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NOTE

Nocturnal moths as potential pollinators of *Marsdenia formosana* (Apocynaceae: Asclepiadoideae)

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ABSTRACT: Asclepiad flowers transfer the pollinaria, including pollen masses, to diverse body parts of flower visitors in various ways. In this study, we observed the insects visiting the flowers of *Marsdenia formosana* (Apocynaceae: Asclepiadoideae) at a forest in Taiwan in early May, 2016. Nocturnal moths of ten species (Lepidoptera: Pyralidae, Crambidae, Geometridae, Erebidae, and Noctuidae) were observed visiting *M. formosana* flowers. A pollinarium was found attached to the proboscis' tip of a *Bertula hadenalis persimilis* (Lepidoptera: Erebidae) specimen. This suggests that nectar-seeking moths visit *M. formosana* flowers and involuntarily transfer the pollinaria on the tip of the proboscis.

KEY WORDS: Erebidae, Lepidoptera, Nocturnal pollinators, Pollinarium attachment, Taiwan.

INTRODUCTION

Many flowering plants rely on insects and birds as pollen vectors for reproductive success (Proctor *et al*., 1996). Some plants have adaptive apparatuses for pollination by insects and birds (Proctor *et al*., 1996). For example, a pollinarium (a cohesive mass of pollen) is one such adaptive apparatus in the plant subfamily Asclepiadoideae (Apocynaceae; Proctor *et al*., 1996). Asclepiadoid plants can transfer the pollinaria to specific body parts of pollinators such as tongues of birds (Pauw, 1998), legs of moths (Mochizuki *et al*., 2017), and tips of moth proboscises (Sugiura and Yamazaki, 2005).

The Asclepiadoid genus *Marsdenia* Brown has approximately 200 species and is mainly found in the tropical regions (Endress and Bruyns, 2000; Juárez-Jaimes and Lozada-Pérez, 2015). Nocturnal moths and diurnal insects (wasps, flies, beetles, and butterflies) are known pollinators of *Marsdenia* plants (Forster, 1989; Yeoh *et al*., 2013; Ollerton *et al*., 2019). However, the pollinators of many *Marsdenia* species remain undocumented (Ollerton *et al*., 2019). *Marsdenia formosana* Masam. is a perennial vine species growing in the forests of China, Taiwan, and Japan (Ohashi *et al*., 2017). As with other members of the genus, pollinators of *M. formosana* remain unreported. Expanding the list of pollinators in the genus *Marsdenia* would contribute to our understanding of the evolutionary process of pollination systems in Asclepiadoideae (Ollerton *et al*., 2019). In this study, we investigated the flower visitors of *M. formosana* in Taiwan. The potential pollinators of *M. formosana* were identified by investigating the presence of pollinaria on the sampled flower visitors.

MATERIALS AND METHODS

Field observations were conducted at a deciduous forest on Lixing Industry Road, Ren'ai Township, Nantou county, central Taiwan (24°03'N, 121°09'E, 1500 m above sea level). Insects visiting 23 inflorescences (a total of ca. 100 flowers) of a *M. formosana* plant were observed at 30-min intervals during 19:30–5:00 on May 8 and 9, 2016 (a total of 19 observations) and each observation lasted for 10 min. All insect visitors were then captured by an insect net and killed in ethyl acetate. The sampled insects were individually wrapped in paper and brought to our laboratory, where they were identified based on their morphologies (Kishida, 2011; Nasu *et al*., 2013; Butterflies and moths of Taiwan, 2019). The presence of pollinaria on each specimen was investigated under a stereomicroscope. The corolla length of *M. formosana* flowers (n = 10) and the proboscis length for all captured insects were measured using slide calipers to the nearest 0.1 mm to investigate whether visitors can feed on nectar.

