Differentiating Callosobruchus of South India with Special Reference to Callosobruchus maculatus - A Useful Guide for Entomologists and Non-Entomologists

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Abstract

Losses of seed produce in pulses due to seed beetles is a very serious problem faced by farmers and traders during storage. Resistance (or susceptibility) and the degree of damage in legume seeds differ with respect to different bruchid species. The seed beetles, *Callosobruchus chinensis* and *C. maculatus*, are the two most widespread bruchid species in Southern India, which is being potentially misidentified. With this perspective, fundamental studies on their morphology are of great importance for correct identification, species specific resistant variety improvement and management. For a reliable and effective resistance evaluation in breeding program, a known, identified species or biotype must be employed. In this study, dissection of striking external morphological characters of both the species was done for comparisons. Type of antennae and hind femur are two common characters used for distinguishing these allied species. However, the most reliable genus and species indicators are variation in the spines on the underside of hind femur. The possible distinguishing characters of these two widespread species are illustrated in light of previously published literatures including other rare *Callosobruchus* species found in India *viz. C. nigritus* and *C. orientalis*, reported from Manipur, India. Furthermore, Scanning Electron Microscope study of South Indian *C. maculatus* strain revealed the presence of different types of sensilla on mouthparts and antennal regions, which had different functions. It is expected that this paper will provide practical information to both entomologists and non-entomologists working on bruchids especially plant breeders involved in resistant varietal development in the tropics and sub-tropical countries.

Key Messages

- *Callosobruchus maculatus* is more destructive and dominating than *C. chinensis* and other *Callosobruchus* species in South Indian region.
- Variations in the spines present on the underside of hind femur is the most reliable identifying characteristics between *Callosobruchus* species
- Under laboratory conditions, *C. maculatus* occurs in two forms, active and sedentary forms, which may resemble *C. analis*, thus can be misidentified
- Different types of sensilla are more prominent in the mouthparts when compared to antennal region in *Callosobruchus maculatus* adults

Introduction

Seed beetles in the genus *Callosobruchus* are tropical and sub-tropical agricultural insect pests (Southgate 1979) causing severe damage in different legumes mostly during storage. Important *Callosobruchus* species commonly found in India comprises of *C. maculatus* (Fabricius), *C. chinensis* (Linnaeus) and *C. analis* (Fabricius) (Raina 1970). Initial infestation starts from the field with adults laying eggs on mature pods and secondary infestation continuing in storage conditions after the crops are harvested, which accounts for the huge grain loss sometimes reaching up to 99% within six months (Seck 1993). Ovipositon occurs on the surface of seeds i.e. on seed coat and larva(e) burrows directly from egg(s) into the cotyledon through seed coat penetration after 5–6 days (incubation period). Under favourable environmental conditions of 25–30 degree Celsius temperature and 65–70% relative humidity, pupation takes place inside the seeds and emergence of reproductively matured adult beetle(s) occurs within 25–35 days depending on host seeds (CABI 2014a). The minimum developmental period for *C. maculatus* and *C. chinensis* is about 21 days and 22–23 days respectively on susceptible host seeds (CABI 2014a; CABI 2014b). Under these conditions, adults mature within 24 hours after emergence and have an average longevity of 12–14 days during which time mating and oviposition takes place. In case of *C. chinensis*, more than one adult usually emerges from a single seed, whereas only one adult beetle emergence is observed (e.g. from mungbean or urd bean seeds) for South Indian *C. maculatus* biotype (Mitchell 1991; Seram et al. 2016b). The entire life cycle can be successfully completed without the provision of any food source or water other than the dried beans for oviposition alone. Adults will readily mate and the lifetime fecundity of C. *maculatus* females is more than that of *C. chinensis*, ranging from 30–115 eggs compared to 20–65 eggs in laboratory cultures (CABI 2014a; CABI 2014b; Seram et al. 2019). The laboratory conditions provide an environment similar to the natural environment that these beetles would experience. This unusual similarity between the natural and laboratory environments is a consequence of *C. maculatus* naturally infesting stored bean products (Credland 1994; Credland and Dendy 1998).

Only around 20 species in the family Bruchidae are commonly encountered as pests of the stored legume seeds which are cultivated and consumed by human (Johnson & Kistler 1987). Infestation during storage due to bean beetles (*Callosobruchus chinensis* L. and *C. maculatus* Fab.) occurs mainly in *Vigna* species such as *V. unguiculata*, *V. radiata, V. mungo*, *V. angularis* (not common in India), etc. and the infestation level of a particular bruchid species differs with respect to *Vigna* crops. The post-harvest damage to *V. radiata* seeds can reach up to 100% loss (Zhang et al. 2002). Host plant resistance (HPR) is an integral part of IPM programs, and is considered by some to be the foundation of IPM strategies (Smith 2005). HPR is recognized as a cost-effective, environmentally friendly method that has been deployed by breeders for controlling different insect pests. Apart from the use of resistant crop varieties and their subsequent improvements through both conventional and molecular techniques, another alternative method of managing this insect include seed treatment with diatomaceous earth formulations (Badii et al. 2014), which was reported to show resistance towards *C. maculatus* infestation, with reduced female oviposition, adult emergence, and seed weight loss.

For the last few decades, development of mungbean varieties resistant to major diseases such as mungbean yellow mosaic virus (MYMV) disease and pests such as bruchids continues to be important breeding priorities but with little or no success. Generally, insect biological parameters such as egg number, hatching percentage, time and number of adult emergence, larval weight, and larval mortality have been used in order to compare seed resistance and susceptibility against *C. maculatus* (Lale and Efeovbokhan 1991; Crux et al. 2016; Badii et al. 2013; Seram et al. 2016a and b). These factors, however, can vary depending on the insect species (Yadav and Pant 1974; Srinivasan et al. 2008), origin of the insect populations (Appleby and Credland 2003; Seram et al. 2016b), strain (Credland and Dick 1987) and the different forms of a particular species (Utida 1954; Southgate et al. 1957). Varieties with resistance derived from different sources will be required to combat the emergence or evolution of different species, strains or biotypes of important pests like bruchids. The two *Callosobruchus* species are quite common as to which *Vigna* species they infest and cultivars of different pulse seeds vary considerably in their susceptibility to these bruchid attacks (Yadav and Pant 1974). For example, both bruchids are able to feed on *V. radiata* and *V. unguiculata* but rarely on cultivated *V. umbellata* (Tomooka et al. 2000). However, differences in *Vigna* host specific to the bruchid exist, e.g. *C. chinensis* fails to feed on urd bean, while *C.*

maculatus can (Srinives et al. 2007). In addition, a reliable bioassay for bruchid resistance evaluation must always employ a known, identified species, biotype or strain of the insect (Credland 1994). Out of the two *Callosobruchus* species, *C. maculatus* is the most widely distributed one, occurring in Africa, Asia, and Australia (Daglish et al. 1993). In India, it is widespread (Vir and Jindal 1981) and the most predominant *Callosobruchus* species infesting various legume seeds in Southern states (Srinivasan and Durairaj 2007) including Tamil Nadu, where the present study was carried out.

