

Research Article

Biology of *Omaspides pallidipennis* Boheman, 1854 (Coleoptera: Chrysomelidae: Cassidinae)

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The biology and the feeding habits of the subsocial species *Omaspides pallidipennis* were studied at the Floresta Nacional de Passa Quatro, MG, Brazil, during the period from October 2010 to April 2011. The species was bivoltine, beginning its reproductive and food cycle in October (spring) and seeking its diapause sites in April (autumn). The juveniles took 54.4 days on average to complete their development, a period in which the female remained close to offspring, only feeding during the larval stage of the juveniles. It is a monophagous species, feeding only on *Ipomoea alba* Linnaeus (Convolvulaceae). In the first cycle, the average number of eggs was 55.7 ± 15.5 eggs per egg cluster ($n = 1, 837$ eggs in 33 clusters) and in the second it was 61.6 ± 14.2 eggs per egg cluster ($n = 5, 607$ eggs in 91 clusters). Oviposition peaks were observed in the months of November and February. The average durations of the incubation period and the larval and the pupal development in the first cycle were 19.2 ± 1.4 ; 26.0 ± 1.5 ; 8.7 ± 0.8 days, respectively. In the second cycle they were 16.7 ± 1.4 ; 27.0 ± 2.4 ; 10.2 ± 1.5 days, respectively.

1. Introduction

The family Chrysomelidae is one of the largest among the insects of the order Coleoptera [1]. Due to its diversity of representatives it is subdivided into 19 subfamilies [2]. Among these Cassidinae stands out for being the second largest in number of species (ca. 6,000 species), with approximately 16% of the diversity [3]. Its representatives also stand out for having unique morphological, ecological and biological characteristics [4]. However, an evident problem that exists regarding that subfamily is the shortage of information regarding the biology of many of its species. Although the majority is solitary, various species are subsocial. The study of those characteristics can explain the determination of the sequence and exact number of transitions among the way of life of the solitary, gregarious, and subsocial species [5]. Moreover, to know the relationship between the performance of the offspring and the egg laying preference, it is essential to understand the population dynamics of herbivore insects, as well as their distribution [6].

The majority of existing research on Cassidinae about the biology of the species, solitary or subsocial, was conducted in laboratory [7–10]. In field, the biology of subsocial species is described, minutely, for *Acromis sparsa* Boheman, 1854 (see, e.g., [11, 12]) and *Omaspides tricolorata* Boheman, 1854 [13, 14]. However, the number of species that exhibiting that behavior is much higher (16 species described, for the Stolaini and Eugenyssini tribes) and should increase, due higher number of researchers working with this theme.

For the subsocial species *Omaspides pallidipennis* Boheman, 1854, no data was found on its biology. Information about the description of the pupa and adults were given by Costa Lima [15], also registering the presence of the subsocial behavior [11, 15–18]. As for its distribution in Brazil, the species is found in the states of Espírito Santo, Minas Gerais, Paraná, Rio Grande do Sul, Rio de Janeiro, Santa Catarina, and São Paulo [19], in environment of Atlantic forest, riparian forest, and savanna (Fernando Frieiro-Costa, personal information). In relation to the host plant, information is also scarce. Few information exists

of *Ipomoea alba* Linnaeus, 1753 (Convolvulaceae) as host plant [19, 20]. Although most of the subsocial Cassidinae have been observed in only one type of host plant, some species can be found on different host plants genus. For *O. pallidipennis*, this fact has not been observed (Fernando Frieiro-Costa, personal information).

The objective of the present work was describe the biology of *Omaspides pallidipennis* Boheman, 1854 (Coleoptera: Chrysomelidae: Cassidinae) and its relation with host plant, in a natural environment in the Atlantic Forest biome.

2. Material and Methods

2.1. Study Area. The research was conducted in the Floresta Nacional (FLONA) de Passa Quatro, Municipal district of Passa Quatro, Minas Gerais State, Brazil (22° 23' S, 44° 56' O); altitude of 900 m; 335 ha), in an Atlantic Forest recovery area. The Conservation Unit (CU) contains roads that are used by tourists for visitation and by the guards for local patrols. The study was conducted on the host plants that grew on the edge of one of those roadsides.

