Life history notes on *Bicyclus dorothea* Cramer (Nymphalidae: Satyrinae) in Cameroon

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Abstract: *Bicyclus d. dorothea* (Cramer, 1779) is a widely distributed butterfly inhabiting much of the northern part of the tropical African rainforest. The biology of the species has not been well studied despite it being relatively common throughout its distribution. In this study, we report on the life history of *B. d. dorothea* following three years of research on the species in Cameroon. We describe the life cycle of the species and report on key life history aspects such as distribution, habitat, reproduction, and host plant relationships.

INTRODUCTION

The genus *Bicyclus* (Kirby, 1871) constitutes a group of over 100 species and several subspecies found exclusively on the African continent (Aduse-Poku *et al.* 2017). *Bicyclus* species tend to be common in the understory of a variety of forest types, although a fair number of species inhabit savannah ecosystems. Members of the genera *Bicyclus* and *Hallelesis* Condamin, 1961 were first considered to belong to the genus *Mycalesis* before Condamin in 1961 separated them into two distinct genera. These two genera shared a common Asian ancestor before diverging some 25 million years ago (Aduse-Poku *et al.*, 2015). Condamin's (1973) monograph classified the then-known 77 species into 29 species groups based on their morphology. Later work by Aduse-Poku *et al.* (2017) updated the systematics of the genus and arranged the now 103 species into sixteen revised species groups largely following Condamin's earlier work. Despite the genus now being fairly well resolved systematically, identification of many species remains challenging. Many species are very similar in appearance to one another, and in such cases, examination of male androconial brushes located beneath the hindwings are often used for identification. In some cases, females cannot be identified using wing morphology alone.

Bicyclus d. dorothea (Cramer, 1779), or the Light Bush Brown, might be one the most common forest satyrines in tropical African rainforests (Larsen 2005). In contrast to other satyrines in the region, *B. d. dorothea* is paler in color and relatively easy to identify from other satyrines. A recent phylogeny of *Bicyclus* demonstrated that *B. dorothea* can be grouped into a "*dorothea*-complex" consisting of *B. dorothea, B. moyses* Condamin & Fox, 1963 and *B. jeyfferyi* Fox, 1963 based on significant morphological and genetic similarities between the three species (Aduse-Poku *et al.*, 2017). In any case, little is known about the biology or ecology of *B. dorothea*. Apart from a few notes made on its immature stages in a comparison to other immature stages of *Bicyclus* and *Hallelesis* (Sourakov & Emmel, 1997) and some rearing experiments at Lamto, Ivory Coast (Vuattoux, 1994), little has been published with regards to its biology or natural history.

In this paper, we describe life history features of *B. d. dorothea* in Cameroon, with the ultimate goal of providing a background for further study of this species and other African satyrines. We report here on field observations and rearing records from research on the species between 2014 and 2016.

RESULTS AND DISCUSSION

Distribution

Bicyclus d. dorothea is a largely tropical species found in the whole west African forest zone. Its distribution extends to southern Cameroon, the northern part of the republic of Congo, Democratic Republic of Congo and Central African Republic (Aduse-Poku *et al.*, 2017). While Larsen (2005) and vande Weghe (2010) stated its presence in northern Angola, recent studies by Aduse-Poku *et al.* (2017) show that it is *B. moyses* which is present there. In addition, none of these studies noted its presence in Gabon, but it has recently been recorded at the Minkébé National Park, northern Gabon (van de Weghe 2016, pers. comm). The subspecies *B. d. concolor* Condamin & Fox, 1964 inhabits São Tomé, Principe, and Bioko islands (Larsen, 2005). Among species of the "*dorothea*-complex", *B. moyses* has a sympatric distribution with *B. d. dorothea* in southern Cameroon, northern Democratic Republic of Congo and the Republic of Congo, while in Angola, southern Democratic Republic of Congo, Republic of Congo, Gabon and Angola, only *B. moyses* is present. Of the remaining species of the complex, *B. jeyfferyi* is mostly found in eastern Africa. *Bicyclus d. dorothea* seems to be distributed in low to mid elevation habitats. Out of 28 localities sampled in Cameroon (Fig. 1), only one individual has been caught above 1000 m above sea level (1032 m on Mount Cameroon).

