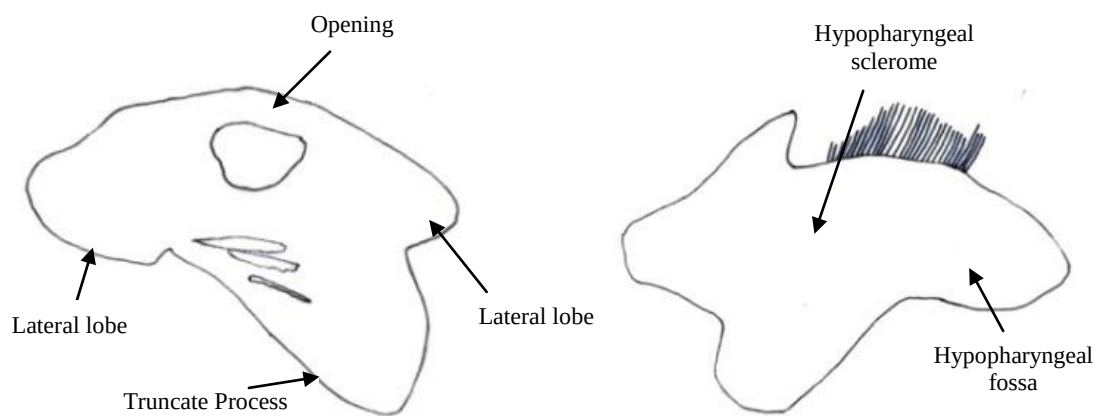


Fig. 4.48 Hypopharynx *M. virescens* (= *P. fullo*)

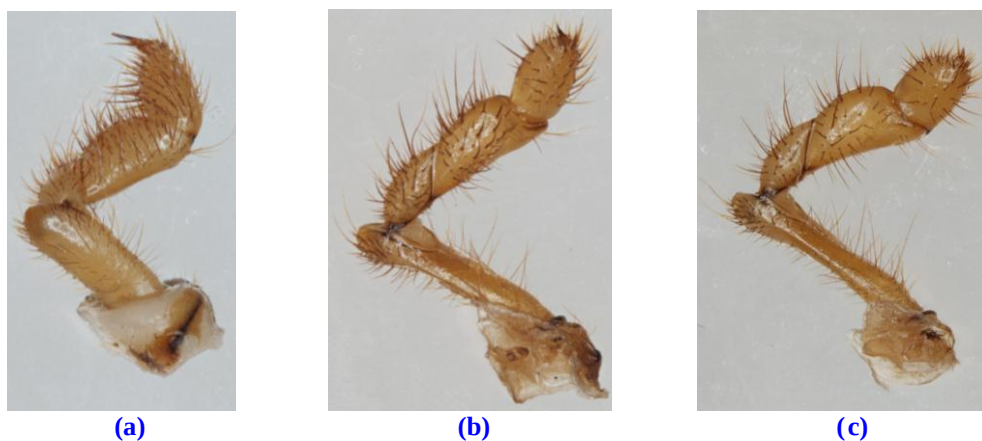


Plate 4.52 Legs of *M. virescens* (= *P. fullo*) grubs (a) Prothoracic legs; (b) Mesothoracic legs; (c) Metathoracic legs

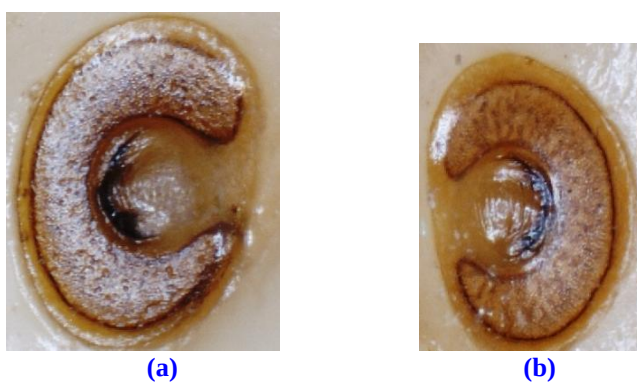


Plate 4.53 Spiracles of *M. virescens* (= *P. fullo*) (a) Prothoracic spiracle; (b) Abdominal spiracle

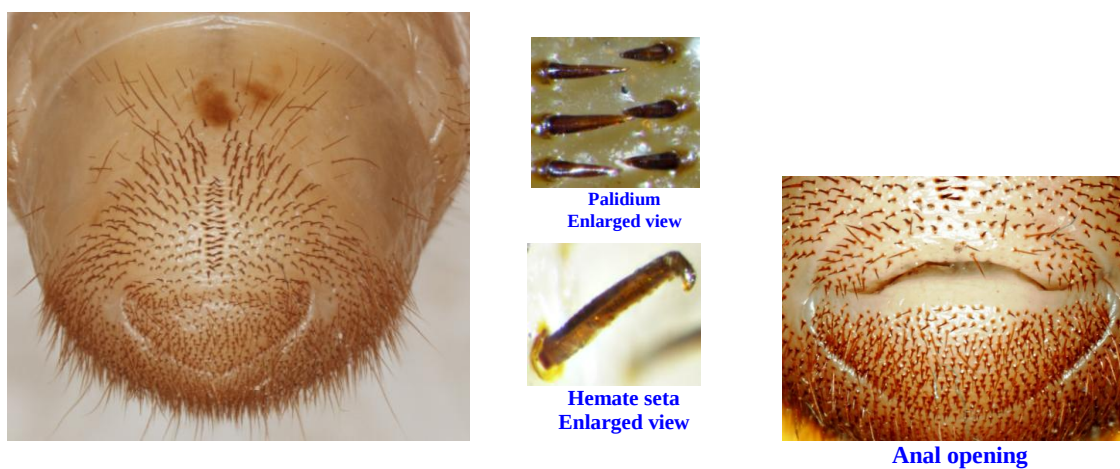


Plate 4.54 Raster pattern of *M. virescens* (= *P. fullo*)

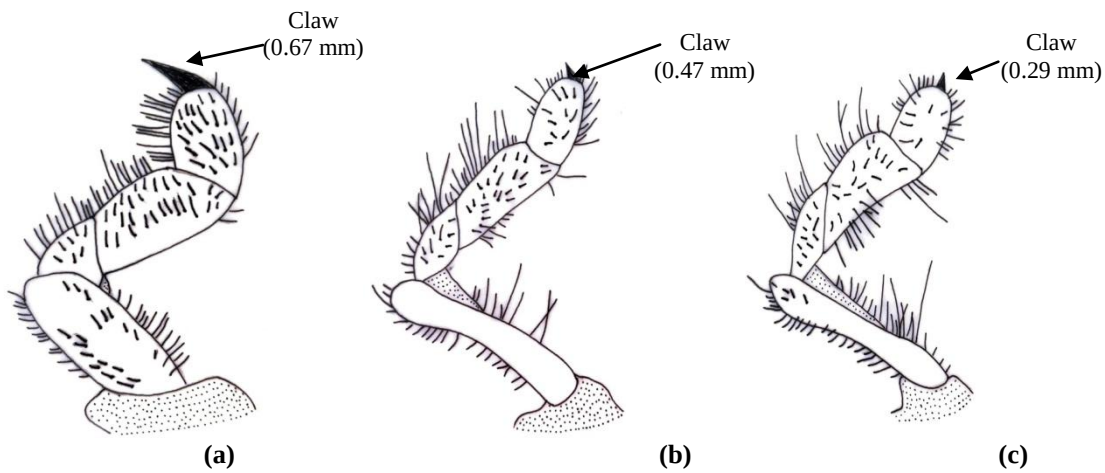


Fig. 4.49 Legs *M. virescens* (= *P. fullo*) grubs (a) Prothoracic leg; (b) Mesothoracic leg; (c) Metathoracic leg

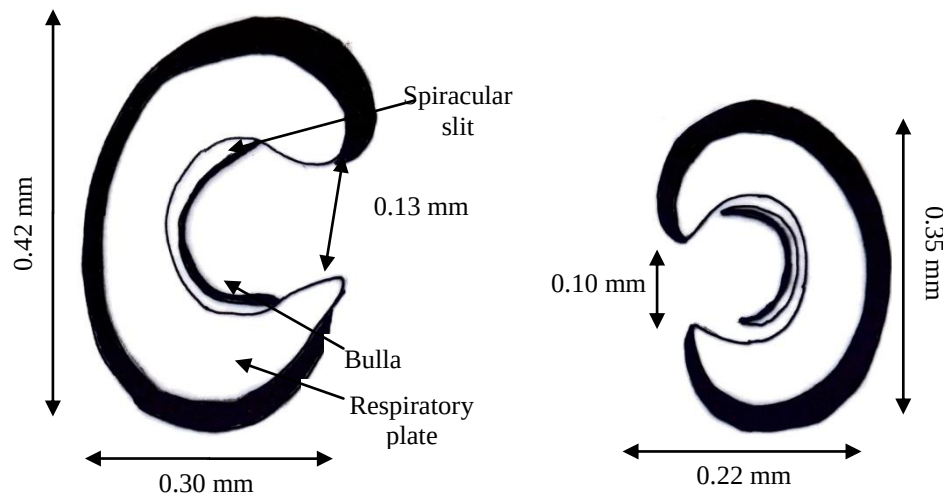


Fig. 4.50 Prothoracic and abdominal spiracles of *P. fullo*

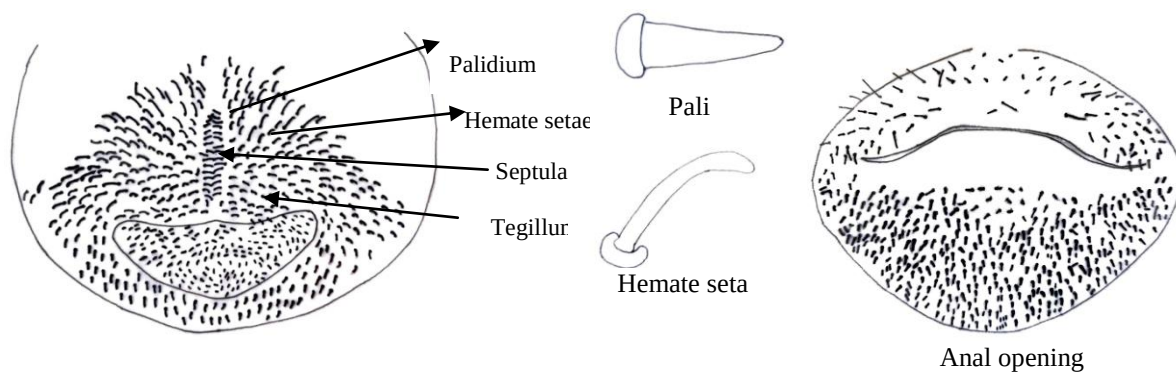


Fig. 4.51 Raster pattern *M. virescens* (= *P. fullo*)



Pre-pupa



Pupae

Plate 4.55 Prepupa and Pupa of *M. virescens* (= *P. fullo*)

of maxillary palps and reach to the base of meso coxa, whereas in females, the antennae are shorter, extend only up to maxillary palps and reach up to the base of pro coxa (Plate 4.56).

In males of *M. virescens* (= *P. fullo*), the caudal appendages are long, slightly swollen at the base and are of uniform width for most part of its length. Near the caudal extremity, the caudal appendages taper in a characteristic manner and are directed outward. The caudal tips are missing. In females, the caudal appendages are swollen at the base and almost abut each other towards the apical end. The caudal tips are conspicuous and pointed. A median glubose projection with a median key hole shape structure is present on last sternum in males. In females, an anal slit is present on last sternum, which is elliptical in shape (Plate 4.56; Fig. 4.52).

Total life cycle: The total life cycle from egg to adult stage was determined to be of 619-652 days with an average of 638.8 ± 3.48 days (Table 4.27). According to Erler and Ates (2015), *P. fullo* has a biennial or triennial life cycle by region in Turkey. These reports are in agreement with our findings observed for *P. fullo* in the present study.

4.5.5 Adult: The adult emergence usually began during second week of May, 2019. The new adult does not necessarily fly at once, but often remained in the soil until a favourable evening, with flight probably dependant on temperature. The flights initiated when the temperature was around 20°C. Young (1988) reported that there is little or no flight below 54°F at 8:30 pm and maximum flight occurs at 60 °F. Flights started around 7:30 pm and lasted about two hours with sporadic catches at light until 9:30 pm.

The adults are elongate, dark brown beetles, clothed very densely and evenly with pale scales (Plate 4.57). The head dark brown and covered laterally with with dense and thin pubescens in males, whereas in females, the pubescens was thick and it was confined more on the lateral sides of the head. In males, the clypeus was trapezoidal in shape, lateral sides were narrower and anterior margin is bifurcated into two lobes. In case of females, the clypeus was heart-shaped with lateral sides swollen in the middle and narrower basally (Plate 4.58). Elytra are densely and evenly covered with scales. The scutellum is devoid of punctuation, and it is small and more rounded posteriorly in males as compared females (Plate 4.59; Fig. 4.53). The pronotum is trapezoidal in shape. The antero-lateral and posterior-lateral margins are less pointed and slightly rounded in appearance (Plate 4.60; Fig. 4.54). In the posterior region, a depression mark is present, and this depression mark is slightly lesser in females as compared to males. Pygidium elongate and projected behind in the caudal end. In males beetles, the apical margins of



(a)



(b)

Plate 4.56 (a) Pupal antennae; (b) Pupal tips *M. virescens* (= *P. fullo*)

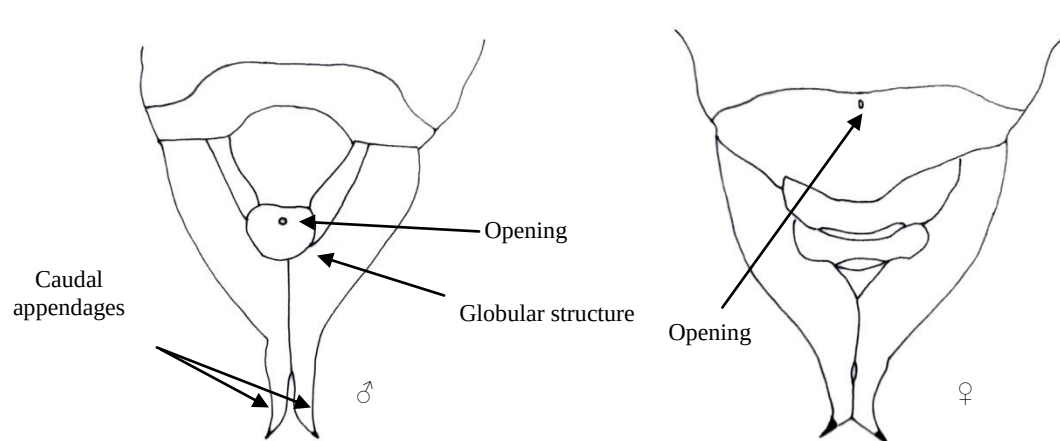


Fig. 4.52 Pupal tips *M. virescens* (= *P. fullo*)



Plate 4.57 Adults of *P. fullo*



Plate 4.58 Head capsule of *P. fullo*



Plate 4.59 Scutellum of *P. fullo*



Plate 4.60 Pronotum of *P. fullo*

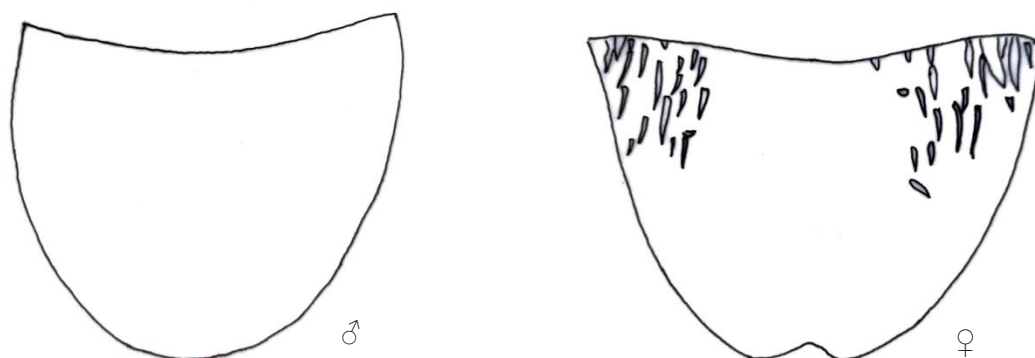


Fig. 4.53 Scutellum of *P. fullo*

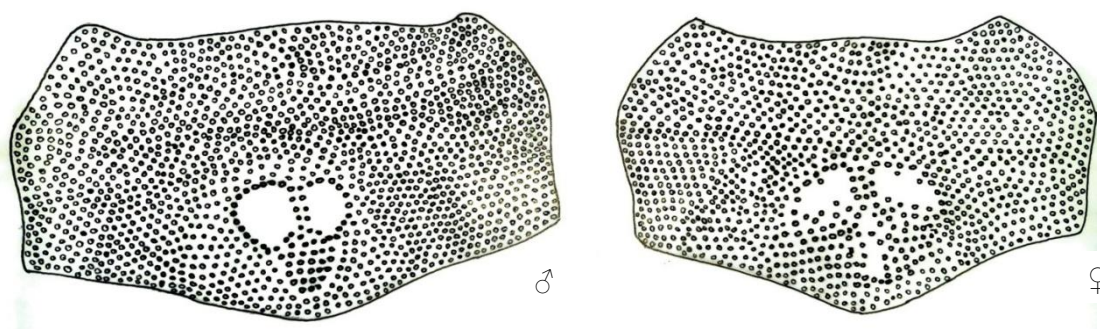


Fig. 4.54 Pronotum of *P. fullo*

the wings are touching the pygidium. Pygidium tapered gradually, and the caudal extremity is rounded. In females, there exists sufficient gap between apical margins of wings and pygidium. As compared to males, the pygidium lobe is narrow in females (Plate 4.61; Fig. 4.55).

The female beetles measured 22.80-26.15 mm (mean = 24.69 ± 0.38 mm) in length and 11.92-13.24 mm (mean = 12.77 ± 0.16 mm) in width (Table 4.27). However, in males, the body length averaged 23.35 ± 0.46 mm (range = 21.59-25.75 mm), and the mean body width was recorded to be 10.89 ± 0.18 mm (range = 10.22-11.89 mm). In Nebraska, the adult beetles of *P. occidentalis* have been reported to measure 24.6 mm in length, and 10.9 mm in width (Young 1988). These reports are in close association with our results.

Antennae: The lamellate antennae were dark brown and 10 segmented in both males and females. In males, the antennal club was 7 segmented, whereas in females, it was 6 segmented. The lamellae of terminal segments in males are larger than in females (Plate 4.62; Fig. 4.56). In females, second segment of flagellum was reduced to plate-like structure. In male beetles, the maximum width of antennal club has been recorded to be 5.22 mm, whereas in females, it was 1.06 mm (Fig. 4.56). According to Young (1988), in *Polyphylla* species, the antennal club is long, 7 segmented and 4-10 mm in length in males, but in females, the antennal club is short, 5 segmented and 2 mm in length.

Legs: In fore legs of *P. fullo*, the tibial spines are small, pointed and 0.82 mm in males, whereas in females, tibial spines are more pointed, slightly curved, and measured 1.20 mm in length. The claws are bifurcated and toothed in both sexes. The length of claw is 1.73 mm in males, and 1.56 mm in females. In mid legs, the tibial spines are long in females (1.12 mm) than in males (0.51 mm). The claws are single, short and toothed in males, having length of 1.21 mm. In females, claws are bifurcated, long and toothed with an average length of 1.55 mm. In hind legs, the femur width is more in females (7.40 mm) than in males (4.87 mm). The tibial spines are conspicuously thick, long and more pointed in females (1.59 mm), whereas in males, the tibial spines are short measuring 0.90 mm in length. The claws were pointed, curved and long in females (1.51 mm), whereas in males, the claws were short and 1.30 mm in length (Plate 4.63; Fig. 4.57).

Male genitalia: Phallobase is shorter than parameres. The attachment of phallobase to parameres showed concavity towards the base of phallobase (Plate 4.64). The length and width of phallobase is 3.23 mm and 2.0 mm, respectively. Parameres asymmetrical, and the left paramere appears broader than right paramere. The parameres are light brown in



♂



♀

Plate 4.61 Pygidium of *P. fullo*



♂



♀

Plate 4.62 Antennae of *P. fullo* adult

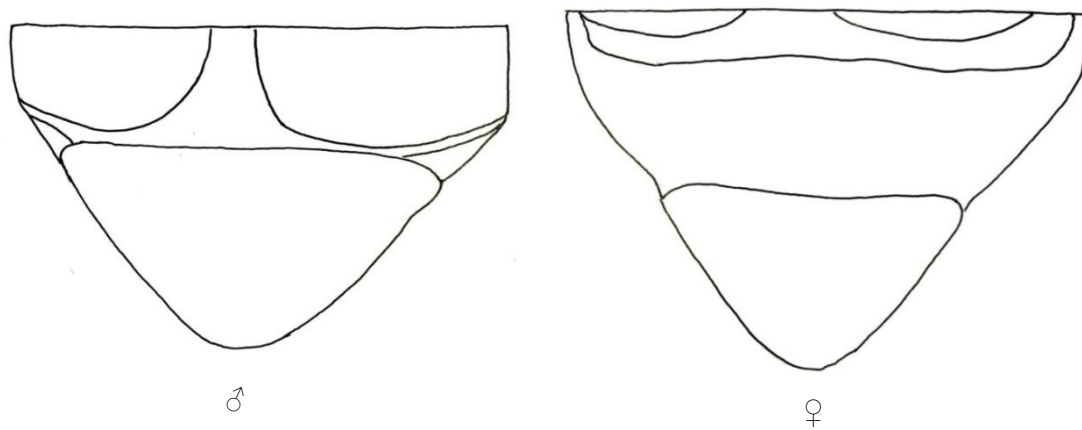


Fig. 4.55 Pygidium of *P. fullo*

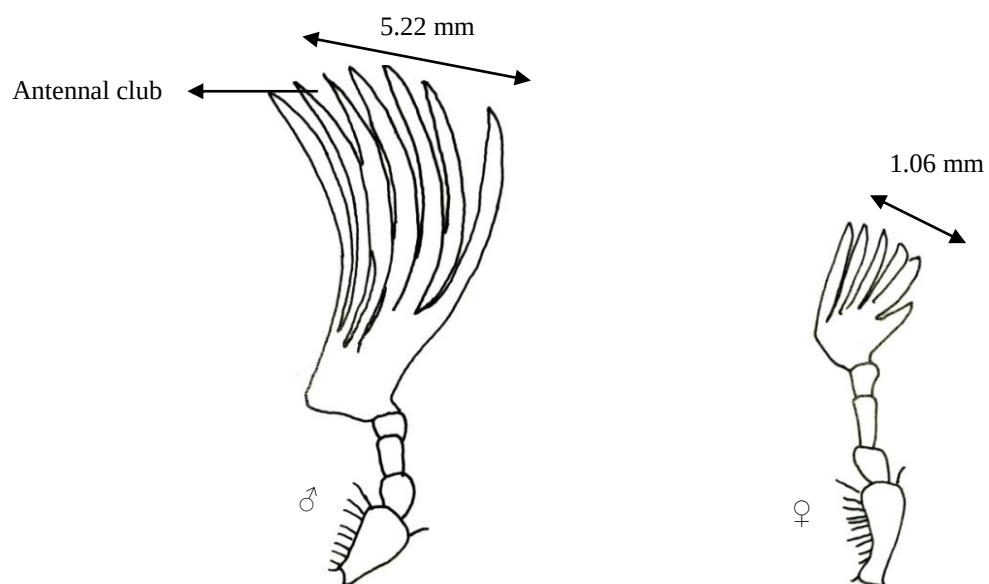


Fig. 4.56 Antennae of *P. fullo* adult



Forelegs



Midlegs



Hindlegs

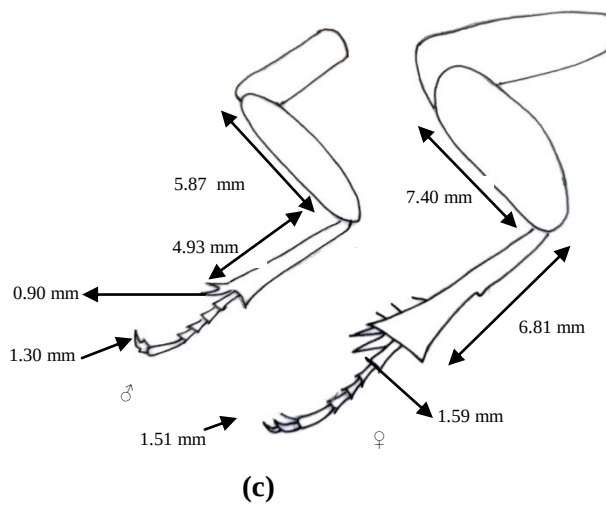
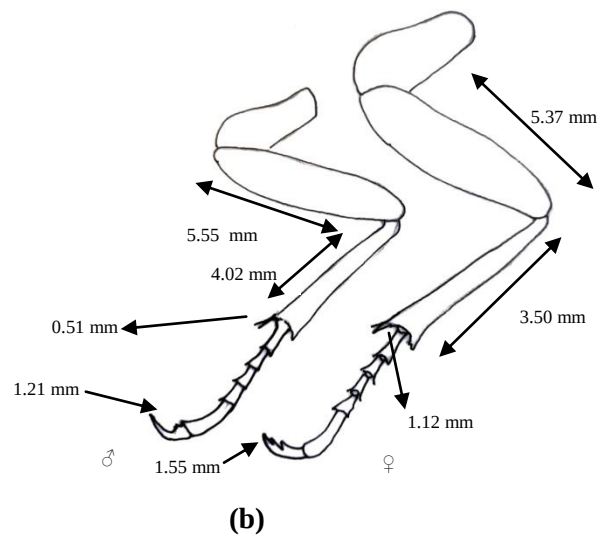
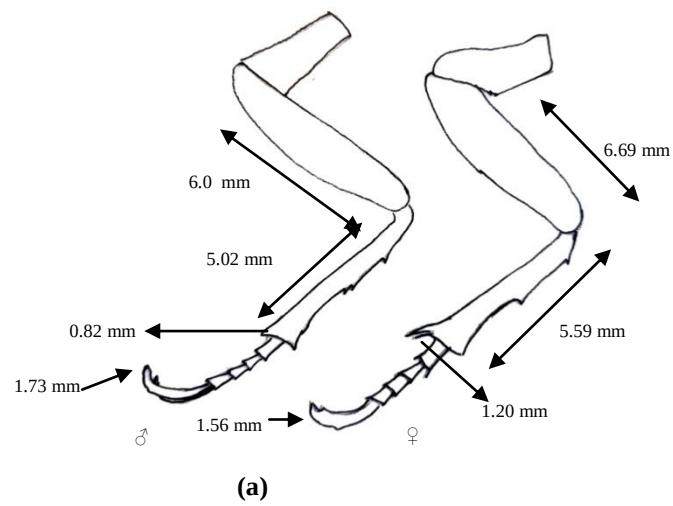
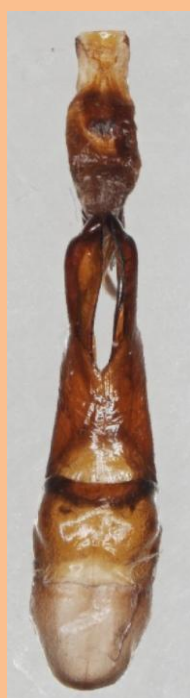


Fig. 4.57 Legs of *P. fullo* adult (a) Forelegs; (b) Midlegs; (c) Hindlegs



Dorsal view



Ventral view



Lateral view



Spiculum gastrale

Plate 4.64 Genitalia of *P. fullo*

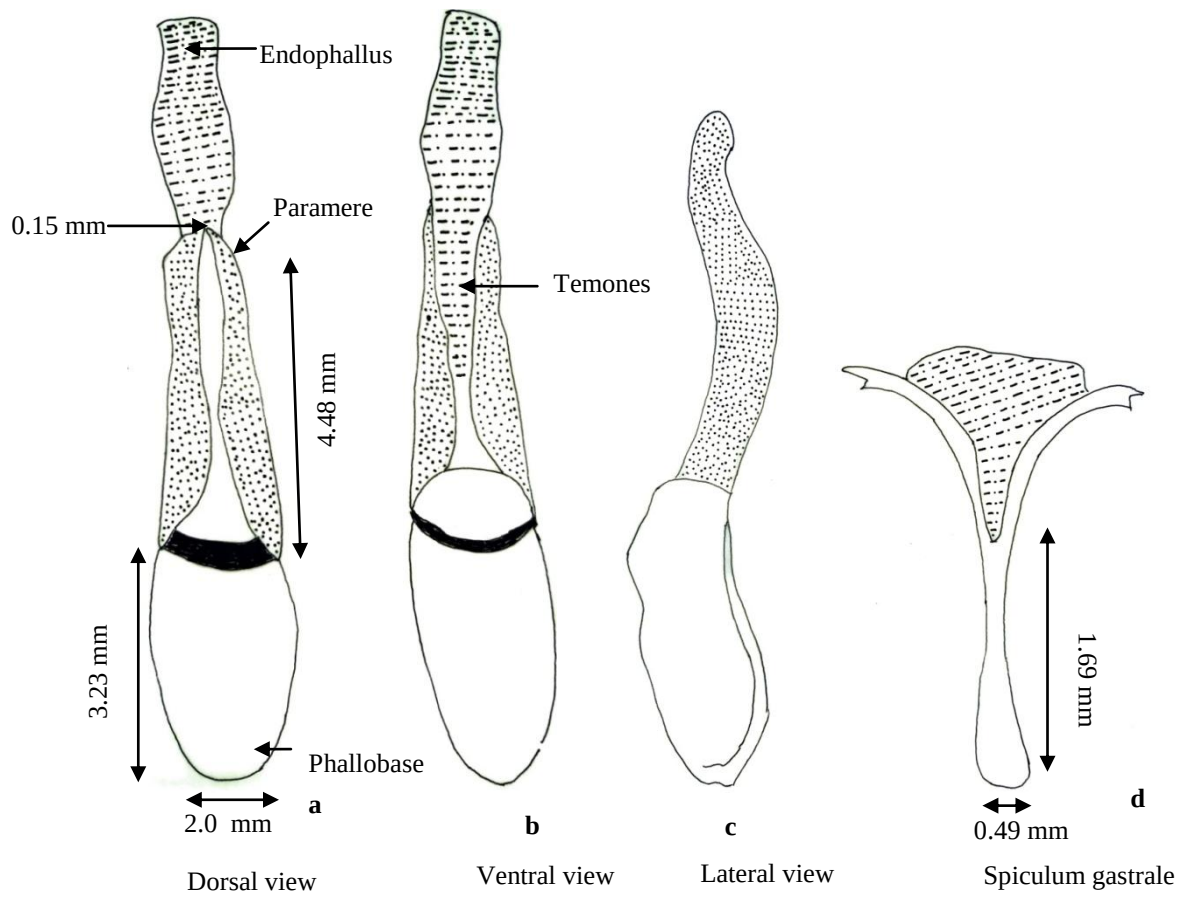


Fig. 4.58 Male genitalia of *P. fullo*

colour, whereas phallobase is dark brown on the edges and creamish in the middle. Parameres measured 4.48 mm in length and 0.15 mm in width. Endophallus flat, elongated and chimney shaped. Spiculum gastrale 'Y' shaped, sclerite, stem short, measuring length of 1.69 mm and width of 0.49 mm. Arms longer than stem. The width of arms measured up to 3.11mm (Fig. 4.58). Arms are further bifurcated into two in the distal region; longer arms are internal in position and larger arms are internal, and pointed outward. The posterior end of the stem is lobe-like.

