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First report of coconut case caterpillar, *Mahasena corbetti* Tams (Lepidoptera: Psychidae) from India

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Abstract Coconut case caterpillar or Bagworm, *Mahasena corbetti* Tams (Lepidoptera: Psychidae), is a destructive polyphagus pest in Southeast Asia and some islands of the Pacific. Considering the rapid expansion of the distribution range of *M. corbetti*, this species is included in the list of dangerous pests in plant quarantine act of Malaysia. *M. corbetti* is also considered as a pest of quarantine importance in many countries. During 2015–2016, severe incidence of *M. corbetti* was observed for the first time on arecanut (*Areca catechu* L.) plants in Sohbar and adjoining villages of East Khasi Hills district of Meghalaya state (India). This is the first report on occurrence of *M. corbetti* in India. We are reporting the infestation, natural field parasitism and description of life stages of *M. corbetti* infesting arecanut plants in India. Considering the taxonomic complexities in identification and destructive nature of bagworms, we have also developed DNA barcode for this species by sequencing standard barcoding region of mitochondrial cytochrome oxidase I (COI) gene. This report would serve as a preliminary foundation for reliable diagnosis of *M. corbetti* and also for creating awareness among the arecanut growers and other related functionaries in India and nearby countries.

Keywords Bagworm moth · Arecanut · Meghalaya · Psychidae

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Introduction

The Coconut case caterpillar or bagworm, *Mahasena corbetti* Tams, 1928 (Lepidoptera: Psychidae), is one of the serious polyphagous pest of many plants in Southeast Asia and some islands of the Pacific (Waterhouse 1993). It is regarded as destructive agricultural pests, as their larvae voraciously feed on the leaves of many economically important plants (Sankaran and Syed 1972; Kamarudin et al. 1994). The larvae of *M. corbetti* are known to attack plants of about 37 genera in 21 families and their most preferred families are 'Fabaceae' and 'Arecaceae' (Kamarudin et al. 1994; Robinson et al. 2011; Leong and Lim 2012). Bagworms including *M. corbetti* are one of the main leaf-eating pests of oil palm in Malaysia and Indonesia. Serious bagworm attack can result in the huge crop losses due to the extensive defoliation they cause. Liau (1987) reported that a severe defoliation by *M. corbetti* in Malaysia resulted in crop losses in excess of 40–50% in two subsequent years. A moderate defoliation of about 10 to 30% may cause a crop loss of about 33 to 40% (Basri 1993). Thus, it is essential to prevent the bagworm infestation reaching a moderate level as it can result in serious economic losses (Darus and Wahid 2000).

Bagworm, *M. corbetti* is previously recorded from Thailand, Peninsular Malaysia, Sumatra, Java, Borneo (Brunei, Sabah), Solomon Islands, Samoa, Papua New Guinea and Philippines (Plate 1) (Waterhouse 1993; Robinson et al. 1994; Batugal et al. 2005; Hättenschwiler et al. 2013). In 2015–2016, severe incidence of *M. corbetti* was observed for the first time on arecanut (*Areca catechu* L.) plants (Plate 2) in Sohbar (25°12′47.006" N and 91°45′4.117" E) and adjoining villages of East Khasi Hills district of Meghalaya State (India). This region is bordering India and Bangladesh, where the farmers are more dependent on natural resources including arecanut farming for their livelihood.

Bagworms are leaf eating caterpillars characterized by the possession of bag, which they construct out of tough silk embedded with materials from dried plants such as leaves, small twigs (Barlow 1982) or in other species with debris of mineralic material (Arnscheid and Weidlich 2017). Larvae construct cases made of large pieces of leaflet that give them their characteristics 'shaggy appearance'. Bagworms attack may not be noticed at the beginning when larvae are small. Leaves that bagworms eat usually turn yellow and die before falling down. Serious attack of bagworms can kill the tree because of severe defoliation (Hisham 2012). Bagworm, *M. corbetti* is considered as a pest of quarantine importance in several countries (Anonymous 1999; Batugal et al. 2005; Anonymous 2016). This investigation aims to report the infestation of *M. corbetti* for the first time on the arecanut plants in India and to provide details on the diagnosis and preparedness for its effective management. Since taxonomic identification of this species is only possible with adult male specimens, which are very less and take at least 4–5 months for emergence. Therefore, we have also developed DNA barcode, which would eventually help the quarantine departments and other agencies across the World for rapid diagnosis at any life stage.