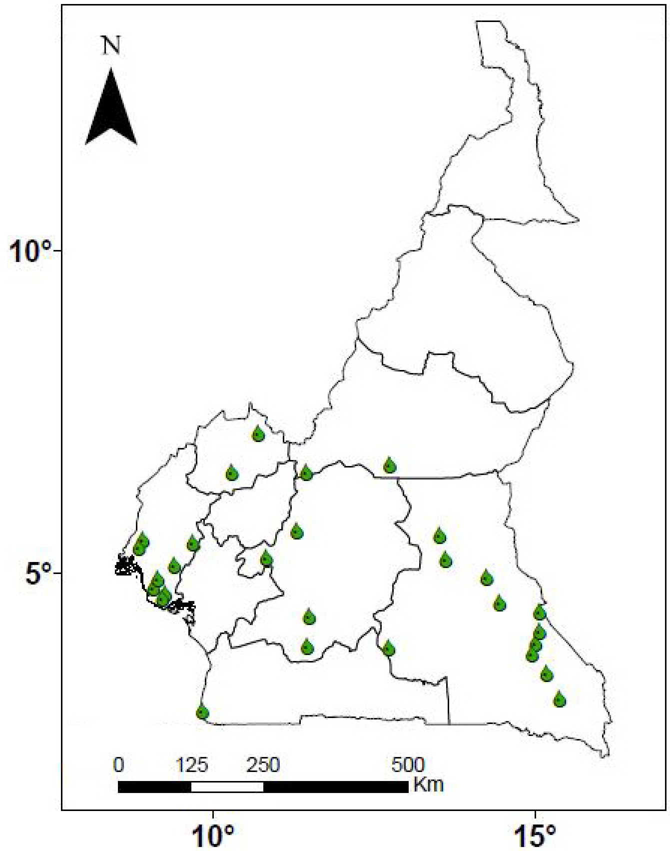

Figure 1. Map showing locations where specimens of *B. d. dorothea* have been collected in Cameroon from 2014 to 2016.

Habitat

Bicyclus d. dorothea inhabits a wide variety of forest types and appears particularly to favor degraded or disturbed forests habitats (Larsen, 2005). It can also be found in forest-savannah ecotones where population sizes appear relatively low compared to forest habitats. From our observations in the field, the species is rarely encountered in dense forest understory habitats though occasionally a female will fly away from a threat into the understory. Generally, *B. d. dorothea* is found in degraded forests where colonies establish in clearings and road tracks receiving sun and where larval host plants and adult food resources are abundant. Agricultural lands harboring canopy structures like palm farms, fruit trees and, cacao farms are also optimal habitats for the species, probably because these microhabitats provide abundant food resources such as the decaying fruits on which they feed.

Flight activity starts as soon as the sun has risen, but under intense sunshine conditions adults will retreat into shade. Males are abundant throughout the day while female numbers increase in the afternoon (from 14:00 to 17:00) when they display mating and oviposition behavior. *Bicyclus dorothea* individuals usually fly alone, although it is common to see contest behaviors in males.

Immature stages

Eggs

Bicyclus dorothea eggs are white in color and similar to other *Bicyclus* species (Sourakov & Emmel, 1997). Eggs are laid singly or in patches of two to ten on the underside of host plant leaves (Fig. 2a) and typically take three to five days to hatch under natural conditions. One day before hatching, the upper pole of the egg will turn black (Fig. 2b), representing the dark head capsule of the developing larva.

First instar larvae

After the egg maturation, the young caterpillar ecloses by nibbling a portion of the egg's shell. The newly emerged first instar larva has a 3 to 3.5 mm long, cylindrical white body covered by dorso-lateral rows of setae (Fig. 2c). At the posterior end of the body, a pair of backward-pointing setae can be easily observed. The head is dark and bears setae, protuberances, and one pair of short horns. First instar larvae are mobile immediately after eclosion and their body color turns greenish over time. After about three to five days, they molt into the second instar.

Second instar

Molting in *B. dorothea* is consistent with other immature stages of *Bicyclus* species (Sourakov & Emmel, 1997). Larvae will outgrow their head capsule, with the cuticle of the body remaining intact. The second instar larva of *B. dorothea* is characterized by a brownish head capsule with a well distinguished pair of horns; the body is greenish with more pronounced setae relative to the first instar (Fig. 2d). This instar lasts four to six days before reaching the next stage.

