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First Report on the Larval Development of *Caryedon furcatus* (Anton & Delobel), the Primary Pest of *Senegalia macrostachya* Reichenb. ex DC. (Kyal & Boatwr.) Seeds in Storage in Burkina Faso

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Abstract

Data on some biodemographic parameters of *Caryedon furcatus* (Anton & Delobel), a beetle belonging to the Chrysomelidae family and the primary pest of *Senegalia macrostachya* Reichenb. seeds, an edible wild legume in Burkina Faso, have been reported by some authors, but they do not include the development times of the immature stages or their descriptions. Our main objective was therefore to document this missing data on the biology of *C. furcatus*. We have therefore updated the biodemographic parameters of *C. furcatus* and identified larval stages which are currently unknown. For this purpose, *Senegalia macrostachya* seeds were exposed to *C. furcatus* adults for twenty-four hours and then incubated. Twenty seeds, each with no more than one egg, were selected and placed in a petri dish to form a seed batch that was stored in a freezer (−18˚C) until adults emerged from the incubated seeds. These seeds were then removed from the freezer and dissected after soaking in water. To determine biological parameters, forty pairs were isolated upon emergence and placed in Petri dishes containing 20 g of seeds. After twenty-four hours, the insects were transferred to new dishes, and this process was repeated daily until the females died. The eggs laid were observed until emergence of adults. The results showed that the average development time for each larval stage was 9 days for egg to first instar larva; 3.25 days for first instar larva to second instar larva; 4 days for second instar larva to third instar larva; 5.1 days for third instar larva to fourth instar larva; 6.07 days for fourth instar larva to pupa; and 10.2 days for pupa to adult. The results also showed that females live longer than males and the sex ratio was female-biased. Females laid during their lifetime an average of 25.4 eggs, of which an average of 21.95 hatched. The pre-oviposition period was approximately one day, and the peak of female oviposition occurred on the second and third day after emergence. The population of *C. furcatus* doubles every 9.63 days. These results will contribute to a better understanding of the biology of *C. furcatus* and hence provide cues for further studies towards effective control using parasitoids.

Keywords

Caryedon furcatus, Development Time, Immature Stages, Oviposition, Biodemographic Parameters

1. Introduction

In many tropical African countries, legumes are a vital food supplement for rural populations, who generally depend on their agricultural production throughout the year [1]. While many leguminous species have been domesticated, a significant number remain wild in forest ecosystems. In Burkina Faso, *Senegalia macrostachya* Reichenb. ex DC (Kyal. & Boatwr.) holds a prominent position among these undomesticated wild food legumes due to the numerous benefits its seeds provide. Commonly known as zamné, they are commonly used in Burkina Faso as a vegetable or food ingredient [2]. With a high protein content (35.76%) and micronutrients such as calcium (68.40 mg/100g), iron (4.77 mg/100g), and zinc (5.34 mg/100g), they make a significant contribution to improving population dietary quality [3]. *S. macrostachya* seeds are consumed by livestock, which helps balance their rations during the dry season when other resources are scarce [4]. Additionally, healthy seeds are essential for the species' assisted natural reproduction, a vital mechanism for the stability and resilience of semi-arid ecosystems [2]. Economically, the marketing of these seeds generates substantial income for rural populations [5]. They also hold potential in the pharmaceutical industry, because *S. macrostachya* seeds contain an extract with natural peptides that improve skin hydration. This opens up prospects for their wider use in the cosmetics industry [6]. However, post-harvest management of *S. macrostachya* seeds faces numerous challenges, the most critical of which are related to damage caused by insect pests [7] [8]. *Bruchidius silaceus*, *Bruchidius* sp., and *Caryedon furcatus* are the primary pest species identified in *S. macrostachya* seed stocks during storage [8]. *C. furcatus* is considered the most significant pest, as it is the only primary pest capable of remaining in stock throughout the storage period, causing substantial damage in both quantity and quality. The rate of perforated seed increased from 16% at the beginning of storage to 35% after eight (8) months of storage [8]. Consequently, it acts as a significant barrier to exploiting the seed potential of this wild legume. Therefore, any control strategy should preferably target this pest. Knowledge of the biological parameters of *C. furcatus* such as longevity, development duration, sex ratio, and larval development duration, etc., will enable better adaptation of control strategies. Several studies have explored various aspects of *C. furcatus* biology [9]-[11]. However, these studies have not addressed the different immature stages of this insect pest. The description of the various immature stages of *C. furcatus*, as well as the duration of their development, is therefore poorly documented. Thus, our study, which additionally considers biodemographic parameters, aims to fill this knowledge gap and contribute to a better understanding of *C. furcatus*. Specifically, we will: i) identify and describe the different larval stages of *C. furcatus*; ii) determine the development time of each larval stage; and iii) determine biodemographic parameters of *C. furcatus*.