Adult longevity: The pre-oviposition period of *P. fullo* females ranged from 7-9 days with an average of 8.1 ± 0.28 days. The oviposition period was very short and it was observed to range from 2-3 days (mean: 2.3 ± 0.15 days). The mean fecundity was recorded to be 21 eggs per female. Rodstrom and Brown (2017) reported that an average of about 12 eggs are laid by mated females of *P. decemlineata* in the soil near where they emerged. The post-oviposition period was comparatively longer and it averaged 11.1 ± 0.41 days with a range of 10-14 days. The female beetles survived for a longer period than male beetles. The overall longevity of females of *P. fullo* has been recorded to vary from 18-16 days (mean: 21.5 ± 1.0 days), and there was reduction in longevity of male beetles, the maximum and minimum being 11 and 19 days (mean: 15.1 ± 0.90) as shown in table 4.27.

Table 4.27 Duration and morphometrics of different stages of *M. virescens* (= *P. fullo*)

Stage	Duration in days		Length (mm)		Width (mm)	
	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE	Range	Mean $\pm$ SE
Egg	16-18	17.2 $\pm$ 0.35	-	-	-	-
Freshly laid	-	-	3.62-4.21	3.88 $\pm$ 0.07	2.57-3.09	2.81 $\pm$ 0.06
Prior to hatch	-	-	4.73-4.98	4.86 $\pm$ 0.03	3.10-3.38	3.23 $\pm$ 0.03
Larvae I	46-51	47.9 $\pm$ 0.57	10.16-13.97	12.08 $\pm$ 0.78	2.60-2.84	2.72 $\pm$ 0.05
II	214-229	221.7 $\pm$ 1.71	23.65-27.70	25.89 $\pm$ 0.71	4.33-5.13	4.68 $\pm$ 0.17
III	309-320	314.1 $\pm$ 1.19	48.33-54.17	50.17 $\pm$ 1.03	7.92-8.48	8.19 $\pm$ 0.11
Total larval period	574-594	583.7 $\pm$ 2.10	-	-	-	-
Pre-pupal period	15-23	19.3 $\pm$ 0.83	33.33-38.32	35.99 $\pm$ 0.59	-	-
Pupa	14-22	18.6 $\pm$ 0.82	30.92-34.10	32.72 $\pm$ 0.41	14.20-14.27	14.23 $\pm$ 0.01
Total duration	619-652	638.8 $\pm$ 3.48	-	-	-	-
Adult longevity						
Male	11-19	15.1 $\pm$ 0.90	21.59-25.75	23.35 $\pm$ 0.46	10.22-11.89	10.89 $\pm$ 0.18
Female	18-26	21.5 $\pm$ 1.0	22.80-26.15	24.69 $\pm$ 0.38	11.92-13.24	12.77 $\pm$ 0.16
Pre-oviposition period	7-9	8.1 $\pm$ 0.28	-	-	-	-
Oviposition period	2-3	2.3 $\pm$ 0.15	-	-	-	-
Post-oviposition period	10-14	11.1 $\pm$ 0.41	-	-	-	-

4.6 Sequencing of barcode regions of *Cox1* and 28s *rRNA* genes of different *Melolontha* species

4.6.1 Isolation and estimation of quality and quantity of DNA

DNA of four *Melolontha* species was extracted from head capsule and legs of adult or larval stages using CTAB method. The quality of DNA (Plate 4.65) extracted from four species was estimated by agarose gel electrophoresis (1%) method, and the quantity was estimated by using spectrophotometer at 260 nm. The yield of DNA was 1.5- 2.0 µg/µl for all the samples.

4.6.2 Amplification and transformation of *Cox1* and 28S *rRNA* genes

The *Cox1* and 28S *rRNA* genes were amplified using gene specific primers. The barcode regions of *Cox1* and 28S *rRNA* genes were amplified using primers, Pat (5'-TCCAATGCACTAATCTGCCATATTA-3') and Jerry (5'-CAACATTATTTTGATTTTTTGG-3') (for *Cox1*) and DD (5'-GGGACCCGTCCTTGAAACAC-3') and FF (5'-TTACACACTCCTTAGCGGAT-3') (for 28s *rRNA*). The amplified products of *Cox1* and 28S *rRNA* genes were visualized using Agarose gel electrophoresis (1%) (Plate 4.66; 4.67; 4.68). Amplified products of both the genes were cloned in pGEM[®]-T EASY vector and the vector was transformed into competent cells (JM109). The transformation was confirmed by blue and white colony screening method (Sambrook et al. 1989) as indicated in plate 4.69. The white coloured colonies were designated as transformants with insert. The presence of gene sequences in the white colonies was confirmed by colony PCR. The selected colonies were cultured in LB broth containing ampicillin antibiotic, and incubated for 16 hours at 37⁰C followed by plasmid isolation and insert sequencing.

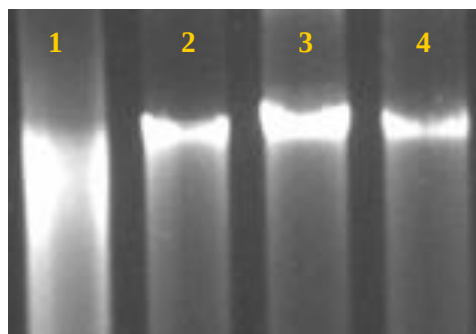


Plate 4.65 Agarose gel electrophoresis (1%) showing quality of DNA
 Lane 1: *M. indica*, Lane 2: *P. fullo*, Lane 3: *M. cuprescens*, Lane 4: *M. furcicauda*

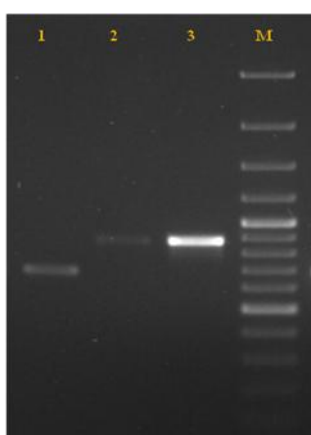


Plate 4.66 Agarose gel electrophoresis (1%) showing amplified product of Cox 1 gene
 Lane 1: *M. indica*, Lane 2: *P. fullo*,
 Lane 3: *M. cuprescens*, M: 100 bp DNA ladder

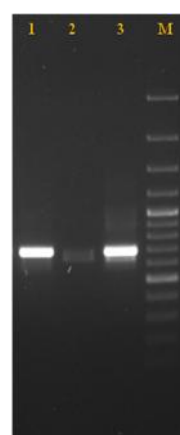


Plate 4.67 Agarose gel electrophoresis (1%) showing amplified product of 28S rRNA gene
 Lane 1: *M. indica*, Lane 2: *P. fullo*, Lane 3: *M. cuprescens*, M: 100 bp DNA ladder

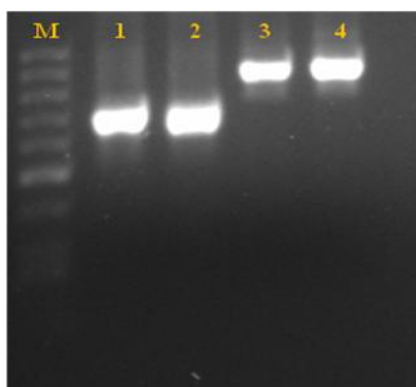
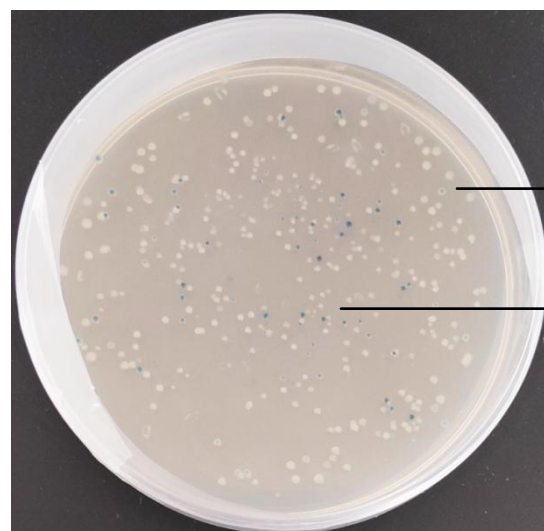
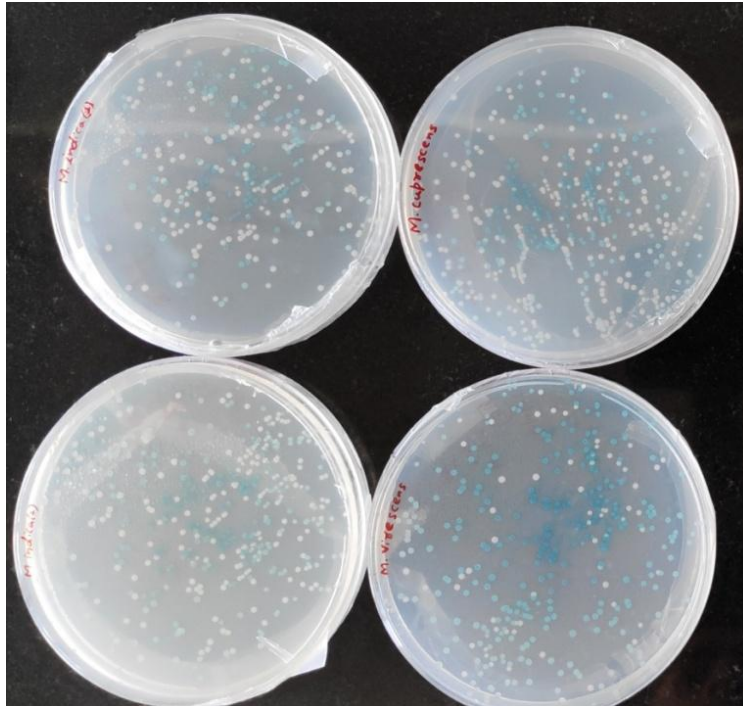


Plate 4.68 Agarose gel electrophoresis (1%) showing amplified product of 28S rRNA and Cox 1 genes.
 M: 100 bp DNA ladder, Lane 1 and 2: *M. furcicauda* (28s rRNA gene), Lane 3 and 4: *M. furcicauda* (Cox1 gene)



→ White colonies with vector and insert

→ Blue colonies without insert

Plate 4.69 Transformed bacterial cells

4.6.3 Sequencing and phylogenetic analysis of *Melolontha* species

The length of amplified *Cox1* gene sequences (Table 4.28) of *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*) were recorded to be 873 bp, 874 bp, 678 bp and 873 bp, respectively. The amplified 28S *rRNA* gene sequences (Table 4.29) in *M. cuprescens*, *M. furcicauda*, *M. indica* were 686 bp, 684 bp and 686 bp, respectively.

Table 4.28 Sequences of amplified products of *Cox1* gene of *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*)

Insect	Sequence
<i>M. cuprescens</i>	TCCAATGCACTAATCTGCCATATTAGAAATTAGTTAATATGGGGAGTTCAGAGTAGCTATGTTTCAG CAGGGGGTATTAGTTGGAATCATTCAATTGAAGTAGTTATACTTAGGGGCACTAAGCTTTTTCGGT GTGAAATAAATCTGTCTCAAATAACAAATAAAAAACATAATAATTCTAATAAGGGAAATTAGGGAT CCGATTGAGGAGATCACATTTCAAGAAGTATAGGCATCTGGGTAATCAGAGTATCGTCGGGGTAT TCCACTAAGTCCCAGAAAATGTTGGGGAAAGAAGGTTATATTAACCTCCAATAAATATGGTTATAA ATTGGATTTTAAAAATTTCCATTAAAGGGTTAAGCCAGAGAATAGGGGGAATCAGTGAGTGAAT CCTGCTATAATTCTAAAAACGGCTCCTATAGAAAAGGACATAATGGAAGTGTGCTACGACATAGTA GGTGTCTGTAGATAAATGTCTGATTGAAGAGTTAGCTAAGATTACTCCAGTCAATCCTCCCACTGT AAATAAAAAATACGAATCCTAGGGCTCACAGAAGTGATGGGGAGTAGTTAATTGGGTCCCATGAA GTGTAGCAATTCATCTGAAAATTTAATACCGGTGGGTACAGCAATGATTATGGTGGCTGAAGTA AAATAAGCCCGAGTATCGACATCTATGCCCACTGTGAATATGTGATGGGCTCAGACAATAAATCC TAATAAGCCAATCGCTATTATAGCATAGATTATTCCTAGTGTTCGAATGTTTCTTTTGTTCCTT TCTTGGCTAATAATGTGGGAGATTATCCAATCCGGGGAGAATTAGAATATAAACTTCAGGGTG TCCAAAAAATCAAAATAATGTTG
<i>M. furcicauda</i>	TCCAATGCACTAATCTGCCATATTAGAAATTAGTTAATATGGGTAGTTCGGAGTACCTATGTTTCAG CTGGGGGTATTATTGGAATCATTTCGATAGAAGTTGTTATGTTTAGGGGGGATAGGCTTTTTCGTA TAGAGATAAATCTATCTCAAATAATAAAAAATAGAATAATACTAATTAAGGAAATTAATGAA CAATAGATGAGATAAATTTTCATACAGTATAGGCGTCGGGGTAATCTGAGTATCGTCGGGGTAT GCCTCTTAATCCTAGAAAATGTTGAGGAAAAAAGGTTATGTTTACTCCAATGAATATTGTAAAAA ATTGAATTTTAAAAAGTTTCTATTAAGTATTAACCTGTAAATAGGGGGAATCAGTGACAAATC CTGCTATAATAGCAAAATACGGCTCCTATTGATAGAACGTAATGAAAGTGGGCTACTACGTAATAA GTATCATGAAGGATAATATCAATTGAAGAATTTGCCAGGATAACTCCCGTTAAACCCCTCACTGT AAATAAGAATACAAATCCTAGGGCTCATAGCAATGATGGGGAATTAATTGAATGAGTTCCATGGA GAGTGGCCAATCATCTGAAAATTTTGATACCAGTAGGTACAGCAATAATTATTGTTGCAGAGGTA AAATAAGCTCGGGTATCTACATCTATTCTACTGTAAATATATGATGGGCCCATACGATAAAGCCT AATAGGCCAATAGCTATTATGGCATAAATTATTCCTAAAGTGCCGAATGTTTCTTTTGTGCTTT CTGTCTAATAATGTGGGAAATTATTCAAATCCCGGGAAGAAATAAAATATAAACTTCTGGGTG TCCAAAAAATCAAAATAATGTTG
<i>M. indica</i>	TCCAATGCACTAATCTGCCATATTAGAAATTAGTTAATATGGGTAGTTCAGAATATCTATGTTTCAG CTGGTGGTATTATTGAAATCATTCAATAGATGTTGTTATTCTTACTGGAGAAAGTCTTTTTCGTAT TGAAATTTGAAATATTTGAATAGTTTCTATTTAATGTTAGTCTGTAAATAACGGAAACCAATG GACAAATCTAGCTATGATAGCAAACTAGTCTCTATTGAAAGTACGTAATGAAAATGGGTTGCAA CATAGTAAGTATCATGAAGGTTGATTAATTGAAGAGTTTGCAAGAATAACTCCTGTTAATCCTC CCACAGTAAATAAGACTACAAAACCTAGGGCCGATAGTAAGGATGGGGAATAGTTTAGTTGAGTT CCGCGTAGAGTAGCCAGTCACTGAAAATTTAATACCCGTAGGTACTGCAATAATTATGGTTGCT GAGGTAAAGTAGGCTACATCTATTCTACAGTAAATATGTGATGAGCTCATACAATGAAGCCTAA CAATCCAAGAGCTATTATTGCATAGATTATCCCTAAAGTACCGAATGTTTCTTTTCTGTTTCTTCT TGTCTAATAATATGAGAAATTATTCCAATCTTGGGAGGATTAATAATAAACTTTTGGATGTCCA AAAAATCAAAATAATGTTG
<i>M. virescens</i> (= <i>P. fullo</i>)	TCCAATGCACTAATCTGCCATATTAGAAATTAGTTAATATGGGGAGTTCAGAGTAGCTATGTTTCAG CAGGGGGTATTAGTTGGAGTCATTCAATTGAAGTAGTTATACTTAGGGGCACTAAGCTTTTTCGGT GTGAAATAAATCTGTCTCAAATAACAAATAAAAAACATAATAATTCTAATAAGGGAAATTAGGGAT CCGATTGAGGAGATCACATTTCAAGAAGTATAGGCATCTGGGTAATCAGAGTATCGTCGGGGTAT TCCACTAAGTCCCAGAAAATGTTGGGGAAAGAAGGTTATATTAACCTCCAATAAATATGGTTATAA ATTGGATTTTAAAAATTTCCATTAAAGGGTTAAGCCAGAGAATAGGGGGAATCAGTGAGTGAAT CCTGCTATAATTCTAAAAACGGCTCCTATAGAAAAGGACATAATGGAAGTGTGCTACGACATAGTA GGTGTCTGTAGATAAATGTCTGATTGAAGAGTTAGCTAAGATTACTCCAGTCAATCCTCCCACTGT AAATAAAAAATACGAATCCTAGGGCTCACAGAAGTGATGGGGAGTAGTTAATTGGGTCCCATGAA GTGTAGCAATTCATCTGAAAATTTAATACCGGTGGGTACAGCAATGATTATGGTGGCTGAAGTA AAATAAGCCCGAGTATCGACATCTATGCCCACTGTGAATATGTGATGGGCTCAGACAATAAATCC TAATAAGCCAATCGCTATTATAGCATAGATTATTCCTAGTGTTCGAATGTTTCTTTTGTTCCTT TCTTGGCTAATAATGTGGGAGATTATCCAATCCGGGGAGAATTAGAATATAAACTTCAGGGTG TCCAAAAAATCAAAATAATGTTG

Table 4.29 Sequences of amplified products of 28S rRNA gene of *M. cuprescens*, *M. furcicauda* and *M. indica*

Insect	Sequence
<i>M. cuprescens</i>	GGGACCCGTCCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGGACTCTGAC GAAACCTAAAGGCGTAATGAAAGTGAAGGTCCGCCTGGCGCGGACCGAGGGAGGATGGGGCGGAC CCGAGGGGGCCGCTCGCACTCCCGGGGCGTCTCGTTCTCATCGCGAGAAGAGGCGCACCCAGAGC GTACACGCTGGGACCCGAAAGATGGTGAACATATGCCTGGTCAGGACGAAGTCAGGGGAAACCCCT GATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTCGGAACCTGGGTATAGGGGCGAAAGA CTAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGTTCCCTCAGGATAGCTGGCGCTCGCTTCT TGCGAGTCTCATTCGGTAAAGCGAATGATTAGAGGCATTGGGGTTCGAAACGGCCTCAACCTATTCT TCAAACCTTTAAATGGGTGAGATCTCCGGCTTGCTTGAACCTGTGAAGCCGCGAGATATCGGATCAG AGTGCCAAGTGGGGCACTTTTGGTAAGCAGAAGTGGCGCTGTGGGATGAACCAAACGCCGAGTTA AAGCGCCTAAATCGACGCTTATGGGATACCATGAAAGGCGTTGGTAACTTAAGACAGCAGGACGG TGCCCATGGAAGTCGGAATCCGCTAAGGAGTGTGTAA
<i>M. furcicauda</i>	GGGACCCGTCCTTGAAACACGGACCAAGGTGTTGGGCCCCCTTTCCGAAACACACGGACCAGGGG TTTTGTTAAATGCGTAAGTAAACCGTCTTGTATTAGGCTGTGGAGGGAAAGAGAATGCATTGGTTC GATTCTTCGGTCCGGACGCTGTTGAAAGAGCGCAGCAACGAGGTCTTAACAGGCACTCATAGCGC ATTTACCGGTACCCGAAAAATGGTGATCTATGCCTGACCAAGACGAAGCCAGAGGAAACTCTGGT GGAGGTCTGTAACGGTCTGACGTGCAAATCGATCGTCATACTTGGGTATAGGGGCGAAAGTA ATCGAACCATCTGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGCAGGGGTGGCCAACCGAA GTTTCTCTTTGTTCGGTGAAGCGAAAGGATTGAGGGCTTGGGGTCCAAAAGAACTTCATCCAATC CCCAACTTTTAAATGGGTCCCAAGACCTATTTTCCTCCTTGGATGGGTGATCATCACAATAACCCCT CCTTGTGGGGCCCTTTTGGTAACAAAACTGGCGCCGGGTATGAACCAAACGTTGAATTAATCG TCCAAAATTACGCTAACAAACCCATTGAAAGTGGTTAATTCTTCACTCAGCAGGACGTTGACCTGG GATGTTGAATCCCCCTACGAGTGGGGTAAAACATGAGTCGGATCCGCTAAGGAGTGTGTAA
<i>M. indica</i>	GGGACCCGTCCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGGACTCTGTT GAAGCCTAAAGGCGTAATGAAAGTGAAGGTCCGCCCTGCGCGGACCGAGGGAGGATGGGTGGAC CCGAGGGGGCCGCCCCGCACTCCCGGGGCGTCTCGTTCTCATCGCGAGAAGAGGCGCACCCAGAGC GTACACGCTGGGACCCGAAAGATGGTGAACATATGCCTGGTCAGGACGAAGTCAGGGGAAACCCCT GATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTCGGAACCTGGGTATAGGGGCGAAAGA CTAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCGCTTCT TGCGAGTCTCATTCGGTAAAGCGAATGATTAGAGGCATTGGGGTTCGAAACGGCCTCAACCTATTCT TCAAACCTTTAAATGGGTGAGATCTCCGGCTTGCTTGAACCTGTGAAGCCGCGAGATATCGGATCAG AGTGCCAAGTGGGGCACTTTTGGTAAGCAGAAGTGGCGCTGTGGGATGAACCAAACGCCGAGTTA AAGCGCCTAAATCGACGCTTATGGGATACCATGAAAGGCGTTGGTAACTTAAGACAGCAGGACGG TGCCCATGGAAGTCGGAATCCGCTAAGGAGTGTGTAA

4.6.4 The sequences of *Cox1* gene from *Melolontha* species and *Polyphylla* species

The sequences of *Cox1* gene from *Melolontha* species and *Polyphylla* species were retrieved from National Centre for Biotechnology Information (NCBI), database (<https://www.ncbi.nlm.nih.gov/>). The sequences were subsequently used for phylogenetic analysis along with sequences obtained in the present study. The sequences of *Cox1* gene retrieved from NCBI are as follows:

Melolontha hippocastani (HM120757)

GAAGTTTATATTTTAAATCTCCAGGATTTGGAATAATTTCCACATCATTAGACAAGAAAGTAACAAAAAGGAAACATT
TGGCACTTTAGGAATAATTTATGCCATAATAGCCATTGGTTTATTAGGCTTTATTGTATGGGGCCACCATATATTTCACTGT
AGGTATAGATGTTGATACTCGAGCCTACTTTACCTCAGCAACCATAATTATCGCAGTACCTACTGGTATTTAAATTTTCA
GATGATTAGCTACTCTCCATGGAACCTCACTAAATTTATTTCCCATCCCTATTATGAGCCCTCGGCTTTGTATTTTATTCA
CAGTAGGAGGACTAACAGGAGTTATTCTCGCAAATTTCTCAATTGACATTATTCTTCATGATACTTATTATGTCGTAGCTC
ATTTTCATTATGTACTTTCAATGGGTGCAGTATTTGCAATTATAGCGGGATTGTCCATTGATTCCCTTTATTCACAGGAC
TAGTATTAACAGAAAAATTATTAATAATCCAAATTTTGGCAATATTATTGGCGTAAATATAACCTTCTTTCCCAACACT
TTTATAGGATTAAGGGGTATACCACGCCGATACTCAGATTACCCTGATGCCTATACAACCTTGAAATGTTGTTTCATCTATCG
GATCACTAATTTCCCTAATCAGTATTATTTTATTCTTATTATTATTGAGACAGATTTAT

Melolontha hippocastani (KX087316)

CAACATTTATTTTGATTTTGGTCAACCCAGAAAGTTTATATTTAATTTCTCCAGGATTTGGAATAATTTCCACATCATTA
GACAAGAAAGTAACAAAAAGGAAACATTGGCACTTTAGGAATAATTTATGCCATAATAGCCATTGGTTTATTAGGCTTT

ATTGTATGGGCCCCACCATATATTTACTGTAGGTATAGATGTCGATACTCGAGCCTACTTTACCTCAGCAACCATAATTATC
GCACTACCTACTGGTATTAAAAATTTTAGATGATTAGCTACTCTCCACGGAACCTCAACTAAATTTATCCCCATCCCTATTA
TGAGCCCTCGGCTTTGTATTTTATTCACAGTAGGAGGACTAACAGGAGTTATTTCTCGCAAATTTCTCAATTGACATTATT
CTTCATGATACTTATTATGTCGTAGCTCATTTCATTATGTACTTTCAATGGGTGCAGTATTTGCAATTATAGCGGGATT
GTCCATTGATTCCCTTTATTCACAGGACTAGTATTAACAGAAAAATTTAAAAATCCAATTTTGTCAATATTTATTGGC
GTAAATATAACCTTCTTTCCCAACCTTTTAGGATTAAGGGGTATACCACGCCGATACCTCAGATTACCCTGATGCCTAT
ACAACCTGAAATGTTGTTTCATCTATCGGATCACTAATTTCCCTAATCAGTATTATTTATTCTTATTTATTTAGACA
GATTTATTTCTATACGAAAAAGACTATCCCCCTAAATATGACAACCTCCATTGAATGATTTCAATCTATACCACCAGCT
GAACATAGATACTCTGAACCTACCAATATTAATAATTTCTAATATGGCAGACTAGTGCAATGGA

***Polyphylla gracilicornis* (MW143080)**

CAACATTTATTTTGATTTTGGACACCTGAAGTTTACATCCTAATTTCTCCCTGGATTGGAATAATCTCCACATCATT
AGCCAAGAGAGAAACAAAAAGGAAACCTTTGGAACACTAGGCATAATCTATGCTATAATAGCAATTGGTTTATTAGGAT
TCATTGTCTGAGCTCACCACATATTTACAGTGGAATAGATGTTGATACCCGAGCTTATTTACCTCAGCCACCATAATC
ATTGCTGTGCCCACTGGTATTAAAAATTTTAGATGACTAGCCCACTCCATGGAACCCAATTAACCTACTCCCATCACT
CTTATGAGCACTAGGATTTGTCTTTTATTTACAGTAGGTGGCTTAACCTGGAGTAGTCCTAGCTAACTCTCCATTGATAT
TATTTTACACGATACTTATTATGTAGTAGCACATTTCCATTATGTCTTTCCATAGGAGCCGTTTGTAGAATTATAGCAGG
ATTACACATTGATTCCCCCTATTCTCTGGCTTAACCTTAATGGAATAATTTTAAAAATTCATTTATAACCATAATTTAT
TGGAGTTAATATAACCTTTTCCCCAACATTTCTGGGACTTAACGGAATACCCCGCCGATACTCGGATTACCCTGATG
CTTATACATCTTGAAATGTGATCTCTCAGTCGGATCCCTAATCTCCCTAATTAGAATTATATATTTTATTTATTTTATTTG
AGAAAGACTTATTTACACCGAAAAAGATTAGTATCATTAATATAAATACTTCAATTGAATGATTTTCACTAATACCC
CCGCTGAACATAGCTACTCTGAGCTTCCCATATTAATAATTTCTAATATGGCAGATTAGTGC

***Polyphylla laticollis* (KF544959)**

CAACATCTATTCTGATTTTTCGGCCATCCTGAAGTTTATATTTTAAATTTTACCTGGATTGGAATAATCTCTCACATCATT
GTCAAGAAAGTAACAAAAAGAACCTTTGGAACCTCTGGGTATAATTTATGCTATAATGGCAATCGGTTTACTAGGATTT
ATTGTATGGGCTCATCATATATTCACAGTGGGTATAGATGTTGATACTCGTGCTTATTTTACTTCAGCCACTATAATTATT
GCAGTGCCAACAGGAATTAATAATCTTTAGATGATTGGCCACTCTACATGGGGCCCACTAAATTAACCTTCATTACT
ATGGGCTTTAGGTTTGTGTTTTTATTTACAGTAGGCGGATTAACAGGAGTAATCTGGCTAATTCATCAATTGATATTAT
TTTACATGACACTTACTATGTGTGCGACATTTTCATTATGTCTCTCCATAGGCGCAGTATTTAGTATCATAGCAGGATT
TACACATTGATTCCCCCTATTCACTGGTTAATACTCAACGAAAAATCTTAAAAATTCATTTATCACTATATTTATTGG
TGTAATATGACATTTTTCCTCAACATTTTCTAGGATTAAGGGGAATGCCTCGCCGATACTCCGACTACCCAGATGCCT
ATACTTCTTGAAATATCGTCTCATCTATTGGATCTCTAATCTCTTAAATAGAATTATCATATTCCTATTTATTATCTGAGA
TAGATTTACTTCAAAACGAAAAAGATTGATATCATTAATATAAATACTCAATTGAGTGATTCCAATTAATGCCCCAG
CTGAACATAGTTACTCTGAACCTACCTATGCTAACTAATTTCTAATATGGCAGATTAGTGCAATGGA

4.6.5 The sequences of 28s rRNA gene from different scarab species

The sequences of 28s rRNA gene from *Melolontha* species were retrieved from National Centre for Biotechnology Information (NCBI), database (<https://www.ncbi.nlm.nih.gov/>). The sequences were subsequently used for phylogenetic analysis along with sequences contained in the present study. The sequences of 28s rRNA gene retrieved from NCBI are as follows:

***Melolontha melolontha* (EU084231)**

GGGACCCGTCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGACTCTGATGAAACCTAAAGGCGTA
ATGAAAGTGAAAGGTCCGCCCTGCGCGGACCGAGGGAGGATGGGTGGACCCGAGGGGCCGCCCGCACTCCCGGGGCGT
CTCGTTCTCATCGCGAGAAGAGGCGCACCCAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACCTATGCCTGGTCAGG
ACGAAGTCAGGGGAAACCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTGGAACTGGGTATAGGGGC
GAAAGACTAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCGCTTCTTGCAGATC
TCATTCGGTAAAGCGAATGATTAGAGGCATTGGGGTCGAAACGGCCTCAACCTATTCTCAAACTTTAAATGGGTGAGAT
CTCCGGCTTGCTTGAACCTGTGAAGCCGCGAGATATCGGATCAGAGTGCCAAGTGGGGCACTTTTGGAAGCAGAACTGG
CGCTGTGGGATGAACCAACGCCGAGTTAAAGCGCCTAAATCGACGCTTATGGGATACCATGAAAGGCGTTGGTAACCT
AAGACAGCAGGACGGTGGCCATGGAAGTCGGAATCCGCTAAGGAGTGTGTAA

***Lepidiota albistigma* (DQ524590)**

GGGACCCGTCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGACTCTGACTAAACCTAAAGGCGTA
ATGAAAGTGAAAGGTCCGCCCTGCGCGGACCGAGGGAGGATGGGCGGACCCGAGGGGCCGCCCTCGCACTCCCGGGGCGT
CTCGTTCTCATCGCGAGAAGAGGCGCACCCAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACCTATGCCTGGTCAGG
ACGAAGTCAGGGGAAACCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTGGAACTGGGTATAGGGGC
GAAAGACTAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCGCTTCTTGCAGATC
TCATTCGGTAAAGCGAATGATTAGAGGCATTGGGGTCGAAACGACCTCAACCTATTCTCAAACTTTAAATGGGTGAGAT
CTCCGGCTTGCTTGAACCTGTGAAGCCGCGAGATATCGGATCAGAGTGCCAAGTGGGGCACTTTTGGAAGCAGAACTGG

CGCTGTGGGATGAACCAAACGCCGAGTTAAAGCGCCTAAATCGACGCTTATGGGATACCATGAAAGGCGTTGGTAACTT
AAGACAGCAGGACGGTGGCCATGGAAGTCGGAATCCGCTAAGGAGTGTGTAA

***Lepidiota stradbrensis* (EU084209)**

GGGACCCGCTCTTGAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGACTCAGACTAAACCTAAAGGCGTA
ATGAAAGTGAAGGTCCGCCCTGCGCGGACCGAGGGAGGATGGGCGGACCCGAGGGGCCGCTCGCACTCCCGGGGCGT
CTCGTTCTCATCGCGAGAAGAGGCGCACCCAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACCTATGCCTGGTCAGG
ACGAAGTCAGGGGAAACCCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTCGGAACCTGGGTATAGGGGC
GAAAGACTAATCGAACCATCTAGTAGCTGGTTCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCGCTTCTGCGAGTC
TCATTGCGTAAAGCGAATGATTAGAGGCATTGGGGTCGAAACGACCTCAACCTATTCTCAAACCTTTAAATGGGTGAGAT
CTCCGGCTTGCTTGAACCTGTGAAGCCGCGAGATATCGGATCAGAGTGCCAAGTGGGCCACTTTTGGTAAGCAGAACTGG
CGCTGTGGGATGAACCAAACGCCGAGTTAAAGCGCCTAAATCGACGCTTATGGGATACCATGAAAGGCGTTGGTAACTT
ATGACAGCAGGACGGTGGCCATGGAAGTCGGAATCCGCTAAGGAGTGTGTAA

***Maladera holosericea* (EU084216)**

GGGACCCGCTCTTGAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGATTTTTACTAAACCTAAAGGCGCA
ATGAAAGTGAAGGTCCGCCCTTGGCGGACCGAGGGAGGATGGACCGTCCCGAGGGACGGCCTCGCACTCCCGGGGCGT
CTCGTTCTCATCGCGAGAAGAGGCGCACCCAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACCTATGCCTGGTCAGG
ACGAAGTCAGGGGAAACCCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTCGGAACCTGGGTATAGGGGC
GAAAGACTAATCGAACCATCTAGTAGCTGGTTCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCGCTTCTGCGAGTC
TCATTGCGTAAAGCGAATGATTAGAGGCATTGGGGTCGAAACGGCCTCAACCTATTCTCAAACCTTTAAATGGGTGAGAT
CTCCGGCTTGCTTGAACCTGTGAAGCCGCGAGATATCGGATCAGAGTGCCAAGTGGGCCACTTTTGGTAAGCAGAACTGG
CGCTGTGGGATGAACCAAACGCCGAGTTAAAGCGCCTAAATCGACGCTTATGGGATACCATGAAAGGCGTTGGTAACTT
AAGACAGCAGGACGGTGGCCATGGAAGTCGGAATCCGCTAAGGAGTGTGTAA

***Maladera significans* (EU084221)**

GGGACCCGCTCTTGAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGATTTTTACTAAACCTAAAGGCGCA
ATGAAAGTGAAGGTCCGCCCTTGGCGGACCGAGGGAGGATAGACCGTACCGAGGTACGGCCTCGCACTCCCGGGGCGT
TCGTTCTCATCGCGAGAAGAGGCGCACCCAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACCTATGCCTGGTCAGGA
CGAAGTCAGGGGAAACCCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTCGGAACCTGGGTATAGGGGC
AAAGACTAATCGAACCATCTAGTAGCTGGTTCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCGCTTCTGCGAGTCT
CATTCCGTAAAGCGAATGATTAGAGGCATTGGGGTCGAAACGGCCTCAACCTATTCTCAAACCTTTAAATGGGTGAGATC
TCCGGCTTGCTTGAACCTGTGAAGCCGCGAGATATCGGATCAGAGTGCCAAGTGGGCCACTTTTGGTAAGCAGAACTGGC
GCTGTGGGATGAACCAAACGCGAGATTAAAGCGCCTAAATCGACGCTTATGGGATACCATGAAAGGCGTTGGTAACTTA
AGACAGCAGGACGGTGGCCATGGAAGTCGGAATCCGCTAAGGAGTGTGTAA

***Adoretus lasiopygus* (DQ524609)**

GGGACCCGCTCTTGAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGACTCTAACGAAACCTAAAGGCGTA
ATGAAAGTGAAGGTCCGCCCTTGGCGGACCGAGGGAGGATGGGCGATCTTTAGGGATCGTCCCGCACTCCCTGGGCGTC
TCGTTCTCATCGCGAGAAGAGGCGCACCAAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACCTATGCCTGGTCAGGA
CGAAGTCAGGGGAAACCCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTCGGAACCTGGGTATAGGGGC
AAAGACTAATCGAACCATCTAGTAGCTGGTTCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCGCTTCTGCGAGTCT
CATTCCGTAAAGCGAATGATTAGAGGCATTGGGGTCGAAACGGCCTCAACCTATTCTCAAACCTTTAAATGGGTGAGATC
TCCGGCTTGCTTGAACCTGTGAAGCCGCGAGATATCGGATCAGAGTGCCAAGTGGGCCACTTTTGGTAAGCAGAACTGGC
GCTGTGGGATGAACCAAACGCTGAGTTAAAGCGCCTAAATCGACGCTTATGGGATACCATGAAAGGCGTTGGTAACTTA
AGACAGCAGGACGGTGGCCATGGAAGTCGGAATCCGCTAAGGAGTGTGTAA

***Holotrichia seticollis* (DQ524596)**

GGGACCCGCTCTTGAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGACTCTGACGAAACCTAAAGGCGCA
ATGAAAGTGAAGGTTCGCCCTAGCGCGGACCGAGGGAGGATGGGTGGTCTCGCGGGACACCTCGCACTTCCGGGCGTC
TCGTTCTCACTCGCGAGAAGAGGCGCACCCAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACCTATGCCTGGTCAGGA
CGAAGTCAGGGGAAACCCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTCGGAACCTGGGTATAGGGGC
AAAGACTAATCGAACCATCTAGTAGCTGGTTCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCGCTTCTGCGAGTCT
CATTCCGTAAAGCGAATGATTAGAGGCATTGGGGTCGAAACGGCCTCAACCTATTCTCAAACCTTTAAATGGGTGAGATC
TCCGGCTTGCTTGAACCTGTGAAGCCGCGAGATATCGGATCAGAGTGCCAAGTGGGCCACTTTTGGTAAGCAGAACTGGC
GCTGTGGGATGAACCAAACGCTGAGTTAAAGCGCCTAAATCGACGCTTATGGGATACCATGAAAGGCGTTGGTAACTTA
AGACAGCAGGACGGTGGCCATGGAAGTCGGAATCCGCTAAGGAGTGTGTAA

***Chiloloba acuta* (DQ524581)**

GGGACCCGCTCTTGAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGACTCTAACGAAACCTAAAGGCGTA
ATGAAAGTGAAGGTTCGCCCTAGCGCGGGCCGAGGGAGGATGGGTGACCCGAGGGGCGCCCCGCACTCCAGGGGCGTC
TCGTTCTCATTGCGAGAAGAGGCGCACCAAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACCTATGCCTGGTCAGGA
CGAAGTCAGGGGAAACCCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTCGGAACCTGGGTATAGGGGC
AAAGACTAATCGAACCATCTAGTAGCTGGTTCCTCCGAAGTTTCCCTCAGGATAGCTGGCGCTCGCTTCTGCGAGTCT
CATTCCGTAAAGCGAATGATTAGAGGCATTGGGGTCGAAACGGCCTCAACCTATTCTCAAACCTTTAAATGGGTGAGATC
TCCGGCTTGCTTGAACCTGTGAAGCCGCGAGATATCGGATCAGAGTGCCAAGTGGGCCACTTTTGGTAAGCAGAACTGGC
GCTGTGGGATGAACCAAACGCTGAGTTAAAGCGCCTAAATCGACGCTTATGGGATACCATGAAAGGCGTTGGTAACTTA
AGACAGCAGGACGGTGGCCATGGAAGTCGGAATCCGCTAAGGAGTGTGTAA

***Cetonia aurata aurata* (EU084146)**

GGGACCCGCTCTTGAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGACTCTAACGAAACCTAAAGGCGTA
ATGAAAGTGAAGGTTCGCCCTAGCGCGGGCCGAGGGAGGATGGGTGTCCCGAGGGGACGCCTCGCACTCCAGGGGCGT

CTCGTTCTCATTGCGAGAAGAGGGCGACCATGAGCGTACACGCTGGGACCCGAAAGATGGTGAACCTATGCCTGGTCAGG
ACGAAGTCAGGGGAAACCCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTGGAACTGGGTATAGGGGC
GAAAGACTAATCGAACCATCTAGTAGCTGGTTCCTCCGAAAGTTTCCCTCAGGATAGCTGGCGCTCGCTTCTCGCGAGTC
TCATTCCGTAAGCGAATGATTAGAGGCATTGGGGTCGAAACGGCCTCAACCTATTCTCAAACCTTTAAATGGGTGAGAT
CTCCGGCTTGCTTGAACCTGTGAAGCCGCGAGATATCGGATCAGAGTGCCAAAGTGGGCCACTTTTGGTAAGCAGAAGCTGG
CGCTGTGGGATGAACCAAACGCTGAGTTAAAGCGCTAAATCGACGCTTATGGGATACCATGAAAGGCGTTGGTAACCT
AAGACAGCAGGACGGTGGCCATGGAAGTCGGAATCCGCTAAGGAGTGTGTAA

Anomala sp. (DQ524588)

GGGACCCGTCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGACTCTAACGAAACCTAAAGGCGTA
ATGAAAGTGAAGGTTCCGCACGCGCAACCGAGGAGGATGGACGGTCGATAGTGCCGTCCTCCGCACTCCCTGGGCGTC
TCGTTCTCATCGCGAGAAGAGGGCGACCAAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACCTATGCCTGGTCAGGA
CGAAGTCAGGGGAAACCCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAATCGATCGTGGAACTGGGTATAGGGGCG
AAAGACTAATCGAACCATCTAGTAGCTGGTTCCTCCGAAAGTTTCCCTCAGGATAGCTGGCGCTCGCTTCTCGCGAGTCT
CATTCGGTAAGCGAATGATTAGAGGCATTGGGGTCGAAACGGCCTCAACCTATTCTCAAACCTTTAAATGGGTGAGATC
TCCGGCTTGCTTGAACCTGTGAAGCCGCGAGATATCGGATCAGAGTGCCAAAGTGGGCCACTTTTGGTAAGCAGAAGCTGGC
GCTGTGGGATGAACCAAACGCGAGTTAAAGCGCCTAAATCGACGCTTATGGGATACCATGAAAGGCGTTGGTAACCTTA
AGACAGCAGGACGGTGGCCATGGAAGTCGGAATCCGCTAAGGAGTGTGTAA

4.6.6 Multiple sequence alignment of *Cox1* gene sequences

The *Cox1* sequences of *M. cuprescence*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*) were aligned with *Cox1* sequences of *Melolontha hippocastani* (HM120757), *Melolontha hippocastani* (KX087316), *Polyphylla gracilicornis* (MW143080) and *Polyphylla laticollis* (KF544959). The multiple sequence alignment of *Cox1* gene from all these species is given as follows:

<i>M. indica</i>	-----	0
<i>M. furcicauda</i>	TCCAATGCACTAATCTGCCATATTAGAAATTAGTTAATATGGGTAGTTCGGAGTACCTAT	60
<i>M. cuprescens</i>	TCCAATGCACTAATCTGCCATATTAGAAATTAGTTAATATGGGGAGTTCAGAGTAGCTAT	60
<i>P. fullo</i>	TCCAATGCACTAATCTGCCATATTAGAAATTAGTTAATATGGGGAGTTCAGAGTAGCTAT	60
HM120757- <i>M. hippocastani</i>	-----	0
KX087316- <i>M. hippocastani</i>	-----CAACATTTATTTTG-----	14
MW143080- <i>P. gracilicornis</i>	-----CAACATTTATTTTG-----	14
KF544959- <i>P. laticollis</i>	-----CAACATCTATTCTG-----	14
<i>M. indica</i>	-----	0
<i>M. furcicauda</i>	GTTTCAGCTGGGGGTATTATTGGAATCATTC-GATAGAAGTTGTTATGTTTGGGGGGAT	119
<i>M. cuprescens</i>	GTTTCAGCAGGGGTATTAGTTGGAATCATTC-AAATTGAAGTAGTTATACTTAGGGGCACT	119
<i>P. fullo</i>	GTTTCAGCAGGGGTATTAGTTGGAGTCATTC-AAATTGAAGTAGTTATACTTAGGGGCACT	119
HM120757- <i>M. hippocastani</i>	-----GAAGTTTATATTTTAAATCTCCAGG-ATT	29
KX087316- <i>M. hippocastani</i>	-----ATTTTGTGTCACCCAGAGTTTATATTTTAAATCTCCAGG-ATT	59
MW143080- <i>P. gracilicornis</i>	-----ATTTTGTGACACCCGTAAGTTTACATCCTAATTCTCCCTGG-ATT	59
KF544959- <i>P. laticollis</i>	-----ATTTTTCGGCCATCCTGAAGTTTATATTTTAAATTTTACCTGG-ATT	59
<i>M. indica</i>	-----	0
<i>M. furcicauda</i>	AGGCTTTTTCGTATAGAGATAAATCTATCTC-AAATAATAAATAAATAAGATAAATACT	178
<i>M. cuprescens</i>	AAGCTTTTTCGGTGTGAAATAAATCTGTCTC-AAATAACAAATAAATAACATAAATACT	178
<i>P. fullo</i>	AAGCTTTTTCGGTGTGAAATAAATCTGTCTC-AAATAACAAATAAATAACATAAATACT	178
HM120757- <i>M. hippocastani</i>	TGG-----AATAAATTTCCACATCATTAGACAAGAAAGTAACAAAAAGGAACATTGG	83
KX087316- <i>M. hippocastani</i>	TGG-----AATAAATTTCCACATCATTAGACAAGAAAGTAACAAAAAGGAACATTGG	113
MW143080- <i>P. gracilicornis</i>	TGG-----AATAATCTCCACATCATTAGCCAAGAGAGAAACAAAAAGGAACCTTGG	113
KF544959- <i>P. laticollis</i>	TGG-----AATAATCTCTCACATCATTAGTCAAGAAAGTAACAAAAAGGAACCTTGG	113
<i>M. indica</i>	-----TCCAATGCACTAATCTGCCATATTA-GAAATTAGTTAATATGGGTAGTTC	49
<i>M. furcicauda</i>	AATTAAGGAAATTAATGAACCAATAGATGAGATAA-TATTTATACAGTATAGGCG--TC	235
<i>M. cuprescens</i>	AATAAGGGAATTAGGGATCCGATTGAGGAGATCA-CATTTCAAGAAGTATAGGCA--TC	235
<i>P. fullo</i>	AATAAGGGAATTAGGGATCCGATTGAGGAGATCA-CATTTCAAGAAGTATAGGCA--TC	235
HM120757- <i>M. hippocastani</i>	CACTTTAGGAATAATTTATGCCATAATAGCCATTGGTTTATTAGGCTTTATTGTA---TG	140
KX087316- <i>M. hippocastani</i>	CACTTTAGGAATAATTTATGCCATAATAGCCATTGGTTTATTAGGCTTTATTGTA---TG	170
MW143080- <i>P. gracilicornis</i>	AACACTAGGCATAATCTATGCTATAATAGCAATGGTTTATTAGGATTCATTGTC---TG	170
KF544959- <i>P. laticollis</i>	AACTCTGGGTATAATTTATGCTATAATGGCAATCGGTTTACTAGGATTTATTGTA---TG	170

* ** *** * *** *

<i>M. indica</i>	AGAATATCTATGTTTCAGCTGGTGGTATTATTTGAAATCATTCAAATAGATGTTGTTATTCT	109
<i>M. furcicauda</i>	GGGGTAATCTGAGTATCGTCGGGGTATGCCTCTTAATCCTAGAAAATGTTGAGGAAAAAA	295
<i>M. cuprescens</i>	TGGGTAATCAGAGTATCGTCGGGGTATCCACTAAGTCCCAGAAAATGTTGGGGAAAAGAA	295
<i>P. fullo</i>	TGGGTAATCAGAGTATCGTCGGGGTATCCACTAAGTCCCAGAAAATGTTGGGGAAAAGAA	295
HM120757- <i>M. hippocastani</i>	GGCCCACCATATATTTCACTGTAGGTATAGATGTTGATACTCGAGCCTACTTTA-----	193
KX087316- <i>M. hippocastani</i>	GGCCCACCATATATTTCACTGTAGGTATAGATGTCGATACTCGAGCCTACTTTA-----	223
MW143080- <i>P. gracilicornis</i>	AGCTCACCACATATTTACAGTGGGAATAGATGTTGATACCCGAGCTATTTTA-----	223
KF544959- <i>P. laticollis</i>	GGCTCATCATATATTTACAGTGGGTATAGATGTTGATACTCGTGCTTATTTTA-----	223
	* * * * *	
<i>M. indica</i>	TACTGGAGAAAGTCTTTTTCGTATTGAAATTTGAAATATTTTGAATAGTTTCT-----	163
<i>M. furcicauda</i>	GGTTATGTTTACTCCAATGAATATTGTAATAAATGAATTTTAAAAAGTTTCT-----	349
<i>M. cuprescens</i>	GGTTATATTAACCTCAATAAATATGTTTATAAATGGATTTTAAAAATTTTCC-----	349
<i>P. fullo</i>	GGTTATATTAACCTCAATAAATATGTTTATAAATGGATTTTAAAAATTTTCC-----	349
HM120757- <i>M. hippocastani</i>	-----CCTCAGCAACCATAATTATCGCAGTACCTACTGGTATTAATAATTTTATAGATGATT	248
KX087316- <i>M. hippocastani</i>	-----CCTCAGCAACCATAATTATCGCAGTACCTACTGGTATTAATAATTTTATAGATGATT	278
MW143080- <i>P. gracilicornis</i>	-----CCTCAGCAACCATAATTATCGTGTGCCACTGGTATTAATAATTTTATAGATGATT	278
KF544959- <i>P. laticollis</i>	-----CTTCAGCCACTATAATTATGTCAGTGCCACAGGAATTAATAATCTTTAGATGATT	278
	* * * * *	
<i>M. indica</i>	-----ATTT	167
<i>M. furcicauda</i>	-----ATTA	353
<i>M. cuprescens</i>	-----ATTA	353
<i>P. fullo</i>	-----ATTA	353
HM120757- <i>M. hippocastani</i>	AGCTACTCTCCATGGAACCTCACTAAATTTATCCCATCCCTATTATGAGCCCTCGGCTT	308
KX087316- <i>M. hippocastani</i>	AGCTACTCTCCACGGAACCTCACTAAATTTATCCCATCCCTATTATGAGCCCTCGGCTT	338
MW143080- <i>P. gracilicornis</i>	AGCCACACTCCATGGAACCCAAATTAACACTACTCCCATCACTCTTATGAGCACTAGGATT	338
KF544959- <i>P. laticollis</i>	GGCCACTCTACATGGGGCCCACTAAATTTACTCACCTTCATTACTATGGGCTTTAGGTTT	338
	* * * * *	
<i>M. indica</i>	AATGTTAGTCCTGTAATAAACGGAACCAATGGACAAATCTAGCTATGATAGCAAA-TAC	226
<i>M. furcicauda</i>	AGTATTAAACCTGTAAATAGGGGGAATCAGTGATCAAAATCCTGCTATAATAGCAAA-TAC	412
<i>M. cuprescens</i>	AGGGTTAAGCCAGAGAAATAGGGGGAATCAGTGAGTGAATCCTGCTATAATTTCTAAA-AAC	412
<i>P. fullo</i>	AGGGTTAAGCCAGAGAAATAGGGGGAATCAGTGAGTGAATCCTGCTATAATTTCTAAA-AAC	412
HM120757- <i>M. hippocastani</i>	TGTATTTTATTACAGTAGGAGGACTAACAGGAGTTATTCTCGCAAATTTCTCAATTGA	368
KX087316- <i>M. hippocastani</i>	TGTATTTTATTACAGTAGGAGGACTAACAGGAGTTATTCTCGCAAATTTCTCAATTGA	398
MW143080- <i>P. gracilicornis</i>	TGTCTTTTATTACAGTAGGTTGGCTTAACCTGGAGTAGTCTTAGCTAACTCTTCCATTGA	398
KF544959- <i>P. laticollis</i>	TGTTTTTTTATTACAGTAGGCGGATTAACAGGAGTAATTCTGGCTAATTCATCAATTGA	398
	* * * * *	
<i>M. indica</i>	TGCTCCTATTGAAAGTACGTAATGAAATGGGTGCAACATAGTAAGTATCATGAAGGTT	286
<i>M. furcicauda</i>	GGCTCCTATTGATAGAACGTAATGAAAGTGGGCTACTACGTAATAAGTATCATGAAGGAT	472
<i>M. cuprescens</i>	GGCTCCTATAGAAAGGACATAATGGAAGTGTGCTACGACATAGTAGGTGTCTGTAGAAAT	472
<i>P. fullo</i>	GGCTCCTATAGAAAGGACATAATGGAAGTGTGCTACGACATAGTAGGTGTCTGTAGAAAT	472
HM120757- <i>M. hippocastani</i>	CATTATTCTTCATGATACTTATTATGTCGTAGCTCATTTCATTATGTACTTTCAATGGG	428
KX087316- <i>M. hippocastani</i>	CATTATTCTTCATGATACTTATTATGTCGTAGCTCATTTCATTATGTACTTTCAATGGG	458
MW143080- <i>P. gracilicornis</i>	TATTATTTTACACGATACTTATTATGTAGTAGACATTTCCATTATGTCTTTCCATAGG	458
KF544959- <i>P. laticollis</i>	TATTATTTTACATGACACTTACTATGTTGTGTCACATTTTCATTATGTCTCTCCATAGG	458
	* * * * *	
<i>M. indica</i>	GATATTAATGAAGAGTTTGCAAGAATAACTCCTGTTAATCCTCCACAGTAAATAAGAC	346
<i>M. furcicauda</i>	AATATCAATTGAAGAATTTGCCAGGATAACTCCCGTTAAACCCCCACTGTAAATAAGAA	532
<i>M. cuprescens</i>	AATGTCGATTGAAGAGTTAGCTAAGATTACTCCAGTCAATCCTCCCACTGTAAATAAAAA	532
<i>P. fullo</i>	AATGTCGATTGAAGAGTTAGCTAAGATTACTCCAGTCAATCCTCCCACTGTAAATAAAAA	532
HM120757- <i>M. hippocastani</i>	TGCAGT-ATTTGCAATTATAGCGGGATTGTCCATTGATTCCCTTTATTCACAGGACTAG	487
KX087316- <i>M. hippocastani</i>	TGCAGT-ATTTGCAATTATAGCGGGATTGTCCATTGATTCCCTTTATTCACAGGACTAG	517
MW143080- <i>P. gracilicornis</i>	AGCCGT-TTTTAGAATTATAGCAGGATTACACATTGATTCCCCCTATTCTCTGGCTTAA	517
KF544959- <i>P. laticollis</i>	CGCAGT-ATTTAGTATCATAGCAGGATTACACATTGATTCCCCCTATTCTACTGGTTTAA	517
	* * * * *	
<i>M. indica</i>	TACAAAACCTAGGGCGCATAGTAAGGATGGGGAATAGTTTAGTTGAGTTCCGCGTAGAGT	406
<i>M. furcicauda</i>	TACAAATCCTAGGGCTCATAGCAATGATGGGGAATAATTTAATTGAGTTCCATGGAGAGT	592
<i>M. cuprescens</i>	TACGAATCCTAGGGCTCACAGAAGTGTAGGGAGTAGTTAATTGGGTCCCATGAAGTGT	592
<i>P. fullo</i>	TACGAATCCTAGGGCTCACAGAAGTGTAGGGAGTAGTTAATTGGGTCCCATGAAGTGT	592
HM120757- <i>M. hippocastani</i>	TA-----	489
KX087316- <i>M. hippocastani</i>	TA-----	519
MW143080- <i>P. gracilicornis</i>	CC-----	519
KF544959- <i>P. laticollis</i>	TA-----	519
	* * * * *	
<i>M. indica</i>	AGCCAGTCATCTGAAAATTTTAATACCCGTAGGTACTGCAATAATTATGGTTGCTGAGGT	466
<i>M. furcicauda</i>	GGCCAATCATCTGAAAATTTTGATACCAAGTAGGTACAGCAATAATTATGTTGCGAGAGT	652
<i>M. cuprescens</i>	AGCAATTTCATCTGAAAATTTTAATACCCGTGGGTACAGCAATGATTATGGTGGCTGAAGT	652
<i>P. fullo</i>	AGCAATTTCATCTGAAAATTTTAATACCCGTGGGTACAGCAATGATTATGGTGGCTGAAGT	652
HM120757- <i>M. hippocastani</i>	----TTAAACAGAAAATTTTAAAAATCCAATTTTGTGCAATATTATGGCGTAAATAT	545
KX087316- <i>M. hippocastani</i>	----TTAAACAGAAAATTTTAAAAATCCAATTTTGTGCAATATTATGGCGTAAATAT	575
MW143080- <i>P. gracilicornis</i>	----CTTAATGGAAAATTTTAAAAATCCAATTTTATAACCATATTATTTGGAGTTAATAT	575
KF544959- <i>P. laticollis</i>	----CTCAACGAAAATTTTAAAAATCCAATTTTATCACTATATTATTTGGTGTAAATAT	575
	* * * * *	