Due to the lack of adequate published reports and scattered literatures on the systematics of *Callosobruchus*, there have been incorrect identifications of important species in the past, when. Advanced studies on the species differentiation of bruchid beetles have been analyzed through mitochondrial DNA polymorphism by Tuda et al. (1995). They were able to differentiate four bruchid species *viz. Callosobruchus chinensis, C. rhodesianus, C. phaseoli* and *Zabrotes subfasciatus* based on variations in the pattern of a mitochondrial DNA region including cytochrome oxidase I and II genes using PCR based RFLP markers. However, molecular level species identification requires expertise and takes time, thus making it a challenging task for the entomologists and plant breeders in differentiating the *Callosobruchus* species during pulse varietal improvement programmes. There are no reports of the development and availability of species specific markers which can be used in molecular characterization except for RAPD markers. RAPD markers have been employed for the species differentiation between *C. maculatus* and *C. chinensis* (Emam et al. 2013).

With this prospect in mind, an attempt was made to differentiate noticeable characters between the potentially misidentified species commonly found in Southern India (especially Tamil Nadu), *C. maculatus* (Vir and Jindal 1981) and *C. chinensis* (Haines 1981; Khandwe et al. 1997) with an aim to clear the doubts and difficulties faced during species identification by those researchers (including the first author during the initial research period) working on bruchid related research, except taxonomic studies, such as management, host plant resistance evaluation, inheritance of seed resistance studies, identification of molecular markers linked with the resistance, QTL mapping of the resistance gene in different pulses, etc. (Young et al. 1993; Miyagi et al. 2004; Somta et al. 2006b; Sun et al. 2008; Somta et al. 2008; Souframanien et al. 2010; Pavithravani 2012; Pavithravani et al. 2015; Chotechung et al. 2016). QTL mapping for bruchid resistance have been extensively investigated in recent years by several workers to develop markers for marker assisted selection (MAS) strategies for crop improvement and several QTLs have been identified in mungbean and other *Vigna* species that contribute to bruchid resistance (Kaga and Ishimoto 1998; Somta et al. 2006b; Mei et al. 2007; Somta et al. 2008b; Chotechung et al. 2016; Mariyammal et al. 2019). Comparisons between certain striking morphological characters of these two bruchid species which will be useful in better and easy identification are made in regard to some of the original descriptions and subsequent published literatures, including those of the other rare *Callosobruchus* species found in India *viz. C. nigritus* and *C. orientalis*, reported only from Tamenglong district of Manipur by Bhubaneshwari and Victoria (2014a and 2014b). This paper gives a brief overview of the morphological differences between commonly found and studied *Callosobruchus* species, which will particularly be useful in their identification to non-entomologists, mostly working on development of bruchid resistant pulse varieties, especially in the tropics and sub-tropical countries.

Materials And Methods

Insect source and maintenance

The bruchid specimens under study were obtained from separate locations (**Table 1**) and rearing was done on bruchid susceptible mungbean seeds (var. Co6) inside an incubator (30±2°C and 70% RH) at Insect Pheromone Research Laboratory, Department of Agricultural Entomology, TNAU, Coimbatore by following the procedure of Strong et al. (1968). Soybean and pigeon pea seeds infested with *C. chinensis* were collected and stored in the laboratory for dissection.

Table.1 Details of bruchid (Callosobruchus **spp.) specimen under study**

Bruchid Insect	Location	Host seed	Nature of sample
C. maculatus	1) IICPT, Thanjavur, Tamil Nadu (TN)	*Vigna radiata*	Laboratory culture
	2) DSST, TNAU, Coimbatore, TN	*Vigna mungo*	Laboratory culture
C. chinensis	1) Imphal, Manipur	*Glycine max*	Local Market
	2) D of T, TNAU, Coimbatore, TN	*Pisum sativum*	Laboratory culture
IICPT - Indian Institute of Crop Processing Technology, DSST - Department of Seed Science and Technology, TNAU – Tamil Nadu Agricultural University, D of T – Department of Toxicology; TN – Tamil Nadu, India			

Dissection and examination of *Callosobruchus* body parts for identification

Ten adult bruchid beetles from each species (*C. maculatus* and *C. chinensis*) were isolated from the main cultures and preserved in separate vials containing 10% KOH solution for dissection. External morphological parts including male genitalia (aedaegus) were dissected under a stereo zoom microscope. The external genitalia were extracted from the abdomen by gently tearing the inter-segmental membranes from and around the organ by using fine forceps and micro needles. After careful cleaning, the genitalia were stained with acid fuschin dissolved in acetic acid and transferred to carbol-xylol for clearing (Carbolic acid and xylene 2:3) and mounted in Canada balsam. The same clearing, staining and mounting process was done for the other insect body parts. Identification and fixing of identity for major characters was done with the help of available literatures. Permanent slides of the hind legs, forewings, antennae,

pygidium and male genitalia of both the species were prepared following the procedure described by Dobie et al. (1984) and microphotographs were taken with the help of Leica Microscope at the Insect Biosystematics Laboratory, TNAU, Coimbatore.

Measurement of *Callosobruchus* spp. adult size

Morphometric measurement of adults (♀ and ♂) and mature larvae of the two *Callosobruchus* species were made with the help of digital vernier calipers with ten replications each. 2–3 days old bruchids were immobilized at low temperatures for taking measurements. At about 20 days after insect infestation (release), seeds with mature larvae developing inside were carefully cut open and larval measurements were taken. Measurement of adult weight was recorded according to Vir (1983) and Srinivasan (2005). This was recorded by weighing a known number of 0–24 hrs old adult beetles in an electronic top balance. Values were expressed as weight in milligram (mg) / single bruchid adult.

Damage potential of *Callosobruchus maculatus* and *C. chinensis*

In order to determine the damage potential between the two species, bioassays with “no-choice” test method described by Seck (1993) and Giga (1995) were conducted on a bruchid susceptible Indian mungbean variety “Co-8” obtained from the Department of Pulses, TNAU, Coimbatore. Fifty healthy seeds of mungbean were separately infested for three days (for oviposition) with one pair each of freshly emerged *C. maculatus* and *C. chinensis* adults. Experiments were conducted at constant conditions maintained inside an incubator (30 degree Celsius and 60% RH) with three replications. About 24 days after infestation (DAI) when F_1 *C. maculatus* adults (progenies) started emerging, a daily count of emerged bruchid adults was performed until 45 DAI for calculating the mean developmental period (MDP in days). Similar records were taken for the emerging *C. chinensis* adults. Examination on various bruchid parameters were taken according to Badii et al. (2003), Soumia et al. (2015) and Seram et al. (2016a). *C. maculatus* population from Thanjavur and Coimbatore population of *C. chinensis* were used in this study.

SEM analysis of South Indian *Callosobruchus maculatus* strain

Different parts of the South Indian bruchid strain (*C. maculatus*) were examined separately by scanning electron microscope (SEM) to by following the procedure of Joseph et al. (1993) at Department of Nanoscience and Technology (DNST), TNAU, Coimbatore. Bruchid samples preserved in 70% alcohol were pat dried on tissue paper, mounted on aluminium stubs and light gentle press was given. Scanning electron micrographs of some specific regions were taken such as mouthparts, antennae, body surface, etc. (Fig. 3,4,5). Different types of sensilla present on these regions were classified based on Hu et al. (2009), which are presented in **Table 9**. Samples were examined individually in a scanning electron microscope (LEICA, Germany) with LEO software and a magnification of 1000x (most of the samples), operated under 7.00 kV voltage and 60 Pa pressure.