The vegetation of CU is characterized by the insertion of a Semideciduous Seasonal Forest in the Atlantic Forest Biome, with a prevalence of planted plant coverings of pine, araucaria, and eucalyptus. Regionally, besides the Semideciduous Seasonal Forest, the Dense Ombrophylous Forest and Mixed Ombrophylous Forest typologies are found in the area [21]. The climate of the area, according to the Köppen classification, is Cwa-moderate temperatures with hot and rainy summers and dry winters. The climatic data were supplied by the National Institute of Meteorology (INMET) and presented an average temperature of 21.4°C, with precipitation and relative humidity of 291.9 mm and 76%, respectively, for the first life cycle of the species (October/January). For the second cycle (February/April) the temperature, precipitation, and relative humidity averages were 21.6°C, 116.9 mm, and 75%, respectively.

2.2. Biological Study of *O. pallidipennis*. The population of *O. pallidipennis* was observed daily, in the morning and in the afternoon (at alternate times), during the period between the months of October 2010 to April 2011. In this period 170 females with egg masses were accompanied and marked. The females received a mark on their elytron, facilitating the observation of parental care, of number of eggs deposited in each cycle, and of the development duration of the juvenile stages. For the marking of the females the Frieiro-Costa and Vasconcellos-Neto methodology was used [14]. Photographs of the egg masses, when the guardian was not over them, facilitated the obtaining of the average number of eggs. The oviposition and eclosion times were logged. Daylight saving time was not taken into account at any time.

2.3. Host Plant. The latescent *I. alba* vine frequently occurs in forest borders. It can also be found in crop areas, where it is a serious competitor of cultivated plants [22]. The flowers are solitary or gathered in groups, with a white or pinkish coloration [23–26]. In the lamina/petiole intersection there are extrafloral nectaries (EFNs) which are constantly visited

by various insect species, especially ants. In Brazil this plant can be found in the states of Bahia, Rio de Janeiro, São Paulo, Paraná, Santa Catarina, Rio Grande do Sul, and Ceará [23].

2.4. Statistical Analysis. The data were submitted to the Kolmogorov-Smirnov test, to verify the distribution type, being expressed as the average \pm standard deviation (SD). To compare the data of number of eggs and developmental time of immatures between one cycle and another, the Student's *t*-test was used for normal distribution data and the Mann-Whitney test for free distribution. For these analysis the Bioestat version 5.3 software was used [27].

3. Results and Discussion

3.1. General Aspects of Biology of *O. pallidipennis*. Bivoltine Coleoptera, *O. pallidipennis* began their reproductive and feeding activities in October (spring) and they sought the diapause sites in the middle of April (autumn). During the whole cycle the juveniles only received care by the female that protected them from any imminent danger.

Species of subsocial tropical Cassidinae, like *O. pallidipennis*, *O. tricolorata* [14] and *Omaspides brunneosignata* Boheman, 1854, do not usually present more than two annual generations, because they spend much time and energy taking care of a single group of offspring. For not being exposed to the seasonal extremes that impede reproduction and growth, tropical and subtropical Cassidinae, subsocial or not, can present a greater number of generations [28, 29], if compared to temperate region species that are usually univoltine [30, 31]. Nevertheless, they are exposed to the alterations of the dry and rainy stations, related to the adequate availability of food [32]. In some of those tropical species, the synchronization of the life cycle with the variable conditions is enabled through the diapause [32].

In the FLONA of Passa Quatro, *O. pallidipennis* presented monophagous habits. Adults as well as juveniles only fed on *I. alba*. Although other plants of the same family and same genus have been found in the CU, those Cassidinae were never observed on another host plant species. Besides *O. pallidipennis*, egg masses and adults of the solitary species *Chelymorpha inflata* Boheman, 1854 (Cassidinae: Stolinae) were found also feeding on *I. alba*. At no time were both species observed feeding on the same leaf. Besides *C. inflata*, grasshoppers and Chrysomelinae and Lepidoptera larvae were found feeding on the leaves of the chosen host.