<i>M. indica</i>	AAAGTAGG-----CTACATCTATTCTACAGTAAA--TATGTGATGAGCTCATACA	515
<i>M. furcicauda</i>	AAAAAAGCTCGGGTATCTACATCTATTCTACTGTAAA--TATATGATGGGCCCATACG	710
<i>M. cuprescens</i>	AAAAAAGCCCCGAGTATCGACATCTATGCCCACTGTGAA--TATGTGATGGGCTCAGACA	710
<i>P. fullo</i>	AAAAAAGCCCCGAGTATCGACATCTATGCCCACTGTGAA--TATGTGATGGGCTCAGACA	710
HM120757- <i>M. hippocastani</i>	AACCTTCT-----TTCCCCAACACTTTTTAGGATTAAGGGGTATACCACGCCGATACT	598
KX087316- <i>M. hippocastani</i>	AACCTTCT-----TTCCCCAACACTTTTTAGGATTAAGGGGTATACCACGCCGATACT	628
MW143080- <i>P. gracilicornis</i>	AACCTTTT-----TCCCCAACACTTTCTGGGACTTAACGGAATACCCCGCGATACT	628
KF544959- <i>P. laticollis</i>	GACATTTT-----TTCCTCAACATTTTCTAGGATTAAGGGGAATGCCTCGCCGATACT	628
	* * * * *	
<i>M. indica</i>	ATGAAGCCTAACAAATCCAA-----GAGCTATTATTGCATAGATTATCC	558
<i>M. furcicauda</i>	ATAAAGCCTAATAGGCCAA-----TAGCTATTATGGCATAAATATATTC	753
<i>M. cuprescens</i>	ATAAATCCTAATAAGCCAA-----TCGCTATTATAGCATAGATTATTC	753
<i>P. fullo</i>	ATAAATCCTAATAAGCCAA-----TCGCTATTATAGCATAGATTATTC	753
HM120757- <i>M. hippocastani</i>	CAGATTACCCTGATGCCATACAACTTGAAATGTTGTTTCATCTATCGGATCACTAATTT	658
KX087316- <i>M. hippocastani</i>	CAGATTACCCTGATGCCATACAACTTGAAATGTTGTTTCATCTATCGGATCACTAATTT	688
MW143080- <i>P. gracilicornis</i>	CGGATTACCCTGATGCTTATACATCTTGAAATGTGATCTCCTCAGTCGGATCCCTAATCT	688
KF544959- <i>P. laticollis</i>	CCGACTACCCAGATGCCATCTCTTGAAATATCGTCTCATCTATGGATCTCTAATCT	688
	* * * * *	
<i>M. indica</i>	CTAAAGTACCGAATGTTTCTTTTCTGTTTCTTTCTTGT-----CTAATAATATG	607
<i>M. furcicauda</i>	CTAAAGTGCCGAATGTTTCTTTTCTGTTTCTTTCTTGT-----CTAATAATGTG	802
<i>M. cuprescens</i>	CTAGTGTTCCGAATGTTTCTTTTCTGTTTCTTTCTTGG-----CTAATAATGTG	802
<i>P. fullo</i>	CTAGTGTTCCGAATGTTTCTTTTCTGTTTCTTTCTTGG-----CTAATAATGTG	802
HM120757- <i>M. hippocastani</i>	CCCTAATCAGTATTATTTTATTCTTATTTATTATTTGAGACAGATTTAT-----	707
KX087316- <i>M. hippocastani</i>	CCCTAATCAGTATTATTTTATTCTTATTTATTATTTGAGACAGATTTATTTCTATACGAA	748
MW143080- <i>P. gracilicornis</i>	CCCTAATTAGAATTATTTATTTTATTATTTTATTATTTGAGAAAGACTTATTTTCAACCCGAA	748
KF544959- <i>P. laticollis</i>	CTTTAATTAGAATTATCATATTCCTATTTTATTATCTGAGATAGATTTACTTCAAAACGAA	748
	* * * * *	
<i>M. indica</i>	AGAAATTATTCCAAATCT-----TGGG-AGGATTAAATATAAACTTTTGG	652
<i>M. furcicauda</i>	GGAAATTATTCCAAATCC-----CGGGAAGAAATATAAACTTCTGG	848
<i>M. cuprescens</i>	GGAGATTATTCCAAATCC-----GGGG-AGAATTAGAATATAAACTTCAGG	847
<i>P. fullo</i>	GGAGATTATTCCAAATCC-----GGGG-AGAATTAGAATATAAACTTCAGG	847
HM120757- <i>M. hippocastani</i>	-----	707
KX087316- <i>M. hippocastani</i>	AAAGACTATCCCCCTAAATATGACAACCTCCATTGAATGATTTCAATCTATACCACCAG	808
MW143080- <i>P. gracilicornis</i>	AAAGATTAGTATCATTAATATAAATACTTCAATTGAATGATTTTCACTAATACCCCCG	808
KF544959- <i>P. laticollis</i>	AAAGATTGATATCATTAATATAAATACTTCAATTGAGTGATTCCAATTAAATGCCCCAG	808
<i>M. indica</i>	A-----TGTCCTCAAAAAA-----TCAAAAATAATGT	676
<i>M. furcicauda</i>	G-----TGTCCTCAAAAAA-----TCAAAAATAATGT	872
<i>M. cuprescens</i>	G-----TGTCCTCAAAAAA-----TCAAAAATAATGT	871
<i>P. fullo</i>	G-----TGTCCTCAAAAAA-----TCAAAAATAATGT	871
HM120757- <i>M. hippocastani</i>	-----	707
KX087316- <i>M. hippocastani</i>	CTGAACATAGTACTCTGAACTACCAATATTAACTAATTTCTAATATGGCAGACTAGTGC	868
MW143080- <i>P. gracilicornis</i>	CTGAACATAGTACTCTGAGCTTCCCATATTAACTAATTTCTAATATGGCAGATTAGTGC	868
KF544959- <i>P. laticollis</i>	CTGAACATAGTTACTCTGAACTACCTATGCTAACTAATTTCTAATATGGCAGATTAGTGC	868
<i>M. indica</i>	TG----	678
<i>M. furcicauda</i>	TG----	874
<i>M. cuprescens</i>	TG----	873
<i>P. fullo</i>	TG----	873
HM120757- <i>M. hippocastani</i>	-----	707
KX087316- <i>M. hippocastani</i>	AATGGA	874
MW143080- <i>P. gracilicornis</i>	-----	868
KF544959- <i>P. laticollis</i>	AATGGA	874

4.6.7 Multiple alignment of 28s rRNA gene sequences

The 28s rRNA sequences of *M. cuprescence*, *M. furcicauda* and *M. indica* were aligned with 28s rRNA sequences of *Melolontha melolontha* (EU084221), *Lepidiota albistigma* (DQ524590), *Lepidiota stradbokensis* (EU084209), *Holotrichia seticollis* (DQ524596), *Maladera holosericea* (EU084216), *Maladera significans* (EU084221), *Adoretus lasiopygus* (DQ524609), *Anomala* sp. (DQ524588), *Chiloloba acuta* (DQ524581) and *Cetonia aurata aurata* (EU084146). The multiple sequence alignment of 28s rRNA gene from all these species is given as follows:

<i>M. furcicauda</i>	GGGACCCGTCCTTGAAACACGGACCAAGGTGTTGGGCCCCCTTCCGAAACACACGGAC	60
DQ524581-C. acuta	-GGGACCCGTCCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGG-GACT	58
EU084146-C. aurata	-GGGACCCGTCCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGG-GACT	58
DQ524609-A. lasiopygus	-GGGACCCGTCCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGG-GACT	58
DQ524588-Anomala	-GGGACCCGTCCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGG-GACT	58
EU084216-M. holosericea	-GGGACCCGTCCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGG-GATT	58
EU084221-M. significans	-GGGACCCGTCCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGG-GATT	58
DQ524596-H. seticollis	-GGGACCCGTCCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGG-GGACT	58
EU084231-M. melolontha	-GGGACCCGTCCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGG-GGACT	58
DQ524590-L. albistigma	-GGGACCCGTCCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGG-GGACT	58
EU084209-L. stradbokensis	GGGACCCGTCCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGGACT	60
<i>M. cuprescens</i>	GGGACCCGTCCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGGACT	60
<i>M. indica</i>	GGGACCCGTCCTTGAAACACGGACCAAGGAGTCTAGCATGTGCGCGAGTCATTGGGGACT	60
* * * * *		
<i>M. furcicauda</i>	AGGGGTTTGTAAATGCGTAAACCGTTCTTGATTAGGCTGTGGAGGGAAGAGAA	120
DQ524581-C. acuta	CTAACGAAACCTAAAGGCGTAATGAAAGTGAAGGTCCGCCTAGCGCGGGCCGAGGGAGGA	118
EU084146-C. aurata	CTAACGAAACCTAAAGGCGTAATGAAAGTGAAGGTCCGCCTAGCGCGGGCCGAGGGAGGA	118
DQ524609-A. lasiopygus	CTAACGAAACCTAAAGGCGTAATGAAAGTGAAGGTCCGCCTTGCGCGGACCGAGGGAGGA	118
DQ524588-Anomala	CTAACGAAACCTAAAGGCGTAATGAAAGTGAAGGTTCGCCACGCGCAACCGAGGGAGGA	118
EU084216-M. holosericea	TTTACTAAACCTAAAGGCGCAATGAAAGTGAAGGTCCGCCTTGCGCGGACCGAGGGAGGA	118
EU084221-M. significans	TTTACTAAACCTAAAGGCGCAATGAAAGTGAAGGTCCGCCTTGCGCGGACCGAGGGAGGA	118
DQ524596-H. seticollis	CTGACGAAACCTAAAGGCGCAATGAAAGTGAAGGTTCGCCACGCGCAACCGAGGGAGGA	118
EU084231-M. melolontha	CTGATGAAACCTAAAGGCGTAATGAAAGTGAAGGTCCGCCTTGCGCGGACCGAGGGAGGA	118
DQ524590-L. albistigma	CTGACTAAACCTAAAGGCGTAATGAAAGTGAAGGTCCGCCTTGCGCGGACCGAGGGAGGA	118
EU084209-L. stradbokensis	CAGACTAAACCTAAAGGCGTAATGAAAGTGAAGGTTCGCCACGCGCAACCGAGGGAGGA	118
<i>M. cuprescens</i>	CTGACGAAACCTAAAGGCGTAATGAAAGTGAAGGTCCGCCTTGCGCGGACCGAGGGAGGA	120
<i>M. indica</i>	CTGTTGAAGCGTAAGGCGTAATGAAAGTGAAGGTCCGCCTTGCGCGGACCGAGGGAGGA	120
* * * * *		
<i>M. furcicauda</i>	TGCATTGGTTCGATTCTTCGGTCCGGACGCTGTTGAAAGAGCGCAGCAACGAGGTCTTAA	180
DQ524581-C. acuta	TGGGTGACCCCGAGG-GGCGCCCGCACTCCAGGGCGTCTCGTTCTCATTTCCGAGAAGA	177
EU084146-C. aurata	TGGGTGTCGCCGAGGGGACGCTCGCACTCCAGGGCGTCTCGTTCTCATTTCCGAGAAGA	178
DQ524609-A. lasiopygus	TGGGCGATCTTTAGGGATCGTCCCGCACTCCCTGGGCGTCTCGTTCTCATTTCCGAGAAGA	178
DQ524588-Anomala	TGGACGCTCGATAGTGGCCGTCCTCCGCACTCCCTGGGCGTCTCGTTCTCATTTCCGAGAAGA	178
EU084216-M. holosericea	TGGACCGTCCCGAGGGACGCGCTCGCACTCCCGGGGCGTCTCGTTCTCATTTCCGAGAAGA	178
EU084221-M. significans	TAGACCGTACCGAGGTACGCGCTCGCACTCCCGGGGCGTCTCGTTCTCATTTCCGAGAAGA	178
DQ524596-H. seticollis	TGGGTGGTCTCGCGGGACCACTCGCACTCCCGGGGCGTCTCGTTCTCATTTCCGAGAAGA	178
EU084231-M. melolontha	TGGGTGGACCCGAGGGGCGCGCCCGCACTCCCGGGGCGTCTCGTTCTCATTTCCGAGAAGA	178
DQ524590-L. albistigma	TGGGCGGACCCGAGGGGCGCGCTCGCACTCCCGGGGCGTCTCGTTCTCATTTCCGAGAAGA	178
EU084209-L. stradbokensis	TGGGCGGACCCGAGGGGCGCGCTCGCACTCCCGGGGCGTCTCGTTCTCATTTCCGAGAAGA	178
<i>M. cuprescens</i>	TGGGCGGACCCGAGGGGCGCGCTCGCACTCCCGGGGCGTCTCGTTCTCATTTCCGAGAAGA	180
<i>M. indica</i>	TGGGTGGACCCGAGGGGCGCGCCCGCACTCCCGGGGCGTCTCGTTCTCATTTCCGAGAAGA	180
* * * * *		
<i>M. furcicauda</i>	CAGGCACTCATAGCGCATTTACCGGTACCCGAAAGATGGTGATCTATGCCTGACCAAGAC	240
DQ524581-C. acuta	GGCGCACCAAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAGGAC	237
EU084146-C. aurata	GGCGCACCATGAGCGTACACGCTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAGGAC	238
DQ524609-A. lasiopygus	GGCGCACCAAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAGGAC	238
DQ524588-Anomala	GGCGCACCAAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAGGAC	238
EU084216-M. holosericea	GGCGCACCAAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAGGAC	238
EU084221-M. significans	GGCGCACCAAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAGGAC	238
DQ524596-H. seticollis	GGCGCACCAAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAGGAC	238
EU084231-M. melolontha	GGCGCACCAAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAGGAC	238
DQ524590-L. albistigma	GGCGCACCAAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAGGAC	238
EU084209-L. stradbokensis	GGCGCACCAAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAGGAC	238
<i>M. cuprescens</i>	GGCGCACCAAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAGGAC	240
<i>M. indica</i>	GGCGCACCAAGAGCGTACACGCTGGGACCCGAAAGATGGTGAACTATGCCTGGTCAGGAC	240
* * * * *		
<i>M. furcicauda</i>	GAAGCCAGAGGAAACTCTGGTGGAGGTCTGTAACGGTTCTGACGTGCAAAATCGATCGTC	300
DQ524581-C. acuta	GAAGTCAGGGGAAACCCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAAATCGATCGTC	297
EU084146-C. aurata	GAAGTCAGGGGAAACCCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAAATCGATCGTC	298
DQ524609-A. lasiopygus	GAAGTCAGGGGAAACCCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAAATCGATCGTC	298
DQ524588-Anomala	GAAGTCAGGGGAAACCCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAAATCGATCGTC	298
EU084216-M. holosericea	GAAGTCAGGGGAAACCCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAAATCGATCGTC	298
EU084221-M. significans	GAAGTCAGGGGAAACCCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAAATCGATCGTC	298
DQ524596-H. seticollis	GAAGTCAGGGGAAACCCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAAATCGATCGTC	298
EU084231-M. melolontha	GAAGTCAGGGGAAACCCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAAATCGATCGTC	298
DQ524590-L. albistigma	GAAGTCAGGGGAAACCCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAAATCGATCGTC	298
EU084209-L. stradbokensis	GAAGTCAGGGGAAACCCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAAATCGATCGTC	298
<i>M. cuprescens</i>	GAAGTCAGGGGAAACCCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAAATCGATCGTC	300
<i>M. indica</i>	GAAGTCAGGGGAAACCCCTGATGGAGGTCCGTAGCGATTCTGACGTGCAAAATCGATCGTC	300
* * * * *		
<i>M. furcicauda</i>	TACTTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGT	360
DQ524581-C. acuta	GAAGTCGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGT	357
EU084146-C. aurata	GAAGTCGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGT	358
DQ524609-A. lasiopygus	GAAGTCGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGT	358
DQ524588-Anomala	GAAGTCGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGT	358
EU084216-M. holosericea	GAAGTCGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGT	358
EU084221-M. significans	GAAGTCGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGT	358
DQ524596-H. seticollis	GAAGTCGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGT	358
EU084231-M. melolontha	GAAGTCGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGT	358
DQ524590-L. albistigma	GAAGTCGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGT	358
EU084209-L. stradbokensis	GAAGTCGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGT	358

<i>M. cuprescens</i>	GAAC TGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTCCTCCGAAAGT	360
<i>M. indica</i>	GAAC TGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTCCTCCGAAAGT	360
* * * * *		
<i>M. furcicauda</i>	TTCCCTCAGGATAGCAGGGGTGGCCAAACGGAAGTTTCTCTTTGTTCCGGTGAAGCGAAAG	420
DQ524581- <i>C. acuta</i>	TTCCCTCAGGATAGCTGGCGCTCGCTTCTCGCGAGTCTCA----TTCGGTAAAGCGAATG	413
EU084146- <i>C. aurata</i>	TTCCCTCAGGATAGCTGGCGCTCGCTTCTCGCGAGTCTCA----TTCGGTAAAGCGAATG	414
DQ524609- <i>A. lasiopygus</i>	TTCCCTCAGGATAGCTGGCGCTCGCTTCTCGCGAGTCTCA----TTCGGTAAAGCGAATG	414
DQ524588- <i>Anomala</i>	TTCCCTCAGGATAGCTGGCGCTCGCTTCTCGCGAGTCTCA----TTCGGTAAAGCGAATG	414
EU084216- <i>M. holosericea</i>	TTCCCTCAGGATAGCTGGCGCTCGCTTCTCGCGAGTCTCA----TTCGGTAAAGCGAATG	414
EU084221- <i>M. significans</i>	TTCCCTCAGGATAGCTGGCGCTCGCTTCTCGCGAGTCTCA----TTCGGTAAAGCGAATG	414
DQ524596- <i>H. seticollis</i>	TTCCCTCAGGATAGCTGGCGCTCGCTTCTCGCGAGTCTCA----TTCGGTAAAGCGAATG	414
EU084231- <i>M. melolontha</i>	TTCCCTCAGGATAGCTGGCGCTCGCTTCTCGCGAGTCTCA----TTCGGTAAAGCGAATG	414
DQ524590- <i>L. albistigma</i>	TTCCCTCAGGATAGCTGGCGCTCGCTTCTCGCGAGTCTCA----TTCGGTAAAGCGAATG	414
EU084209- <i>L. stradbokensis</i>	TTCCCTCAGGATAGCTGGCGCTCGCTTCTCGCGAGTCTCA----TTCGGTAAAGCGAATG	414
<i>M. cuprescens</i>	TTCCCTCAGGATAGCTGGCGCTCGCTTCTCGCGAGTCTCA----TTCGGTAAAGCGAATG	416
<i>M. indica</i>	TTCCCTCAGGATAGCTGGCGCTCGCTTCTCGCGAGTCTCA----TTCGGTAAAGCGAATG	416
* * * * *		
<i>M. furcicauda</i>	GATTGAGGGCTTGGGGTCCAAAAGAACTTCATCCAATCCCCAACTTTTAATGGGTCCCAA	480
DQ524581- <i>C. acuta</i>	ATTAGAGGCATTGGGGTTCGAAACGGCCCTCAACCTATTCTCAAACCTTTAAATGGGTGAGAT	473
EU084146- <i>C. aurata</i>	ATTAGAGGCATTGGGGTTCGAAACGGCCCTCAACCTATTCTCAAACCTTTAAATGGGTGAGAT	474
DQ524609- <i>A. lasiopygus</i>	ATTAGAGGCATTGGGGTTCGAAACGGCCCTCAACCTATTCTCAAACCTTTAAATGGGTGAGAT	474
DQ524588- <i>Anomala</i>	ATTAGAGGCATTGGGGTTCGAAACGGCCCTCAACCTATTCTCAAACCTTTAAATGGGTGAGAT	474
EU084216- <i>M. holosericea</i>	ATTAGAGGCATTGGGGTTCGAAACGGCCCTCAACCTATTCTCAAACCTTTAAATGGGTGAGAT	474
EU084221- <i>M. significans</i>	ATTAGAGGCATTGGGGTTCGAAACGGCCCTCAACCTATTCTCAAACCTTTAAATGGGTGAGAT	474
DQ524596- <i>H. seticollis</i>	ATTAGAGGCATTGGGGTTCGAAACGGCCCTCAACCTATTCTCAAACCTTTAAATGGGTGAGAT	474
EU084231- <i>M. melolontha</i>	ATTAGAGGCATTGGGGTTCGAAACGGCCCTCAACCTATTCTCAAACCTTTAAATGGGTGAGAT	474
DQ524590- <i>L. albistigma</i>	ATTAGAGGCATTGGGGTTCGAAACGGCCCTCAACCTATTCTCAAACCTTTAAATGGGTGAGAT	474
EU084209- <i>L. stradbokensis</i>	ATTAGAGGCATTGGGGTTCGAAACGGCCCTCAACCTATTCTCAAACCTTTAAATGGGTGAGAT	474
<i>M. cuprescens</i>	ATTAGAGGCATTGGGGTTCGAAACGGCCCTCAACCTATTCTCAAACCTTTAAATGGGTGAGAT	476
<i>M. indica</i>	ATTAGAGGCATTGGGGTTCGAAACGGCCCTCAACCTATTCTCAAACCTTTAAATGGGTGAGAT	476
* * * * *		
<i>M. furcicauda</i>	GACCTATTTTCCTCTTGG----ATGGGTGATCATCACAATAACCCCTCCCTTGTGGGCC	536
DQ524581- <i>C. acuta</i>	CTCCGGCTTGCTTGAACGTGTGAAGCCGCGAGATATCGGATCAGAGTGCCAAAGTGGGCCAC	533
EU084146- <i>C. aurata</i>	CTCCGGCTTGCTTGAACGTGTGAAGCCGCGAGATATCGGATCAGAGTGCCAAAGTGGGCCAC	534
DQ524609- <i>A. lasiopygus</i>	CTCCGGCTTGCTTGAACGTGTGAAGCCGCGAGATATCGGATCAGAGTGCCAAAGTGGGCCAC	534
DQ524588- <i>Anomala</i>	CTCCGGCTTGCTTGAACGTGTGAAGCCGCGAGATATCGGATCAGAGTGCCAAAGTGGGCCAC	534
EU084216- <i>M. holosericea</i>	CTCCGGCTTGCTTGAACGTGTGAAGCCGCGAGATATCGGATCAGAGTGCCAAAGTGGGCCAC	534
EU084221- <i>M. significans</i>	CTCCGGCTTGCTTGAACGTGTGAAGCCGCGAGATATCGGATCAGAGTGCCAAAGTGGGCCAC	534
DQ524596- <i>H. seticollis</i>	CTCCGGCTTGCTTGAACGTGTGAAGCCGCGAGATATCGGATCAGAGTGCCAAAGTGGGCCAC	534
EU084231- <i>M. melolontha</i>	CTCCGGCTTGCTTGAACGTGTGAAGCCGCGAGATATCGGATCAGAGTGCCAAAGTGGGCCAC	534
DQ524590- <i>L. albistigma</i>	CTCCGGCTTGCTTGAACGTGTGAAGCCGCGAGATATCGGATCAGAGTGCCAAAGTGGGCCAC	534
EU084209- <i>L. stradbokensis</i>	CTCCGGCTTGCTTGAACGTGTGAAGCCGCGAGATATCGGATCAGAGTGCCAAAGTGGGCCAC	534
<i>M. cuprescens</i>	CTCCGGCTTGCTTGAACGTGTGAAGCCGCGAGATATCGGATCAGAGTGCCAAAGTGGGCCAC	536
<i>M. indica</i>	CTCCGGCTTGCTTGAACGTGTGAAGCCGCGAGATATCGGATCAGAGTGCCAAAGTGGGCCAC	536
* * * * *		
<i>M. furcicauda</i>	TTTTGGTAAACAAAACCTGGCGCCGGGGTATGAACCAAACGTTGAATTAATCGTCCAAA	596
DQ524581- <i>C. acuta</i>	TTTTGGTAAAGCAGAACTGGCGCTGTGGGATGAACCAAACGCTGAGTTAAAGCGCCTAAAT	593
EU084146- <i>C. aurata</i>	TTTTGGTAAAGCAGAACTGGCGCTGTGGGATGAACCAAACGCTGAGTTAAAGCGCCTAAAT	594
DQ524609- <i>A. lasiopygus</i>	TTTTGGTAAAGCAGAACTGGCGCTGTGGGATGAACCAAACGCTGAGTTAAAGCGCCTAAAT	594
DQ524588- <i>Anomala</i>	TTTTGGTAAAGCAGAACTGGCGCTGTGGGATGAACCAAACGCTGAGTTAAAGCGCCTAAAT	594
EU084216- <i>M. holosericea</i>	TTTTGGTAAAGCAGAACTGGCGCTGTGGGATGAACCAAACGCTGAGTTAAAGCGCCTAAAT	594
EU084221- <i>M. significans</i>	TTTTGGTAAAGCAGAACTGGCGCTGTGGGATGAACCAAACGCTGAGTTAAAGCGCCTAAAT	594
DQ524596- <i>H. seticollis</i>	TTTTGGTAAAGCAGAACTGGCGCTGTGGGATGAACCAAACGCTGAGTTAAAGCGCCTAAAT	594
EU084231- <i>M. melolontha</i>	TTTTGGTAAAGCAGAACTGGCGCTGTGGGATGAACCAAACGCTGAGTTAAAGCGCCTAAAT	594
DQ524590- <i>L. albistigma</i>	TTTTGGTAAAGCAGAACTGGCGCTGTGGGATGAACCAAACGCTGAGTTAAAGCGCCTAAAT	594
EU084209- <i>L. stradbokensis</i>	TTTTGGTAAAGCAGAACTGGCGCTGTGGGATGAACCAAACGCTGAGTTAAAGCGCCTAAAT	594
<i>M. cuprescens</i>	TTTTGGTAAAGCAGAACTGGCGCTGTGGGATGAACCAAACGCTGAGTTAAAGCGCCTAAAT	596
<i>M. indica</i>	TTTTGGTAAAGCAGAACTGGCGCTGTGGGATGAACCAAACGCTGAGTTAAAGCGCCTAAAT	596
* * * * *		
<i>M. furcicauda</i>	TTACGCTAACCAACCC--ATTGAAAGTGGTTAATTCTCACTCAGCAGGACGTTGACCTG	654
DQ524581- <i>C. acuta</i>	CGACGCTTATGGGATACCATGAAAGGCGTTGGTAACTTAAGACAGCAGGACGGTGGCCAT	653
EU084146- <i>C. aurata</i>	CGACGCTTATGGGATACCATGAAAGGCGTTGGTAACTTAAGACAGCAGGACGGTGGCCAT	654
DQ524609- <i>A. lasiopygus</i>	CGACGCTTATGGGATACCATGAAAGGCGTTGGTAACTTAAGACAGCAGGACGGTGGCCAT	654
DQ524588- <i>Anomala</i>	CGACGCTTATGGGATACCATGAAAGGCGTTGGTAACTTAAGACAGCAGGACGGTGGCCAT	654
EU084216- <i>M. holosericea</i>	CGACGCTTATGGGATACCATGAAAGGCGTTGGTAACTTAAGACAGCAGGACGGTGGCCAT	654
EU084221- <i>M. significans</i>	CGACGCTTATGGGATACCATGAAAGGCGTTGGTAACTTAAGACAGCAGGACGGTGGCCAT	654
DQ524596- <i>H. seticollis</i>	CGACGCTTATGGGATACCATGAAAGGCGTTGGTAACTTAAGACAGCAGGACGGTGGCCAT	654
EU084231- <i>M. melolontha</i>	CGACGCTTATGGGATACCATGAAAGGCGTTGGTAACTTAAGACAGCAGGACGGTGGCCAT	654
DQ524590- <i>L. albistigma</i>	CGACGCTTATGGGATACCATGAAAGGCGTTGGTAACTTAAGACAGCAGGACGGTGGCCAT	654
EU084209- <i>L. stradbokensis</i>	CGACGCTTATGGGATACCATGAAAGGCGTTGGTAACTTAAGACAGCAGGACGGTGGCCAT	654
<i>M. cuprescens</i>	CGACGCTTATGGGATACCATGAAAGGCGTTGGTAACTTAAGACAGCAGGACGGTGGCCAT	656
<i>M. indica</i>	CGACGCTTATGGGATACCATGAAAGGCGTTGGTAACTTAAGACAGCAGGACGGTGGCCAT	656
* * * * *		
<i>M. furcicauda</i>	GGATGTTGAATCCCCCTACGAGTGGGGTAA	684
DQ524581- <i>C. acuta</i>	GGAAGTCGGAATCCGCTAAGGAGTGTGTAA	683
EU084146- <i>C. aurata</i>	GGAAGTCGGAATCCGCTAAGGAGTGTGTAA	684
DQ524609- <i>A. lasiopygus</i>	GGAAGTCGGAATCCGCTAAGGAGTGTGTAA	684
DQ524588- <i>Anomala</i>	GGAAGTCGGAATCCGCTAAGGAGTGTGTAA	684
EU084216- <i>M. holosericea</i>	GGAAGTCGGAATCCGCTAAGGAGTGTGTAA	684
EU084221- <i>M. significans</i>	GGAAGTCGGAATCCGCTAAGGAGTGTGTAA	684
DQ524596- <i>H. seticollis</i>	GGAAGTCGGAATCCGCTAAGGAGTGTGTAA	684
EU084231- <i>M. melolontha</i>	GGAAGTCGGAATCCGCTAAGGAGTGTGTAA	684
DQ524590- <i>L. albistigma</i>	GGAAGTCGGAATCCGCTAAGGAGTGTGTAA	684
EU084209- <i>L. stradbokensis</i>	GGAAGTCGGAATCCGCTAAGGAGTGTGTAA	684
<i>M. cuprescens</i>	GGAAGTCGGAATCCGCTAAGGAGTGTGTAA	686
<i>M. indica</i>	GGAAGTCGGAATCCGCTAAGGAGTGTGTAA	686
* * * * *		

4.6.8 Phylogenetic analysis

Cox1 gene

The *M. cuprescens* sequence showed 99.89 per cent similarity to *M. virescens* (= *P. fullo*), whereas *M. furcicauda* sequence showed 89.47 per cent similarity to *M. indica* (Fig. 4.59). This is the first report of *Cox1* sequences from *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*). The sequences of gene from these four species were also compared with closely related species *Melolontha* i.e. *M. hippocastani* (HM120757) and *M. hippocastani* (KX087316) and two species of *Polyphylla* i.e. *Polyphylla gracilicornis* (MW143080) and *Polyphylla laticollis* (KF544959). The *Melolontha* species and *Polyphylla* species used in the present study did not show high similarity to *M. hippocastani* (HM120757), *M. hippocastani* (KX087316), *P. gracilicornis* (MW143080) and *P. laticollis* (KF544959) whose sequences were retrieved from the database. The *Cox1* sequences of *M. furcicauda* showed 86.02 and 86.51 per cent similarity to *M. hippocastani* (HM120757) and *M. hippocastani* (KX087316), and 82.34 and 81.64 per cent similarity to *P. gracilicornis* (MW143080) and *P. laticollis* (KF544959), respectively. The *M. indica* had 84.15 and 84.69 per cent similarity to *M. hippocastani* (HM120757) and *M. hippocastani* (KX087316), whereas it showed 82.84 and 83.33 per cent similarity to *P. gracilicornis* (MW143080) and *P. laticollis* (KF544959), respectively. The *M. cuprescens* showed 81.47 per cent similarity to *M. hippocastani* (HM120757), 81.69 per cent to *M. hippocastani* (KX087316), 80.32 per cent to *P. gracilicornis* (MW143080) and 82.49 per cent to *P. laticollis* (KF544959). *P. gracilicornis* (MW143080) and *P. laticollis* (KF544959) were distantly related to our species in the present study. The *M. hippocastani* strains were from France, *P. gracilicornis* from China and *P. laticollis* from Korea. The beetles of *Melolontha* and *Polyphylla* used in the present study were from north western Himalayan state of Himachal Pradesh, India, and represented geographically distinct regions. In confirmation to identification based on morphology, the *Melolontha* species were close to each other with the exception of *P. fullo*, which showed high similarity to *M. cuprescens*. The study further suggested that *Cox1* sequences could be used as barcode for identification purposes in insects. The species identification of *M. melolontha* and *M. hippocastani* was earlier confirmed by sequencing *Cox1* gene with the help of MelCOIF (5'-AAGGAAACATTTGGCACTTT-3') and MelCOIR (5'-GGGAAATTAGTGATCCGATAGA-3') primers (Tereba and Niemczyk 2018). The sequencing of *Cox1* region of *M. melolontha*, *M. hippocastani* and *Melolontha pectoralis*

also showed that *M. melolontha* and *M. pectoralis* were in close proximity to each other (Giannoulis et al. 2011). Similarly, *Leucopholis lepidophora* have shown 88 per cent identity to *L. lepidophora* (KX351390), and *Leucopholis burmeisteri* sequences showed 100 per cent identity to *L. burmeisteri* (JN126323) based on NCBI-BLASTn searches (Swamy et al. 2019). Kalpana (2015) reported 99 per cent identity between *cox1* sequences of *Holotrichia longipennis* and *Holotrichia consanguinea*, whereas 80 per cent identity between *Holotrichia sikkimensis* and *cox1* sequences of *Jekelius brullei*, 99 per cent identity between *H. consanguinea* and *Holotrichia serrata* and 98 per cent identity between *H. serrata* and *Holotrichia nagpurensis*.