Data Analysis

Data on the adult morphometrics were analysed using descriptive statistics. Analysis of variance (ANOVA) was carried out on the bioassay data in order to determine significant differences on seed infestation of the two bruchid species on susceptible mungbean variety. Means were separated using LSD at 5% level of significance.

Results And Discussion

Taxonomy section

Genus Callosobruchus

The name *Callosobruchus* was proposed by Pic (1902) for a new sub-genus of *Bruchus*. Out of the 58 genera of seed beetles, the genus *Callosobruchus* differs from all other genera of Bruchidae in the presence of sub-apical spines in both external and internal carina of hind femur (Borowiec 1987). A pair of distinct ridges (inner and outer) on the ventral side of each hind femur, and each ridge bearing a tooth near the apical end which was also observed in the present study (**Table 5**; Fig. 1d). Earlier, the species name “*maculatus”* was described by Fabricius (1775) from America and placed it in the genus *Bruchus.* Later, it was changed to the present name, *Callosobruchus maculatus.* The genus *Callosobruchus* can be characterized by the following combination of common characters as described by Borowiec (1987) supporting the findings in the present study:

- Antennae subserrate, serrate or pectinate - consisting of scape, pedicel and 9 segments of flagellomeres (Mbata et al. 1997; Hu et al. 2009; **Fig. 1c**); Frons with sharp median carina
- Pronotum campaniform or conical, without lateral carina; Elytra with or without basal tubercle; Pygidium elongated in females than males (**Fig. 1a**); Hind femur moderately swollen, bicarinate ventrally, both external and internal carina with sub-apical spine or denticle; Hind tibia enlarged, straight, carinate, mucro longer than lateral coronal denticle (**Fig. 1d**);
- Aedaegus - Median lobe more or less elongate, ventral valve triangular, internal sac with or without large sclerites; Lateral lobes depressed, not modified apically, deeply divided (**Fig. 1f**).

Distribution and host range

Different *Callosobruchus* species are distributed in almost all the warm parts of the world, such as African, Asian, Australian countries, as stored pests (Southgate 1958). In new world, these species are present as natural emigrants or introduced (Southgate et al. 1957; Southgate 1958). Tuda et al. (2005) reported the occurrence of five *Callosobruchus* species infesting seeds of wild (or inedible) and cultivated edible legumes from different fields of Taiwan, Thailand, continental China, Myanmar, Nepal and the Philippines. *Callosobruchus* species generally infests crops of the family Fabaceae of Papilionoideae super-family which consists of genera such as *Glycine*, *Pueratria*, *Macrotyloma*, *Shenostylis*, *Dolichos*, *Lablab*, *Vigna*, *Phaseolus*, *Cajanus*, *Vicia*, *Lathyrus*, *Lens*, *Pisum* and *Cicer* (Yadava and Pant 1974; Southgate 1979; Vir and Jindal 1981; Borowiec 1987). In India (Southern part mainly Tamil Nadu), the two major bruchid species, *C. maculatus* and *C. chinenesis*, mainly attack *Vigna* crops *viz*. *V. radiata*, *V. unguiculata*, *V. mungo*, etc. During the present investigation, *C. maculatus* was found to cause more damage than *C. chinensis*, especially on munbean or green gram (**Table 2**). Srinivasan (2005) and Srinivasan and Durairaj (2007) reported that both the *Callosobruchus* species are able to infest almost all the major pulse crops and out of these, *C. maculatus* was predominant over the other species in Coimbatore, Tamil Nadu, where the current study was conducted (Tamil Nadu Agricultural University, Coimbatore). For other hosts, refer **Table 1**.

Table.2 Records on the host plants of different Callosobruchus **species from India**

Callosobruchus species	Host plants	References
C. maculatus Fabricius	Pigeon pea - *Cajanus cajan* (L.) Millsp.	Arora (1977)
(1775)	Chana dal - *Cicer arietinum* Linnaeus	Pavithravani (2013)
	Pea - *Pisum sativum* Linnaeus	Seram et al. (2016a)
	Black gram (urdbean) - Vigna mungo **(L.) Hepper**	
	Green gram (mungbean) - V. radiata **(L.) Wilczek**	
	Cowpea - *V. unguiculata* var. *sesquipedalis* (L.) Ohashi	
	Rice bean - *V. umbellata* (Thunb.) Ohwi and Ohasi	
C. chinensis Linnaeus	**Pigeon pea -** Cajanus cajan **(L.) Millsp.**	Mathur et al. (1958);
(1758)	Chana dal - *Cicer arietinum* Linnaeus	Arora (1977);
	Cyamopsis tetragonoloba (L.) Taubert	Mukherji and Chatterjee (1951);
C. analis Fabricius	Pigeon pea - *Cajanus cajan* (L.) Millsp.	Arora (1977)
(1781)	Chana dal - *Cicer arietinum* Linnaeus	
	Soybean - *Glycine max* (L.) Merr.	
	Vigna aconitifolia (Jacq.) Marechal	
	Green gram - *V. radiata* (L.) Wilczek	
	Cowpea - *V. unguiculata* (L.) Ohash	
C. nigritus Anton (2000)	**Soybean -** Glycine max **(L.) Merr.**	Anton (2000)
		Bhubaneshwari and Victoria 2014(a)
C. orientalis Anton (2000)	**Soybean -** Glycine max **(L.) Merr.**	Anton (2000) Bhubaneshwari and Victoria 2014(b)
C. theobromae	Pigeon pea - *Cajanus cajan* (L.) Millsp.	Mukherji and Chatterje (1951); Vazirani (1975)
Linnaeus		

Only the first records of hosts in India are listed (Tuda et al. 2005 with additional information). All host species belong to Leguminosae. Species relevant to the present study are typed in **bold.**

Damage potential of *Callosobruchus* spp.

Colegrave (1993) observed that even two larvae per mungbean seed would lead to competition and subsequent reduction in the weight of emerging bruchid adults. Reduction in weight of adults that emerged from resistant varieties was also observed by Vir (1983) and Dongre et al. (1996). Most studies on grain legume resistance to this group of insect pests have been undertaken at various international institutes in Asian and South-Asian countries such as China, Japan, Korea, Taiwan, Thailand, India and other warm countries like Australia, Nigeria, etc. to find the natural sources of resistance in various pulse germplasms. Almost all the cultivated legume crops, especially the genus *Vigna*, are prone to heavy damage by these bruchid species (Raina 1970; Dongre et al. 1996; Swella and Mushobozy 2009) on different stored pulses; Nwanze et al. (1975); Giga and Smith (1983); Jackai and Asante (2003) on *Vigna unguiculata*; Ponnusamy et al. (2014); Khattak et al. (1987) on *V. radiata* and *V. mungo*] except *V. umbellata*, which is an underutilized and less cultivated legume (Tomooka et al. 2000; Kashiwaba et al. 2003; Somta et al. 2006a; Seram et al. 2016a). During the present study, the ovipositional behaviour differed significantly on 50 mungbean seeds by the two species (Table 3). This was in corroboration with many studies reported earlier (Yadav and Pant 1974; Giga