Material and methods

Study location, sample collection and taxonomic characterization of different stages

The infestation of bagworm, *M. corbetti* was studied in Sohbar (25°12′47.006" N and 91°45′4.117" E) and adjoining villages of East Khasi Hills district of Meghalaya State (India). The affected area was surveyed and bagworm specimens (larval cases) were collected from infested arecanut plants. This bagworm species was later confirmed to be a *Mahasena corbetti* (see result section). The arecanut

Plate 1 Distribution map of *M. corbetti*. Red color shows its distribution in Southeast Asian and pacific region and single red dot (in India's map) shows the recent detection of *M. corbetti* in northeast India (Source of blank map template: https://free-editable-worldmap-for-powerpoint.en.softonic.com/download)

Plate 2 Damage of bagworm caterpillars to arecanut plants. Area inside the red circle shows defoliated leaves

plants in the affected area were inspected and per cent infestation by *M. corbetti* was determined. According to the resident farmers (arecanut growers) of this locality, *M. corbetti* has been observed for the first time on arecanut plants. Thus, the basic information on sudden occurrence and seasonal incidence of *M. corbetti* was collected from the local residents and the Chairman of the Tyllilang Progressive Multi-purpose Co-operative Society Ltd. H.Q. Ladsohbar (Ruiong).

A total of 200 bagworm samples were collected at quarterly interval from the infested plants and reared on arecanut leaves inside the cages (45x45x45cm) in Integrated Pest Management (IPM) laboratory of Division of Crop Protection, ICAR Research Complex for North-eastern Hilly Region, Umiam, Meghalaya State (India). The insects were reared on arecanut branches till the emergence of the adults. Newly emerged male moths were separated for the species identification. Adult females remain inside the pupa throughout the life, where they live, reproduce and lay eggs. Moreover, the females are poorly described in subfamily Oiketicinae of the family Psychidae as females pass their whole life inside the pupa and hence only males are used for description of the species. Therefore, the species level identity in this group had to be determined based on male characteristics. The taxonomic identity at genitalia level was established by treating abdomens of five male moths (Plate 3). Genitalia were prepared by treating abdomen with boiling 10% KOH as described before (Firake et al. 2014). Images of genitalia were captured by using camera mounted on Leica EZ-4D stereozoom microscope. The external genitalic features of male adults were compared with original male genitalia description of *M. corbetti* (Kamarudin et al. 1994).

Plate 3 Male moth of *M. corbetti* in natural sitting posture

DNA extraction, PCR amplification, sequencing and DNA barcoding

Newly emerged adults (two) were used for extraction of DNA. The genomic DNA (gDNA) was extracted from the legs of male adults by using the DNeasy® Blood and Tissue Kit (Qiagen, Valencia, USA) as described in Behere et al. (2015). Additional specimens have been deposited as voucher specimens in Insect Museum of Crop Protection Division, ICAR Research Complex for NEH Region, Umiam, India. For amplification of the standard barcoding region of Cytochrome oxidase I (COI) gene of mtDNA, we used the procedure used for rose saw fly as described in Firake et al. (2013). The standard insect DNA barcoding primers (LepF1 and LepF2) used in this study. The successful PCR amplicons (40 μL post PCR) from the two representative individuals were sent for direct sequencing to M/S Eurofins Genomics India Pvt. Ltd., Bengaluru, India. Both samples were sequenced from both the ends (5′ and 3′). The DNA sequences obtained were analyzed using Pregap4 and Gap4 programs within the Staden

Molecular Biology analysis software (Staden et al. 2000). Nucleotide sequences were aligned using the sequence alignment program Clustal X (Thompson et al. 1997) and then checked manually. A final good quality sequence was subjected to BLASTN search (Altschul et al. 1997) against the nucleotide collection database in NCBI GenBank vide accession number MG574312.

Studies on infestation and natural parasitism of *M. corbetti*

Information on different ecological aspects, especially about native natural enemies is very much crucial for the development and multiplication of any alien species in new habitat. To study the natural parasitism, the representative samples of bagworms (along with leaves) were collected during early January 2016 from infested plants of arecanut grown in Sohbar area. The bagworms collected were reared in the cages (45x45x45cm) until the emergence of either bagworm adults or any parasitoids. Emerged parasitoids were separated and per cent parasitism was measured. The representative samples of each parasitoid were preserved in 70% alcohol for identification.