2. Materials and Methods

2.1. Materials

2.1.1. Plant Material

The *Senegalia macrostachya* seeds used for mass rearing and various tests were purchased from local farmers in the town of Toma, latitude 12˚45'45" longitude 2˚54'01", located in the Boucle du Mouhoun region of the Nayala province (located 315 km from Ouagadougou). These seeds were not pre-treated after harvesting. They were then sorted manually to eliminate those that were malformed or showed signs of insect emergence holes. The apparently healthy seeds obtained after this sorting process were stored in a laboratory freezer at −18˚C throughout the duration of the experiment to eliminate any potential prior infestations.

2.1.2. Animal Material

The animal material used consisted of a strain of *Caryedon furcatus* isolated from *S. macrostachya* seeds obtained from pods harvested directly from spontaneous vegetation in Laye, 35 km from Ouagadougou on the Ouaga-Ouahigouya road. This strain was maintained at the Laboratoire d'Entomologie Fondamentale et Appliquée (LEFA) at the Université Joseph KI-ZERBO using a mass rearing system under the following conditions: 35.42˚C ± 4.31˚C and 40.97% ± 2.26% relative humidity. The procedure involved placing six 50 g batches of healthy seeds in contact with 15 pairs of *C. furcatus* insects in six Plexiglas rearing boxes (length = 14 cm; width = 10.5 cm; height = 3.5 cm). The boxes were then covered with mosquito netting and incubated until adult emergence. To maintain the strain in the laboratory, this same process is repeated as soon as the adults emerge.

2.2. Methods

2.2.1. Determination of the Different Larval Stages and Duration of Larval Development of *Caryedon furcatus*

One hundred (100) pairs of *C. furcatus* up to 48 hours old were brought into contact with 500 g of healthy *S. macrostachya* seeds introduced into a Plexiglas box (length = 30 cm; width = 19 cm; height = 14.5 cm). The box was then incubated at 35.42˚C ± 4.31˚C and 40.97% ± 2.26% relative humidity for monitoring until adult emergence. The contact time between insects and seeds was 24 hours. At the end of this period, the insects were removed from the Plexiglas box. After the insects were removed, 20 seeds, with a maximum of one egg each, were collected daily from the Plexiglas box and placed in a Petri dish to form a batch of seeds, which were then stored in a freezer at −18˚C. This operation stopped when the adults emerged. The 20 seeds in each batch constituted 20 replicates. As soon as emergence began, all seed batches stored in the freezer were removed for dissection after soaking in water for 24 to 48 hours. Seed dissection was carried out using a dissecting kit, with the aim of collecting the larva attached to each seed to describe it and measure its length and width. A binocular magnifier (Leica EZ4HD) equipped with a measurement function was used for this purpose. The distinction between the different larval stages was based on the morphological changes observed in the most representative individuals of each stage. Ten individuals from each post-embryonic developmental stage were selected for these measurements.

