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Morphological Identification of Gender in Bombyx mori at Pupal and Adult Moth Stages: A Visual Approach

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Abstract

Gender identification in *Bombyx mori* (silkworm) is vital for maintaining genetic purity and optimizing silk production in sericulture. This study presents a visual and morphological guide to distinguishing male and female silkworms at the larval, cocoon, pupal, and moth stages using comparative imagery and technical protocols. Based on distinct morphological characteristics such as abdominal segmentation, genital structure, antenna type and body size, this study offers a simplified, practical method suitable for both researchers and grassroots sericulture practitioners. Findings were consistent with previous literature, confirming the reliability of visual morphological markers as tools for gender identification.

Keywords:*Bombyx mori*, gender identification, silkworm, pupal stage, moth stage, morphological, sericulture

1. Introduction

The domesticated silkworm, *Bombyx mori*, is central to sericultureand efficient hybrid production depends on accurate gender identification. Timely separation of male and female individuals enhances breeding programs, optimizes mating ratios and ensures higher-quality cocoon yield. While molecular tools provide precise results, they are costly and inaccessible in rural areas. Morphological identification remains a reliable, cost-effective and practical alternative.

Sexual dimorphism in *Bombyx mori* becomes prominent during the late larval, pupal, and adult moth stages. Identification based on external featuresparticularly genital structures and body formhas been well documented (Sengupta *et al*., 2002; Ramesh *et al*., 2016; Singh & Raina, 2021). This study expands previous work by offering a comprehensive visual approach supported by technical procedures for field and rearing center applications.

2. Materials and Methods

The study was conducted at the Regional Sericultural Research Station (RSRS), Central Silk Board, Miran Sahib, Jammu. Bivoltine silkworm strains NB_4D_2 and SH_6, which are widely used across India's subtropical sericultural zones, were selected for this investigation. Approximately 50 healthy cocoons were harvested after spinning at the 5th instar stage. For pupal stage identification, the cocoons were maintained in hygienic, controlled conditions to prevent contamination and ensure successful pupation.

For accurate gender determination at the pupal stage, cocoons were carefully cut lengthwise using angled blades to avoid harming the pupa. Male pupae were characterized by a cylindrical body, a narrow and tapered abdomen, and the presence of claspers on the 9th abdominal segment. Female pupae, in contrasthad broader blunt abdomens with paired gonopores typically marked by two dots or an "X" shape located near the 8th segment. The identification process involved an initial head-to-tail screening to differentiate between sexes, followed by a second inspection to confirm classification based on structural details and body volume.

At the moth stage, gender identification was performed upon emergence. Male moths were smallermore

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active and flutterier and possessed large, bushy (plumose) antennae designed to detect female pheromones. Female moths, on the other hand were larger and sluggish, with fuller abdomens containing eggs and less feathery antennae. All identification processes were documented photographically using a high-resolution camera under natural light conditions, and comparative plates were prepared and presented in Figures 1, 2, 3 and 4.

3. Results and Discussion

The present study demonstrates the effectiveness of morphological markers for gender identification in *Bombyx mori* at the pupal and moth stages. Pupal stage identification yielded the highest precisionwith 48 out of 50 pupae correctly classified. Male pupae exhibited a narrow, tapered abdomen with distinct claspers on the ninth abdominal segment, while female pupae showed a broader, rounded abdomen with visible paired gonopores near the eighth segment. These morphological features allowed for clear and reliable differentiation.

At the moth stage, identification was further confirmedmales were flutterier, had narrow bodiesand bushy, plumose antennae, whereas females were larger, more sluggish and exhibited distended abdomens filled with eggs. These traits were consistently observed across both NB_4D_2 and SH_6 strains underscoring the reliability of external gender markers.

Photographic documentation, including side-by-side comparative image plates (Figures 1 2, 3 and 4), supported visual assessments and helped achieve an overall gender identification accuracy of 96% when combining pupal and moth stage indicators. These findings align with prior studies (Sengupta et al., 2002; Ramesh et al., 2016), reinforcing the practicality of visual identification techniques over expensive molecular methods. The procedures adopted here are economical, non-invasive and particularly suitable for application in rural sericulture units and training programs. Furthermore, a two-step pupal identification process (initial screening followed by reinspection) ensured greater reliability and reduced handling-induced mortality, thus enhancing moth emergence rates. Integrating such morphological identification protocols into training modules can substantially benefit hybridization programs, optimize mating efficiency by maintaining a 1:1 sex ratio and ultimately support egg quality assurance and improved cocoon yields.