The nucleotide frequencies in amplified *Cox1* sequences of three *Melolontha* species and one *Polyphylla* species were Adenine 34.4 per cent (A), Thymine 30.6 per cent (T), Cytosine 14.4 per cent (C), and Guanine 20.6 per cent (G). From these results it is inferred that across all sequences there existed an excess of Adenine and Thymine over Cytosine and Guanine. The transition/transversion rate ratios are $k1 = 3.152$ (purines) and $k2 = 2.043$ (pyrimidines). The overall transition/transversion bias is $R = 1.264$. The excess of Adenine and thymine have also been reported for *Cox1* sequences of *L. lepidophora*, *Leucopholis coneophora* and *L. burmeisteri* by Swamy et al. (2019). They reported nucleotide frequencies for Adenine, Thymine, Cytosine and Guanine as 29.96 per cent, 32.92 per cent, 28.87 per cent and 16.25 per cent for *Cox1* gene. The present results are in conformity with those of Swamy et al. (2019).

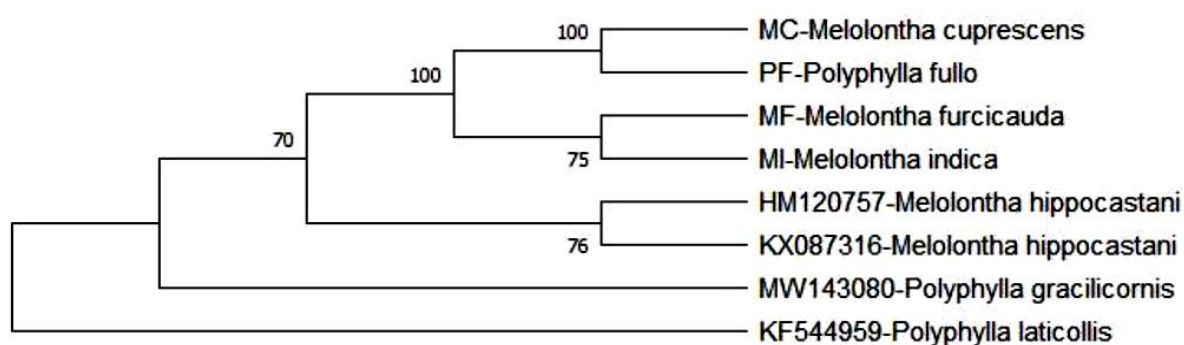


Fig. 4.59 Phylogenetic relationships between five *Melolontha* and three *Polyphylla* species based on *Cox1* gene sequences. The tree was constructed using maximum likelihood method. The Boot Strap values are given on the branches

28s rRNA gene

Based on the 28s rRNA gene sequence, *M. indica* showed maximum identity of 99.42 per cent to *M. melolontha* (EU084231) as compared to *M. cuprescens* and *M. furcicauda* which were distantly related (Fig. 4.60). The *M. cuprescens* and *M. furcicauda*

showed 98.88 and 89.02 per cent similarity to *M. melolontha*, respectively. *M. indica* is closer *L. albistigma* (DQ524590) and *L. stradbokensis* (EU084209) as compared to *M. cuprescens* and *M. furcicauda* suggesting limited utility of 28s *rRNA* gene in barcoding and identification of whitegrubs. According to Kalpana (2015), the 28S *rRNA* sequences of *H. longipennis* and *H. sikkimensis* have 98 per cent and 99 per cent identity to *Hoplochelus piliger*, whereas the sequences of *H. consanguinea* and *H. serrata* were 99 per cent identical to 28S *rRNA* from *Haplidia transversa*.

The nucleotide frequencies for 28s *rRNA* gene sequences were Adenine 25.4 per cent (A), Thymine 21.4 per cent (T), Cytosine 23.4 per cent (C), and Guanine 29.8 per cent (G). A cursory look on the data clearly reflected an excess of Guanine and Adenine over Thymine and Cytosine across all sequences. The transition/transversion rate ratios were $k1 = 1.086$ (purines) and $k2 = 16.956$ (pyrimidines). The overall transition/transversion bias was $R = 3.786$.

The result obtained in the present study implies limited utility of 28s *rRNA* gene sequences in taxonomical identification of white grubs especially *Melolontha* and *Polyphylla* species. It is pertinent to mention that the genera and species included for phylogenetic analysis were from India, Germany and Great Britain.

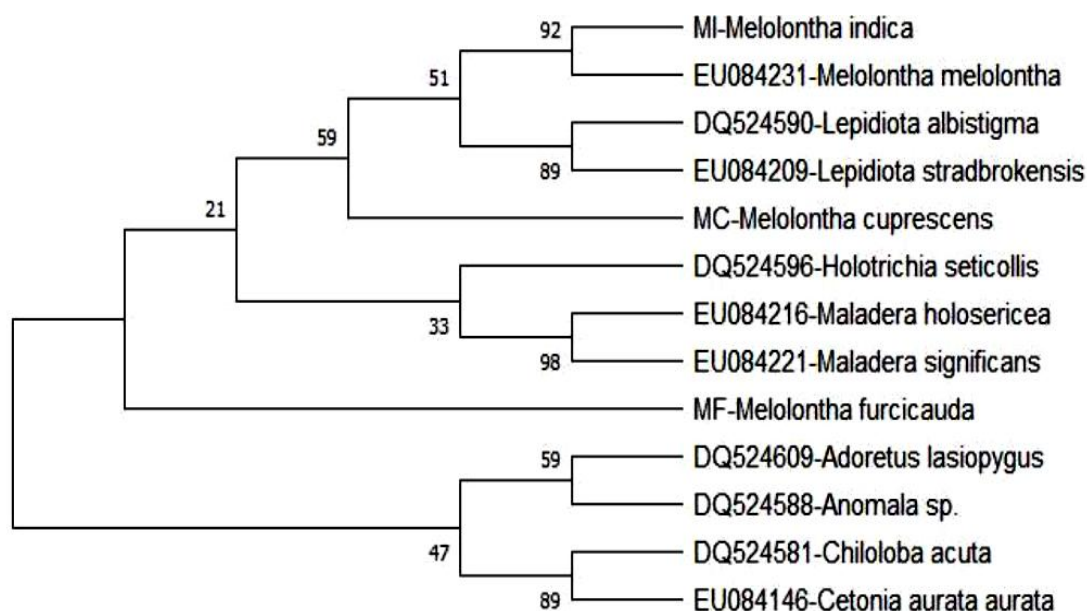


Fig. 4.60 Phylogenetic relationships between four *Melolontha* and nine different white grub species based on 28s *rRNA* gene sequences. The tree was constructed using maximum likelihood method. The Boot Strap values are given on the branches

4.7 Evaluation of Chemicals against *Melolontha* species

The chemicals were tested against first, second and third instar grubs of *Melolontha* species under laboratory conditions at Palampur. The first instar grubs were very delicate and when first instar grubs were released into the soil, there was complete mortality even in control. Hence, it was not possible to record data on the efficacy of chemicals against first instar grubs. When the adult beetles of *M. indica* and *M. virescens* (= *P. fullo*) were released into the insecticide treated soil, there was neither beetle mortality nor egg laying both in treated and untreated soil. In the present study, six chemicals were tested against second and third instar grubs and the data on their mortality are presented hereunder.

4.7.1 Chlorantraniliprole

Second instar: When second instar grubs of *P. fullo* were treated with chlorantraniliprole @ 0.02-0.32 g a.i./kg soil, the mortality varied from 23.33-86.67 per cent after 48 hours of treatment (Table 4.30). In control, mortality was not recorded. The LC_{50} and LC_{90} values were calculated to be 0.057 and 0.411 g a.i./kg soil, with 95 per cent fiducial limits of LC_{50} ranging from 0.033 to 0.078 g a.i./kg soil. The 95 per cent fiducial limits of LC_{90} values were recorded in the range of 0.264 to 0.558 g a.i./kg soil. The slope (b) of regression line was determined to be 1.49 and it demonstrated a best fit (Fig 4.61). The χ^2 value was calculated to 0.18 showing that the data were homogeneous ($p = 0.05$; 3 df).

Table 4.30 Concentration-mortality response of chlorantraniliprole against second instar grubs of *M. virescens* (= *P. fullo*)

Concentration (g a.i./kg soil)	Log conc. (x 1000)	No. of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical probit	Expected probit
0.02	1.30	30	23.33	23.33	4.271	4.30
0.04	1.60	30	43.33	43.33	4.831	4.80
0.08	1.90	30	60.00	60.00	5.253	5.30
0.16	2.20	30	73.33	73.33	5.622	5.60
0.32	2.51	30	86.67	86.67	6.112	6.10
0	-	30	0.00	0.00	0.000	-

$LC_{50} = 0.057$ g a.i./kg soil

$LC_{90} = 0.411$ g a.i./kg soil

$Y = 2.373 + 1.49 x$

$\chi^2_{cal} = 0.18$

95% Fiducial limits = 0.033– 0.078 a.i./kg soil

95% Fiducial limits = 0.264 – 0.558 g a.i./kg soil

Slope = 1.49

4.7.2 Bifenthrin

Second instar: Data contained in table 4.31 showed that mortality of second instar grubs of *P. fullo* to bifenthrin varied from 16.67-90.0 per cent at concentrations ranging from 0.02-0.32 g a.i./kg soil. There was gradual increase in mortality with increase in

concentration, and @ 0.04, 0.08 and 0.16 g a.i./kg soil, 33.33, 56.67 and 76.67 per cent mortality was recorded, after 48 hours of treatment. In control, no mortality was recorded. The regression line depicting best fit ($b = 1.88$) is given in fig 4.61. The LC_{50} and LC_{90} values were calculated to be 0.066 g a.i./kg soil and 0.315 g a.i./kg soil, respectively. The 95 per cent fiducial limits for LC_{50} and LC_{90} values were found to range between 0.044-0.086 g a.i./kg soil and 0.230 – 0.399 g a.i./kg soil, respectively. The data were homogenous as calculated value of χ^2 (0.03) was less than the tabulated value.

Table 4.31 Concentration - mortality response of bifenthrin against second instar grubs of *M. virescens* (= *P. fullo*)

Concentration (g a.i./kg soil)	Log conc. (x 1000)	No. of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical probit	Expected probit
0.02	1.30	30	16.67	16.67	4.03	4.00
0.04	1.60	30	33.33	33.33	4.57	4.60
0.08	1.90	30	56.67	56.67	5.17	5.20
0.16	2.20	30	76.67	76.67	5.73	5.70
0.32	2.51	30	90.00	90.00	6.28	6.30
0	-	30	0.00	0.00	0.00	-

$LC_{50} = 0.066$ g a.i./kg soil

$LC_{90} = 0.315$ g a.i./kg soil

$Y = 1.578 + 1.88 x$

$\chi^2_{cal} = 0.03$

95% Fiducial limits = 0.044 - 0.086 g a.i./kg soil

95% Fiducial limits = 0.230 – 0.399 g a.i./kg soil

Slope = 1.88

4.7.3 Clothianidin

Second instar: Clothianidin was applied at concentrations ranging from 0.02-0.32 g a.i./kg soil against second instar grubs of *P. fullo*. At tested doses, the mortality ranged from 16.67 – 80.0 per cent, after 48 hours of treatment (Table 4.32). In control, the grub mortality was recorded up to 3.33 per cent after 48 hours of treatment. The LC_{50} and LC_{90} values were computed to be 0.098 g a.i./kg soil and 0.661 g a.i./kg soil, respectively, with 95 per cent fiducial limits ranging from 0.060-0.139 g a.i./kg soil and 0.394-0.928 g a.i./kg

Table 4.32 Concentration - mortality response of clothianidin against second instar grubs of *M. virescens* (= *P. fullo*)

Concentration (g a.i./kg soil)	Log conc. (x 1000)	No. of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical probit	Expected probit
0.02	1.30	30	16.67	13.79	3.91	3.90
0.04	1.60	30	30.00	27.59	4.34	4.30
0.08	1.90	30	46.67	44.83	4.87	4.90
0.16	2.20	30	63.33	62.07	5.31	5.30
0.32	2.51	30	80.00	79.31	5.82	5.80
0	-	30	3.33	0.00	0.00	-

$LC_{50} = 0.098$ g a.i./kg soil

$LC_{90} = 0.661$ g a.i./kg soil

$Y = 1.931 + 1.54 x$

$\chi^2_{cal} = 0.02$

95% Fiducial limits = 0.060– 0.139 g a.i./kg soil

95% Fiducial limits = 0.394 – 0.928 g a.i./kg soil

Slope = 1.54

soil, respectively. The regression line gave best fit and the value of slope was determined to be 1.54 (Fig. 4.61). The calculated value of χ^2 (0.02) clearly indicates homogenous nature of data.

4.7.4. Thiamethoxam

Second instar: Second instar grubs of *P. fullo* when exposed to different concentrations of thiamethoxam changing from 0.02-0.32 g a.i./kg soil, the mortality occurred in the range of 20.0-80.0 per cent after 48 hours of treatment (Table 4.33). In control, 3.33 per cent mortality was observed. After correcting the mortality data using Abbott's correction, and further probit analysis of the corrected mortality data revealed LC₅₀ of 0.095 g a.i./kg soil and LC₉₀ of 0.632 g a.i./kg soil, after 48 hours of treatment. The 95 per cent fiducial limits of LC₅₀ and LC₉₀ values ranged between 0.059-0.134 g a.i./kg soil, and 0.385-0.879 g a.i./kg soil, respectively. The value of slope was computed to be 1.56, and regression line depicting best fit is given in fig. 4.61. The data were homogenous at $p = 0.05$ at 3 degree of freedom.

Table 4.33 Concentration - mortality response of thiamethoxam against second instar grubs of *M. virescens* (= *P. fullo*)

Concentration (g a.i./kg soil)	Log conc. (x 1000)	No. of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical probit	Expected probit
0.02	1.30	30	20.00	17.24	4.1	4.00
0.04	1.60	30	26.67	24.14	4.3	4.40
0.08	1.90	30	46.67	44.83	4.9	4.90
0.16	2.20	30	66.67	65.52	5.4	5.40
0.32	2.51	30	80.00	79.31	5.8	5.80
0	-	30	3.33	0.00	0.00	-

LC₅₀ = 0.095 g a.i./kg soil

LC₉₀ = 0.632 g a.i./kg soil

Y = 1.923 + 1.56 X

$\chi^2_{cal} = 0.44$

95% Fiducial limits = 0.059– 0.134 g a.i./kg soil

95% Fiducial limits = 0.385 – 0.879 g a.i./kg soil

Slope = 1.56

4.7.5. Chlorpyrifos

Second instar: The mortality of second instar grubs of *P. fullo* was found to range between 13.33-73.33 per cent after 48 hours of treatment when the grubs were exposed to different doses of chlorpyrifos ranging from 0.04-0.64 g a.i./kg soil. In control, 6.67 per cent of grub mortality was recorded after 48 hours of treatment. The 48 hour mortality data were corrected by using Abbott's formula and subjected to probit analysis. The data in table 4.34 revealed that LC₅₀ and LC₉₀ values were estimated to be 0.256 g a.i./kg soil and 1.674 g a.i./kg soil, respectively. The range of 95 per cent fiducial limits of LC₅₀ and LC₉₀ values were 0.162 – 0.374 g a.i./kg soil, and 0.956 – 2.392 g a.i./kg soil, respectively.

The mortality data were homogenous ($p = 0.05$; 3 degree of freedom), and probit kill (Y) followed straight line related with log concentration (x) as $Y = 1.219 + 1.57 x$ (Fig. 4.61).

Table 4.34 Concentration - mortality response of chlorpyrifos against second instar grubs of *M. virescens* (= *P. fullo*)

Concentration (g a.i./kg soil)	Log conc. (x 1000)	No. of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical probit	Expected probit
0.04	1.60	30	13.33	7.14	3.53	3.70
0.08	1.90	30	30.00	25.00	4.33	4.20
0.16	2.20	30	43.33	39.29	4.73	4.70
0.32	2.51	30	60.00	57.14	5.18	5.20
0.64	2.81	30	73.33	71.43	5.57	5.60
0	-	30	6.67	0.00	0.00	-

$LC_{50} = 0.256$ g a.i./kg soil

$LC_{90} = 1.674$ g a.i./kg soil

$Y = 1.219 + 1.57 x$

$\chi^2_{cal} = 0.73$

95% Fiducial limits = 0.162 – 0.374 g a.i./kg soil

95% Fiducial limits = 0.956 – 2.392 g a.i./kg soil

Slope = 1.57

4.7.6. Imidacloprid

Second instar: When second instar grubs of *P. fullo* were treated with imidacloprid at concentrations ranging from 0.04-0.64 g a.i./kg soil, the mortality to the tune of 13.33-73.33 per cent was noticed after 48 hours of treatment as shown in table 4.35. In control, there was no mortality up to 48 hours of treatment. The LC_{50} and LC_{90} values were calculated as 0.239 g a.i./kg soil (95 % fiducial limits: 0.140-0.362 g a.i./kg soil) and 1.918 g a.i./kg soil (95 % fiducial limits: 0.974-2.863 g a.i./kg soil), respectively. The probit kill (Y) was linearly related to log concentration (x) as $Y = 1.636 + 1.41 x$ (Fig. 4.61). The data were homogenous at $p = 0.05$ as calculated value of χ^2 (0.87) was less than the tabulated value.

Table 4.35 Concentration - mortality response of imidacloprid against second instar grubs of *M. virescens* (= *P. fullo*)

Concentration (g a.i./kg soil)	Log conc. (x 1000)	No. of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical probit	Expected probit
0.04	1.60	30	13.33	13.33	3.89	3.90
0.08	1.90	30	23.33	23.33	4.27	4.30
0.16	2.20	30	46.67	46.67	4.92	4.70
0.32	2.51	30	53.33	53.33	5.08	5.20
0.64	2.81	30	73.33	73.33	5.62	5.60
0	-	30	0.00	0.00	0.00	-

$LC_{50} = 0.239$ g a.i./kg soil

$LC_{90} = 1.918$ g a.i./kg soil

$Y = 1.636 + 1.41 x$

$\chi^2_{cal} = 0.87$

95% Fiducial limits = 0.140– 0.362 g a.i./kg soil

95% Fiducial limits = 0.974 – 2.863 g a.i./kg soil

Slope = 1.41

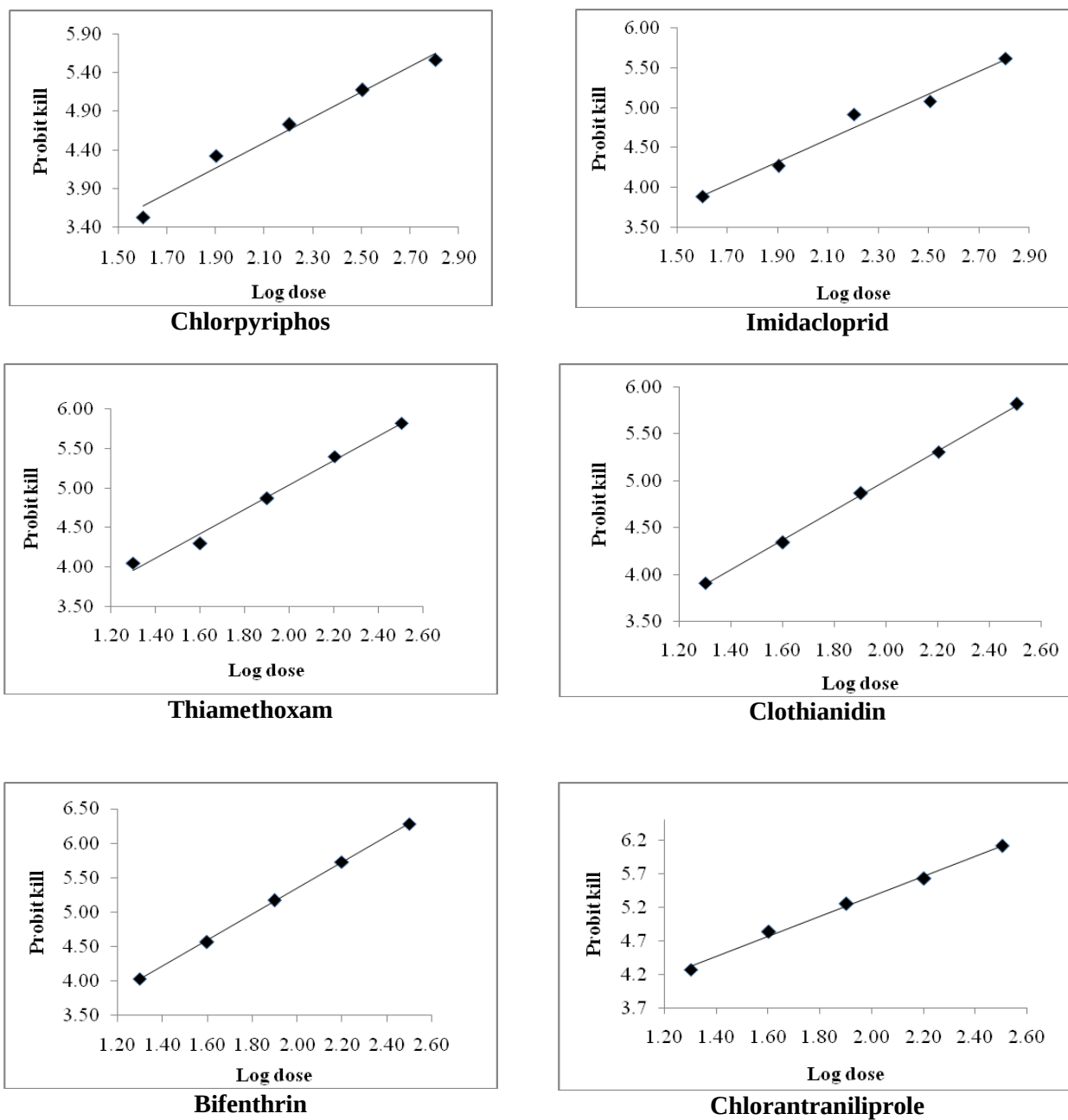


Fig. 4.61 Dose - mortality response of different insecticides tested in soil against second instar grubs of *M. virescens* (= *P. fullo*)

4.7.7 Chlorantraniliprole

Third instar: Consequent upon treatment of third instar grubs of *M. virescens* (= *P. fullo*) with chlorantraniliprole @ 0.02-0.32 g a.i./kg soil, 20.0-90.0 per cent mortality was recorded after 48 hours of treatment as shown in table 4.36. In control, the corresponding grub mortality averaged 3.33 per cent. The LC₅₀ and LC₉₀ values were worked out to be 0.071 g a.i./kg soil (95% Fiducial limits = 0.045-0.096 g a.i./kg soil) and 0.419 g a.i./kg soil (95% Fiducial limits = 0.285-0.553 g a.i./kg soil), respectively. The regression line is depicted in fig 4.62. The value of slope was calculated to be 1.66. The data were homogenous at $p = 0.05$ as $\chi^2_{(cal)} = 1.14$ is less than the tabulated value.

Table 4.36 Concentration - mortality response of chlorantraniliprole against third instar grubs of *M. virescens* (= *P. fullo*)

Concentration (g a.i./kg soil)	Log conc. (x 1000)	No. of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical probit	Expected probit
0.02	1.30	30	20.00	17.24	4.05	4.10
0.04	1.60	30	40.00	37.93	4.69	4.60
0.08	1.90	30	53.33	51.72	5.04	5.10
0.16	2.20	30	66.67	65.52	5.40	5.60
0.32	2.51	30	90.00	89.66	6.26	6.00
0	-	30	3.33	0.00	0.00	-

LC₅₀ = 0.071 g a.i./kg soil

LC₉₀ = 0.419 g a.i./kg soil

Y = 1.915 + 1.66 x

$\chi^2_{cal} = 1.14$

95% Fiducial limits = 0.045 – 0.096 g a.i./kg soil

95% Fiducial limits = 0.285 – 0.553 g a.i./kg soil

Slope = 1.66

4.7.8 Bifenthrin

Third instar: Bifenthrin resulted in 13.33 – 86.67 per cent mortality of third instar grubs of *P. fullo* after 48 hours of treatment at concentrations ranging from 0.02-0.32 g a.i./kg soil in a concentration - dependent manner (Table 4.37). There was, no mortality was recorded during this period. The doses of 0.076 g a.i./kg soil and 0.350 g a.i./kg soil were required to kill 50 and 90 per cent of treated population, respectively. The 95 per cent fiducial limits of LC₅₀ and LC₉₀ values were calculated to be 0.052-0.098 g a.i./kg soil and 0.259-0.441 g a.i./kg soil, respectively. Probit kill (Y) followed a linear relationship with log concentration (x), and regression line (Fig 4.62.) had a slope value of $b = 1.93$. The $\chi^2_{(cal)} = 0.31$ represented homogeneity of data.

Table 4.37 Concentration - mortality response of bifenthrin against third instar grubs of *M. virescens* (= *P. fullo*)

Concentration (g a.i./kg soil)	Log conc. (x 1000)	No. of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical probit	Expected probit
0.02	1.30	30	13.33	13.33	3.89	3.90
0.04	1.60	30	30.00	30.00	4.48	4.50
0.08	1.90	30	50.00	50.00	5.00	5.00
0.16	2.20	30	76.67	76.67	5.73	5.60
0.32	2.51	30	86.67	86.67	6.11	6.10
0	-	30	0.00	0.00	0.00	-

LC₅₀ = 0.076 g a.i./kg soil

LC₉₀ = 0.350 g a.i./kg soil

Y = 1.381 + 1.93 x

$\chi^2_{cal} = 0.31$

95% Fiducial limits = 0.052 – 0.098 g a.i./kg soil

95% Fiducial limits = 0.259 – 0.441 g a.i./kg soil

Slope = 1.93

4.7.9 Clothianidin

Third instar: Against third instar grubs of *P. fullo*, clothianidin was applied at concentrations ranging from 0.04-0.64 g a.i./kg soil, and after 48 hours of treatment, the mortality ranged from 16.67-76.67 per cent at tested concentrations (Table 4.38). There was no mortality of grubs in control upto 48 hours of treatment. The LC₅₀ value was calculated to be 0.205 g a.i./kg soil with 95 per cent fiducial limits varying from 0.119-0.305 g a.i./kg soil, and the LC₉₀ value was computed to be 1.645 g a.i./kg soil with fiducial limits ranging from 0.876-2.414 g a.i./kg soil. The value of slope was 1.42 as shown in fig. 4.62. The value of χ^2 was calculated to be 0.10, which was less than the tabulated value, thereby suggesting homogeneity of data.

Table 4.38 Concentration - mortality response of clothianidin against third instar grubs of *M. virescens* (= *P. fullo*)

Concentration (g a.i./kg soil)	Log conc. (x 1000)	No. of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical probit	Expected probit
0.04	1.60	30	16.67	16.67	4.03	4.00
0.08	1.90	30	26.67	26.67	4.38	4.40
0.16	2.20	30	43.33	43.33	4.83	4.80
0.32	2.51	30	60.00	60.00	5.25	5.30
0.64	2.81	30	76.67	76.67	5.73	5.70
0	-	30	0.00	0.00	0.00	-

LC₅₀ = 0.205 g a.i./kg soil

LC₉₀ = 1.645 g a.i./kg soil

Y = 1.723 + 1.42 x

$\chi^2_{cal} = 0.10$

95% Fiducial limits = 0.119 – 0.305 g a.i./kg soil

95% Fiducial limits = 0.876 – 2.414 g a.i./kg soil

Slope = 1.42

4.7.10 Thiamethoxam

Third instar: Third instar grubs of *P. fullo* exhibited mortality ranging from 16.67-73.33 per cent after 48 hours of treatment, when they were exposed to different doses of thiamethoxam ranging from 0.04-0.64 g a.i./kg soil is given in table 4.39. There was no mortality in control. Probit analysis revealed that 0.223 g a.i./kg soil and 1.984 g a.i./kg soil of thiamethoxam is required to kill 50 and 90 per cent of the treated population. The regression line showed the best fit and log concentration (x) was linearly related to probit kill (Y) as $Y = 1.833 + 1.35 x$. The value of χ^2 was calculated to be 0.63 (Fig. 4.62) indicating the homogeneity of data.

Table 4.39 Concentration - mortality response of thiamethoxam against third instar grubs of *M. virescens* (= *P. fullo*)

Concentration (g a.i./kg soil)	Log conc. (x 1000)	No. of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical probit	Expected probit
0.04	1.60	30	16.67	16.67	4.03	4.00
0.08	1.90	30	23.33	23.33	4.27	4.40
0.16	2.20	30	46.67	46.67	4.92	4.80
0.32	2.51	30	56.67	56.67	5.17	5.20
0.64	2.81	30	73.33	73.33	5.62	5.60
0	-	30	0.00	0.00	0.00	-

LC₅₀ = 0.223 g a.i./kg soil

LC₉₀ = 1.984 g a.i./kg soil

Y = 1.833 + 1.35 x

$\chi^2_{cal} = 0.63$

95% Fiducial limits = 0.125 – 0.342 g a.i./kg soil

95% Fiducial limits = 0.955 – 3.012 g a.i./kg soil

Slope = 1.35

4.7.11 Chlorpyrifos

Third instar: Chlorpyrifos at concentrations of 0.04, 0.08, 0.16, 0.32 and 0.64 g a.i./kg soil resulted in 10.0, 26.67, 46.67, 56.67 and 70.0 per cent mortality of third instar grubs of *P. fullo* after 48 hours of treatment. Probit analysis of data after Abbott's correction revealed that the concentrations of 0.235 g a.i./kg soil and 1.886 g a.i./kg soil were required to kill 50 (LC₅₀) and 90 per cent (LC₉₀) of the treated population of third instar grubs of *P. fullo*. The 95 per cent fiducial limits of LC₅₀ and LC₉₀ were in the range of 0.136-0.356 g a.i./kg soil and 0.956-2.815 g a.i./kg soil, respectively (Table 4.40). The probit kill (Y) followed a straight line equation with log concentration (x) of $Y = 1.643 + 1.42 x$ (Fig. 4.62). The data were homogenous as the value of χ^2 was calculated to be 1.09 which was less than the tabulated value.