To determine the duration of the different larval stages, the various dates from oviposition to the date when the seeds were placed in the freezer were recorded. Additionally, to monitor the overall development of the insects, the dates from oviposition to insect emergence (from the first to the last) were noted. Sex determination was performed using a Leica EZ4HD magnifying glass, which provided better visualization of sternite V. This sternite is distinctly emarginated in males but not in females.

2.2.2. Determination of *C. furcatus* Biodemographic Parameters

As soon as they emerged, insects under 24 hours old were isolated and placed in pairs in Petri dishes containing 20 g of *S. macrostachya* seeds. A total of forty pairs was formed to constitute 40 replications. After twenty-four hours, the insects were transferred to new dishes. This process was repeated daily until the females died. The average temperature and relative humidity during this test were 35.42˚C ± 4.31˚C and 40.97% ± 2.26%, respectively. Monitoring the eggs from hatching to adult emergence allowed us to determine some biodemographic parameters that constitute key indicators for assessing the proliferation potential of this pest population, which will enable its impact to be anticipated and control or management strategies to be adapted. These parameters include:

Development time (DD): It represents the time elapsed between egg laying and adult emergence. This parameter provides insight into the speed of infestations and the resulting damage to stocks. This makes it possible to anticipate periods of high risk and implement more effective pest management strategies. It is estimated using the equation:

$$DD = \sum nixi \Big/ \sum xi$$

xi : number of insects emerging per day; ni : corresponding number of days.

Intrinsic rate of natural increase (Rm): This is the population growth rate attributable to natural population movement. It is a key parameter that will enable us to understand this insect's ability to proliferate under our conditions. It was obtained using the formula of [12]:

$$R_m = \ln(NS)/T + 1/2L$$

L = female lifespan; N = average number of eggs laid per female; S = larval survival rate; T = development time; ln = natural logarithm

Finite growth rate (λ):

$$\lambda = e^{R_m}$$

The net reproduction rate (R_0): it is the average number of female offspring produced by each female during her lifetime. It is estimated by the formula:

$$R_0 = \sum lxmx$$

l_x is the probability of survival from the egg stage to the beginning of class x, m_x is the average number of female offspring per female of age x, x is the age class.

Population doubling time (DT): It is the time required for the population to double in size. It was determined by using the formula of [13]

$$DT = \ln 2/Rm$$

3. Data Analysis

The data were initially organized using an Excel spreadsheet, where means and standard deviations were also calculated. All statistical tests were performed using R software version 4.2.1 (2022-06-23). Data were first tested for normality using the shapiro.test function, followed by Bartlett's test for homogeneity of variances. Depending on the distribution, either a permutation analysis of variance (ANOVA) (parametric test) or the Kruskal-Wallis test (non-parametric test) was used. The pairwise.perm.t.test function was employed for pairwise comparisons of means in the permutation ANOVA, and the dunn.test function was used for pairwise comparisons in the Kruskal-Wallis test. A significance level of 5% was applied.

4. Results

In addition to the embryonic stage, *Caryedon furcatus* passes through four larval stages, followed by a pupal stage. The different larval stages vary in shape, colour, and size. The duration of development from one immature stage to the next also varies.

4.1. Embryonic Stage of *Caryedon furcatus*

The freshly laid egg is translucent and sticks to the seed wall, with an oblong shape. The egg measures approximately 1.00 ± 0.08 mm in length and 0.65 ± 0.03 mm in width (**Table 1**). Hatching occurs on average 72 hours (3.33 ± 0.58 days) after laying. At this stage, a reddish (or black) spot can be seen inside the egg, representing the head capsule of the first-stage larva.

Table 1. Size variations of eggs and the main post-embryonic developmental stages of *C. furcatus*.