Results

Detection and monitoring of bagworm, *M. corbetti* infestation on arecanut

Initially during April 2015, the larvae of bagworm, *M. Corbetti* were found feeding on few arecanut plants and subsequently 1/3rd leaves of those plants were found to be defoliated within next 2–3 months. By the month of September, several areacanut plants (about 59) were found to be affected by this pest. About 26 plants were found completely defoliated (Plate 4) by the mid December 2015 in Sohbar area. The growing larvae (Plate 5) remain inside the cases and molted inside, since their head capsules were found visible near the front portion of the cases (Plate 6). The affected plants had very few number and poor quality fruits compared to unaffected plants. Besides severe defoliation, necrotic patches were also formed on infested leaves which gave them 'burnt appearance' to the plants.

Two parasitoids were found naturally parasitizing the *M. corbetti* larvae in the affected area during January 2016. A total of 74.31% bagworm larvae ($n = 253$) were observed to be parasitized by the unidentified tachinid fly (order: Diptera) (Plate 7); while 2.5% bagworm larvae ($n = 253$) were parasitized by the parasitoid, *Echthromorpha* spp. (Plate 8) (Hymenoptera: Ichneuomonidae). Since February 2016 to till date, no further infestation of bagworm was observed in Sohbar area and the infested plants were completely recovered with fresh leaves.

Plate 4 Completely defoliated arecanut plants in Sohbar area of Meghalaya state, India

Identification and taxonomic description of male *M. corbetti*

Based on the structure of male genitalia, the identity of the bagworm species infesting arecanut plants in Meghalaya state (India) was confirmed to be *M. corbetti*. The morphology of male moth specimens and other stages of *M. corbetti* from our study were found matching with those previously illustrated (Kamarudin et al. 1994). Full grown larva (without case) of

Plate 5 Larvae of bagworm, *M. corbetti* feeding on arecanut leaves

Plate 6 Mature larva of bagworm, M. corbetti along with the head capsules of different instars attached at the frontal portion of the bag

M. corbetti (Plate 9) was about 15–20 mm long, head and thorax were brown in colour, while the abdomen was light yellowish brown in colour. Bags or cases of male *M. corbetti* were smaller than female cases. The male pupae (Plate 10) were 12–15 mm length and 3–4 mm diameter), usually found attached to the bottom of the case or bag. The female pupae (Plate 11) were larger in size (20–25 mm length and 10–12 mm diameter) and dark brown in colour and found inside the cases. The adult female remains inside the pupa throughout the life. Adult females were apterous, cylindrical in shape and creamy-white in colour. All the body appendages were strongly reduced or absent. Interestingly mouthparts were absent in both adult male and females.

Plate 7 Adult of tachnid fly (unidentified) recovered from the larvae of *M. corbetti*

Plate 8 Ichneumonid wasp, *Echthromorpha* spp. recovered from the larvae of *M. corbetti*

Taxonomic description of the male moth reared from arecanut plants in India

Forewings stretched, costal margin almost straight, apex roundish, termen oblique. Wingspan 20–25 mm, darkish brown without any pattern, densely covered with medium broad scales (classes 3–4 after Sauter 1956) (Plate 12). Venation with 10 veins from discal cell, r3 + r4 and m2 + m3 long stalked, intercalary cell present. Hindwings small, termen distinctly straight; venation with 7 veins from discal cell, intercalary cell present. Antennae short, of ¼ of the forewing length, bipectinate with 21–28 ciliated pecten. Long epiphysis present on forelegs, reaching the end of the tibia.

Male genitalia resemble slightly to the genitalia of the west Palearctic distributed genus *Ptilocephala* Rambur. Male genitalia are long and stretched (Plate 13).

Plate 9 Larva of *M. corbetti* removed outside the case or bag

Plate 10 Male pupa (vacated) of *M. corbetti*

Tegumen (te) hemispherical, folded on both sides, valvae (va) long, extending the distal end of tegumen. Clasper (cl) of sacculus (sa) distinctly sclerotized with 2 or 3 hook shaped extensions. Vinculum (vi) trapezoidal, saccus (sc) very long, of valva length (Plate 14). Phallus very long, longer than entire genitalia, curved, distinctly thinner towards distal end (Plate 15).