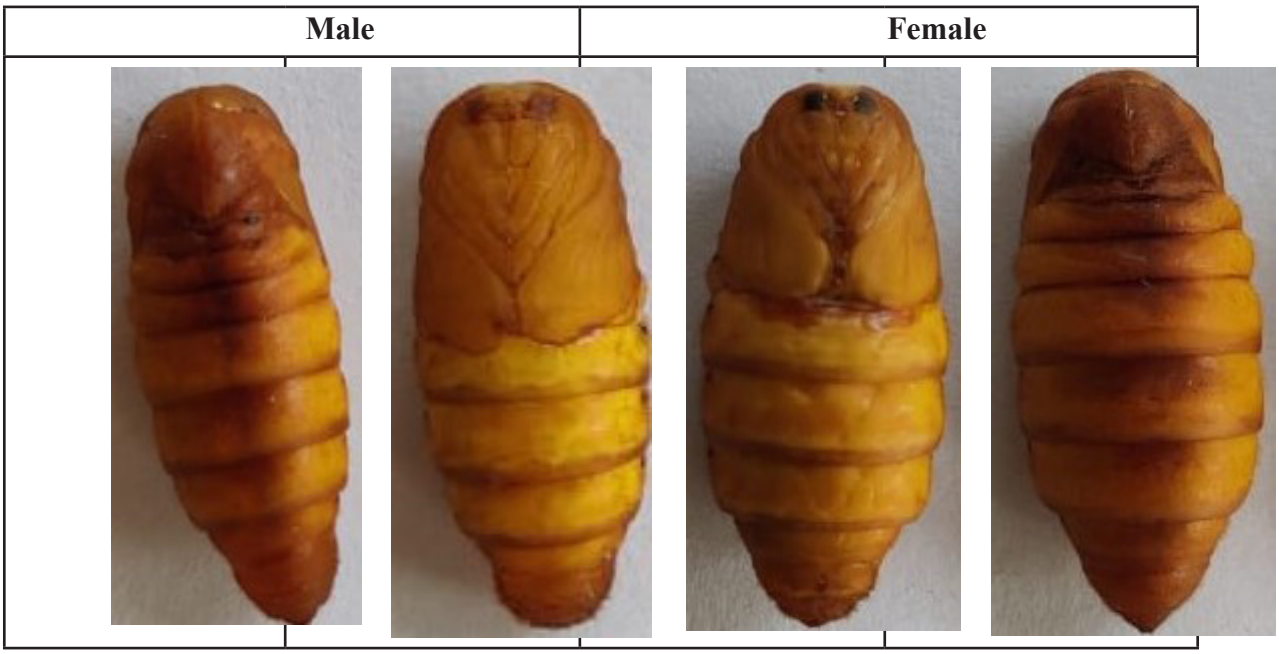

Figure 1: Morphological identification of male and female pupae of *Bombyx mori*

(The male pupa (left two images) shows a narrower abdomen with a pointed tip and a distinct slit-like genital opening near the posterior end. The female pupa (right two images) displays a broader and more rounded abdomen with a larger, curved genital mark located mid-abdominally).

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Figure 2: Male pupa of *Bombyx mori*: Characterized by a narrow abdomen and visible claspers at the posterior end, which are distinctive morphological features used for sex identification during the pupal stage.

Male	Female

Figure 3: Morphological identification of male and female moths of *Bombyx mori*.
(The male moth (left) is smaller with a slender abdomen and has large, feathery antennae used for detecting female pheromones. The female moth (right) is larger with a broader abdomen and smaller, less feathery antennae, adapted for egg-laying).**Figure 4:**Sexual dimorphism in Bombyx mori moths: The male (left) shows a slender abdomen and broader antennae, while the female (right) has a larger, more rounded abdomen and smaller antennaekey traits used for gender differentiation in silkworm moths.

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4. Conclusion

Morphological gender identification in Bombyx mori proves to be a practical, reliable, and cost-effective method across the larval, pupal, and moth stages. The findings reinforce its applicability in hybrid production, efficient moth pairing, and quality assurance within sericulture systems. This visual and technique-supported approach offers a valuable tool for farmers, trainers, researchers, and breeding stations, contributing meaningfully to both field-level practices and structured breeding programs.

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