	Length (mm)	Width (mm)
Immatures stages	Mean (±SD) Min-Max	Mean (±SD) Min-Max
Egg	1 ± 0.08 0.91 - 1.15	0.65 ± 0.03 0.6 - 0.72
first larval stage (L1)	0.99 ± 0.09 0.81 - 1.08	0.56 ± 0.1 0.37 - 0.66
second larval stage (L2)	2.38 ± 0.21 2.07 - 2.81	1.20 ± 0.12 1.06 - 1.41
third larval stage (L3)	3.68 ± 0.25 3.22 - 3.96	1.53 ± 0.19 1.19 - 1.88
Fourth larval stage (L4)	5.7 ± 0.58 4.81 - 6.54	2.62 ± 0.3 2.06 - 3.19
pupa	4.92 ± 0.29 4.26 - 5.3	2.63 ± 0.15 2.33 - 2.87

4.2. Immature Stages during Post-Embryonic Development of *Caryedon furcatus*

The length and width of the larvae increase significantly from L1 to L4 (Table 1 and Table 2). However, the length of the pupa is less than that of the L4 larva, contrary to their width (Table 1). The development time also increases gradually from L1 to L4, as well as from L4 to the pupa and from the pupa to the adult (Table 3).

The first instar larva (L1) is whitish, globular, and characteristic of the chrysomelid type (Figure 1), measuring approximately 0.99 ± 0.09 mm in length and 0.56 ± 0.1 mm in width (Table 1). Forty-eight hours after hatching, the larva begins to actively burrow into the seed. During this process, the larva ejects tegument and cotyledon debris into the chorion, which turns a whitish-yellow colour. After an average of 96 hours, it has completely penetrated the seed. The average time from the egg stage to the L1 stage is nine (9) days (Table 3).

Once inside the seed, the 1st instar larva does not significantly change shape. It undergoes two molts and gradual growth, progressing through the second (L2) and third (L3) instars (Figure 1). The second instar larva measures an average of 2.38 ± 0.21 mm in length (Table 1). It is strongly curved and yellowish-white in colour. The average time for a larva to transition from the 1st to the second instar is 3.25 days (Table 3). The third instar larva (Figure 1) is also curved, yellowish in colour, and begins to show segmentation on its body. Its average length and width are 3.68 ± 0.25 mm and 1.53 ± 0.19 mm, respectively (Table 1). The L2 larva takes an average of four (4) days to develop into the L3 stage (Table 1).

The fourth larval stage (L4) (Figure 1) weaves a translucent membranous cocoon on the outside of the seed. This cocoon is yellowish-white in colour and morphologically distinct from the L1, L2, and L3 stages. It is covered with a reddish pubescence, clearly visible under a magnifying glass. Body segmentation is also clearly visible, and the legs and antennae appear as translucent outlines. The average L4 larva measures 5.7 ± 0.58 mm in length and 2.62 ± 0.30 mm in width (Table 1). The average time for L3 larvae to transition into L4 is 5.1 ± 0.88 days (Table 3).

Table 2. Comparison of the size of different larval stages.

Larval stages	P-value (length)	P-value (width)
L1 - L2	1.28e−10	1.19e−10
L1 - L3	1.37e−12	1.38e−9
L1 - L4	4.92e−10	4.04e−10
L1 - pupa	3.61e−13	1.74e−16
L2 - L3	4.05e−10	3.65e−4
L2 - L4	2.03e−9	1.41e−8
L2 - pupa	1.38e−13	2.38e−14
L3 - L4	2.62e−7	6.47e−8
L3 - pupa	9.16e−9	5.24e−11
L4 - pupa	2e−3	9.12e−1