Molecular characterization of bagworm moth, *M. corbetti*

According to external morphological characters of larvae and adults, the bagworms infesting arecanut crop in Meghalaya were found to be *M. corbetti*. Considering the destructive nature, we have also developed DNA barcode for this species. The standard insect DNA barcoding primers (LepF1 and LepF2) used in this study successfully amplified the targeted fragment of COI gene. A final good quality 670 bp sequences were obtained for two specimens of *M. corbetti* after trimming the ambiguous 5′ and 3′ ends of the sequences. Both the sequences were identical and no mutation was detected. BLASTN search of 670 bp sequence as a query in NCBI shown 88 to 90% homology with two species of the genus *Mahasena* viz., the *M. oolona* (MF596484) and *M. aurea* (LC094209) reported from Japan. Since there was no variation among two sequences of *M. corbetti*, a representative sequence has been deposited to the NCBI with accession number MG574312.

Plate 11 Female pupa along with female of *M. corbetti*

Plate 12 Male moth of *M. corbetti* (stretched view)

Plate 13 Long and stretched male genitalia of *M. corbetti*

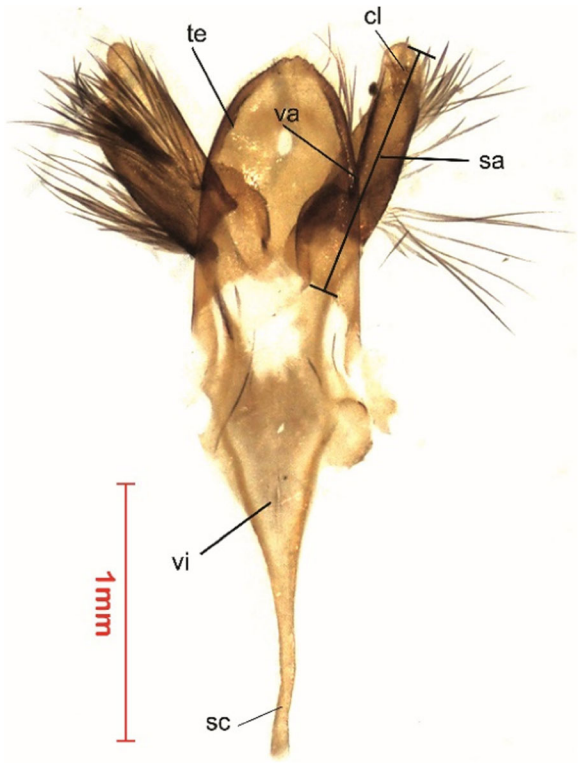

Plate 14 Male genitalia of *M. corbetti* without adeagus (phallus)

Discussion

M. corbetti was detected feeding on arecanut plants for the first time during 2015–2016 in Sohbar area of Meghalaya (India). Arecanut is one of the important plantation crop of India, occupying 0.47 million ha area, which produced 0.74 million tones arecanut fruits during 2015–2016 (Anonymous 2017). The Meghalaya state (Districts: East Khasi Hills, Jaintia Hills, West Garo Hills, East Garo Hills) is one of the major arecanut growing belt of India (Anonymous 2017).

Even moderate defoliation by bagworms usually results into significant crop losses (Basri 1993). So far three species of psychids viz., *Manatha albipes* Moore; *Cryptolhelia* sp. and *Thyridopteryx* sp. are known to attack arecanut in India (Pillai and Kurian 1959; Nair and Menon 1963). Since the infestation of *M. corbetti* was limited to Sohbar and nearby villages during 2015–16, significant losses in arecanut production have not been reported from Meghalaya state (Anonymous 2017). It is not clear if this species has invaded from neighboring countries to our country recently or it existed here without notice. A very high per cent of natural parasitism observed in our study, indicates that, this species might have entered earlier without achieving pest status and hence might have gone unnoticed. The main reason behind the ignorance could also be due to difficulties in identification of *M. corbetti*, since its taxonomic identification is only relied on adult male specimens, which are less in numbers and take at least 4–5 months for emergence.

Accurate identification of species is fundamental requisite for biological research and species conservation. Determining the species of many bagworm moths is extremely difficult using traditional taxonomy because many species look remarkably alike and share similar ecological characteristics. Moreover, the morphological identification of females is more difficult because they lack wings and other important organs (Chevasco et al. 2014). In general, species identification in family Psychidae is complex as well as daunting task and thus molecular data are being given due importance in species recognition and studying evolution in family Psychidae (Yen et al. 2004). DNA barcoding is one of the molecular technique which is now becoming popular among researchers working in the field of taxonomy and systematic. DNA barcoding has recently facilitated detecting and characterization of several invasive pest species in northeast India (Manger et al. 2017; Sankarganesh et al. 2017; Firake et al. 2017). However, BLASTN search in NCBI GenBank did not find matching sequence of *M. corbetti* in database; indicating that, this DNA sequence has been submitted for the first time to NCBI GenBank for this species. The COI sequences for only two species of the genus *Mahasena*