Table 4.40 Concentration - mortality response of chlorpyrifos against third instar grubs of *M. virescens* (= *P. fullo*)

Concentration (g a.i./kg soil)	Log conc. (x 1000)	No. of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical probit	Expected probit
0.04	1.60	30	10.00	10.00	3.72	3.80
0.08	1.90	30	26.67	26.67	4.38	4.30
0.16	2.20	30	46.67	46.67	4.92	4.80
0.32	2.51	30	56.67	56.67	5.17	5.20
0.64	2.81	30	70.00	70.00	5.52	5.60
0	-	30	0.00	0.00	0.00	-

LC₅₀ = 0.235 g a.i./kg soil

LC₉₀ = 1.886 g a.i./kg soil

Y = 1.643 + 1.42 x

$\chi^2_{cal} = 1.09$

95% Fiducial limits = 0.136 – 0.356 g a.i./kg soil

95% Fiducial limits = 0.956 – 2.815 g a.i./kg soil

Slope = 1.42

4.7.12 Imidacloprid

Third instar: Imidacloprid at 0.04, 0.08, 0.16, 0.32 and 0.64 g a.i./kg soil resulted in 13.33, 26.67, 43.33, 53.33 and 70.0 per cent mortality of third instar grubs of *P. fullo*, 48 hours after the treatment. In control, 3.33 per cent mortality occurred within 48 hours (Table 4.41). Probit analysis of the data revealed that the concentrations of imidacloprid required to kill 50 and 90 per cent population of third instar grubs of *P. fullo* were 0.273 g a.i./kg soil (95 % fiducial limits: 0.160-0.425 g a.i./kg soil) and 2.229 g a.i./kg soil (95 % fiducial limits: 1.065-3.393 g a.i./kg soil), respectively. The χ^2 test showed that data were homogenous at $p = 0.05$; $\chi^2_{(cal)} = 0.39$. Probit kill (Y) followed a straight line equation with log concentration (x) of $Y = 1.581 + 1.40 x$ as shown in fig. 4.62.

Table 4.41 Concentration - mortality response of imidacloprid against third instar grubs of *M. virescens* (= *P. fullo*)

Concentration (g a.i./kg soil)	Log conc. (x 1000)	No. of insects treated	Observed mortality (%)	Corrected mortality (%)	Empirical probit	Expected probit
0.04	1.60	30	13.33	10.34	3.74	3.80
0.08	1.90	30	26.67	24.14	4.30	4.20
0.16	2.20	30	43.33	41.38	4.78	4.70
0.32	2.51	30	53.33	51.72	5.04	5.10
0.64	2.81	30	70.00	68.97	5.49	5.50
0	-	30	3.33	0.00	0.00	-

LC₅₀ = 0.273 g a.i./kg soil

LC₉₀ = 2.229 g a.i./kg soil

Y = 1.581 + 1.40 x

$\chi^2_{cal} = 0.39$

95% Fiducial limits = 0.160 – 0.425 g a.i./kg soil

95% Fiducial limits = 1.065 – 3.393 g a.i./kg soil

Slope = 1.40

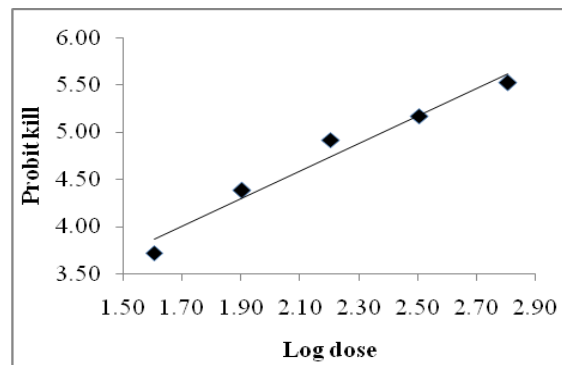
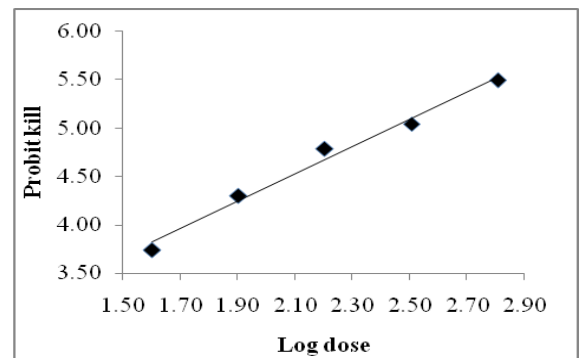
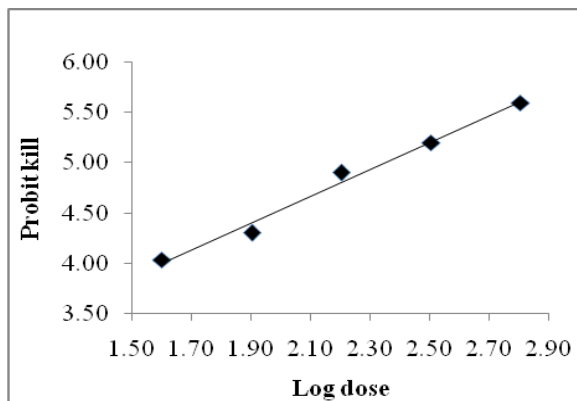
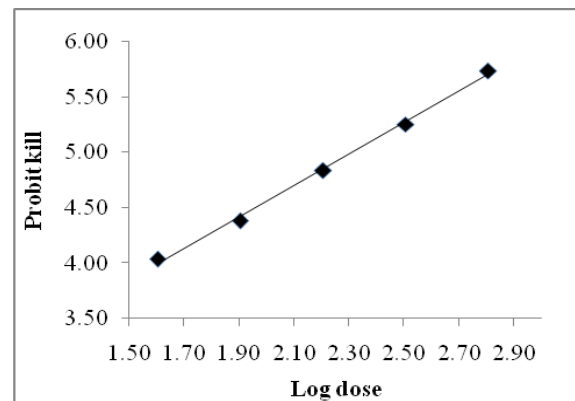
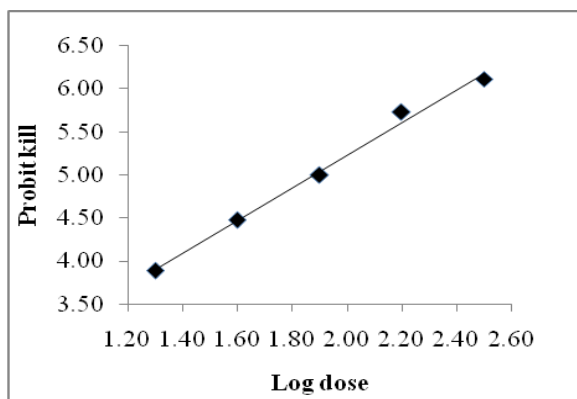
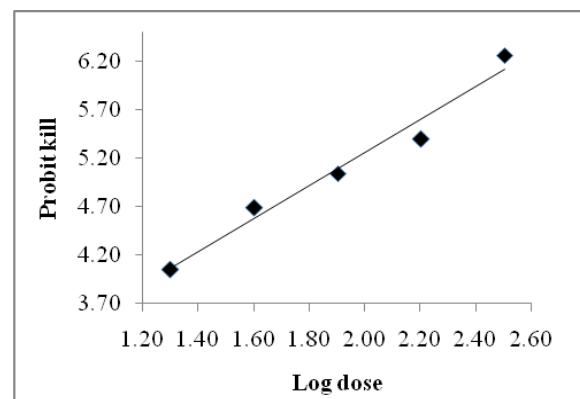
**Chlorpyrifos****Imidacloprid****Thiamethoxam****Clothianidin****Bifenthrin****Chlorantraniliprole**

Fig. 4.62 Dose - mortality response of different insecticides tested in soil against third instar grubs of *M. virescens* (= *P. fullo*)

The response of *M. virescens* (= *P. fullo*) grubs varied considerably to different tested insecticides. Irrespective of chemical, there was increase in mortality with increase in concentration. This increase in LC₅₀ value with increase in instar was due to increase in size of grubs with advancement of age. The second instar grubs of *M. virescens* (= *P. fullo*) were 23.65-27.70 mm in length. In third instar, the length was almost double as the fully fed grubs were reported to measure 48.33-54.17 mm. Therefore, it seems possible that there is proportional increase in LC₅₀ values in relation to age and size of grubs.

Chlorpyrifos is widely used to control different soil insects. In India, chlorpyrifos has been recommended to control white grubs in potato, groundnut and sugarcane (Chandel et al. 1993; Manjula and Sulochanamma 2001; Anitha et al. 2005). In Polish forestry, Dursban 480 EC @ 2.5-3.0 litres in 450-600 litres of water was mainly used in surface treatments before planting to control *Melolontha* grubs (Malinowski et al. 1998). According to Pozenel et al. (2015), the efficacy of chlorpyrifos against *M. melolontha* grubs at a dose rate of 15 kg/ha was 68.0 per cent.

Comparison of LC₅₀ values revealed differential susceptibility of second instar grubs of *M. virescens* (= *P. fullo*) to tested insecticides. Chlorantraniliprole was found highly effective, and was 1.16, 1.67, 1.72, 4.20 and 4.49 times more toxic than bifenthrin, thiamethoxam, clothianidin, imidacloprid, and chlorpyrifos. Bifenthrin showed increase in LC₅₀ by 1.44, 1.49, 3.63 and 3.88 times respectively to thiamethoxam, clothianidin, imidacloprid, and chlorpyrifos. The increase in LC₅₀ value of thiamethoxam in comparison to clothianidin, imidacloprid, and chlorpyrifos was calculated to be 1.03, 2.51 and 2.69 times. There was 2.44 and 2.61 times increase in LC₅₀ value of clothianidin as compared to imidacloprid and chlorpyrifos, and 1.07 times for imidacloprid with respect to chlorpyrifos.

The relative toxicity was determined by dividing the LC₅₀ value of chlorpyrifos (0.256 a.i. g/kg soil) which was least toxic in comparison to other tested insecticides. The order of toxicity (Table 4.42) on the basis of LC₅₀ value in relation to chlorpyrifos was chlorantraniliprole (4.49 times toxic) > bifenthrin (3.88 times toxic) > thiamethoxam (2.69 times toxic) > clothianidin (2.61 times toxic) > imidacloprid (1.07 times toxic). The 95 % fiducial limits of LC₅₀ revealed statistically non-significant differences in toxicity among chlorpyrifos and imidacloprid, and clothianidin and thiamethoxam as their 95 % fiducial limits showed distinct overlap among them. Imidacloprid and thiamethoxam indicated significant differences between them on the basis of 95 % fiducial limits of LC₅₀ values. Chlorantraniliprole and bifenthrin showed their superiority on the basis of their LC₅₀

values, and toxicity of both the insecticides showed non significant difference from each other on the basis of their LC₅₀ values.

Table 4.42 Relative toxicity of insecticides against second instar grubs of *M. virescens* (= *P. fullo*)

S. No.	Insecticides	LC ₅₀ (g a.i./kg soil)	Relative toxicity
1.	Chlorantraniliprole	0.057	4.49
2.	Bifenthrin	0.066	3.88
3.	Thiamethoxam	0.095	2.69
4.	Clothianidin	0.098	2.61
5.	Imidacloprid	0.239	1.07
6.	Chlorpyrifos	0.256	1

As compared to second instar grubs, third instar grubs were 1.02-2.35 times less susceptible to different tested insecticides. This decrease in susceptibility was attributed to increase in size of third instar grubs. Comparison of susceptibility of third instar grubs to the tested insecticides revealed that third instar grubs were highly susceptible to chlorantraniliprole as indicated by the LC₅₀ value. The LC₅₀ value of bifenthrin, clothianidin, thiamethoxam chlorpyrifos and imidacloprid was 1.07, 2.89, 3.16, 3.32 and 3.85 times more in comparison to chlorantraniliprole. Bifenthrin demonstrated an increase in LC₅₀ values by 2.70, 2.94, 3.09 and 3.59 times respectively to clothianidin, thiamethoxam chlorpyrifos and imidacloprid. Increase in LC₅₀ value of clothianidin in relation to thiamethoxam, chlorpyrifos and imidacloprid was worked out to be 1.09, 1.15 and 1.33 times. Malinowski (2011) has also reported low sensitivity of third instar grubs of *Melolontha* spp. to the contact action of neonicotinoid insecticides viz., actamiprid, clothianidin and imidacloprid under laboratory conditions. There was 1.05 and 1.22 times increase in LC₅₀ value of thiamethoxam with respect to chlorpyrifos and imidacloprid, and 1.16 times for chlorpyrifos with respect to imidacloprid. According to Nuri et al. (2010), imidacloprid (0.003 %) had the best effect for preventive control of *P. olivieri* (Castelnau) in ornamental plants in Qazvin. The chlorpyrifos was found to be least toxic on the basis of its LC₅₀ values calculated for third instar grubs of *M. virescens* (= *P. fullo*).

The order of toxicity for third instar grubs on the basis of LC₅₀ values in relation to imidacloprid were chlorantraniliprole (3.85 times toxic) > bifenthrin (3.59 times toxic) > clothianidin (1.33 times toxic) > thiamethoxam (1.22 times toxic) > chlorpyrifos (1.16 times toxic) (Table 4.43). Koranga (2018) has reported chlorantraniliprole, bifenthrin and clothianidin to be 1.80-2.77 times more toxic as compared to thiamethoxam against third

instar grubs of *Brahmina coriacea* (Hope) in Himachal Pradesh. This report confirmed the results of our experiments. The 95 % fiducial limits of LC_{50} revealed statistically non significant difference in toxicity among chlorpyrifos, thiamethoxam, clothianidin and imidacloprid. In case of bifenthrin and chlorantraniliprole, there was overlap in 95 % fiducial limits of LC_{50} values showing thereby non significant difference in their toxicity to third instar grubs of *M. virescens* (= *P. fullo*).

Table 4.43 Relative toxicity of insecticides against third instar grubs of *M. virescens* (= *P. fullo*)

S. No.	Insecticides	LC_{50} (g a.i./kg soil)	Relative toxicity
1.	Chlorantraniliprole	0.071	3.85
2.	Bifenthrin	0.076	3.59
3.	Clothianidin	0.205	1.33
4.	Thiamethoxam	0.223	1.22
5.	Chlorpyrifos	0.235	1.16
6.	Imidacloprid	0.273	1

4.8 Evaluation of insecticides as seed treatment against grubs of *M. indica*

The experiment was conducted during the month May, the normal sowing time for rajmash in high hills of Himachal Pradesh. The results pertaining to the mortality of plants have been presented in table 4.44. After 10 days of treatment, very high plant mortality to the extent of 41.67 per cent was recorded in control. Seed treatment with chlorantraniliprole was found to be highly effective showing least plant mortality (20.0 %), followed by chlorpyrifos (21.67 %) and bifenthrin (26.17 %). All these treatments were found statistically superior to control. Jakhar et al. (2020) found seed treatment with imidacloprid 600 FS @ 6.5 ml/ kg seed superior to chlorantraniliprole 18.5 SC @ 2.0 ml/ kg seed in reducing damage of *H. consanguinea* Blanchard grubs in groundnut. The plant mortality (20.0-36.67%) with chlorantraniliprole, chlorpyrifos, clothianidin, thiamethoxam, and bifenthrin was statistically at par with each other. In Rajasthan, imidacloprid 17.8 SL @ 3.0 ml/ kg seeds and with clothianidin 50WDG @ 2.0 g/ kg seed were superior to chlorantraniliprole 18.5 SC @ 3.0 ml/ kg seed against grubs of *H. consanguinea* in groundnut (Jakhar et al. 2020). This difference in mortality can be due to differences in pest species, crop, location and the dose of the pesticide used. Seed treatment with imidacloprid showed 36.67 per cent plant mortality after 10 days of sowing and was significantly at par with control. Similarlry Thiamethoxam and clothianidin were also found to be at par with control. The plant mortality in imidacloprid, clothianidin, thiamethoxam and bifenthrin varied from 26.67-36.67 per cent, and the plant mortality

data exhibited non significant differences, but clothianidin, thiamethoxam and bifenthrin were statistically at par with chlorantraniliprole and chlorpyrifos.

After 20 days of treatment, 76.67 per cent plants succumbed to grub attack in control. The trend of efficacy was almost similar with chlorantraniliprole showing least plant mortality (30.0 %), followed by chlorpyrifos (33.33 %). Both these treatments were statistically superior to all other treatments. There was 46.67-55.0 per cent plant mortality when seeds were treated with imidacloprid, clothianidin, thiamethoxam and bifenthrin, and all these treatments were statistically at par with each other. Sood et al. (2010) tested five insecticides as seed treatment in rajmash in Sangla valley and reported 55.96 per cent reduction in plant mortality due to white grubs with chlorpyrifos 20 EC @ 4.0 ml/ kg seed. Contrary to their results, seed treatment with imidacloprid and thiamethoxam were not very effective in present study. Mishra and Singh (1994) have also recommended seed treatment with chlorpyrifos @ 25 ml/ kg seed for protection against *Anomala dimidiata* (Hope) in soybean.

Table 4.44 Mortality of rajmash plants due to attack of *M. indica* grubs

Treatments	Dose (ml or g/ kg seed)	Mean mortality of plants after indicated days of sowing (%)		Reduction in plant mortality over control (%)	
		10 days	20 days	10 days	20 days
Chlorpyrifos 20 EC	5 ml	21.67 (27.45) ^c	33.33(35.12) ^c	48.00	56.52
Imidacloprid 17.8 SL	5 ml	36.67 (37.07) ^{ab}	55.00 (48.07) ^b	12.01	28.26
Clothianidin 50 WDG	3.5 g	31.67 (34.00) ^{abc}	51.67 (46.12) ^b	24.01	32.61
Thiamethoxam 25 WDG	3.5 g	33.33 (33.52) ^{abc}	55.00 (47.92) ^b	20.01	28.26
Bifenthrin 10 EC	2.5 ml	26.67 (29.34) ^{bc}	46.67 (43.20) ^b	36.01	39.13
Chlorantraniliprole 18.5 SC	2.5 ml	20.00 (26.33) ^c	30.00 (33.03) ^c	52.00	60.87
Control	-	41.67 (40.13) ^a	76.67 (61.44) ^a	-	-
CD(p=0.05)	-	(8.29)	(7.27)		

Figures in parentheses are arcsin transformed values

After 20 days of sowing, the soil was tipped out of trays to record grub mortality. There was 13.33 per cent mortality in control. Maximum mortality was recorded in chlorantraniliprole treatment (56.67 %), followed by chlorpyrifos (46.67 %) and bifenthrin (40.0 %). The mortality data registered in chlorantraniliprole treatment was at par with chlorpyrifos and bifenthrin, but mortality response in bifenthrin treatment was also statistically at par with imidacloprid and clothianidin. The grub mortality with thiamethoxam was recorded to be 26.67 per cent and was statistically at par with control (Table 4.45). The grubs of *Melolontha* sp. caused damage at the time of sowing, leading to plant mortality. The grubs of *Melolontha* spp. remained deep in the soil and hence were

Table 4.45 Mortality response of *M. indica* grubs to different seed treatments

Treatments	Total number of grubs	Number of dead grubs observed after 20 days of sowing	Per cent grub mortality after 20 days of sowing
Chlorpyrifos 20 EC	30	14	46.67 (43.04) ^{ab}
Imidacloprid 17.8 SL	30	9	30.00 (31.72) ^{bc}
Clothianidin 50 WDG	30	10	33.33 (32.09) ^{bc}
Thiamethoxam 25 WDG	30	8	26.67 (26.62) ^{cd}
Bifenthrin 10 EC	30	12	40.00 (39.14) ^{abc}
Chlorantraniliprole 18.5 SC	30	17	56.67 (50.46) ^a
Control	30	4	13.33 (14.09) ^d
CD(p=0.05)	-	-	(13.68)

Figures in parentheses are arcsin transformed values

unaffected by the soil treatment (Chandel et al. 2019). Therefore, seed treatment with chlorpyrifos and chlorantraniliprole would be effective in the management of white grubs infesting rajmash in high hills of north western Himalayas.

5. SUMMARY AND CONCLUSIONS

Studies on “Bioecology and management of *Melolontha* spp. (Scarabaeidae: Coleoptera) in Himachal Pradesh” were conducted during the years 2017 and 2018.

In Himachal Pradesh, five species of *Melolontha* beetles viz., *Melolontha cuprescens* Blanchard, *Melolontha furcicauda* Ancy, *Melolontha indica* Hope, *Melolontha* sp.1 and *M. virescens* (Brenske) [= *P. fullo* (Linnaeus)] were collected on UV light traps, and from host trees during 2017-18. At Palampur, *M. furcicauda* and *M. indica* were recorded to be the predominant species on light trap constituting 48.91-50.19 and 48.67-50.0 per cent of total *Melolontha* beetle catch during 2017-18. At Chhatanseri, three species viz; *M. cuprescens*, *M. furcicauda* and *M. indica* were collected on light trap, and *M. cuprescens* constituted 69.17 and 73.33 per cent of total *Melolontha* beetle catch during 2017 and 2018, respectively. Total five *Melolontha* species were collected on light trap from Badagaon and Janjhli, and *M. cuprescens* (27.93-36.11 %) and *M. virescens* (= *P. fullo*) (23.89-32.40 %) were found to be the predominant species at Badagaon, whereas at Janjhli, *M. cuprescens* was predominant during both years. The catch for *M. cuprescens* was 43.62-48.11 per cent higher at Ahla as compared to other *Melolontha* species, and peak period of its activity was observed in July, followed by August during both the years. In Sangla area, *M. virescens* (= *P. fullo*) was found to be the predominant species constituting 72.73 and 80.0 per cent of total *Melolontha* beetle catch during 2017 and 2018, respectively. The Shannon index ($H' = 1.35-1.36$), Simpson index of diversity ($D = 0.73-0.76$) and Pielou's evenness index ($J' = 0.97-0.98$) were calculated to be maximum at Badagaon. The species richness and abundance showed total similarity among *Melolontha* species between Badagaon and Janjhli as indicated by the values of Sorensen's index and Jaccard's index, which were calculated to be 1.0 during both the years. Maximum dissimilarity was recorded among *Melolontha* assemblages from Palampur and Sangla as indicated by the values of Bray Curtis distance index (0.93-0.94).

On host trees, *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*) were collected on apple, peach, plum, persimmon, walnut, wild rose, *bathua* and Indian siris at different locations. At Palampur, Chhatanseri and Badagaon, 54.55-100.0 per cent beetles of *M. cuprescens*, *M. furcicauda* and *M. indica* were taken on apple, whereas *M. cuprescens* and *M. virescens* (= *P. fullo*) at Badagaon displayed distinct

preference for persimmon (56.25-80.0%). At Ahla, maximum collection for different species was done on wild rose (25-100%) and Indian siris (66.67-100 %).

In laboratory, five hosts were tested for their food preference in multi-choice and no-choice tests. Both in multi and no-choice tests, rose was found to be the most preferred host for *M. indica* and *M. furcicauda*, and apple for *M. cuprescens*. In multi-choice tests, leaf area consumption was recorded to be 33.73 and 29.55 cm² on rose for the beetles of *M. indica* and *M. furcicauda*, respectively. In no-choice test, maximum leaf area consumption of 31.24 and 30.16 cm² was registered by *M. indica* and *M. furcicauda* beetles, whereas *M. cuprescens* beetles showed maximum consumption (45.23 cm²) on apple leaves. Peach and pecan nut were almost rejected by the beetles of these species in both tests.

In the present study, data on abundance and infestation of *Melolontha* grubs were recorded on potato, cabbage, rajmash, onion and apple at seven locations in five districts of Himachal Pradesh. In potato, maximum tuber damage was recorded at Badagaon (47.50 % during 2017 and 42.50 % during 2018). In cabbage, the plant mortality as a result of *Melolontha* grub attack varied between 11.25-28.75 per cent during 2017-18 at different locations. Maximum plant mortality (26.25 % during 2017 and 28.75 % during 2018) was recorded at Badagaon. In Rajmash, maximum plant infestation to the tune of 18.7 and 20.0 per cent was recorded at Badagaon during 2017 and 2018, respectively. At Chhatanseri, 6.25-8.75 per cent onion bulbs were found to be infested by the *Melolontha* grubs during 2017-18. At Sangla and Pangna, fully established apple trees were found to be attacked by grubs of *M. indica* and *M. virescens* (= *P. fullo*), respectively. The severely damaged plants showed wilting and drying of leaves.

Studies on the biology of *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*) were conducted under laboratory conditions at Palampur. The average length x width of newly laid eggs of *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*) was recorded to be 4.58 ± 0.06 x 3.33 ± 0.04 mm, 3.94 ± 0.06 x 2.90 ± 0.06 mm, 3.63 ± 0.02 x 2.60 ± 0.09 mm and 3.88 ± 0.07 x 2.81 ± 0.06 mm, respectively. After about three weeks, the average length x width of eggs increased to 5.11-5.50 x 3.61-3.80 mm, 5.23 ± 0.06 x 3.81 ± 0.02 mm, 4.49 ± 0.05 x 3.20 ± 0.05 mm and 4.86 ± 0.03 x 3.23 ± 0.03 mm in *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*), respectively. The incubation period varied from 19-22, 22-25, 24-30 and 16-18 days in *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*),

respectively. Fecundity was recorded to be 19, 26, 23 and 21 eggs/female in *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*), respectively.

The fully fed first instar grubs of *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*) averaged 15.33 ± 0.66 mm, 11.15 ± 0.37 mm, 13.49 ± 0.70 mm and 12.08 ± 0.78 mm, respectively. The width of head capsule of first instar grubs was measured to be 3.65 ± 0.22 mm, 3.21 ± 0.19 mm, 2.37 ± 0.05 mm and 2.72 ± 0.05 mm, respectively. The duration of first instar grubs of *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*) ranged from 56-64 days, 54-56 days, 53-58 days and 46-51 days, respectively. The mean body length of second instar grubs of *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*) was recorded to be 22.99 ± 0.32 mm, 23.20 ± 0.36 mm, 22.61 ± 0.35 mm and 25.89 ± 0.71 mm, respectively, and the width of head capsule averaged 4.88 ± 0.17 mm, 5.04 ± 0.09 mm, 4.63 ± 0.20 mm and 4.68 ± 0.17 mm, respectively. The duration of second instar grubs of *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*) was recorded to be 226-230, 218-224, 234-237 and 214-229 days, respectively. The third instar grubs of *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*) were robust and most active. In *M. furcicauda* and *M. virescens* (= *P. fullo*), the body colour was creamish-white to slightly yellow, whereas third instar grubs of *M. cuprescens* were more yellowish in colour as compared to *M. furcicauda*. In *M. indica*, the third instar grubs appeared greyish in colour. The third instar grubs of *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*) varied from 51.12-58.20 mm, 50.11-53.39 mm, 40.13-50.03 mm and 48.33-54.17 mm in body length, and 7.68-8.99 mm, 6.89-7.13 mm, 6.98-7.58 mm, and 7.92 ± 8.48 mm in head capsule width, respectively. The duration of third instar grubs ranged from 325-329 days in *M. cuprescens*, 299-311 days in *M. furcicauda*, 310-312 days in *M. indica* and 309-320 days in *M. virescens* (= *P. fullo*). In *M. cuprescens* and *M. virescens* (= *P. fullo*), third instar grubs occurred from May, 2018 to April, 2019, and in *M. furcicauda*, the third instar grubs occurred from May, 2018 to March, 2019. In *M. indica*, the third stage grubs were recorded between July, 2018 to June, 2019.

Certain structures of the larvae were of taxonomic importance. A detailed description of the mouth parts (clypeus, labrum, mandibles, maxilla, epipharynx, labium, hypopharynx), raster, spiracles, thoracic legs of the third instar larva and last abdominal segment of pupa has been given with the help of suitable diagrams. In case of *M. cuprescens* and *M. indica*, there were 11 sensilla basiconica in total, whereas in *M.*

furcicauda and *M. virescens* (= *P. fullo*), only nine sensilla basiconica were present in apical sensory area of antennae. The epipharynx of *M. cuprescens* showed protuberant haptomerum, having 18 heli, and six minute sensilla basiconica. In *M. indica*, the haptomerum had 15 heli and eight sensilla basiconica. In *M. virescens* (= *P. fullo*), 12 heli and five sensilla basiconica were present, whereas in *M. furcicauda*, there was reduction in number of heli (7) and sensilla basiconica (3). In mandibles, the width of scissorial area was recorded to be maximum in *M. indica* (1.58 mm), and minimum in *M. cuprescens* (1.04 mm). The raster was well developed with two widely separated and prominent palidia. The paired palidia consisted of 28 or 29 pali in *M. cuprescens*, 26 pali in *M. furcicauda*, 31 or 33 pali in *M. indica* and 15 pali in *M. virescens* (= *P. fullo*). The anal slit was Y-shaped in *M. cuprescens*, *M. furcicauda* and *M. indica*, but it was V-shaped in *M. virescens* (= *P. fullo*).

In *M. cuprescens*, the pre-pupal period ranged from 22-25 days. The pre-pupal period was relatively short in *M. furcicauda* and *M. indica* where the duration of pre-pupal stage has been recorded to be 12-14 days and 11-12 days. In *M. virescens* (= *P. fullo*), the pre-pupal period lasted for 15-23 days. The pupae were of exarate adecticious type. In *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*), the mean body length x width of pupa was recorded to be 32.22 ± 0.37 mm x 14.56 ± 0.29 mm, 32.47 ± 0.11 mm x 14.55 ± 0.13 mm, 31.97 ± 0.15 mm x 12.71 ± 0.22 mm, and 32.72 ± 0.41 mm x 14.23 ± 0.01 mm, respectively. The clear-cut differences existed in caudal appendages in male and female pupae. The average pupal period under laboratory conditions was 20.3 ± 0.7 , 30.0 ± 0.47 , 25.1 ± 0.67 and 18.6 ± 0.82 days in *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*), respectively.

The adult formation was noticed during first week of June in *M. cuprescens* and *M. furcicauda*, whereas in *M. indica*, the adult formation occurred during second week of July. In *M. virescens* (= *P. fullo*), the adult emergence began during second week of June. In *M. cuprescens*, the body was elongate, convex, blackish in colour with brownish tinge, and covered all over with dirty white scales. In *M. furcicauda*, the adult beetles were reddish brown with creamish-white scales, whereas in *M. indica*, the body colour was yellowish-brown with pale scales. The adult beetles of *M. virescens* (= *P. fullo*) were elongate, dark brown, clothed very evenly with pale scales. The female beetles were slightly larger in size than the male beetles in all studied species. Body length x width of male beetles of *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*) ranged from 30.0-35.20 mm x 19.2-20.30 mm, 27.81-30.72 mm x 12.78-14.35 mm, 23.30-

29.21 mm x 12.30-14.0 mm, and 21.59-25.75 mm x 10.22-11.89 mm, respectively. The corresponding body length x width of female beetles has been recorded to be 32.0-35.10 mm x 19.10-22.10 mm, 28.90-30.80 mm x 13.70-14.56 mm, 24.40-30.10 x 13.30-15.90 mm, and 22.80-26.15 x 11.92-13.24 mm, respectively. All species had biennial life cycle ranging from 619-685 days.