and Singh 1983; Kashiwaba et al. 2003; Srinivasan et al. 2008). The damage potential of the two *Callosobruchus* species under study was evaluated by assessing the per cent damage to seeds according to the formula given by Khattak et al. (1987), i.e. [(number of seeds damaged / number of seeds taken) x 100], which was manifested by the presence of round exit hole with the 'flap' of seed coat made by the emerging adults. Mitchell (1991) described one adult emergence per seed for South Indian *C. maculatus* strain which was evident in the present study (Table 3). In case of *C. chinensis*, more than one adult emergence from a single seed was observed (Table 3). Seed damage per cent was significantly higher for *C. maculatus* (92.33%) than *C. chinensis* (701.67%) indicating that the former species is more destructive and dominating than the later. The larval stage is the only feeding stage in case of seed beetles and it is a measure of both the physiological and usefulness of the food and the total amount of food ingested (Hovanitz and Chang 1962). Although mungbean seeds used for assessing damage in this study were highly susceptible with 71.67% (*C. chinensis*) and 92.33% (*C. maculatus*) damage (HS; score 7; Table 3) to both the bruchid species, the mean developmental period (MDP in days) were found to be significantly different for the two species (29.98 days and 24.89 days for *C. chinensis* and *C. maculatus* respectively). A shorter mean developmental time suggests that the insect may complete its life cycle in less time; resulting in more generations per year and thus causing more seed damage (Srinivasan et al. 2008). According to Utida (1953), the difference in the MDP is due to larval stage competition. Similarly, Thanthianga and Mitchell (1990) discovered that larval competition during the early stages of development inside the seeds tends to lengthen the developmental period, which corresponds to the emergence of more than one *C. chinensis* adult from one seed.

The developmental suitability of the food material/genotype is determined on the basis of Growth Index (GI) or Index of Suitability (IS), which is generally regarded as an important parameter of insect growth and development. It is a criterion for comparing the growth responses of insects to different plants (Saxena 1969; Howe 1971). Genotypes with a low GI are considered as resistant and those with a high GI are considered as susceptible by various researchers (Howe 1971; Jackai and Asante 2003; Soumia et al. 2015; Ponnusamy et al. 2014).

Table.3 Damage potential of Callosobruchus chinensis **and** C. maculatus **on mungbean seeds** (susceptible *var*. CO-8)

Callosobruchus Species	Eggs/ 50seeds	Eggs Hatched	% Hatch	Adults emerged	Damaged seeds	Damage %	% Survival	MDP (Days)	IS
C. chinensis	64.34 ± 1.55[a]	44.33 ± 1.14[a]	67.43 ± 1.24[a]	38.33* ± 1.15[a]	36.33* ± 1.53[a]	71.67 ± 3.06[a]	61.58 ± 3.20[a]	29.98 ± 0.39[a]	0.058[a]
C. maculatus	112.67 ± 4.74[b]	98.33 ± 6.12[b]	86.12 ± 1.86[b]	45.67** ± 1.53[b]	46.67** ± 1.53[b]	92.33 ± 3.06[b]	42.92 ± 1.45[b]	24.89 ± 0.07[b]	0.065[b]
Mean	**87.6**	**70.34**	**77.76**	**43.33**	**41.52**	**82.98**	**52.75**	**27.65**	**0.064**
SEd	**2.88**	**3.57**	**1.26**	**1.13**	**1.27**	**2.46**	**2.04**	**0.25**	**0.001**
CD (0.05)	**7.98**	**9.96**	**3.58**	**3.06**	**3.48**	**6.95**	**5.67**	**0.68**	**0.005**

Values are mean of 3 replications (mean ± SD); Different letters in the same column indicate significant differences at 5 per cent level by LSD; MDP – Mean developmental period, IS – Index of Suitability (Growth Index); *More than one *C. chinensis* adults emerged from few individual seeds; **Only one adult emergence per seed for South Indian *C. maculatus* strain (Thanthianga,and Mitchell 1990; Mitchell 1991; present study); Scoring (score 1, 0% - completely resistant; score 3, 1 to 9% - resistant; score 5, 10 to 69% - moderately susceptible; score 7, 70 to 99% - highly susceptible and score 9, 100% - completely susceptible) based on seed damage per cent as per Weigand and Tahhan (1990).

Morphological features for identification

Body dimensions

Adult sexes can be distinguished by means of recognizable morphological differences that can be easily seen with the naked eye. Female *C. maculatus* have dark stripes on each side of the posterior dorsal abdomen that is not found in males. Females are generally bigger in size when compared to males (**Fig. 1a**). In the case of *C. chinensis*, males and females can be differentiated based on the antennal shape, which is described in the following section. Adults of different species have an average mass (body weight) of 2–3 mg to 4–6 mg and an average body length ranging from 2–3 up to 4–6 mm (Bhubaneshwari and Victoria 2014c) which is in similar trend with the current findings on morphometric measurements of both the *Callosobruchus* species which are given below:

Table.4 Morphometric differences between C. chinensis **(L.) and** C. maculatus **(Fab.)**

	C. chinensis (L.)			*C. maculatus* (Fab.)		
Parameters*	**Male adults**	**Female Adults**	**Mature Larvae**	**Male adults**	**Female adults**	**Mature Larvae**
Length (mm)	2.41 ± 0.07	2.52 ± 0.06	2.35 ± 0.05	2.61 ± 0.07	3.93 ± 0.12	2.63 ± 0.07
Breadth (mm)	1.52 ± 0.07	1.83 ± 0.10	1.06 ± 0.09	2.39 ± 0.09	2.53 ± 0.36	1.52 ± 0.07
Weight (mg)	2.23 ± 0.08	2.78 ± 0.07	2.15 ± 0.05	4.87 ± 0.06	5.27 ± 0.37	4.91 ± 0.06
*Values are mean of 10 replications (Mean ± SD) (present study); *C. chinensis* from infested soybean and pigeon pea seeds; *C. maculatus* from infested mungbean seeds, Thanjavur, Tamil Nadu						

Antennae

The type and shape of antennae and hind femur are two common characters that are used to easily distinguish these *Callosobruchus* species. In *C. chinensis*, sexual dimorphism of antennae was observed (**Fig. 1c**). In males, the fourth through apical segments are pectinate to highly pectinate whereas in females, the segments are serrate. Whereas in *C. maculatus* male and female, the antennae are slightly serrate through the apical segments. These differences were observed in the present study (**Table 4**; **Fig. 1c**) which was also in confirmation with Mbata et al. (1997) and Hu et al. (2009) who conducted datailed experiments on *Callosobruchus* antennae and reported similar kind of antennal features. For more in-depth details of antennal and sensillar structures, refer Mbata et al. (1997). Comparisons between other striking external morphological characters observed in the present study are listed and described in **Table 4** which were similar and as per the previous literatures cited.