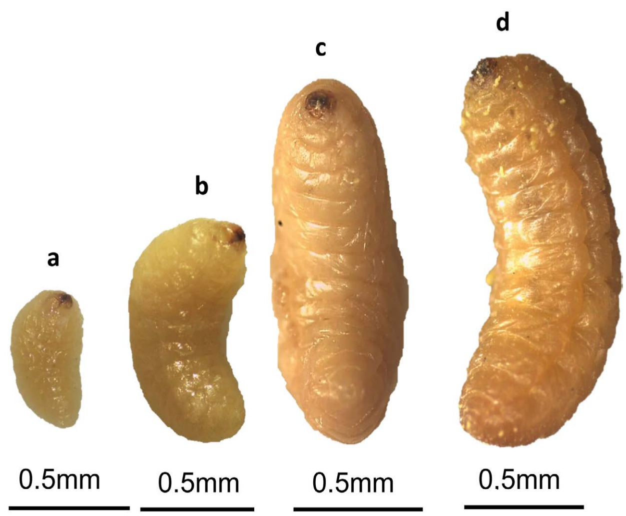


Figure 1. Different immature stages of C. *furcatus*: a: first larval stage L1; b: second larval stage L2; c: third larval stage L3; d: fourth larval stage L4.

Pupae, the final immature stage, are initially whitish-yellow (**Figure 2(a)**) and resemble the adult, with an average length of 4.92 ± 0.29 mm and a width of 2.63 ± 0.15 mm (**Table 1**). As the pupa develops, it gradually turns reddish-brown, eventually maturing into an adult (**Figure 2(b)**). Initially, the pupa has a single dark black spot at the eye level, which soon develops into several black spots. These spots darken over time, and the eyes become large and prominent, while the mandibles also turn dark black. The pupa resembles the adult with an elongated, oval body and strongly dilated, denticulate posterior femora along the ventral edge (**Figure 2(b)**). The antennae are long and translucent in the pre-pupal stage. They are composed of segments. On average, the transition from L4 to pupa takes 6.08 days, while the transition from pupa to adult takes approximately 10.2 days. The mature adult stage begins with light-brown imagos covered in russet pubescence. Under our experimental conditions, the overall development duration was an average of 34.09 ± 4.3 days.

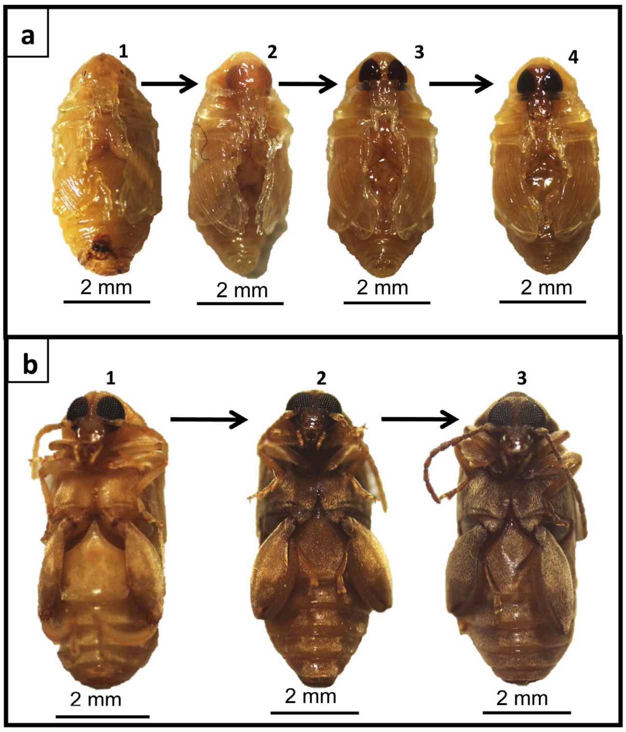


Figure 2. Pupal stage and adult of *Caryedon furcatus*; a: young pupa; b: mature pupa becoming adult.

Table 3. Variation in development time of different immature stages of *C. furcatus*.

