

Biological study of *Epicephala assamica* (Lepidoptera: Gracilariidae) with notes on morphology of immature stages



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ABSTRACT

The biology and immature stages of *Epicephala assamica* Li (Lepidoptera: Gracilariidae) were investigated firstly based on the field observations and laboratory experiments, *E. assamica* pollinate for *Glochidion assamicum* (Muell. Arg.) Hook. (Phyllanthaceae) and then lay eggs in pollinated female flowers. In turn, hosts partly sacrifice seeds for larval growth and keeping some seeds for breeding, thus they form the most strictly integrated one-to-one nursery pollination mutualism. *E. assamica* have two generations correspondingly which are closely consistent with flowering phenology of *G. assamicum*, peak in March–April and September–October respectively. We investigated the benefits that each species obtains from partner. For *G. assamicum*, the ripening rate is 51.14%, the rate of consumed fruits is 79.52%, the average number of seeds consumed by each larva is 2.62, and the proportion of intact seeds is 68.03% that could keep the stabilization of mutualism in each population. The study first reports sex ratio in the genus *Epicephala* and suggests that the male-biased offspring in *E. assamica* can make sure that female moths have more mating opportunities. The eruciform larva bears three pairs of thoracic legs and four pairs of prolegs on abdominal segments III–V and X (caudal proleg), 11 crochets uniserial arranged in proleg. Antenne exceeds segment X in pupa. The study helps to further understand the evolutionary mechanism and driving force of coevolution in the obligate pollination mutualism between *Epicephala* and *Glochidion*.

Introduction

The genus *Epicephala* Meyrick, 1880 (Gracilariidae: Ornixolinae) consists of 64 described species worldwide (Meyrick, 1880; Hu et al., 2011; Zhang et al., 2012a; Li and Yang, 2015; Li et al., 2015; De Prins and De Prins, 2016; Kawakita and Kato, 2016; Li and Zhang, 2016; Kawahara et al., 2017), and 19 species have been described from China in recent few years. *Epicephala* moths have recently become an important issue in ecology and evolutionary biology because of their mutualism with plants in the genera *Glochidion*, *Breynia* and *Phyllanthus* (Kato et al., 2003; Zhang et al., 2012b; Kawakita et al., 2015; Li et al., 2015).

Pollinators and host plants have a variety of relationships. Given the described principle in most obligate pollination interactions, theoretical studies have predicted that cooperative interactions can keep evolutionarily stable only when both participants possess mechanism to prevent overexploitation by the other (Axelrod and Hamilton, 1981; Bull and Rice, 1991; Bronstein, 2001). It has been assumed that excessive exploitation of seeds by pollinators would confer a substantial cost to plants and would subsequently lead to a collapse of the

mutualistic relationship (Bull and Rice, 1991; Herre et al., 1999; Bronstein, 2001). The obligate pollination mutualisms between *Epicephala* moths and Phyllanthaceae plants (including *Glochidion*, *Breynia* and *Phyllanthus*) remain benefit balance by keeping some fruits and seeds (Kato et al., 2003; Kawakita and Kato, 2004, 2006; Zhang et al., 2012b; Zhang et al., 2016; Luo et al., 2017). However, few publications present the ripening rate of hosts and consumption of seeds by larvae in *Glochidion-Epicephala* mutualism.

The study of how sexually reproducing organisms divide their resources between offspring of the two sexes (sex allocation) has proved one of the most successful areas in evolutionary biology (Charnov, 1982; Godfray, 1994; Herre et al., 1997; Peng et al., 2014). Several studies have reported the mating behaviours of *Epicephala* species (Okamoto et al., 2007; Zhang et al., 2012b; Zhang et al., 2016), however, no publications present the of sex ratio in the genus *Epicephala*.

Immature characteristics is important for the phylogenetic classification and the morphological identification in the insect study (Di Giulio et al., 2003; Archangelsky, 2004; Mutanen et al., 2009), in particular larval characteristics. Larval characteristics are used widely in systematics, including for classification and phylogenetic

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reconstruction (Archangelsky, 2004; Lee et al., 2007; Chenoweth et al., 2008; Mutanen et al., 2009; Regier et al., 2013), especially within Lepidoptera. In the mutualism between *Glochidion* and *Epicephala*, *Epicephala* species identification is performed based on male and female genitalia by rearing field-collected larvae until they become adults (Hu et al., 2011; Zhang et al., 2012a; Li and Yang, 2015; Li et al., 2015; Li and Zhang, 2016), however, few publications present the studies of immature stages on *Epicephala* moths. So, the characters of immature stages in *Epicephala* moths, including size, body color, chaetotaxy, proleg and crochets need to be studied.

In this study, we investigated the biological characteristics between *E. assamica* and its obligate host-plant *G. assamicum*, including the life history and habits of *E. assamica*, the ripening rate of host-plant, consumption of seeds by pollinators and sex ratio in *E. assamica*, describing characteristics of some immature stages in order to understand the evolutionary mechanism in the obligate pollination mutualism between *Epicephala* moths and *Glochidion* plants and provide a scientific basis for the protection of the tropical rainforest ecosystem.