Table.4 Morphological differences between Callosobruchus chinensis Linnaeus (1758**) and** C. maculatus Fabricius (1775**) adults for easy identification**

Characters	*C. chinensis*	*C. maculatus*	References
1. Body Colour	Elytra are pale brown with small median dark marks and larger posterior dark patches, which may merge to make the entire posterior part of the elytra dark in colour.	Orange-brown with dark markings; Females often have strong markings on elytra consisting of two large lateral dark patches mid-way along the elytra and smaller patches at the anterior and posterior ends, leaving a paler brown cross-shaped area covering the rest whereas males are much less distinctly marked	CABI (2014a and 2014b); PADIL (2012); Southgate et al. (1957); Present study (**Fig. 1b**)
2. Eyes	Emarginate, small, shallow or less bulging	Deeply emarginate, prominent, Bulbous (spherical)	Beck and Blumer (2006, 2014); PADIL (2012); Present study (**Fig. 1a**)
3.Antennae	Pectinate in male, serrate in female	Serrate in both male and female	Mbata et al. (1997); Hu et al. (2009); Present study (**Fig. 1c**)
4.Scutellum (thorax)	Smaller median lobe not extending posterior margin	Median lobes extending well beyond posterior margin	Southgate et al. (1957); PADIL (2012)
5.Elytral Striae	Reddish brown intercepted by black areas in both sexes	♀ Pale brown with intermittent black patches; ♂ Median black patches with white testaceous space in between assuming two C-shaped areas their bases facing each other	Southgate et al. (1957); Present study (**Fig. 1b**)
6.Pronotum and Pygidium	Pygidium of female (and male) covered with white or silver setae	Pronotum of mature adults with black cuticle, and with golden setae, except on the basal median gibbosites; Female pygidium strongly convex at the sides, projecting well beyond elytra and are covered with white scale-like setae	PADIL (2012); Present study (**Fig. 1e**)
7.Abdominal sterna	The side margins of the abdomen have distinct patches of coarse white setae, a feature that is shared with *C. rhodesianus* and *C. theobromae*.	The abdomen is well extended from the elytra in females with distinct spots; whereas the abdomen and elytra are in same length in case of males	CABI (2014a and 2014b); Present study **Figure 1**(a,e)
8.Other Features	- -	A unique chordotonal structure in the fore coxae; also in *C. subinnotatus* The location and ultrastructure of sex pheromone glands in female	Ramaswamy and Monroe (1997) Pierre et al. (1996)

Spines on hind femur

The common characteristics of the genus *Callosobruchus* spp. include the hind femur with spine or denticle on both internal and external ventral margins (Borowiec 1987). Differences in the sub-apical spines or tooth present on hind femur of *Callosobruchus* species found in India are described in the following table:

Table.5 Differences in hind femur of Callosobruchus **Species of India**:

Callosobruchus species	Hind femur Description	References
C. maculatus Fabricius	Inner carina of hind femur smooth; acute tooth on inner carina of ventral edge; Inner tooth triangular and typically as long as (or slightly longer than) the outer tooth (**Fig. 1d**)	Southgate et al. (1957); Borowiec (1987); CABI (2014a); Present study (**Fig. 1d**)
C. chinensis Linnaeus	Inner tooth of hind femur with sides more or less parallel, converging near apex; The inner tooth is slender, rather parallel-sided, and equal to (or slightly longer than) the outer tooth (**Fig. 1d**)	Borowiec (1987); CABI (2014b); Present study (**Fig. 1d**)
C. analis Fabricius	Inner carina of hind femur with numerous irregularly-spaced small denticles (teeth) along its proximal two-thirds; inner tooth rather shorter than, or as long as, outer tooth	Southgate et al. (1957)
C. nigritus Anton	Hind femora with acute, preapical denticle at lateroventral and mesoventral margins, lateroventral denticle distinctly broader and feebly longer than mesoventral denticle	Anton (2000); Bhubaneshwari and Victoria, 2014(a)
C. orientalis Anton	Hind femora with acute, preapical denticle at lateroventral and mesoventral margins; both denticles of same length, lateroventral denticle distinctly broader than mesoventral denticle.	Anton, 2000; Bhubaneshwari and Victoria, 2014(b)

Reproductive apparatus

The structure of male genitalia is one of the most important and distinctive taxonomic characters in seed beetles, both in genus and species level (Mukherji and Chatterjee 1951; Borowiec 1987; Anton 2000). In the family Bruchidae consisting of *Callosobruchus* spp., the parameres are always well developed and often strongly modified (Borowiec 1987). The male copulatory apparatus (aedaegus) consists of median lobe, lateral lobes and their base. Lateral lobes and base are always fused, forming a ring around the median lobe. Similar features were also noticed on the dissected aedaegus from the two bruchid species under investigation (**Table 6**; **Fig. 1f**). The female genitalial part, bursa corpulatrix in both the species is a pear-shaped bag which bears a pair of thin-walled, cup-shaped structures near the middle (**Fig. 1f**), which were also described by Southgate et al. (1957).

Table.6 Differences in male aedaegus of Callosobruchus **species of India**:

Callosobruchus species	Male Aedaegus Description	References
C. maculatus Fabricius	Male genitalia with the median lobe distinctly shorter, ventral valve triangular, and the internal sac subapically with pair of oblong to longitudinal bands of numerous, minute, plate-like sclerites (denticles); parameres rather stout and broadly spatulate	Mukherji and Chatterjee (1951); Borowiec (1987); CABI (2014a); PADIL (2012); Present study (**Fig. 1f**)
C. chinensis Linnaeus	Median lobe more elongate, apex with exophallic valve spearhead-shaped, and base with two sclerotized plates, parameres normal and rather broadly spatulate	Mukherji and Chatterjee (1951); CABI (2014b); Present study (**Fig. 1f**)
C. analis Fabricius	Median lobe without sclerotized areas near its middle; parameres rather slender and only narrowly spatulate	Mukherji and Chatterjee (1951); Southgate et al. (1957)
C. nigritus Anton	Male genitalia with a pair of elongated lateral lobes, tridal from base. Median lobe shorter, more or less spear shaped. Aedaegus length 1.39–1.40 mm	Anton (2000); Bhubaneshwari and Victoria, 2014(a)
C. orientalis Anton	Lateral lobes strongly elongate, completely separated subspatulate, latero-apically and at basal half stronger sclerotized than at remaining parts, apically arcuate; apex with about 18 setae; setae varying in length, two of them long but median lobe is more elongated than two lateral lobes. Aedaegus length 1.51 mm	Anton (2000); Bhubaneshwari and Victoria 2014(b)

Differences between *Callosobruchus maculatus* and *C. analis*

In India, *C. maculatus* has often been misidentified as *C. analis*, which was also reported by Haines (1989). The original descriptions of *C. maculatus* and *C. analis* are based on the general ground colour and on the pattern formed by the pubescence on the two species (Borowiec 1987). Differences between these two similar species were suggested by Southgate et al. (1957). For instance, in freshly emerged adults, the two species can be separated based on the colour patterns which are sufficiently distinct from one another, i.e. the white scales on the body are conspicuous in *C. analis* whereas in *C. maculatus* they are relatively inconspicuous. The disposition of the darker areas of elytral cuticle and the white and golden pubescence in mature and old adults, however, is almost identical in the two species. Hence, their identification has been confused since it is difficult to differentiate these species on the basis of cuticle colour and pattern. Nonetheless, they can be distinguished most reliably by examining the hind femur [**Fig. 2**(iii)], which bears a large spine on its inner carina in *C. maculatus* and only a small one, or even none at all, in *C. analis.* Moreover, in *C. maculatus*, it is possible to separate the sexes on elytral and pygidial markings, but in *C. analis*, this is much more difficult. The elytral markings in both sexes of *C. analis* are more constant and similar [**Fig. 2**(ii)]. Both sexes also have a median line of white pubescence on the pygidium. However, it is possible to separate them by the cuticular colour of the pygidium, which bears a wide median testaceous stripe or V-shaped area in the male and a much narrower one in the female. *C. analis* was not examined in the present study since this species was not found during the survey. Within *C. maculatus* species, occurrence of two different morphs or forms has been reported under laboratory conditions, sedentary and active forms (Utida 1954; Credland and Dick 1987; CABI 2014), which are likely to be misidentified as different species such as *C. analis*. Active form of *C. maculatus* appears to be less fertile (present study, *unpublished*) and is possibly migratory in nature (Southgate et al. 1957). Sedentary (normal) adults are very easy to isolate from the culture container without anaesthesia, however an aspirator is required for separation of the active (quick fliers) form including gender identification (present study). The fecundity of the sedentary (normal) female adults was higher when compared to the active form (present study, *unpublished*). This may be attributed to the non-consumption of stored energy in the sedentary female adults compared to the active female, which is otherwise utilized during flight or movement. Descriptions of these species given by Southgate et al. (1957) along with current findings are listed in the following **Table 7**:

Table.7 Comparisons of the important distinguishing features of Callosobruchus maculatus **(Fab.) and** C. analis **(Fab.)**

Characters	*Callosobruchus maculatus*	*Callosobruchus analis*
Antennae	Subserrate	Not serrate
Eyes	Deeply emarginate, prominent and buldged	Less deeply emarginate, flattened and less prominent
Thorax	Median lobes extending beyond posterior margin	Median lobes extending only slightly beyond posterior margin
Hind femur	Large, acute tooth on inner carina of ventral edge	Tooth small or absent on inner carina of ventral edge
Median lobe of male genitalia	Aedeagus lined with more numbers of heavily chitinised denticles; chitinised area deeply emarginate apically	Aedeagus lined with few denticles; chitinised area slightly rectangular
Descriptions are in accordance with that of Southgate et al. (1957) (Also refer for more details)		

Rare Callosobruchus **species in India**

C. orientalis and *C. nigritus* were reported for the first time from Tamenglong district (24.9833°N, 93.4833°E) of Manipur, North-East India by Bhubaneshwari and Victoria 2014(a) and 2014(b). They were able to distinguish these species from other *Callosobruchus* members by their peculiar male genitalia (aedeagus) following the descriptions on its structure according to Anton (2000). The specimens were also registered under RRS No. 2117CO012/14 at Network Project on Insects Biosystematics (NPIB), Division of Entomology, Indian Agricultural Research Institute (IARI), New Delhi (Bhubaneshwari and Victoria 2014a and 2014b). Table.8 shows the comparisons between the two rare *Callosobruchus* species.

Table.8 Differentiating characters between Callosobruchus nigritus **and** C. orientalis

Species	Diagnosis	Description	Species confirmation
Callosobruchus nigritus Anton (2000)	Short body, oval, 2.26–2.3.mm in length, 1.03–1.04 mm in width, bluish black colour, 1–4 reddish brown antennal segments and greyish colour in remaining segments; a pair of longer lateral lobes and a short median lobe present in male aedaegus	**Males**: Longer, more serrate, or pectinate antennae. Eyes often more bulging. Abdomen strongly telescoped, pygidium vertical. Median lobe elongated, ventral valve triangular, internal sac in many species with strongly sclerotized dental plates. Depressed lateral lobes not modified **Females**: shorter, subserrate or serrate, never pectinate antennae. Eyes less bulging. Abdomen slightly telescoped, pygidium subvertical.	Based on the male aedeagus structure according to Anton (2000)
Callosobruchus orientalis Anton (2000)	Closely related to *C. chinensis*. Both are morphologically similar, but different in elytral striae at fifth apical region without pattern of pale grayish and dark brown setae. Shorter elytra, less bulging eyes and more distance between two eyes. Lateral lobes shorter and male aedeagus with long median lobe	**Males**: Oval shaped small body; Compound eyes with deep 'U'-shaped cleft opening towards the front. 11-segmented antennae, serrate to often pectinate; Morphologically almost similar with *C. chinenesis* in colour but differs in aedeagus having two elongated lateral lobes and longer median lobe.	Based on the male aedeagus structure according to Anton (2000)
Ref.: Anton (2000); Bhubaneshwari and Victoria, 2014(a); Bhubaneshwari and Victoria, 2014(b)			
These two rare species were not examined under the present study; only the available literatures have been cited			

SEM analysis of South Indian *Callosobruchus maculatus* strain

From the present study, scanning electron microscope (SEM) study of *C. maculatus* egg (Fig. 3a) showed a projection on one end and plain surface on its membrane (Fig. 3b) However, Thakur and Kalpna (2015) reported the presence of hexagonal plates on egg membrane of another bruchid species, *Acanthoscelides macrophthalmus* without any protrusion. This indicates variation in the egg morphology, which may be useful in bruchid species identification. Based on SEM observations of antennae and mouthparts (Fig. 4a and d), morphology of *C. maculatus* sensilla were investigated and was found to have three different types of sensilla on labial palpi (Fig. 5a and b) and antennal segments, especially the flagellum part (Fig. 5c). Observation of three types of sensilla on maxillary and labial palpi was also reported by Mbata et al. (1997). In classifying the sensilla types, terminologies given by Schneider (1964) and Hu et al. (2009) were applied (**Table 9**). For instance, sensilla chaetica (sensory bristles or spines) are bristles distinguished by a specialized and flexible circular membrane at the base, sensilla trichodea (sensory hairs) are trichoid sensilla hairs without any specialized basal cuticular ring serving as articulating membrane, sensilla basiconica (sensory pegs or cones) are mnipresent trichoid sensilla without any specialized basal membrane. Hu et al. (2009) suggested possible functions of all the sensilla with supportive studies. According to them, sensilla trichoidea1 (ST1) may have an olfactory function (smell), whereas sensilla trichoidea2 (ST2) probably function as sex pheromone receptors. Sensilla chaetica (SC) in *C. chinensis* and *C. maculatus* is believed to have a dual function of mechanoreception and contact chemoreception. Also, sensilla basiconica1 (SB1) was implicated to have an olfactory function which was verified by the use of electrophysiological recordings (Hu et al. 2009). In the present SEM study, sensilla trichoidea (ST), sensilla basiconica (SB) and sensilla chaetica (SC) on *C. maculatus* mouthparts were more abundant and pronounced than their antennal sensilla. This finding is in conformity with Mbata et al. (1997), who reported that a greater number of sensilla were present on female maxillary palps than on male maxillary palps and labial palps of both male and female, which may suggest the involvement of these sensilla in stimulus detection, possibly food source and/or oviposition sites. Limited literature is available on the sensilla types of *C. maculatus* and other *Callosobruchus* species. Fouda et al. (2016) reported two sub-types of sensilla basiconica (SB1 & SB2) and one type of sensilla chaetica (SC) in the mouthparts of rice storage beetles, *Sitophilus oryzae* and *S. granarius*, which supported the present findings. Sensilla trichoidea1 (ST1) are described on the antennae of most investigated insects (Schneider 1964). Hu et al. (2009) reported that ST1 is the most abundant sensilla type on the whole antennae of *C. maculatus* which was evident and confirmed in the present investigation (Fig. 5c). The types of sensilla on the antennae of *C. maculatus* recorded are largely in conformity with those reported for other bruchid and beetle species; *C. chinensis* (Hu et al., 2009), *Acanthoscelides macrophthalmus* (Thakur and Kalpna, 2015), *Sitophilus oryzae* and *Sitophilus granarius* (Fouda et al., 2016). Thanjavur *C. maculatus*female ovipositor had sensilla trichoidea1 (ST1) surrounding the region which is also supported by Mbata et al. (1997), who stated that sensilla found on ovipositor lobes resemble trichoid sensilla on the ovipositor of several insects. Although these sensilla are hypothesized to function in different roles based on their distributional pattern and observed morphological characteristics, the actual physiological role may be attributed only after studying their ultrastructure and with detailed electrophysiological and behavioral investigations.