	Mean (±SD) (in days)	Min - Max
Egg-to-hatch stage	3.33 ± 0.58	3 - 4
Egg to L1 stage	9 ± 3.31	4 - 14
Stage L1 to L2	3.25 ± 0.46	3 - 4
Stage L2 to L3	4 ± 0.91	3 - 6
Stage L3 to L4	5.1 ± 0.87	4 - 7
Stage L4 to pupa	6.07 ± 0.86	5 - 8
Pupa to adult	10.2 ± 1.09	9 - 12
Egg to adult	34.09 ± 4.30	27 - 40

4.3. The Average Lifespan of Adult *C. furcatus*

The average lifespan of *Caryedon furcatus* is 7.52 ± 2.06 days. However, this lifespan varies significantly by gender ($P = 0.0001$). Thus, the lifespan of females is relatively longer (around 8.6 days) than that of males (around 6.45 days).

4.4. The Reproductive Potential and Oviposition Activity of *C. furcatus*

On average, female *C. furcatus* lay 25.40 ± 10.85 eggs during their lifetime. The average laying period is 7 days. The number of eggs laid, which was zero on the first day, increases to a median of 7.85 eggs on the second day. However, the median number of eggs laid on the 2nd and 3rd days is not statistically different. This number then gradually decreases to a median of 2 eggs on the 5th day and then does not vary significantly until the 6th day, after which no eggs were laid (Figure 3). The estimated average egg hatching rate is 86.42 ± 25.67%. Ninety-two point zero three percent (92.03%) of larvae from hatched eggs complete their development until emergence, representing an emergence rate of 79.53% (Table 4).

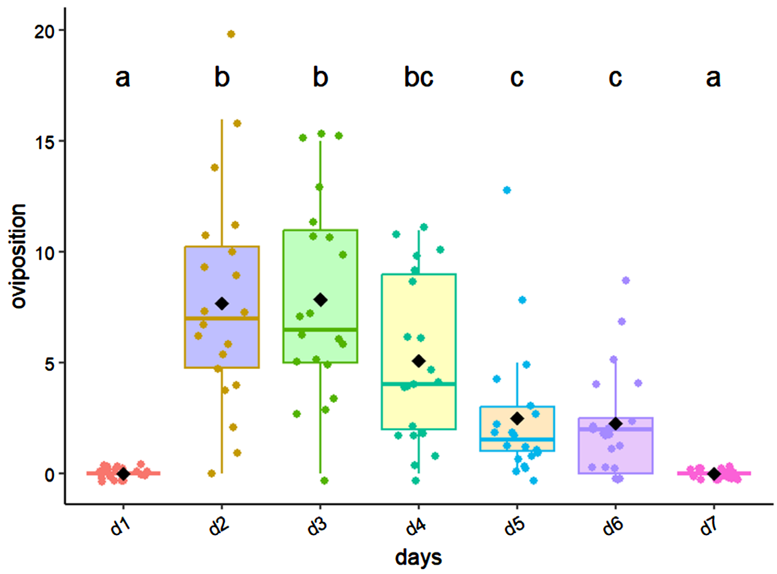


Figure 3. The evolution of the average number of eggs laid per day by *C. furcatus* females throughout her life. Groups with the same letter do not have significantly different averages. However, groups with different letters have significantly different averages.

Table 4. Average number of eggs laid and hatching rate.

Parameters	Mean
Number of eggs laid	25.4 ± 10.86
Number of eggs hatched	21.95 ± 13.04
Number of sterile eggs	2.25 ± 11.22
Hatching rate (%)	86.42 ± 25.67
Larval mortality rate (%)	7.97 ± 29.43
Larval survival rate (%)	92.03 ± 29.43
Emergence rate	79.53 ± 20.47
Total number of individuals emerged	20.2 ± 14.85

