“Female Spot" of Stag Beetle Larvae is a Symbiont Organ

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Stag beetles are highly popular insects as pets in the world. During the larval stage, a pair of orange spot is observed exclusively on the abdomens of female larvae. These structures commonly referred to as “female spot”, have long been considered as ovary primordia and widely used for sex identification during larval period. However, no anatomical evidence has been presented to support this assumption. In this study, we tested the hypothesis that female spots are not ovary primordia but instead function as organs harboring symbiotic microorganisms. First, we examined the presence and size of female spot in both sexes and found that some male larvae also possessed these structures. Second, Analysis based on paraffin sections and SEM revealed that the female spots are matrix-like structure on the digestive tract, indicating that they are unlikely to be ovary primordia. Third, we identified microorganisms within the female spots and the mycangia by sequencing PCR-amplified 26S rRNA genes. The detected microorganisms were xylose-fermenting yeasts, which were the same species as those detected from the mycangia of adult females. Overturning the conventional view documented in classification guides, our results strongly suggest that female spots are not ovary primordia but symbiotic organs that harbor symbiotic yeasts. Our findings call for a revision of the conventional interpretation of female spots and provide new insights into symbiosis in stag beetles.

Awards Won:
Fourth Award of $600