Table.9 Morphological types and structures of sensilla in *Callosobruchus chinensis* and *C. maculatus*

Types of sensilla	Tip	Wall	Shape	Socket
BB	Blunt	Smooth	Straight	Wide
ST 1	Sharp	Grooved	Straight or slightly curved	Tight
ST 2	Blunt	Smooth	Straight	Tight
SC	Blunt	Grooved	Straight	Wide
SB 1	Blunt	Smooth	Straight	Tight
SB 2	Blunt	Grooved	Curved	Wide
GP	Blunt	Grooved	Straight	Wide
SCa	-	-	-	-

BB - Bo¨hm bristles; ST1 - Sensilla trichoid 1; ST2 - Sensilla trichoid 2; SC - Sensilla chaetica; SB1 - Sensilla basiconic 1; SB2 - Sensilla basiconic 2; GP - Grooved pegs and SCa - Sensilla cavity

Conclusion

A proper understanding of the taxonomic characteristics, morphology and biology of any insect pest is important in planning an effective management operation and for the varietal development of crops imparting resistance against insect pests. Two of the most common species of *Callosobruchus, C. maculatus* (F.) and *C. chinensis* (L.), are compared and identified based on the visual differences, damage potential, morphometrics and the previous literatures, with descriptions of important morphological characteristics for easy identification in both entomological and allied studies. *C. maculatus* under laboratory conditions occurs in two forms, active and sedentary forms, which may be potentially misidentified with another *Callosobruchus* species, *C. analis*. The distinctions should be examined prior to any studies related to this pest. Furthermore, with the increasing polymorphic nature of *C. maculatus* as reported by Credland (1994), George and Verma (1999) and from the present study, detailed study on the identification of the forms or morphs is essential for proper documentation.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All the relevant data and pictures are included in the paper. There are no other raw data to deposit for this article.

Competing interests

The authors report that there is no conflict of interest to declare.

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Authors' contributions

DS conducted the experiments and wrote the first draft of the manuscript. JSK, SN and PM conceived and planned this study and article. DS conducted the dissection of the insect, drew diagrams and analyzed the recorded data. AK contributed to the SEM analysis study. All authors read, contributed to and approved the final manuscript.