4.5. Some Parameters of the *C. furcatus* Life Table

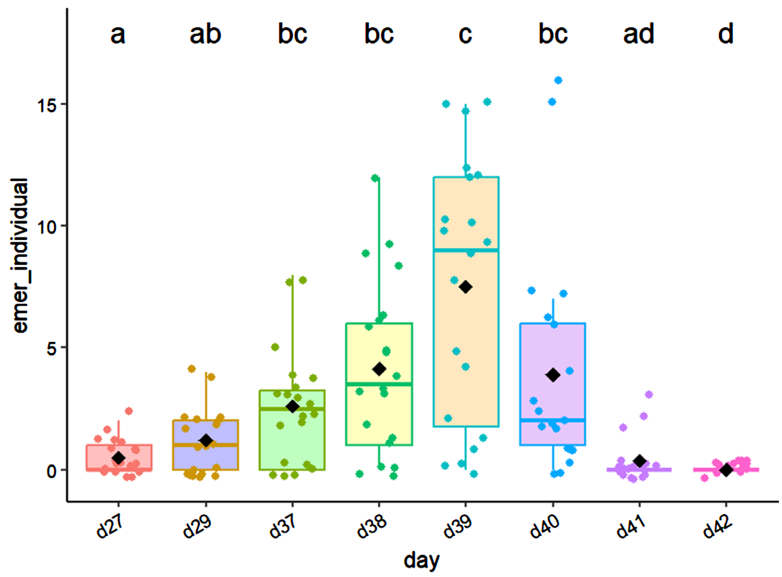
Table 5 shows a natural growth rate of 0.072 per day. Thus, *C. furcatus* population multiplies 1.074 times per day (λ) with a doubling time (DT) of 9.63 days. A female is capable of producing 11 female offspring (R0) in one generation.

Table 5. Biodemographic parameters.

Intrinsic rate of natural increase (R_m)	0.072
Reproduction rate (R_0)	11
Finite growth rate (λ)	1.074
Doubling time (DT)	9.63 (days)

4.6. Trends in Adult Emergence and Sex Ratio of *C. furcatus*

The first adult emergences were observed on day 27 (minimum) and ended on day 41 (maximum) after oviposition. There was low emergence on day 41, with less than 1% of individuals emerging. There was no emergence from day 30 to 36. Emergences mainly occurred between the 37th and 40th day. The maximum number of individuals emerged (7 individuals) on the 39th day (Figure 4). After the 41st day, no more individuals emerged.



Groups with the same letter do not have significantly different averages. However, groups with different letters have significantly different averages.

Figure 4. Trends in *C. furcatus* adult emergence.

5. Discussion

The genus *Caryedon* includes species within the Bruchinae subfamily. *Caryedon furcatus*, initially identified as *Caryedon mauritanicus*, was reported on *S. macrostachya* seeds in Senegal in 1995 by [9] and in Burkina Faso in 1981 by [14].