Later larval instars

The next instars (third, fourth and fifth) differ from the previous essentially in terms of body length and head capsule colors (Fig. 2e-g). The body color is quite uniform and there is an increase of the body length as they grow. The third instar has a dark cephalic capsule with green striations in front. Horns are distinguishable and this stage takes about four to six days to complete. The next two molts bring the caterpillar to fourth and fifth instars.

Pre-pupal and pupal stages

At the pre-pupal stage the body of the caterpillar gradually shrinks in length and the caterpillar finds a spot on the underside of a leaf blade where it spins a silk pad (Fig. 2h). It then brings its head towards the anal end to form a pre-pupa. This stage lasts one day at ambient temperature but can reach up to three days when reared at temperatures lower than 20°C.

The pupa of *B. dorothea* is green in color and approximately cylindrical (Fig. 2i). Pupation takes place as follows: once the pre-pupa is matured, a slot opens on the head capsule and the hanging caterpillar makes uncoordinated movements to facilitate shedding of the body cuticle toward the anal end. In most cases, the shed cuticle is removed from the fresh, soft pupa which hardens a few hours later. This stage lasts six to eight days under ambient conditions, and the pupa darkens in coloration one day before the imago's emergence (Fig. 2j).

Adult variation and identification

One of the most visible wing features in *Bicyclus* is the presence of eyespots. Many *Bicyclus* species display strong phenotypic variation of these traits over seasons (Brakefield & Reistma, 1991; Roskam & Brakefield, 1999; Brakefield *et al.*, 1998). *B. dorothea* adults have among the lightest colored eyespots of the group, although the species is very similar to