The meso-metasternal process was originated from metathorax and it was more broad and slightly more elongated in males as compared to females. The caudal processes of pygidium were elongated and bifurcated into two lobes in males of *M. cuprescens* and *M. furcicauda*, whereas in females, the caudal processes were short, pointed and widely separated. In *M. indica* males, the caudal processes were less projected. In females of *M. indica*, the caudal extremity was slightly bifid without any further extension. In *M. virescens* (= *P. fullo*), the pygidium lobe was narrow in females as compared to males. The antennal club was 7-segmented in males, whereas in females, it was 6-segmented in all *Melolontha* species. In legs, the differences in male and female beetles were evident in tibial spine and tarsal claws. The male genitalia showed clear-cut difference among different species. In *M. cuprescens*, the phallobase was broader anteriorly, slightly narrow at the base, both parameres symmetrical, separated widely and lobe-like. In *M. furcicauda*, the phallobase tapered gradually from anterior region to the base, which was more narrower and the parameres look elongated and sickle shaped. In *M. indica*, the phallobase was broader at the base. Parameres typically joined in the centre in the form of two sub-triangular plates in dorsal mid-posterior region. In *M. virescens* (= *P. fullo*), the phallobase is shorter than parameres. The attachment of phallobase to parameres showed concavity towards the base of phallobase. The overall survival of female beetles was significantly more than males in *M. indica* and *M. furcicauda*. In *M. cuprescens*, the longevity of female and male beetles did not show significant difference. It was recorded to be 25.0 ± 0.71 days for male beetles, and 25.1 ± 0.71 days for female beetles. In *M. furcicauda* and *M. indica*, the average survival of male beetles was recorded to be 17.4 ± 0.40 and 27.4 ± 0.34 days, whereas the female beetles of *M. furcicauda* and *M. indica* survived for 23.0 ± 0.49 and 36.6 ± 1.18 days, respectively. The overall longevity of females and males of *M. virescens* (= *P. fullo*) has been recorded to vary from 16-18 days and 11-19 days, respectively.

Phylogenetic analysis of mitochondrial gene (*Cox1*) and nuclear gene (*28s rRNA*) from *M. cuprescens*, *M. furcicauda*, *M. indica* and *M. virescens* (= *P. fullo*) were amplified and sequenced. The *Cox1* sequence from *M. cuprescens* showed 99.89 per cent

similarity to *M. virescens* (= *P. fullo*), whereas *Cox1* sequence from *M. furcicauda* showed 89.47 per cent similarity to *M. indica*. Among retrieved *Cox1* sequences from NCBI database, *M. hippocastani* (HM120757) and *M. hippocastani* (KX087316) were more close to *M. furcicauda* (86.02-86.51 %) and *M. indica* (84.15-84.69 %) as compared to *M. cuprescens* (81.47-81.69 %) and *M. virescens* (= *P. fullo*) (81.47-81.58 %). Based on the 28s rRNA gene sequence, *M. indica* showed maximum identity of 99.42 per cent to *M. melolontha* (EU084231) as compared to *M. cuprescens* and *M. furcicauda* which were distantly related.

The efficacy of six insecticides viz., chlorantraniliprole 18.5 SC, bifenthrin 10 EC, clothianidin 50 WDG, thiamethoxam 25 WDG, imidacloprid 17.8 SL and chlorpyrifos 20 EC was tested against second and third instar grubs of *M. virescens* (= *P. fullo*) in laboratory, and the LC₅₀ values were 0.057, 0.066, 0.095, 0.098, 0.239 and 0.256 g a.i./kg soil for second instar grubs, respectively. The corresponding LC₅₀ values of these insecticides against third instar grubs were calculated to be 0.071, 0.076, 0.205, 0.223, 0.235 and 0.273 g a.i./kg. The order of toxicity for third instar grubs was chlorantraniliprole (3.85) > bifenthrin (3.59) > clothianidin (1.33) > thiamethoxam (1.22) > chlorpyrifos (1.16). The efficacy of these insecticides by seed treatment was tested against grubs of *M. indica* under laboratory conditions. Seed treatment in rajmash indicated better efficacy of chlorantraniliprole 18.5 SC (2.5 ml/kg seed), followed by chlorpyrifos 20 EC (5.0 ml/kg seed).

Conclusion: The survey of *Melolontha* beetles on light trap and host trees revealed a complex of five species in Himachal Pradesh, and out of these, *M. cuprescens*, *M. indica* and *M. virescens* (= *P. fullo*) were of more economic importance at altitudes ranging from 1189-2588 m amsl. There was lesser collection of *Melolontha* beetles both from light traps and host trees even in areas where grubs of *Melolontha* were present in high numbers in soil and inflicted severe crop losses. The beetles in genus *Melolontha* were large in size as compared to other species of scarabaeid beetles and these species take high flight during swarming settling on tall trees for feeding and mating where adult sampling was not possible. It seems that beetles of *Melolontha* sp. were less phototactic, which could be the reason of low beetle catch on light trap. The grubs caused economic losses in crops like potato, rajmash, cole crops, onion and apple in Badagaon, Janjehli, Kothikohar, Chhatanseri, Ahla, Pangna and Sangla areas. The life cycle was studied for four species, and all of them had biennial life cycle. The third instar grubs of these species remained

active from May-October and attacked various crops extending over a period of 5-6 months, whereas in other species having annual life cycle, the damage was caused only during one month between September-October. In high hill temperate areas, the crops are sown in the month of April-May and cropping season was up to October-November indicating complete synchronization between damaging stage of the pest and the stage of the crop. Therefore, proper strategy is required to tackle these grubs in soil right from sowing till harvest of crop. The larvae of different *Melolontha* species can be easily distinguished on the basis of mandibles, epipharynx and raster pattern. The caudal extremity of pupae also allows differentiation among species. In beetles, shape of pronotum and pygidium can be used to separate different *Melolontha* species and sexes. Male genitalia is the most stable character for differentiation of species in adult stage. The *Cox1* was a better DNA barcode for identification of *Melolontha* species, whereas *28s RNA* has the limited utility in DNA barcoding of *Melolontha* species. Very high doses of insecticides were required to kill second and third instar grubs. Although, eggs and first instar grubs were very delicate, yet they could not be killed due to the poor translocation of chemicals. Therefore, either alternative chemicals with higher efficacy or alternative biorational strategies needed to be developed to control *Melolontha* grubs in soil.

LITERATURE CITED

-
- Abbott WS. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* 18: 265-267
- Ahmad M and Faisal M. 2012. Status of insect pests of poplar in India with special reference to *Clostera* spp. *Forestry Bulletin* 12(1): 105-122
- Alao JS, Sale FA and Ojo AS. 2011. Problems and management of insect pests in social forestry in Nigeria. *African Journal of Agricultural Research* 6(33): 6755-6758
- Alekseev VI and Shapoval AP. 2019. Beetles (Insecta, Coleoptera), sampled with use of light trap on the Curonian Spit: the materials of the sixth season. *Acta Biologica Sibirica* 5(2): 68–82
- Ali ATM. 2001. Biosystematics of phytophagous scarabaeidae - An Indian Overview. In: Indian Phytophagous Scarabs and their Management: Present Status and Future Strategies (G Sharma, YS Mathur and RBL Gupta, eds) eds), *Agrobios Jodhpur, India*, p 5-37
- Altaf S and Ahad I. 2020. Insect pest complex of apple nurseries at Wadura Sopore in North Kashmir. *International Journal of Entomology Research* 5(3): 96-98
- Altaf S, Ahad I, Pathania SS, Lone GM, Peer FA and Maqbool S. 2019. Insect pest complex of apple nurseries in north Kashmir. *Journal of Entomology and Zoology Studies* 7(3): 697-700
- Anitha V, Wightman J and Rogers DJ. 2005. Management of white grubs (Coleoptera: Scarabaeidae) on groundnut in southern India. *International Journal of Pest Management* 51(4): 313-320
- Anonymous. 2008. T.C. Tarım ve Koyisleri Bakanligi Tarımsal Arastirmalar Genel Mudurlugu, Zirai Mucadele Teknik Talimatları. Cilt 4, Ankara, p 316-318
- Anonymous. 2009. *Melolontha melolontha* L. Common Cockchafer, May-bug, May beetle. Interactive Agricultural Ecological Atlas of Russia and Neighboring countries. http://www.agroatlas.ru/en/content/pests/Melolontha_melolontha/
- Anonymous. 2011. June beetles. Available online: <http://www.bahcesel.net/forumsel/genel-zararlilar/12225-haz304ran-bocekler304-polyphylla-fullo/>

- Aragon A and Moron MA. 2000. Description of third-instar larva of two species of *Phyllophaga* (Coleoptera: melolonthidae). *The Canadian Entomologist* 132: 323-332
- Arif U, Bano P, Ahad I, Singh P, Dar ZA, Badri Z, Maqbool S, Aafreen S and Kumar R. 2019. Insect pests of maize at different altitudes of north Kashmir, J&K. *Journal of Entomology and Zoology Studies* 7(2): 1123-1128
- Barwal RN. 1998. Record of Scarabaeoidea from the temperate regions of India. *Journal of Applied Zoological Research* 9(1&2): 36-37
- Beeson CFC. 1941. The ecology and control of the forest insects in India and the neighbouring countries. Publications of forest Research, Division. Government of India, p 1007
- Benker U and Leuprecht B. 2007. The swarming flight of Common cockchafer *Melolontha melolontha* L., 1758 (Coleoptera, Scarabaeidae) in two different areas of Bavaria and an approach to control the egg deposition. Integrated Control of Soil Insect Pests. *IOBC/wprs Bulletin* 30(7): 91-94
- Bhadauria AS and Nigam PM. 1982. Food preference to *Holotrichia consanguinea* (Coleoptera: Scarabaeidae). *Entomon* 7(3): 31-32
- Bhat OK, Bhat AA and Kaul VK. 2005. Faunistic studies of white grubs in Kashmir. *Insect Environment* 11(1): 14-15
- Bhat ZH. 2007. Development of Integrated Pest Management (IPM) capsule for white Grub and cutworm. Ph.D. Thesis, p 120. Aligarh Muslim universality Aligarh, India
- Billamboz A. 2014. Dendroarchaeology and cockchafers north of the Alps: Regional patterns of a middle frequency signal in oak tree-ring series. *Environmental Archaeology* 19(2): 114-123
- Blaisinger P, Casanova M, Gentet C, Habert M, Pelletier L, Riffiod G, Robert P and Varlet G. 1978. New chemical control tests against white grubs, the larvae of the common cockchafer *Melolontha melolontha* L., in permanent meadowland. *Phytiatrie-Phytopharmacie* 27(4): 297-306
- Blum M. 1985. Fundamentals of Insect Physiology. John Wiley and Sons, New York.

- Boas JEV and Thomsen M. 1922. Oldenborrernes Optraeden Danmark Aarene 1904-1919. *Kgl. Veterinaer-og Landbohojskole*, pp 56-65
- Boving AG. 1942. Description of the larva of some West Indian Melolonthinae beetles and a key to the known larvae of the tribe. *Proceedings of the United States National Museum* 92: 167-175
- Boving AGE and Craighead FC. 1931. An illustrated synopsis of the principal larval forms of the order Coleoptera. *Entomologica Americana* 11: 351
- Britton E.B. 1978. A revision of the Australian chafers (Coleoptera: Scarabaeidae: Melolonthinae) Vol. 2. Tribe Melolonthini. *Australian Journal of Zoology, Supplementum Series* 60: 1-150
- Buchi R and Jossi W. 1979. On the action of the growth regulator Dimilin on the cockchafer *Melolontha melolontha* L. and the dock beetle *Gastroidea viridula* Deg. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 52(1): 75-81
- Bulmer MG. 1977. Periodical Insects. *The American Naturalist* 3: 1099-1117
- Burakowski B. 1993. Laboratory methods for rearing soil beetles (Coleoptera). *Memorabilia Zoologica* 46: 1-66
- Buss EA. 2006. White grub biology and management. EDIS. <http://edis.ifas.ufl.edu/LH037>
- Cardiff J. 2001. *Melolontha melolontha*, Animal Diversity Web. https://animaldiversity.org/accounts/Melolontha_melolontha
- Chandel RS and Kashyap NP. 1997. About white grubs and their management. *Farmer and Parliament* 37(10): 29-30
- Chandel RS, Chander R and Gupta PR. 1993. Toxicity of some insecticides to immature stages of *Brahmina coriacea* (Hope). *Journal of Soil Biology and Ecology* 13(2): 103-107
- Chandel RS, Gupta PR and Chander R. 1994. Diversity of scarabaeid beetles in mid hills of Himachal Pradesh. *Himachal Journal of Agricultural Research* 20(1&2): 98-101

- Chandel RS, Gupta PR and Chander R. 1995. Behaviour and biology of the defoliating beetle, *Brahmina coriacea* (Hope) (Coleoptera: Scarabaeidae) in Himachal Pradesh. *Journal of Soil Biology and Ecology* 15(1): 82-89
- Chandel RS, Mehta PK, Verma KS and Sharma PC. 2010. A survey study of white grubs of economic importance in Himachal Pradesh. In: Proceedings of National Conference on Plant Protection in Agriculture through Ecofriendly Techniques and Traditional Farming Practice, Durgapur Rajasthan, pp 441-442
- Chandel RS, Pathania M, Verma KS, Bhattacharyya B, Vashisth S and Kumar V. 2015. The ecology and control of potato whitegrubs of India. *Potato Research* 58: 147-164
- Chandel RS, Verma KS and Rana A. 2019. *Melolontha virescens* Brenske: An emerging pest of wheat in high hills and dry temperate zone of Himachal Pradesh. In: Proceedings of Conference on Wheat Productivity Enhancement through Climate Smart Practices, 14-16 February 2019, CSKHPKV, Palampur, H.P, p 71
- Chandel Y. 2020. Studies on phytophagous white grubs in Mandi district of Himachal. M.Sc. Thesis, p 107. Chaudhary Sarvan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh, India
- Chandra K and Gupta D. 2012. Pleurostict scarabs (Coleoptera: Scarabaeidae): New distributional records in Arunachal Pradesh, north-east India. *Check List: Journal of species lists and distribution* 8(5): 889-893
- Chandra K and Uniyal VP. 2007. On a collection of pleurostict scarabaeidae (Coleoptera) from the great Himalayan National Park, Himachal Pradesh, India. *Zoo's Print Journal* 22: 2821-2823
- Chandra K, Gupta D, Uniyal VP, Bharadwaj M and Sanyal AK. 2012. Studies on Scarabaeid Beetles (Coleoptera) of Govind Wildlife Sanctuary, Garhwal, Uttarakhand, India. *Biological Forum - An International Journal* 4(1): 48-54
- Chandra K. 2005. Insecta: Coleoptera: Scarabaeidae. *Zoological Survey of India Faun of Western Himalayas* (Part 2), pp 141-155
- Colwell RK and Coddington JA. 1994. Estimating terrestrial biodiversity through extrapolation. *Philosophical Transactions of the Royal Society B: Biological Sciences* 345: 101-118

- Couturier A and Jacky F. 1970. Complementary observations on flight stimulation and sensitivity to the spectrum of *M. melolontha*. *Bulletin de la Societe entomologique de France* 75(5-6): 113- 116
- Couturier A. 1967. Sensibilite differentielle du hanneton commun (*Melolontha melolontha* L.) (Coleopt., Scarabeidae) vis-a-vis de certaines longueurs d'onde du spectre visible. *Annales de la Societe entomologique de France* 3: 767- 770
- Craighead FC. 1950. Insect enemies of eastern forests. U.S. Department of Agriculture Miscellaneous Publication 657: 115
- Daly Howell V. 1985. Insect morphometric. *Annual Review of Entomology* 30: 41-438
- Deka TN, Gudade BA, Harsha KN and Vijayan AK. 2014. New record of *Melolontha* sp. nr. *furcicauda* Anczy, *Anomala dimidiata* (Hope) and *Brahmina* Sp. from large cardamom ecosystem. *Life Sciences Leaflets* 49: 128-130
- Delb H. 2004. Monitoring der Waldmaikafer (*Melolontha hippocastani* F.)-Populationen und der Schaden durch Engerlinge in der nordlichen Oberrheinebene, BadenWurttemberg und Rheinland-Pfalz. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* 56: 108-116
- Demir S, Karagoz M, Hazir S and Kaya HK. 2014 Evaluation of entomopathogenic nematodes and their combined application against *Curculio elephas* and *Polyphylla fullo* larvae. *Journal of Pest Science* 88: 163-170
- Devi N, Desh R and Kashyap NP. 1994. Relative abundance of some white grub beetles in mid hill zone of Himachal Pradesh (India). *Journal of Entomological Research* 18(2): 139-142
- Dhoj GC. 2006. White grubs (Coleoptera: Scarabaeidae) associated with Nepalese agriculture and their control with the indigenous entomopathogenic fungus *Metarhizium anisopliae* (Metsch.) Sorokin. PhD Thesis, p 250. The University of Basel, Switzerland
- D'Hotman and D'Scholtz CH. 1990. Comparative morphology of the male genitalia of derived groups of Scarabaeoidea (Coleoptera). *Elytron* 4: 3-39
- Dittrich G, Conlong DE and Mitchell A. 2006. Molecular identification of South African Sugarcane white grubs (Coleoptera: Scarabaeidae). *Proceedings of the South African Sugarcane Technologists Association* 80: 264-268

- Dittrich-Schroder G, Conlong D, Way M, Harrison J and Mitchell A. 2009. Identification key to Scarabaeid beetle larvae attacking sugarcane in South Africa using DNA barcoding and integrative taxonomy. *Proceedings of the South African Sugarcane Technologists Association* 82: 500-524
- Doane RW, Van Dyke EC, Chamberlin WJ and Burke HE. 1936. Forest insects. McGraw-Hill Book Co Inc. New York, p 233
- Dominik J. 1954. Proba zwalczania trzyletniego pedraka chrabaszcz *Melolontha melolontha* L. preparatem, Intox 8. *Sylwan* 3: 204-209
- Dominik J. 1958. Proba zwalczania dwuletniego pedraka chrabaszcz majowego (*Melolontha melolontha* L) przez dwukrotne traktowanie gleby preparatem "Intox 8". Warsaw University of Life Sciences, Poland 1: 77- 82
- Doskocil JP. 2007. Evaluating the occurrence, seasonal history, species composition and impact of *Phyllophaga* and *Cyclocephala* grubs infesting Bermudagrass (*Cynodon* spp.) in Oklahoma. M.Sc. Thesis, p 65. Oklahoma State University Stillwater, Oklahoma, USA
- Downes W and Andison H. 1941. Notes on the life history of the June beetle *Polyphylla perverse* Casey. *Journal of the Entomological Society of British Columbia* 37: 5-8
- Doyle JJ and Doyle JL. 1990. Isolation of plant DNA from fresh tissue. *Focus* 12:13–15
- Emden FIV. 1941. Larvae of British beetles, II: A key to the British *Lamellicornia* larvae. *Entomologists Monthly Magazine* 75: 117-127, 181-192
- Erler F and Ates AO. 2015. Potential of two entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* (Coleoptera: Scarabaeidae), as biological control agents against the June beetle. *Journal of Insect Science* 15(1): DOI: 10.1093/jisesa/iev029
- Faber W. 1961. Ergebnisse zehnjähriger Erhebungen über die Flugjahre des Maikafers (*Melolontha melolontha* L. und *M. hippocastani* F.) in Österreich. *Pflanzenschutz Berichte* 27: 101-146
- Feroz M, Tara JS, Azam M and Ramamurthy VV. 2015. Staphylinidae, Scarabaeidae, Dermestidae and Curculionidae (Insecta: Coleoptera) first record, from Kargil (Ladakh), J&K, India. *Munis Entomology and Zoology* 10(1): 172-182

- Ferron P. 1971. Modification of the development of *Beauveria tenella* mycosis in *Melolontha melolontha* larvae, by means of reduced doses of organophosphorus insecticides. *Entomologia Experimentalis et Applicata* 14(4): 457-466
- Finney DJ. 1971. Computer and other techniques. Probit Analysis. 3rd edition. Cambridge University Press 81-97.
- Flerov SK, Ponomarevova EN, Kljusnik PI and Voroncov AI. 1954. *Ochranalesu Praha*, SZN: 352
- Fraival A. 1998. "HYPP Zoology" (On-line). Accessed April 15, 2001 at [http:// www. inra. fr/ Internet/ Produits/ HYPPZ/ RAVAGEUR/ 6melmel. htm](http://www.inra.fr/Internet/Produits/HYPPZ/RAVAGEUR/6melmel.htm)
- Froeschle M and Glas M. 2000. The 1997 control campaign of *Melolontha melolontha* (L.) at the Kaiserstuhl area (Baden-Wurtemberg): field trials and practical experiences *Bulletin OILB/SROP* 23(8): 27-32
- Gardner JCM. 1935. Immature stages of Indian Coleoptera (196). *Indian Forest Records* 1: 1-33
- Gaugler R, Wang Y and Campbell JF. 1994. Aggressive and evasive behaviors in *Popillia japonica* (Coleoptera: Scarabaeidae) larvae: defenses against entomopathogenic nematode attack. *Journal of Invertebrate Pathology* 64: 193-199
- Giannoulis T , Dutrillaux AM, Mamuris Z, Montreuil O, Stamatis C and Dutrillaux B. 2011. Evolution of European cockchafers (Melolonthinae: Scarabaeidae: Coleoptera): a morphological, molecular and chromosomal study of intra and inter-specific variations. *Bulletin of Entomological Research* 101(3): 345-52
- Glowacka B and Sierpinska A. 2012. Control of adult cockchafers *Melolontha* spp. with Mospilan 20 SP. *Folia Forestalia Polonica* 54(2): 109–115
- Goffau LJW. 1990. Problems caused by indigenous and exotic Scarabaeidae (Coleoptera) in the Netherlands. *Proceedings of Experimental and Applied Entomology of the Netherlands Entomological Society* 1: 152-157
- Gorres CM and Chesmore D. 2019. Stridulations of *Melolontha* spp. larvae open up new possibilities for species specific pest monitoring in soils. doi: <http://dx.doi.org/10.1101/520734>
- Gressitt JL. 1953. *Bishop Museum Bulletin* 212: 157

- Gupta BP, Joshi NK and Joshi LD. 1977. Some important defoliating and fruit eating beetles of temperate fruit trees-A Survey Study and Control. *Progressive Horticulture* 8: 53-60
- Gupta RK. 2019. Bioecology and Management of *Maladera insanabilis* (Brenske) in Himachal Pradesh. M.Sc. Thesis, p127. Chaudhary Sarvan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh, India
- Hafsi A, Facon B, Ravigne V, Chiroleu F, Quilici S, Chermiti B and Duyck PF. 2016. Host plant range of a fruit fly community (Diptera: Tephritidae): Does fruit composition influence larval performance? *BMC Ecology* 16: 1-12
- Hall TA. 1999. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>
- Hann P, Grunbacher EM, Trska C and Kromp B. 2008. Effect of climate change on the dispersion of white grub damages in the Austrian grassland. *Bioacademy*, pp 62-65
- Haq A. 1962. Notes on the bionomics of *Lachnosterna longipennis* Bl. (Melolonthidae: Coleoptera). *Indian Journal of Entomology* 24(3): 220-221
- Hauss R and Schutte F. 1976. Zur Polyphagie der Engerlinge von *Melolontha melolontha* L. an Pflanzen aus Wiese und Odland. *Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz* 49: 129-132
- Hauss R and Schutte F. 1978. Studies on the oviposition of the cockchafer (*Melolontha melolontha* L.) in relation to the larval food-plants. *Zeitschrift für Angewandte Entomologie* 86: 167-74
- Hauss R. 1975. Methoden und erste Ergebnisse zur Bestimmung der Wirtspflanzen des Maikaferengerlings (*Melolontha melolontha* L.) Mitteilungen aus der Biol. Bundesanstalt für Land- und Forstwirtschaft Berlin Dahlen, pp 73-77
- Hayes WP. 1925. A comparative study of the life history of certain polyphagous scarabaeid beetles. Technical Bulletin, Kansas Agriculture Experimental Station 16: 146
- Hayes WP. 1928. The epipharynx of lamellicorn larvae (Coleop.), with a key to common genera. *Annals of the Entomological Society of America* 21: 282-306

- Haynes KF, Potter DA and Collins JT. 1992. Attraction of male beetles to grubs: evidence for evolution of a sex pheromone from larval odor. *Journal of Chemical Ecology* 18: 1117-1124
- Hebert PDN, Cywinska A, Ball SL and deWaard JR. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society, Series B* 270: 313-321
- Hinson KR. 2011. Species diversity and seasonal abundance of scarabaeoidea at four locations in South Carolina. M.Sc. Thesis, p 102. Clemson University, South Carolina
- Holloway JD, Kibby G and Peggie D. 2001. The families of Malesian moths and butterflies. *Fauna Malesiana Handbook* 3. Brill (Leiden, Boston, Koln)
- Honda K. 2011. Reactions to light in insects and practical applications. *Journal of Biomechanics* 35: 233-236
- Houston WWK and Weir TA. 1992. Melolonthinae. In: Zoological catalogue of Australia. Coleoptera: Scarabaeoidea, (Houston WWK, ed), Australian Government Printing Service, Canberra, pp 174-358
- Huiting HF, Moraal LG, Griepink FC and Ester A. 2006. Biology, control and luring of the cockchafer, *Melolontha melolontha*: literature report on biology, life cycle and pest incidence, current control possibilities and pheromones. Research Report, Applied Plant Research, Wageningen, The Netherland, p 17
- Humayun SD and Ali RZ. 1999. Investigation on important pest of filbert in alamout Area. *AGRIS. Qazvin Agricultural and Natural Resources Research Center*, p 21
- Hurpin B. 1955. Developpment des larves du hanneton *Melolontha melolontha* L. (Coleoptera: Scarabeidae) a differentes temperatures constantes. *Annales des epiphyties* 4: 529-534
- Hurpin B. 1962. Superfamille des Scarabaeoidea. Les *Melolontha*. In: Entomologie Appliquee a l' Agriculture, Tome 1: Coleopteres (Balachowsky AS, ed), Masson, Paris, pp 59-122
- Instruction of Forest Protection (Instrukeja ochrony lasu). 2012. Tom II. CIPL, Warsaw (in Polish)

- Jaccard P. 1912. The distribution of the flora of the alpine zone. *New Phytologist* 11(2): 37-50
- Jackson TA and Klein MG. 2006. Scarabs as pests: a continuing problem. *Coleopterists Society Monograph* 5: 102-119
- Jakhar BL, Baloda AS, Saini KK and Yadav T. 2020. Evaluation of some insecticides as seed dresser against white grubs in groundnut crop. *Journal of Entomology and Zoology Studies* 8(3): 1468-1469
- Jander R. 1963. Insect orientation. *Annual Review of Entomology* 8: 95-114
- Jarfás J and Toth J. 1977. Forecast and protection of the damaging *Melolontha* species in vineyard (in Hungarian). *Szotermesztési Agrokémiai Tajekoztató, Kecskemet* 3(1): 2-7
- Jerath ML. 1960. Notes on larvae of nine genera of Aphodiinae in the United States (Coleoptera: Scarabaeidae). *Proceedings of the United States National Museum* 111: 43-94
- Jia ZC, Fang H and Jiang L. 2020. Morphological description of the white grub *Melolontha incana* (Coleoptera: Scarabaeidae: Melolonthinae: Melolonthini). *Microscopy Research Technique*. DOI: 10.1002/jemt.23653
- Johansen NS, Vanninen I, Pinto DM, Nissinen AI and Shipp L. 2011. In the light of new greenhouse technologies: 2. Direct effects of artificial lighting on arthropods and integrated pest management in greenhouse crops. *Annals of Applied Biology* 159: 1-27
- Johnson MW, Molinar A, Wang X, and Nadel H. 2012. The Continuing Struggle to Manage the Tenlined June Beetle. <http://cestanislaus.ucanr.edu/files/141103.pdf>
- Johnson MW, Wang X, Day K, Bentley W, Klein M, Leal W, McKenry M, Stark JD, Zalom F, and Verdun C. 2006. Refining Chemical Controls and Application Methods for Tenlined June Beetle Grubs. California Tree Fruit Agreement. *Annual Research Report* 137-144
- Johnson SN, Read DB and Gregory PJ. 2004. Tracking larval insect movement within soil using high resolution X-ray microtomography. *Ecological Entomology* 29: 117-122

- Joshi KC and Joshi R. 1980. Insect pests of fruits trees in Kumaon hills. *Indian Horticulture* 25(1): 21-24
- Kalpana HS. 2015. Bioecology of *Holotrichia longipennis* Blanchard and *Holotrichia sikkimensis* Brenske in Himachal Pradesh. M.Sc. Thesis, p 111. Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh, India
- Karimi J, Darsoule R, Fatta-hosseini S and Sadeghi H. 2012. Phylogenetic Analysis on Some Iranian White Grubs with New Data about Natural Pathogen of *Polyphylla adspersa*. *Journal of the Entomological Research Society* 14(1): 1-13
- Kaundal P. 2018. Insect pest complex of onion in Himachal Pradesh. M.Sc. Thesis, p 100. Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh, India
- Keen FP. 1952. Insect enemies of western forests. U.S. Department of Agriculture Miscellaneous Publication 273: 28
- Keith D and Li CL. 2005. Notes on the *Melolontha guttigera* group with description of a new species from Java (Coleoptera, Scarabaeidae, Melolonthinae). *Kogane* 6: 1-7
- Keller E. 1986. Wirtschaftliche Bedeutung der Schaden und Fonds zuderen Vergutung, in Neuere Erkenntnisse uber den Maikafer (Keller S and Keller E, Eds). Beiheft Mitteilungen der Thurgauischen Naturforschenden Gesellschaft, pp 49-56
- Keller S and Zimmermann G. 2005. Scarabs and other soil pests in Europe: situation, perspectives and control strategies. *Bulletin OILB/SROP* 28(2): 9-12
- Keller S, Schweizer C and Shah P. 1999. Differential susceptibility of two *Melolontha* populations to infections by the fungus *Beauveria brongniartii*. *Biocontrol Science and Technology* 9: 441-446
- Keller S, Schweizer C, Keller E and Brenner H. 1997. Control of White Grubs (*Melolontha melolontha* L.) by Treating Adults with the Fungus *Beauveria brongniartii*. *Biocontrol Science and Technology* 7(1): 105-116
- Keller S. 1984. Das andere Gesicht des Malikafers. *Tages Anz. Magazine* 19: 27-34