The first detailed description of *C. furcatus* was made in 2004 by [15]. However, this description did not differentiate the various immature stages of the insect. The biology of *C. furcatus* is similar to that of other Bruchinae species associated with legume seeds. The insect develops on *S. macrostachya* seeds through four larval stages and a pupal stage. As with other species of the genus Caryedon, the first-instar (L1) larva enters the seed and remains there until the fourth-instar (L4) stage, at which point it emerges from the seed and spins a silken cocoon outside for pupation [16] [17]. However, there are no data in the literature on the immature stages of this species. This study, the first of its kind in Burkina Faso, allowed us to distinguish the different immature stages of *C. furcatus* during its post-embryonic development, determine the duration of larval development in this bruchid species, and measure the time interval for a larva to transition from one stage to another. The results show that *C. furcatus* larvae are of the Chrysomelidae type, similar to *Spermophagus niger* [18]. The findings reveal that the time from the egg stage to the L1 stage is approximately nine (9) days on average. In *Caryedon serratus*, [17] noted that the egg hatches around one week after oviposition. The second instar larva (L2) is the same color as the L1 larva, but there is a highly significant difference in size between these two larval stages (**Table 3**). The L3 larva is significantly larger than the previous two stages. Similar results were reported by [18] on *S. niger*. It takes an average of 3.25 days to transition from L1 to L2 and 4 days from L2 to L3. L4 larvae appear slightly less curved than the previous three larval stages and, like *C. serratus*, they are highly mobile. L3 larvae transition to L4 after an average of 5.1 days. The distinction between male and female becomes evident from the pupal stage onwards, with the difference observed in sternite V, which is notched in the female but smooth in the male. The same observation was made by [15]. The time taken for the pupa to transform into an adult is the longest compared to other stages. This could be explained by the presence of several pre-pupal stages (six observed in this study) and also by the fact that some insects, even when mature, remain in the cocoon for an extended period before emerging (personal observation). Our results corroborate those of [16], who estimated that the time taken between cocoon formation and adult emergence in *C. serratus* species at 35˚C averages 15 days. However, this duration can vary depending on environmental temperature and humidity. The measurements of the various larval stages of *C. furcatus* indicate that growth is continuous from the 1st to the 4th larval stage, as is the case with *S. niger* [18]. Our results showed that the embryonic development time of *C. furcatus* aligns with those obtained by [19] on the same insect, while the overall development time we recorded is shorter than that reported by the same authors (50.45 days on average). This discrepancy could be due to the higher temperature and relative humidity in our study. According to [20], slight variations in temperature can alter the metabolic activity of organisms. Indeed, insects have a limited capacity to regulate their body temperature as they are ectothermic organisms. As a result, temperature variations induce various changes in their biological activities, such as development, survival, reproduction, and behaviour [21]-[23]. The results of this study also showed that *C. furcatus* females have relatively short lifespans. However, it is longer than that of *C. serratus* females. It is also shorter than the 21.57 and 11 days obtained by [24] and [25] respectively, for *Caryedon serratus* and *Caryedon furcatus* females. The reproductive potential of *C. furcatus* females in this study is moderately high compared to the potential obtained by [26] in the same species. At temperatures ranging from 21˚C to 31˚C and relative humidities ranging from 45 to 95%, these authors obtained an average fecundity of 16.2 eggs per female. However, [25] work has shown that this species has a high reproductive potential. In our study, the pre-oviposition period was approximately one day (24 hours), and the average female laid 37.45 eggs at a mean temperature of 31.09˚C ± 2.18˚C and a relative humidity of 29.78% ± 1.38%. Our results could be explained by the fact that the females' physiological state and geographical origin are factors that likely influence their egg-laying behavior in the laboratory [26].

The average number of emerged females is statistically higher than the number of males. A female-biased sex ratio has already been demonstrated in several stored commodity insects, including *Rhyzopertha dominica* [27], *S. niger* [28], and *C. furcatus* [25]. The population doubling time is lower than the value obtained by [25]. However, our results are consistent with those of [16] regarding an *Arachis hypogaea* strain of *C. serratus* at 30˚C and 35˚C, with respective values of 8.9 and 9.5 days. The discrepancy in biodemographic parameters may be due to our temperature and humidity conditions (35.42˚C ± 4.31˚C and 40.97% ± 2.26%). According to [29] and [30], higher temperatures modify the demographic parameters; thus, a drop in temperature prolongs the development time of insects. In other words, temperature and humidity influence the development of beetles by altering their metabolic reaction speed, fertility, and life cycle length. An increase in temperature stimulates metabolic and developmental processes up to an optimal threshold, resulting in shorter development times. A major gap in the literature is the lack of data on the influence of ecological factors (temperature and humidity) on the development of *C. furcatus*. However, based on the work of [11], we can infer that an increase in temperature affects *C. furcatus* development time.

6. Conclusion

To find effective and sustainable ways to control an insect pest, it is crucial to understand its biology. This study is a significant advance in the scientific context, because it contributes to the precise identification of the different larval stages of *C. furcatus*, which were previously unknown. We were also able to determine the time taken by a larva to pass from one stage to another, as well as the overall larval development time. These data are important, because they should help to determine the best time to implement a strategy to combat this pest, especially one that uses biological control with parasitoids.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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