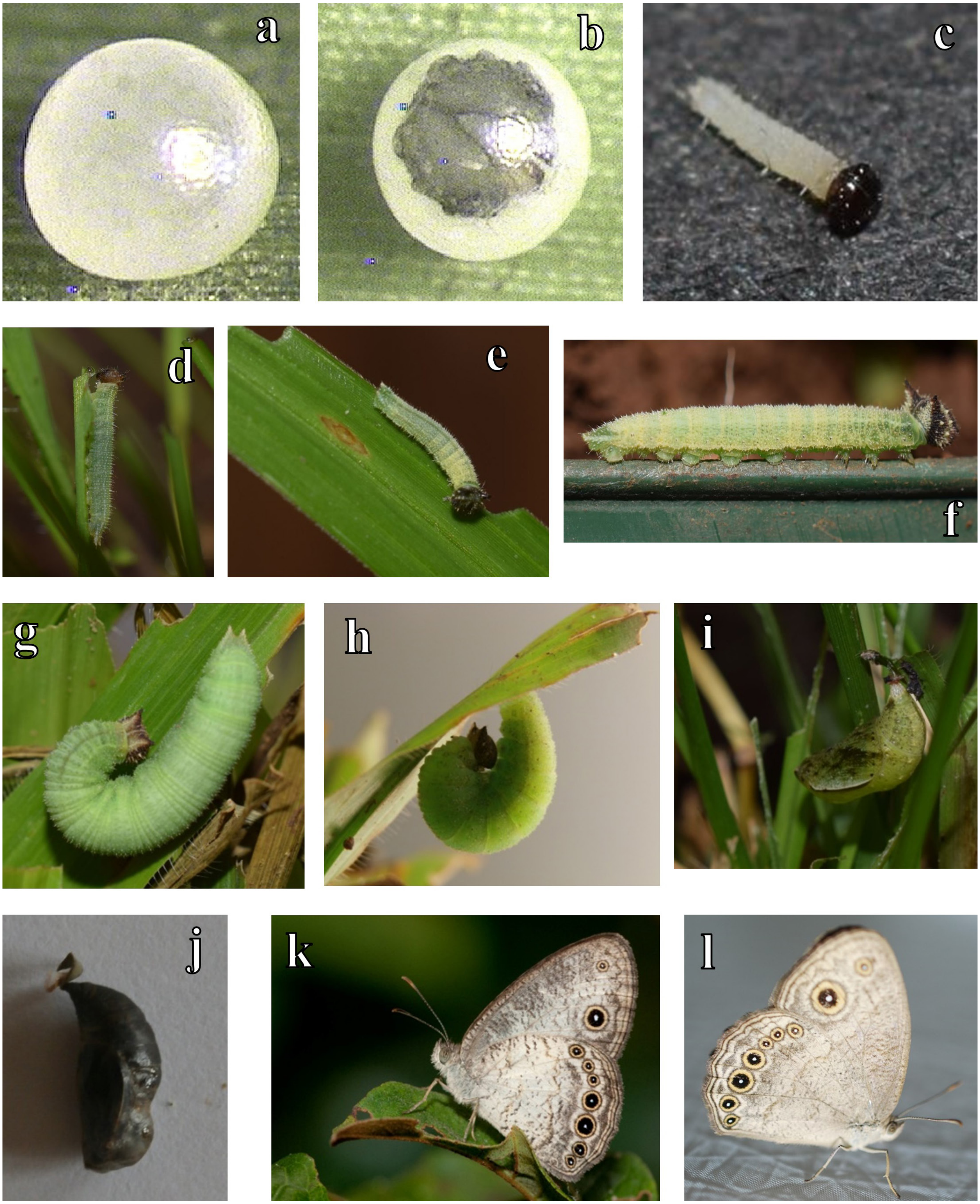

Figure 2. Life cycle of *B. d. dorothea*: (a) newly laid egg; (b) egg on a lawn leaf with the head of the first instar caterpillar visible through the egg shell; (c) first larval instar; (d) second larval instar; (e) third larval instar; (f) fourth larval instar; (g) fifth larval instar; (h) prepupa; (i) early pupa; (j) pupa a few hours before the emergence of the adult; (k) ventral view of an adult male (Photographed by Oskar Brattström in Liberia); (l) ventral view of an adult female.

other members of the "*dorothea*-complex", namely *Bicyclus moyses* and *B. jefferyi*. There is low genetic divergence among these species, but there are consistent differences in wing pattern (Aduse-Poku *et al.*, 2017). *B. dorothea* possess two eyespots on the forewing and seven on the hind wing, although sometimes individuals can display three eyespots on the forewing and eight on the hind wing. The mean wing span is about 40 mm; in males, the discal area of the forewings and the basal half of the hind wings are light grey while the rest of the wing is darker (Fig. 2k, Fig. 3a). The underside of the wing is gray-beige with the eyespots not visible. The male's hind wing bears two androconial brushes which are absent in females; this is one of the main features that can be used to differentiate *B. dorothea*, *B. moyses* and *B. jefferyi*. In fact, males of all three species have two androconial brushes in cell Rs and CuA2 of the hind wings, but in *B. dorothea* the brush is light brown to yellow and weakly developed in cell Rs while in *B. moyses* the brush is darker, and it is almost black in *B. jefferyi* (Aduse-Poku *et al.*, 2017). The female wings are uniformly light grey in *B. dorothea* (Fig. 2l, Fig. 3b).

Laval host plants and adult feeding

Larvae of the genus *Bicyclus* feed primarily on grasses (Poaceae). A semi-artificial diet made of bean flour has been developed and has been used to successfully rear immature stages of *B. anynana*, *B. ena* and *B. safitza* (Holloway *et al.*, 1991). Many species in the family Poaceae are used as host plants by *B. dorothea*. Larvae have been reared on the genera *Oplismenus*, *Paspalum* and *Axonopus* at Lamto, Ivory Coast (Vuattoux, 1994). In our research, we reared *B. dorothea* on millet, *Pinnesetum glaucum*, and *Axonopus*, but all trials with maize (*Zea mays*) were unsuccessful, in contrast to *B. anynana* where maize may be used for rearing (Kooi, 1992).

Whilst a majority of adult butterflies obtain carbohydrates by feeding on nectar (Bonebrake *et al.*, 2010), satyrine species usually feed on decaying fruits (Boggs, 1997a,b). *Bicyclus dorothea* feeds on fallen fruits found in the forest such as guava, overripe banana, umbrella fruit tree, mangoes and many other species. Although we have observed a few individuals exhibiting mud-puddling behaviour, we have never seen the species foraging on dung or on nectar.