Plate 15 Aedeagus (phallus) of male genitalia of *M. corbetti*

(*M. aurea* and *M. oolona*) have been deposited in the Genbank. Therefore, it was not possible to establish the reliable evolutionary relationships in between different species of the genus *Mahasena* using phylogenetic analysis. Nevertheless, the molecular and taxonomic information generated in this study would help researchers, quarantine departments and other agencies across the World for reliable diagnosis of *M. corbetti*. Given the complexities in taxonomic identification, there is an urgent need to generate additional molecular data, which subsequently can be used for development of molecular tools for rapid identification of these species.

The northeastern hilly region of India is very rich in terms of biodiversity of flora and fauna; although much of them are still unexplored and pest complexes of many crop plants are relatively different than other parts of the India (Azad Thakur et al. 2012). *M. corbetti* has wide distribution and broad host range; however the palms (including oilpalms, coconut and arecanut) are the major host of this species. Considering the rapid expansion of the distribution range of *M. corbetti*, this species is under the list of dangerous pests in plant quarantine act of Malaysia (Muthaiyan 2009). *M. corbetti* is also a quarantine pest for India, therefore specific phytosanitary measures are being taken while importing coconut germplasms from Indonesia and Papua New Guinea to India (Batugal et al. 2005). Since *M. corbetti* has not been recorded from other arecanut or palm growing area of India, continuous monitoring for its incidence is very essential for further spread. Moreover, it is also important to develop more resources and information for correct diagnosis of *M. corbetti* and other species of this genus.

Besides high reproductive potential (>3000 eggs/female), *M. corbetti* takes relatively long time to complete active larval period (about 12–17 weeks out of 5 moths for life cycle); thus often creates outbreak situations (Hill 2008). Several natural enemies are known to attack bagworms on different palms (Cheong et al. 2010) and these natural enemy complexes normally regulate the population of *M. corbetti;* however the relative importance of the species of this complex varies with locality (Wood 1968; Sankaran and Syed 1972). Six species of hymenopterous parasitoids (including braconids, ichneumonids, and eulophids) and five species of dipterous parasitoids (all tachinids) were found to be associated with *M. corbetti* in east Malaysia (Sankaran and Syed 1972). Clerid beetle, *Callimerus bellus* has also been reported as a larval predator of *M. corbetti* in Singapore (Leong and Lim 2012). In present study, we observed tachinid fly as a dominant parasitoid of *M. corbetti* larvae followed by ichneuomonid wasp, *Echthromorpha* spp. Different species of tachinid flies are known as important parasitoids of bagworms. A tachinid larval parasite, *Eozenillia equatorialis* Townsend and an Ichneumonid, *Echthromorpha agrestoria* Swed have been reported as major parasitoids of *M. corbetti* in Sabah, east Malyasia (Sankaran and Syed 1972).

The females of *M. corbetti* are apterous and they remain inside pupa for its whole life, where mating and oviposition takes place. The abdomen of male of Oiketicinae is thus highly extensible so it can insert into the larval case of the female during mating. The entire abdomen of males is highly specialized for this requirement and capable of extending itself to three times its normal length (Kozhanchikov 1956; Davis 1964). Also, very long phallus (aedeagus) of male genitalia may perhaps be essential for facilitating mating process in this species.

Bagworm, *M. corbetti* is polyphagus and many host plants of this pest are widely available plant species in northeastern and other states of India e.g. palms, banana, citrus etc. (Dumbleton 1954; Kamarudin et al. 1994); thus regular monitoring and subsequent management would be needed to detect its presence on other host plants and localities. Moreover, northeastern India shares international borders with many countries, including China, Bhutan, Myanmar, Bangladesh and Nepal. Thus, chances of further spread of *M. corbetti* in neighboring countries cannot be ignored and hence this study would also help to create pest alert and awareness within and nearby countries. In Malaysia, *Bt* sprays are being used to control bagworm (including *M. corbetti*) on oil palms (Basri et al. 1996). Information generated on infestation, natural parasitism, DNA barcode and diagnosis of different stages of *M. corbetti* in this report would be useful for further studies including creating awareness among the arecanut growers and other related functionaries in India and nearby countries.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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