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Figures

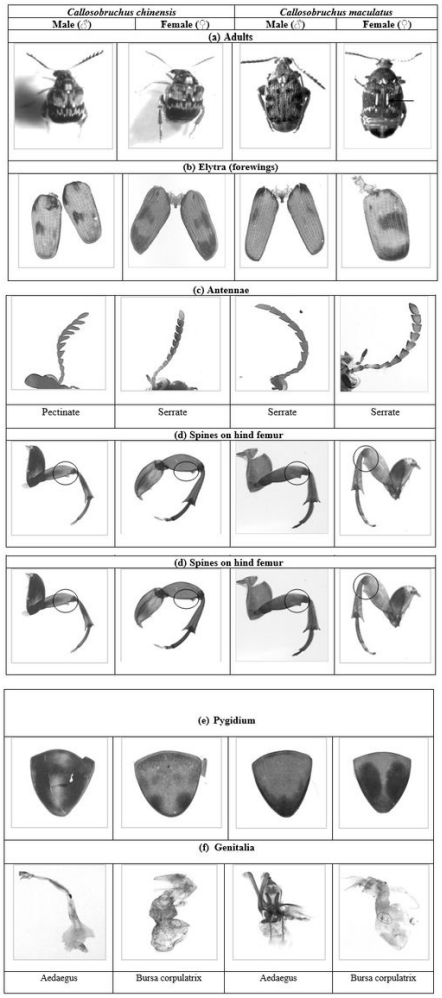

Figure 1

Morphological differences between *Callosobruchus chinensis* and *C. maculatus*

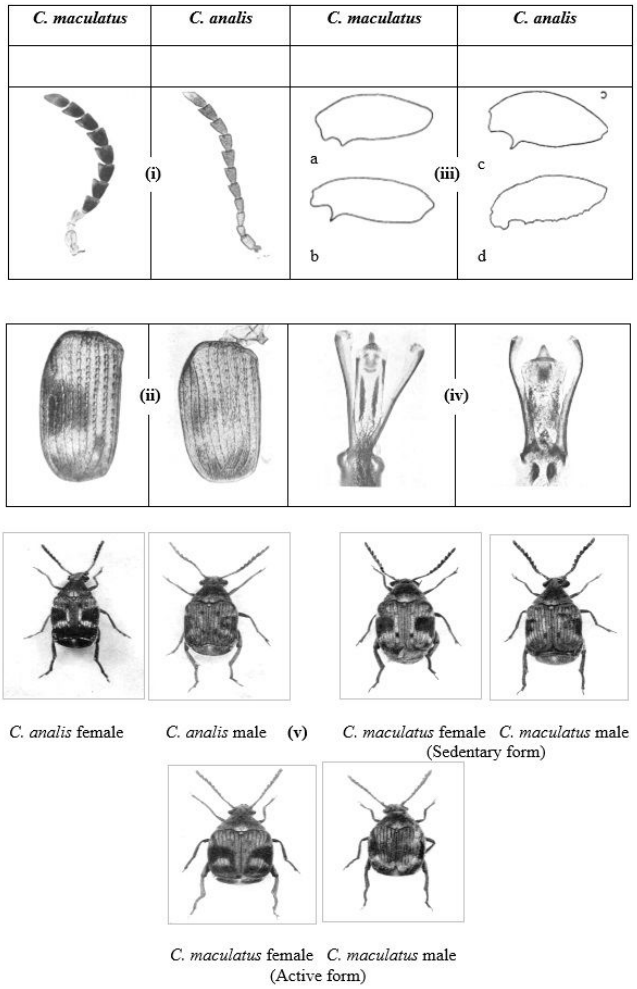

Figure 2

Morphological differences between *Callosobruchus maculatus* and *C. analis*

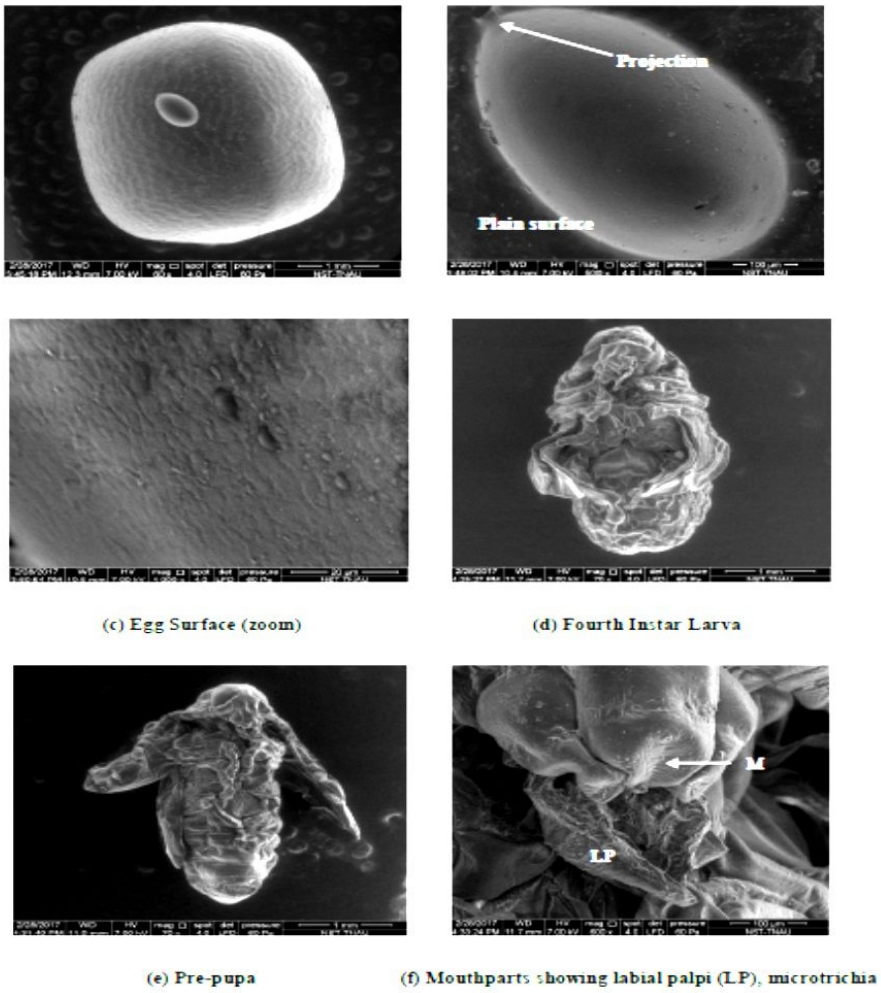

Figure 3

SEM images of *C. maculatus* eggs and developmental stages

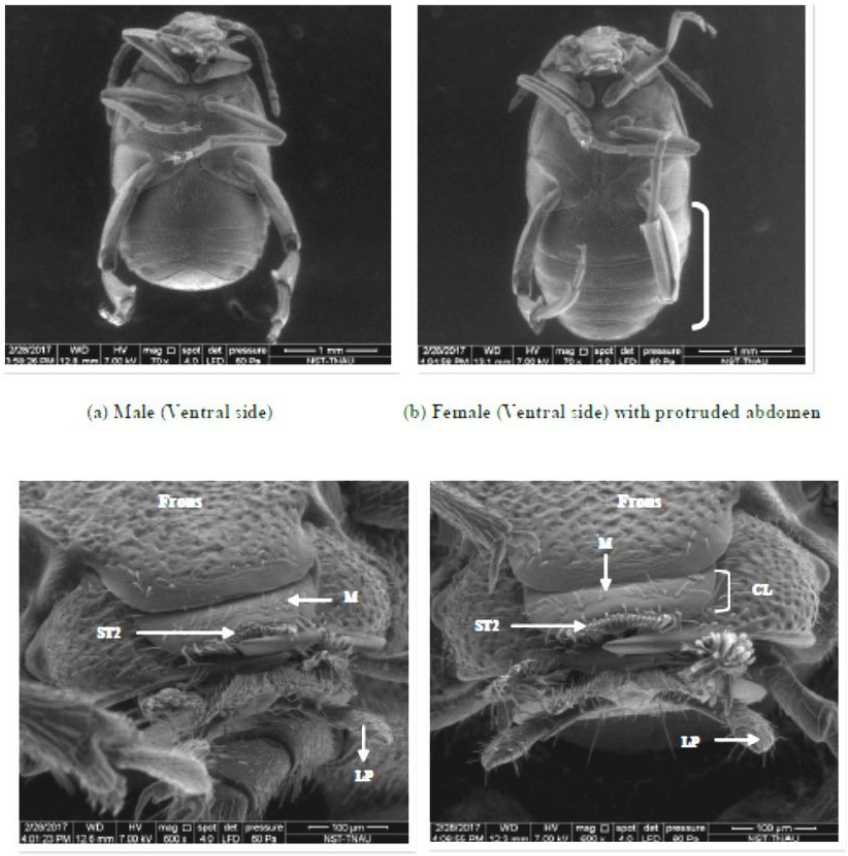

Figure 4

SEM images of *C. maculatus* mouthparts showing different sensilla

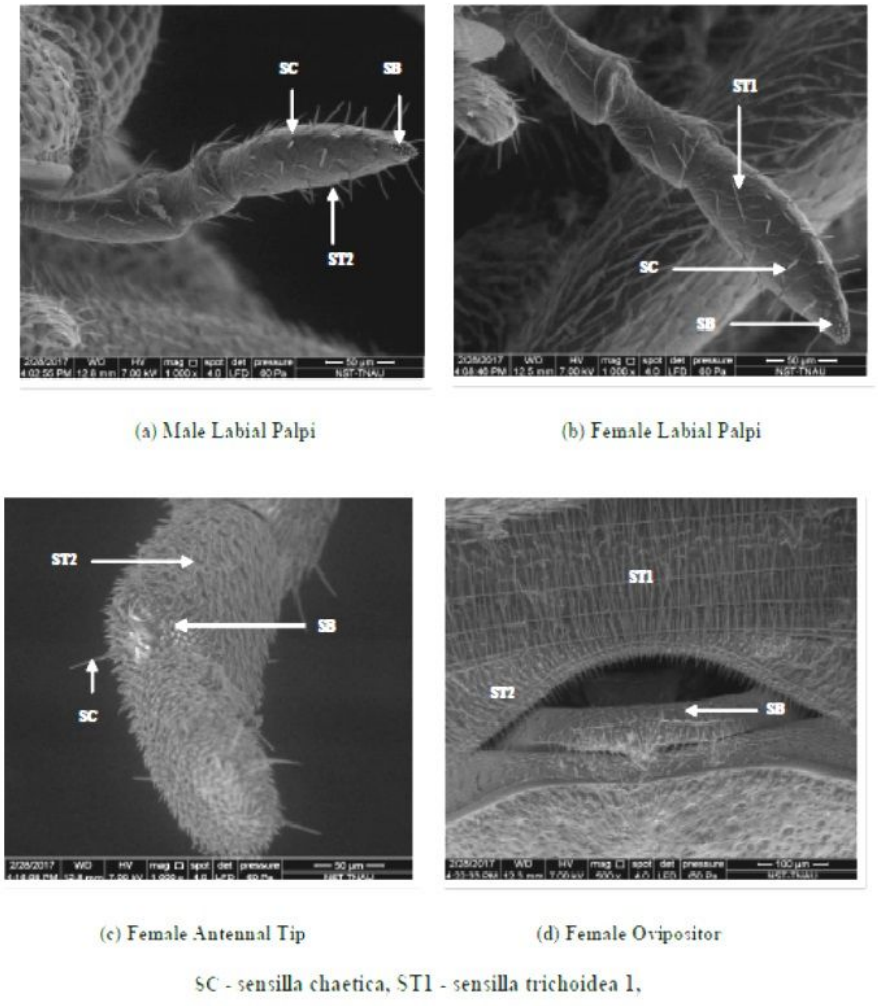

Figure 5

SEM images of *C. maculatus* body parts showing different sensilla