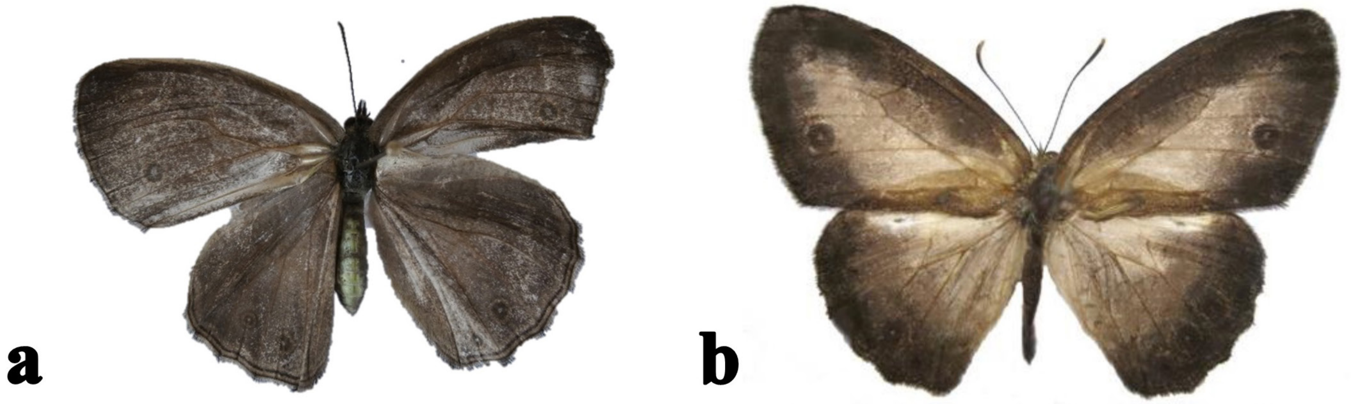

Figure 3. Dorsal view of (a) an adult male and (n) an adult female of *B. dorothea*.

Mating, reproduction and adult life span

Mating, reproduction, and adult life span have been well studied in *B. anynana* (Pijpe, 2007; Geister *et al.*, 2008), and this research serves as a useful comparison with *B. dorothea*. These studies have highlighted the role of experimental set up (Brakefield *et al.*, 2009), dorsal eyespot and wing sizes (Breuker & Brakefield, 2002), sex pheromone production, sexual conflicts in males (Wiklund *et al.*, 2001), sexual selection and the problem of protandry in reproduction (Zwaan *et al.*, 2008; San Martin *et al.*, 2011). In *B. anynana*, males and females are generally pooled together following eclosion in hanging cages for mating purposes. Females tend to be ready to mate after two or three days, after which they lay eggs during the two following weeks (Brakefield *et al.*, 2009). Reproduction in *B. dorothea* appears to be similar to *B. anynana*, although some differences are apparent; for example, *B. dorothea* females generally do not mate for approximately 10 days after eclosion. Moreover, mating seems to be more challenging in *B. dorothea* than in *B. anynana* and we had some difficulty producing viable eggs from lab-mated females. This may be due to the fact that *B. anynana* used in many laboratories in Europe has been selected for many generations in the lab to be quick to mate. Almost all other species tested have a relatively long pre-oviposition period. Another possible explanation is that *B. anynana* exploits a short-lived rainy season, so mating quickly is advantageous, while forest-associated species can breed throughout the year and can afford to be more selective in mate choice and timing.

Despite some difficulties in initiating successful mating, we did eventually observe frequent mating pairs in the lab and also in the field. In the lab, we also frequently observed females laying unfertilized eggs, likely as a consequence of unsuccessful mating. As observed in many insects, female *B. dorothea* lay most of their eggs in the first two weeks following the first oviposition event. Life span, like other traits, varies with environmental conditions, but we observed that at 25°C *B. dorothea* adults can survive up to 60 days in the lab.

CONCLUSION

Research on *B. anynana* has developed the species into a model organism for topics such as development, ecology and evolution. In recent years, additional research on other *Bicyclus* species has provided an opportunity for powerful comparative approaches (Brakefield, 2010). In this paper, we have provided notes on *B. dorothea* life history which we hope will aid further investigation of this and other *Bicyclus* species. Such research is particularly important in Cameroon, where the ecological impacts of anthropogenic change are significant, but remain poorly understood (Lawton *et al.*, 1998).

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