RESULTS

A total of 31 moths belonging to ten species of five families were collected from *M. formosana* flowers at night (Table 1). The moth species *Hypersypnoides punctosa* (Walker) (Lepidoptera: Erebidae) was the most abundant flower visitors at night (Fig. 1A; Table 1). A pollinarium was found on a proboscis of one individual of *Bertula hadenalis persimilis* (Wileman) (Erebidae); a gland (corpusculum) of the pollinarium was found attached to the tip of the proboscis (Fig. 1B). The percentage proboscises with pollinaria was 3.2% (n =

Fig. 1. ***Marsdenia formosana*** and its flower visitors. **A**: A nocturnal moth ***Hypersypnoides punctosa*** sipping the nectar from the flower. The petal length is approximately 3.5 mm. **B**: A gland (corpusculum) of the pollinarium catching on the tip of the proboscis of a nocturnal moth ***Bertula hadenalis persimilis***. The proboscis length is 6.3 mm.

Table 1. Flower visitors on ***Marsdenia formosana***.

Family /Species	Proboscis length	No. of visits
Pyralidae		
Endotricha sp.	5.3	1
Crambidae		
Ceratarcha umbrosa Swinhoe	10.9	1
Nacoleia charesalis (Walker)	4.7 ± 0.3[a]	2
Omiodes sp.	11.1	1
Geometridae		
Alcis taiwanovariegata Sato	6.7	1
Erebidae		
Adrapsa ablualis Walker	5.1	1
Bertula hadenalis persimilis (Wileman)	6.3	1[b]
Hypersypnoides punctosa (Walker)	10.4 ± 0.5[a]	20
Noctuidae		
Dipterygina indica (Moore)	8.3	1
Xylostola indistincta (Moore)	9.4	2

NOTE: a: mean ± SE (mm); b: A pollinarium was attached to the tip of the proboscis.

1/31; Table 1). The corolla length of *M. formosana* was 2.9 ± 0.2 mm (mean ± standard errors). The proboscises of all captured visitors (9.3 ± 0.3 mm; range: 4.4–11.6 mm) were longer than the measured corollas of *M. formosana* (Table 1).

DISCUSSION

Moths visit flowers and feed on floral nectar to gain energy for reproduction and dispersal (Gilbert and Singer, 1975; Kevan and Baker, 1983). Although many studies have clarified the importance of hawkmoths (Sphingidae) as pollinators (e.g., Bawa, 1990; Sazatornil *et al*., 2016; Johnson *et al*., 2017), relatively few studies have focused on the role of settling moths (e.g., Pyralidae, Geometridae, Erebidae and Noctuidae) as nocturnal pollinators (Oliveira *et al*., 2004; Hahn and Brühl, 2016). This study shows that settling moths frequently visit the flowers of *M. formosana* to feed on nectar (Fig. 1A; Table 1). In addition, a gland of the pollinarium caught on the tip of the proboscis of a settling moth (Fig. 1B). Similar attachment of pollinaria on settling moths has been reported in the Asclepiadoid species *Metaplexis japonica* (Thunb.) Makino (Sugiura and Yamazaki, 2005). Therefore, *M. formosana* flowers may induce nectar-feeding moths to pull out the proboscis along a guide rail (anther slit) and thereby attach the pollinaria onto the tip of the proboscis.

Some Asclepiadoid species are pollinated by both diurnal and nocturnal insects (Ollerton *et al*., 2019). However, the relative importance of diurnal and nocturnal insects for pollination varies among Asclepiadoid species (Bertin and Wilson, 1980; Morse and Fritz, 1983; Darrault and Schlindwein, 2005). This could be evaluated by investigating the differences in nectar production and pollinaria removal between daytime and nighttime (Morse and Fritz, 1983). Nocturnal nectar production and pollinaria removal were not investigated in this study. However, the attachment rate of pollinaria on nocturnal visitors was lower in *M. formosana* (3.2%) than in moth-pollinated Asclepiadoid species (12.6–17.6%; Nakahama *et al*., 2013; Mochizuki *et al*., 2017), suggesting that diurnal insects as well as nocturnal moths may transfer the pollinaria of *M. formosana*. Because our observations were conducted only at night, further observations and experiments are needed to clarify the overall pollination ecology of *M. formosana*.

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