- Keller S. 2000. Use of *Beauveria brongniartii* in Switzerland and its acceptance by farmers, In: Integrated Control of Soil Pest-Subgroup “*Melolontha*” (Keller S, ed), IOBC/wprs bulletin, Sion, Switzerland 23: 67-72
- Khramtsov NN. 1979. Cockchafer. The Great Soviet Encyclopedia, 3rd Edition (1970-1979)
- Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111-120
- Klingler J. 1957. Über die Bedeutung des Kohlendioxyds für die Orientierung der Larven von *Otiorrhynchus sulcatus* F., *Melolontha* und *Agriotes* (Col.) im Boden (Vorläufige Mitteilung). *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* 30: 317–322
- Koranga R. 2018. Evaluation of Some Insecticides for the Control of *Brahmina coriacea* (Hope) in Himachal Pradesh. M.Sc Thesis, p 92. Chaudhary Sarvan Kumar Himachal Pradesh Krishi Vishwavidyalaya, Palampur, Himachal Pradesh, India
- Korschevsky VR. 1940. Arbeitsgemeinschaft Physiologischer und Angewandter. *Entomologie Berlin-Dahlem* 7(1): 41-52
- Kratochvil J, Landa V, Novak K and Skuhřavý V. 1953. Chroustia bojů nímí. *Praha, Nakladatelství CSAV*: 156
- Krebs CJ. 1985. Ecology-The experimental Analysis of Distribution and Abundance. New York: Harper and Row
- Krebs CJ. 2001. Species diversity measures of heterogeneity. In: Ecology: The Experimental Analysis of Distribution and Abundances (E Fogarty, H Dutton and W Earl, eds) Wesley Longman, Inc. San Francisco, pp 617-618
- Kumar J, Sharma SD and Lal R. 2007. Scarabaeid beetles of Kullu valley, Himachal Pradesh. *Entomon* 32(2): 103-110
- Kumar J, Sharma SD, Lal R and Deor BS. 2005. White grubs damaging maize and paddy crops in Kullu and Mandi districts of Himachal Pradesh. *Pest Management and Economic Zoology* 13(1): 15-20

- Kumar R, Ahad I, Kumar R, Kumar R and Rasool K. 2019. Occurrence of insect-pests and natural enemies infesting oats (*Avena sativa* L.) in North Kashmir. *Journal of Entomology and Zoology Studies* 7(2): 818-825
- Kumar R, Ahad I, Rehman SA and Dorjey S. 2018. Impact of weather parameters on population dynamics of soil borne insect pests infesting oats (*Avena sativa* L.) in North Kashmir. *Journal of Entomology and Zoology Studies* 6(3): 533-537
- Kumar R. 2019. Bioecology and Management of *Maladera insanabilis* (Brenske) in Himachal Pradesh. M.Sc. Thesis, p127. Chaudhary Sarvan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh, India
- Kumar S and Vishvendra. 2018. Identification of Scarabaeid beetles (Coleoptera) in vicinity of sugarcane crop in the western plain zone of Uttar Pradesh, India. *Journal of Pharmacognosy and Phytochemistry* 5 (Special Issue): 111-115
- Kumar S, Ali Musthak TM and Reddy Aswathanarayana N. 2006. Studies on faunal composition of scarabaeids on rose around Bangalore. *Agriculture Science Digest* 26(4): 257-260
- Kumar S, Kumar V, Bhat S and Tadagi G. 2019. White grub diversity explored from various biodiversity rich areas of Almora, Uttarakhand. *Indian Journal of Pure and Applied Bioscience* 7(4): 530-534
- Kumar V, Sreedevi K and Singh S. 2017. Notes on major white grub species associated with groundnut crop ecosystem in Rajasthan, Andhra Pradesh, India. *Journal of Entomology and Zoology Studies* 5(5): 607-613
- Laengle T, Pernfuss B, Seger C and Strasser H. 2005. Field efficacy evaluation of *Beauveria brongniartii* against *Melolontha melolontha* in potato cultures *Sydowia* 57 (1): 54-93
- Lal M and Sharma PK. 2017. Species richness and abundance of white grubs (Coleoptera: Scarabaeidae) in forest ecosystem of Doon Valley, (Uttarakhand), India. *Journal of Environment and Bio-Sciences* 31(2): 447-452
- Lane A, Gladders P and Buckley D. 2000. Pests and Diseases of Sugar Beet. In: Pest and Disease Management Handbook (Alford DV, ed.). Wiley, Cambridge, UK, pp 166-184

- Li CN, Yang PS and Wang CC. 2010. Revision of the *Melolontha guttigera* group (Coleoptera: Scarabaeidae) with a key and an annotated Checklist of the East and South-East Asian *Melolontha* groups. *Annals of the Entomological Society of America* 103(3): 341-359
- Lisov NA. 1984. Impact of some ecological factors on place choice for oviposition of females of the forest cockchafer. In: Proceedings 9 All-Union Entomological Society (Tsygankova E.N. ed.), Naukova dumka, Russia, p 19
- Lisova R and Petroviene I. 2007. Use of Actara insecticide in potato stands. *Zemes ukio Mokslai* 14(2): 23-30
- Liu GR, Zhang YW and Wang R. 1997. The colour illustrated of common lamellicornia beetles of northern China. China Forestry Publishing House, Beijing, China
- Luisa M and Mauro V. 1996. Presence and diffusion of the common cockchafer (*Melolontha melolontha* L.) in the areas of Mezzocorona and San Michele a/A in Trento Province. *Bulletin OILB SROP* 19(2): 15-20
- Magurran AE. 2004. Measuring Biological Diversity. Blackwell ScienceLTD, USA
- Malinowski H, Woreta D and Stocki J. 1998. Problems of the occurrence and management of Melolonthinae in Polish forestry. *AGRIS* 19(2): 21-26
- Malinowski H. 2011. Susceptibility of third instar *Melolontha* spp. white grubs to the contact action of neonicotinoid insecticides. *Forest Research Papers* 72(1): 17-20
- Malusa E, Tartanus M, Furmanczyk EM and Labanowska BH. 2020. Holistic approach to control *Melolontha* spp. in organic strawberry plantations. *Organic Agriculture* <https://doi.org/10.1007/s13165-020-00295-2>
- Manjula K and Sulochanamma BN. 2001. Efficacy of chlorpyrifos against root grub in groundnut. *Indian Journal of Entomology* 63(4): 481-482
- Manners A. 2017. Managing scarab beetles in production nurseries. Nursery levy at work: Building Resilience and Biosecurity Capacity: Factsheet. Agri-science Queensland, Department of Agriculture and Fisheries, *Ecosciences Precinctn Brisbane*, p 4

- Martinek P, Kula E and Hedbavn J. 2018. Reactions of *Melolontha hippocastani* adults to high manganese content in food. *Ecotoxicology and Environmental Safety* 148: 37-43
- McGavin GC. 2007. Expedition Field Techniques: Insects and other terrestrial arthropods. Geography Outdoors: the centre supporting field research, exploration and outdoor learning. Royal Geographical Society Kensington Gore, London
- Mehta PK, Chandel RS and Mathur YS. 2008. Phytophagous whitegrubs of Himachal Pradesh. Technical Bulletin: Department of Entomology, CSK HPKV, Palampur, p 13
- Meinert G, Glas M, Froschle M, Albert R, Harmuth P and Schmidt K. 2001. Integrated plant protection measures against the European Cockchafer (*Melolontha melolontha* L.) in the Northern Kaiserstuhl area 53: 148-157
- Mico E, Piau O, Galante E, and Lumaretb JP. 2003. Taxonomy of Iberian *Hoplia* (Col., Scarabaeoidea, Hopliinae) based on mtDNA analysis. *Molecular Phylogenetics and Evolution* 26: 348-353
- Mikkola K. 1972. Behavioural and electrophysiological responses of night-flying insects, especially Lepidoptera, to near-ultraviolet and visible light. *Annales Zoologici Fennici* 9: 225-254
- Mishra PN and Singh MP. 1994. Control of white grub *Anomala dimidiata* Hope (Coleoptera: Rutelinae) through insecticidal seed treatment in soybean: A new approach. *Annals of Agricultural Research* 15: 237-239
- Mishra PN and Singh MP. 1997. Determination of predominant species of white grub in Kumaon region of Uttar Pradesh hills and their control. *Annals of Plant Protection Sciences* 5(1): 154-156
- Mishra PN and Singh MP. 1999. Determination of predominant species of white grubs in Garhwal regions of Uttar Pradesh hills (India). *Journal of Entomological Research* 23(1): 65-69
- Monaghan MT, Balke M, Gregory TR and Vogler AP. 2005. DNA-based species delineation in tropical beetles using mitochondrial and nuclear markers. *Philosophical Transactions of the Royal Society B* 360: 1925-1933
- Moutia LA. 1940. *Bulletin of Entomological Research* 31: 193-208

- Murthy KS, Banu SL, Ranjitha A and Pattar S. 2018. DNA Barcoding and Evolutionary Lineage of Some Economically Important Scarabaeid Beetles in South India. *Advances in Biochemistry and Biotechnology* 3(1): DOI: 10.29011/2574-7258.000051
- Muska A. 1975. Results of thirteen years observations of cockchafer (*Melolontha melolontha* L.) swarming on the territory of Czechoslovakia. *Sbornik UVTI - Ochrana Rostlin* 11(4): 283-294
- Niemczyk M, Karwanski M and Grzybowska. 2017. Effect of environmental factors on occurrence of cockchafer (*Melolontha* spp.) in forest stands. *Baltic Forestry* 23(2): 334-341
- Novotny V, Basset Y, Miller SE, Drozd P and Cizek L. 2002. Host specialization of leaf chewing insects in a New Guinea rainforest. *The Journal of Animal Ecology* 71: 400-412
- Nowinszky L, Petranyi G and Puskas J. 2010. The relationship between lunar phases and the emergence of the adult brood of insects. *Applied Ecology and Environmental Research* 8(1): 51-62
- Nowinszky L, Puskas J and Kiss M. 2017. Light Trapping of Coleoptera, Lepidoptera and Heteroptera species in relation to the altitude of the tropopause. *Global Journal of Research and Review* 4(2): 1-4
- Nuri, Ali HF, Ghanadamuz F, Fazal SM and Morteza. 2010. The new method for controlling white grubs, *Polyphylla olivieri* Cast in ornamental plants. *Qazvin Agricultural and Natural Resources Research Center*, p 43
- Oltean I, Bexa A, Florian T and Varga MI. 2015. Monitoring the Species *Melolontha melolontha* L. in the nursery of Lunca Bradului Forestry Department. *Bulletin UASVM Horticulture* 72(1): 237-238
- Pathania M, Chandel RS, Verma KS and Mehta PK. 2015. Diversity and population dynamics of phytophagous scarabaeid beetles (Coleoptera: Scarabaeidae) in different landscapes of Himachal Pradesh, India. *Arthropods* 4(2): 46-68
- Pathania M. 2014. Studies on phytophagous white grubs of Himachal Pradesh. Ph.D Thesis, p 258. Chaudhary Sarvan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur, Himachal Pradesh, India

- Patil AS and Hapase DG. 1991. Biology and chemical control of *Leucopholis lepidophora* (Coloeoptera: Scarabaeidae) in sugarcane. In: Advance in management and conservation of soil fauna (Veeresh GK, Rajagopal C and Viraktamah A, eds), Oxford and IBH Publisher, pp 209-215
- Perju T, Bobarnac B, Costescu C, Duvlea S, Filipescu C, Ghizdavu I and Pasol P. 1983. *Entomologie agricola (Agricultural entomology)*. Edit. Didactica si Pedagogica, Bucuresti
- Potter DA, Patterson CG and Redmond CT. 1992. Influence of turf grass species and tall fescue endophyte on feeding ecology of Japanese beetle and southern masked chafer grubs (Coleoptera: Scarabaeidae). *Journal of Economic Entomology* 85: 900-909
- Pozenel A, Rot M, Zezlina I, Cuk J and Carlevaris B. 2015. The efficacy of soil insecticides against the common cockchafer (*Melolontha melolontha* L.) grubs. Conference paper presented on Zbornik predavanj in referatov 12. Slovenskega posvetovanja o varstvu rastlin z mednarodno udeležbo, 3-4 marec 2015, Ptuj, Slovenija, pp 147-151
- Pozenel A. 2005. A great increase of population of common cockchafer (*Melolontha melolontha* L.) on Idrija region. Conference paper presented on Zbornik predavanj in referatov, Slovensko posvetovanje o varstvu rastlin, 8-10 marec, Slovenija, pp 476-478
- Price DL. 2004. Species diversity and seasonal abundance of scarabaeid dung beetles (Coleoptera: Scarabaeidae, Geotrupidae and Trogidae) attracted to cow dung in central New Jersey. *Journal of New York Entomology Society* 112: 334-347
- Puskas J and Nowinszky L. 2011a. Light trap catch of harmful insects in connection with the height of tropopause. *Advances in Bio Research* 2(2): 101-103
- Puskas J and Nowinszky L. 2011b. Light-trap catch of the common cockchafer (*Melolontha melolontha* L.) depending on the atmospheric ozone concentration. *Acta Silvatica & Lignaria Hungarica* 7: 147–150
- Qiu B, Chen Y, Liu L, Peng W, Li X, Ahmed MZ, Mathur V, Du Y and Ren S. 2009. Identification of three major *Bemisia tabaci* biotypes in China based on morphological and DNA polymorphisms. *Progress in Natural Science* 19: 713-718

- Raina RH and Khan ZH. 2007. Studies on Seasonal activity of insect pests associated with high altitude agriculture, horticulture and forestry ecosystems of Kashmir Himalaya by utilizing fluorescent light trap. *Asian Journal of Biological Sciences* 3(1): 195-203
- Ratcliffe BC and Jameson ML. 2004. The revised classification for Scarabaeoidea: what the hell is going on. *Scarabs* 15: 3-10
- Regnier R. 1950. Eighth International Congress of Entomology, pp 672-678
- Reinecke A, Ruther J, Mayer CJ and Hilker M. 2006. Optimized trap lure for male *Melolontha* cockchafers. *Journal of Applied Entomology* 130(3): 171–176
- Rishi ND. 1981. Insect pests of poplars in Kashmir Valley. In: Symposium on Silviculture, Management and Utilization of Poplars, Srinagar, 15-18 October 1979. Proceedings, Shimla, Government of India Press, pp 123-125
- Ritcher PO. 1958. Biology of Scarabaeidae. *Annual review of Entomology* 3: 311-334
- Ritcher PO. 1966. White grubs and their allies. Oregon State University. Press, Corvallis, p 219
- Rodstrom RA and Brown JJ. 2017. Tenlined June beetle: Insect pest management in hybrid poplars series. Department of Entomology, Washington State University, p 5
- Rosca I, Oltean I, Mitrea I, Talmaciu M and Petanec D. 2011. Tratat de entomologie generala si special (General and special entomology treaty). Edit. Alpha MDN, Buzau
- Sambrook J, Fritsch EF, and Maniatis T. 1989. Molecular cloning: a laboratory manual. New York: Cold spring harbor laboratory press, p 79
- Sawada H. 1991. Morphological and Phylogenetical Study on the Larvae of Pleurostict Lamellicornia in Japan. Tokyo University of Agriculture Press, Tokyo
- Scheerpelz O. 1950. Der Maikafer. Verl. A, Ziemsen, Wittenberg, Lutherstadt
- Scheifinger H, Matulla C, Cate P, Kahrer A and Koch E. 2007. CLIMPHEN-Climate impact on plant and insect phenology in Austria. Project funded by the Global Change Programme of the Austrian Academy of Sciences, final report. http://hw.oeaw.ac.at/0xc1aa500d_0x00167059.pdf.

- Schmidt M and Hurling R. 2014. A spatially-explicit count data regression for modeling the density of forest cockchafer (*Melolontha hippocastani*) larvae in the Hessian Ried (Germany). <http://www.forestecosyst.com/content/1/1/19> 19(1)
- Schnaider Z and Karlikowski T. 1953. Z badan nad chemicznym zwalczaniem pędraków. *Roczniki Nauk Lesnych* 4: 89-107
- Schwenke W. 1978. Die Forstschadlinge Europas, 3. Band: Schmetterlinge. Hamburg: Paul Parey
- Schwerdtfeger F. 1944. Die Waldkrankheiten. Paul Parey, Berlin, Germany, p 479
- Sehnal R and Bezdek A. 2011. Review of the subgenus *Polyphylla* (*Granida*) from continental Asia (Coleoptera, Scarabaeidae, Melolonthinae). *Zookeys* 102: 65-76
- Sezen K, Demir Y and Demirbag Z. 2007. Identification and pathogenicity of entomopathogenic bacteria from common cockchafer, *Melolontha melolontha* (Coleoptera: Scarabaeidae). *New Zealand Journal of Crop and Horticultural Science* 35(1): 79-85
- Shah NK and Garg DK. 1988. Seasonal abundance of white grub beetles on a light trap. *Indian Journal of Ecology* 15(1): 105-108
- Shah NK and Shah L. 1990. Bionomics of *Holotrichia longipennis* BL. (Coleoptera: Melolonthinae) in western Himalayas. *Indian Journal of Forestry* 13(3): 234-237
- Sheikh AH, Thomas M, Bhandari R and Bunkar K. 2016. Light trap and insect sampling: an overview. *International Journal of Current Research* 8(11): 40868-40873
- Shimoda M and Honda K. 2013. Insect reactions to light and its applications to pest management. *Applied Entomology and Zoology* DOI 10.1007/s13355-013-0219-x
- Sierpinski Z. 1963. Jesienne zabezpieczanie upraw przed pędrakami za pomocą insektycydów. *Prace IBL* 266: 52-62
- Sierpinski Z. 1975. Wazniejsze owady-szkodniki korzeni drzew i krzewow lesnych. Panstwowe Wydawnictwo Rolnicze i Lesne, Warszawa, Polska
- Singh P and Singh S. 1986. Insect pests and diseases of poplars. Dehradun, Forest Research Institute, p 74
- Singh V and Banyal HS. 2013. Insect Fauna of Khajjiar Lake of Chamba District, Himachal Pradesh, India. *Pakistan Journal of Zoology* 45(4): 1053-1061

- Sipek P and Ahrens D. 2011. Inferring larval taxonomy and morphology in *Maladera* species (Coleoptera: Scarabaeidae: Sericini) using DNA taxonomy tools. *Systematic Entomology* 36: 628-643
- Slater FM. 2001. Catches of the cockchafer, *Melolontha melolontha* (Linnaeus 1758) (Cloeoptera: Scarabaeidae), at a moth trap in mid-Wales. *Entomologist's Gazette* 52: 189-193
- Sliwa E. 1993. Szkodniki korzeni drzew i krzewow. Warsaw, Poland: Wydawnictwo Swiat (in Polish)
- Sood P, Chandel RS, Mehta PK and Choudhary A. 2010. Diversity of soil insects in kidney bean ecosystem and their management in north-western Himalayas. *Journal of Food Legumes* 23 (1): 66-70.
- Sood P, Mehta PK, Chandel RS and Chandel YS. 2009. Population dynamics of white grubs in the off season pea ecosystem and their management under temperate region of north-western Himalaya. *Journal of Insect Science* 22(4): 370-376
- Sreedevi K and Sakshi T. 2016. Notes on male genitalia and endophallus of five predominant species of *Holotrichia* (Coleoptera: Scarabaeidae: Melolonthinae). *Current Biotica* 9 (4): 328–339.
- Sreedevi K and Tyagi S. 2013 Diagnostic characters of immature stages of flower chafer beetle, *Chiloloba acuta* (Wiedemann) (Coleoptera: Scarabaeidae: Cetoniinae): Taxonomic importance. *Pest Management in Horticultural Ecosystems* 19(2): 185-190
- Sreedevi K, Chandel RS, Pthania M and Stanley J. 2014. Species distribution and larval diagnostic characters of white grub species, *Holotrichia longipennis* (Blanchard), *Holotrichia sikkimensis* (Brenske) and *Holotrichia rosettae* Frey. *Current Biotica* 8(2): 151-156
- Sreedevi K, Tyagi S and Sharma V. 2017. Species diversity of white grubs (Coleoptera: Scarabaeidae) in the sub-Himalayan and northern plains of India. *Current Science* 113(2): 322-329
- Sreedevi S, Tyagi S and Ramamurthy VV. 2015. The morphology of twelve species of melolonthinae and rutelinae (Coleoptera: Scarabaeidae). *The Coleopterist Bulletin* 69(3): 426-434

- Stebbing EP. 1914. Indian forest insects of economic importance, coleoptera 16: 648
- Steenwyk VRA, and Rough D. 1989. Biology of the Tenlined June beetle (Coleoptera: Scarabaeidae). *Journal of Economic Entomology* 82: 1738–1742
- Strasser H. 2004. Biocontrol of important soil-dwelling pests by improving the efficacy of insect pathogenic fungi. *Laimburg Journal* 1: 236-241
- Sukovata L, Jaworski T, Karolewski P and Kolk A. 2015. The performance of *Melolontha* grubs on the roots of various plant species. *Turkish Journal of Agriculture and Forestry* 39: 107-116
- Sushil SN, Mohan M, Selvakumar G and Bhatt JC. 2006. Relative abundance and host preference of white grubs (Coleoptera: Scarabaeidae) in Kumaon hills of Indian Himalayas. *Indian Journal of Agriculture Sciences* 76(5): 338-339
- Svestka M and Drapela K. 2009. The effect of environmental conditions on the abundance of grubs of the cockchafer (*Melolontha hippocastani* F.) *Journal of Forest Science* 55(7): 330-338
- Svestka M. 2006. Distribution of tribes of cockchafers of the genus *Melolontha* in forests of the Czech Republic and the dependence of their swarming on temperature. *Journal of Forest Science* 52(11): 520–530
- Svestka M. 2007. Ecological conditions influencing the localization of egg-laying by females of the cockchafer (*Melolontha hippocastani* F.) *Journal of Forest Science* 53 (Special Issue): 16–24
- Svestka M. 2010. Changes in the abundance of *Melolontha hippocastani* Fabr. and *Melolontha melolontha* (L.) (Coleoptera: Scarabeidae) in the Czech Republic in the period 2003–2009. *Journal of Forest Science* 56(9): 417-428
- Swamy HMM, Ramasamy A, Kalleshwaraswamy CM, and Adarsha SK. 2019. Arecanut white grubs *Leucopholis* species (Melolonthinae: Scarabaeidae: Coleoptera) morphological, molecular identification and phylogenetic analysis. *Journal of Asia-Pacific Entomology* 22: 880-888
- Szczepanska K. 1972. Effectiveness of new soil pesticides applied against larvae of the cockchafer (*Melolontha melolontha* L.) and wireworms (*Agriotes* sp.). *Biuletyn Instytutu Ochrony Roslin* 54: 129-141

- Szentkiralyi F. 2002. Fifty year long insect survey in Hungary: T. Jermys contribution to light trapping. *Acta zoologica acadamaiae scientarum Huneraicae* 48: 85-105
- Szmidla H, Malecka M, Tkaczyk M, Tarwacki G and Sierota Z. 2019. The Spring Assessing Method of the Threat of *Melolontha* spp. grubs for Scots Pine Plantations. *Forests* 10(5): doi: 10.3390/f10050399
- Tadic M. 1976. Some experiences in work with light-traps. *Zastita Bilja* 27(135): 37-45
- Tartanus M, Malusa E, Labanowska BH, Tkaczuk C, Kowalczyk W, Canfora L, Pinzari F and Chalanska A. 2017. Utilization of non-chemical (mechanical and physical) methods to control soil-borne pests in organic strawberry plantations. *Journal of Research and Applications in Agricultural Engineering* 62: 182–185
- Tereba A and Niemczyk M. 2017. Variability and cross-species amplification of microsatellite loci in *melolontha melolontha* and *melolontha hippocastani* (Coleoptera, Scarabaeidae). *Entomological News* 127(3): 192-197
- Tereba A and Niemczyk M. 2018. A quick PCR-based method for identification of *Melolontha Melolontha* and *Melolontha hippocastani*. *Entomologica Fennica* 29: 141-145
- Thiem H. 1944. *Deutsch Ges Allgem Angew Entomol* 5: 77-95
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F and Higgins DG. 1997. The Clustal X window interface: flexible strategies for multiple sequence alignment aided by quality and analysis tools. *Nucleic Acids Research* 24: 4876-4882
- Toth J. 1998. Damage caused by cockchafer (*Melolontha* spp.) in Hungary during the last 30 years. In: Population Dynamics, Impacts, and Integrated Management of Forest Defoliating Insects (ML McManus and AM Liebhold, eds) USDA Forest Service General Technical Report, Forest Research Institute, Frankel Budapest, Hungary, p 341
- Tripathi GM and Gupta BP. 1985. Studies on collection of beetles at light trap at Chaubattia in Uttar Pradesh hills. *Progressive Horticulture* 17(4): 347-353
- Trotus E, Naie M and Buburuz AA. 2013. Research on the flight evolution of *Melolontha melolontha* L. (Coleoptera, Scarabaeidae) species in the Central of Moldavia conditions, Romania. *Cercetari Agronomice in Moldova* 46(2): 65-71

- Tsai DS. 1965. Studies on the life history of the large chestnut chafer *Melolontha* L. *Acta Entomologica Sinica* 3: 274-284
- Turkmenoglu H. 1967. Investigations on the June beetle, *Polyphylla fullo* turkmenoglui Petr. *Turkish Bulletin of Plant Protection* 7: 67-77
- Varga MI, Oltean I, Muntean V, Florian T and Mandru M. 2014. Monitoring the species *Melolontha melolontha* L. in the Nursery of Gurghiu Forestry High School. *Bulletin UASVM Horticulture* 71(1): 128-129
- Veeresh GK, Vijayendra M, Reddy NV and Rajanna C. 1982. Bioecology and management of areca white grubs (*Leucopholis* spp.) (Coleoptera: Scarabaeidae: Melolonthinae). *Journal of Soil Biology and Ecology* 2(2): 78-86
- Veeresh GK. 1977. Studies on root grubs in Karnataka with special reference to bionomics and control of *Holotrichia serrata* F. (Coleoptera: Melolonthinae). *UAS Monograph Series No. 2*, Bangalore, p 87
- Vishwanath. 1979. Bionomics and control of whitegrub *Holotrichia consanguinea* In: National Symposium on Bionomics and control of whitegrub, CS Azad University of Agriculture and Technology, Kanpur
- Vlug HJ. 1996. Occurrence and biocontrol of grass grubs, especially of *Melolontha melolontha*. *Bulletin OILBSROP* 19(2): 35-36
- Vogel W. 1950. Eibildung und Embryonalentwicklung von *Melolontha vulgaris* F. und ihre Auswertung für die chemische Maikäferbekämpfung. *Zeitschrift für Angewandte Entomologie* 31: 537-582
- Vogel W. 1950. Eibildung und Embryonalentwicklung von *Melolontha vulgaris* F. und ihre Auswertung für die chemische Maikäferbekämpfung. *Zeitschrift für Angewandte Entomologie* 31: 537-582
- Wagenhoff E, Blum R and Delb H. 2014. Spring phenology of cockchafer, *Melolontha* spp. (Coleoptera: Scarabaeidae), in forests of south-western Germany: results of a 3-year survey on adult emergence, swarming flights, and oogenesis from 2009 to 2011. *Journal of Forest Science* 60(4): 154-165
- Weissteiner S, Huetteroth W, Kollmann M, Weibbecker B, Romani R, Schachtner J, Schutz S. 2012. Cockchafer larvae smell host root scents in soil. *PLoS ONE* 7(10): e45827

- Wille H and Wildbolz T. 1953. Beobachtungen über die Eiablage des Maikafers und die Entwicklung des Engerlings im Laboratorium. Mitt. Schweiz, ent. Ges., Schaffhausen, Bern, Lausanne 26: 219-224
- Woodruff RE. 1978. Foreign chafers: *Melolontha melolontha* (Coleoptera: Scarabaeidae). Entomology circular No. 189, Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, Florida
- Woreta D and Malinowski H. 1998. Activity of several contact insecticides against selected forest insect pests in Poland. In: Proceedings: Population Dynamics, Impacts, and Integrated Management of Forest Defoliating Insects (McManus ML and Liebhold AM, eds), USDA Forest Service General Technical Report NE-247 p 317-321
- Woreta D, Lipinski S and Wolski R. 2016. Effects of food source quality on the adults of *Melolontha melolontha* and *M. hippocastani*. *Forest Research Papers* 77 (1): 14–23
- Woreta D, Wolski R, Lipinski S and Tkaczyk M. 2018. Effects of food quality on *Melolontha* spp. adults. *Folia Forestalia Polonica* 60(2): 108–121
- Woreta D. 2015. Control of cockchafer *Melolontha* spp. grubs-a review of methods. *Folia Forestalia Polonica, series A* 57 (1): 33-41
- Yadava CPS and Saxena RC. 1977. Bionomics of *Holotrichia consanguinea* Blanchard. *Indian Journal of Agricultural Sciences* 47: 139-42
- Yakir DB, Koren L and Harari AR. 1996. Biometric discrimination of the larval instars and sexes of *Maladera matrida* (Coleoptera: Scarabaeidae: Melolonthinae). *Israel Journal of Entomology* 30: 47-52
- Yanfang M, Zongmou X and Yongqiang Z. 2011. Spatial distribution patterns of *Melolontha hippocastani* larvae. *AGRIS* 35(4): 140-142
- Yan-fang MA, Zong-mou XIE, Yan-lin GUO, Yong-qiang ZHANG, Wei TIAN, Lian-xiao GAO and Hui-ying ZHU. 2010. Comparison test of two application methods in controlling *Melolontha hippocastani mongolica* larvae. *Journal of Gansu Forestry Science and Technology* 35(4)

- Young RM. 1988. A monograph of the genus *Polyphylla* Harris in America North of Mexico (Coleoptera: Scarabaeidae: Melolonthinae). *Bulletin of the University of Nebraska State Museum*, p 106
- Zivanovic V. 1974. The status of the population of the common cockchafer (*Melolontha melolontha* L.) in western Serbia during the flight year 1974. *Acta Entomologica Jugoslavica* 11(1/2): 93-100

Brief Biodata of Student

Name : Abhishek Rana
 Father's Name : Sh. Kamlesh Kumar
 Mother's Name : Smt. Daya Devi
 Date of Birth : 13-12-1992
 Permanent Address : Abhishek Rana s/o Sh. Kamlesh kumar V.P.O. Suan, Tehsil
 Jaisinghpur, District Kangra, Himachal Pradesh-176094

Academic Qualification

Qualification	Year	School/Board/University	Marks (%)	Division	Major Subject
Matriculation (10 th)	2008	HPBOSE, Dharamshala	77.86	First	English, Hindi, Science, Math, Social Science, Sanskrit, Art
Plus two (12 th)	2010	HPBOSE, Dharamshala	78.80	First	Non-Medical
B.Sc. (Agriculture)	2014	CSK HPKV, Palampur	77.10	First	Agriculture
M.Sc. (Agricultural Entomology)	2016	CSK HPKV, Palampur	83.90	First	Agricultural Entomology
PhD. (Agricultural Entomology)	2021	CSK HPKV, Palampur	81.20	First	Agricultural Entomology

Title of M.Sc. Thesis: Effect of dazomet and metham sodium on soil biota under polyhouse conditions

Fellowships/ Scholarships/ Awards: Merit Scholarship during Ph.D.

Publications

Research/review papers : 10
 Popular articles : 11
 Manuals : 03
 Extension/Technical bulletins : 04