Materials and methods

The study was performed from July 2013 to October 2014 in Xishuangbanna Tropical Botanical Garden (XTBG), Chinese Academy of Sciences (Fig. 1A), southwest Yunnan, China. The four seasons are not clear, but there are two obvious seasons, dry season between November and next April and the May to October rainy season. Dry season is divided into foggy-cool and dry-hot seasons, foggy-cool season lasts from November to February, heavy fog, wet in the morning and evening; dry-hot season lasts from February to April, the fog gradually reduce, dry heat, less rainfall (Zhu, 1993).

Epicephala assamica Li, 2016 is a new species for China (Li and Zhang, 2016), the specific name is derived from the name of the obligate host-plant *Glochidion assamicum* (Muell. Arg.) Hook. *G. assamicum*

is a monoecious plant which occurs in China and southeast Asia that flowers in bisexual axillary clusters, with many male and female flowers. The male flowers (Fig. 1B) grow at the base of branchlets, while the female flowers (Fig. 1C, D) tend to occur towards the apex. Fruits pedicels short (Fig. 1E); capsules depressed globose, usually 4-locular, pericarp thinner (Li and Gilbert, 2008).

Biological observation

We made tracking observations of the flower and fruit developmental stages of *G. assamicum* in XTBG for a total 46 individuals. We monitored 5 randomly selected branchlets each plant. We recorded the development of flowers and fruits, and counted the number of flowers and developing fruits of the selected plant individuals bi-week. We observed *E. assamica* during full anthesis and recorded their flower-visiting behaviours in detail, paying particular attention to their nocturnal activities, focused how they used their proboscis to collect pollen and pollinate flowers, and where they oviposited, photographs were taken with a Canon G11 digital camera in field. We recorded the times that the moths spent on pollination, oviposition and pollen collection. After the observations, moths collected from hosts were made dried specimens for species identification and sex determination. The genitalia of adult moths were dissected to identify the species follow the methods introduced by Li and Zheng (1996).

The assessment of mutualism mechanisms

To investigate ripening rate, we estimated based on total quantity of female flowers at peak anthesis, and retention of naturally matured fruits. To assess the seed production, we dissected mature fruits, recorded the number of intact seeds and consumed seeds. We collected developing and mature fruits and put them in a cylindrical plastic box (8.5 × 12 cm) group by group to rear *Epicephala* larvae for descriptive

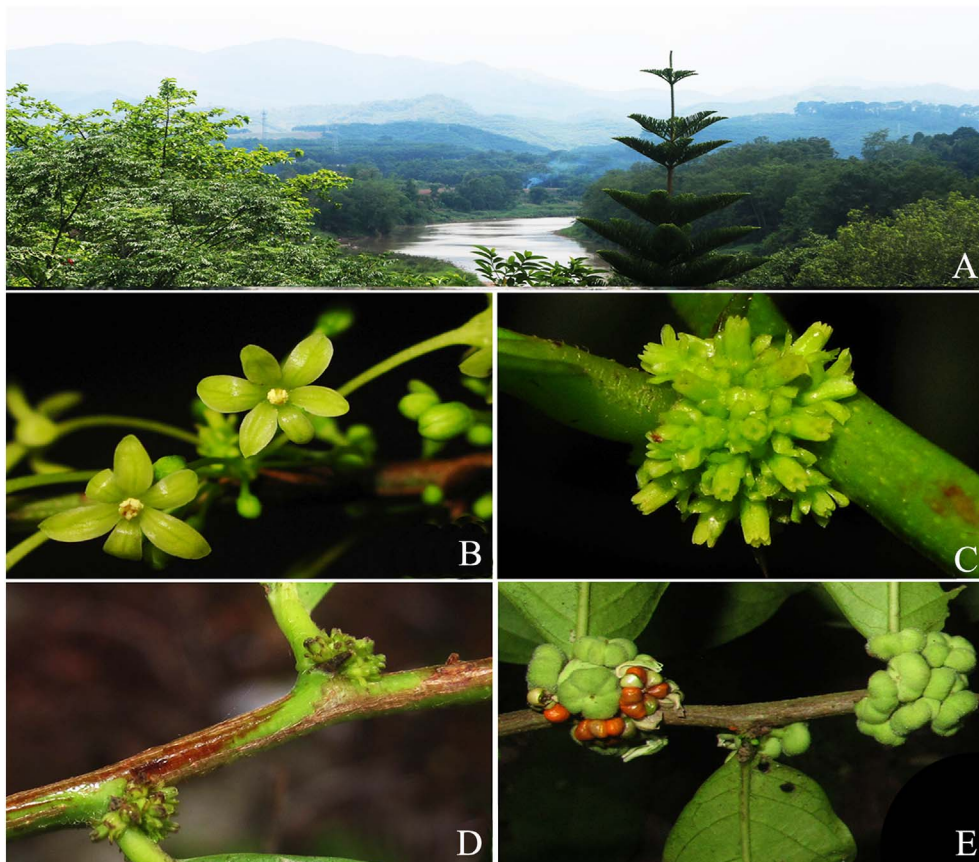


Fig. 1. The environment of researching site and the morphology of *Glochidion assamicum*. A. Landscape of XTBG; B. Male flowers; C. Female flowers; D. Pollinated female flowers during the flower-fruit interval season; E. Fruits.

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