I. alba was observed in FLONA of Passa Quatro, in an open field area as well as roadside. The specimens of the host plant remained under direct sunlight most of the day, with few shaded portions.

3.2. Immature Stages. These insects are holometabolic, their cycle being completed in approximately two months (54.4 days on average, from egg to adult).

3.2.1. Eggs. The egg clusters of *O. pallidipennis* presents a diamond-shaped format that, with elongated eggs, approximately 2.8 times longer than their highest width and without any covering (Figure 1(a)). When recently laid they

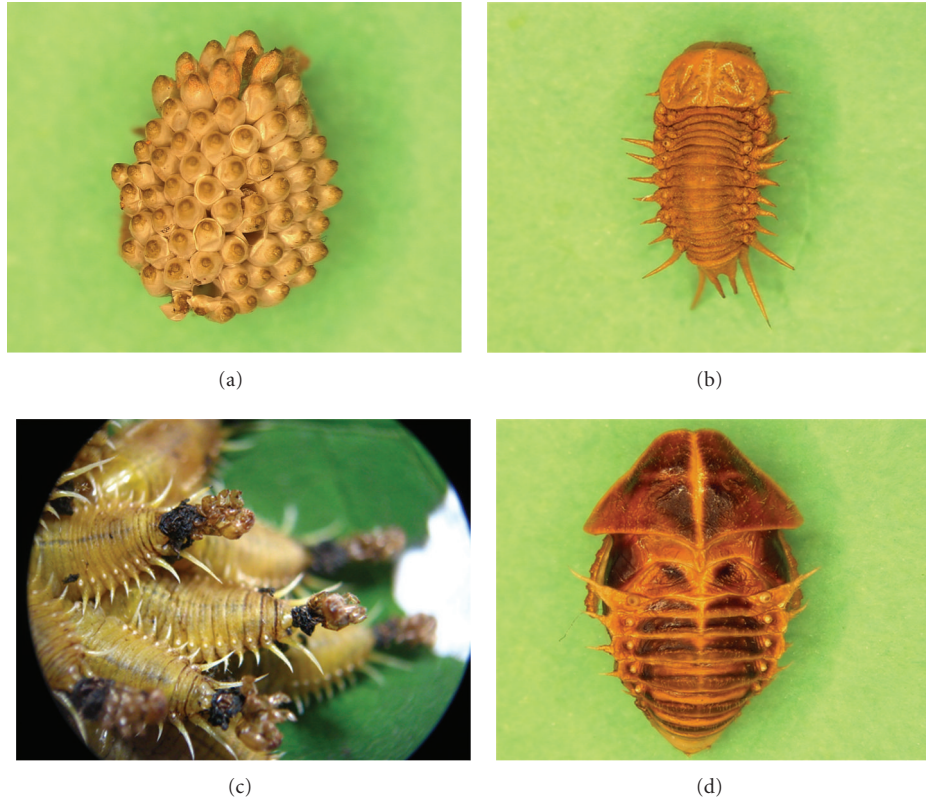


FIGURE 1: Immature stages of *Omaspides pallidipennis* Boheman, 1854 (Chrysomelidae). (a) Egg cluster, (b) dorsal view of last instar larvae, (c) exuvial-fecal shield, (d) pupae in dorsal view. Photos: (a), (b), and (d): Flávia Fernandes.

presented an amber coloration (Figure 2(a)) later becoming straw-yellow as the hardening of the chorion occurred (Figure 2(b)). That difference in the coloration allowed the distinction of the oldest egg clusters from the most recent. In the first cycle (October to December) the oviposition presented, on average, 55.7 ± 15.5 eggs/egg clusters ($n = 1,837$ eggs in 33 clusters; range 12–80 eggs), and in the second cycle (February to April) the average corresponded to 61.6 ± 14.2 eggs/egg clusters ($n = 5,607$ eggs in 91 clusters; range 13–80 eggs). The ratio between the number of egg masses in the first and second cycles was significantly different ($U = 1106.00$; $P = 0.0253$). The factors for this difference can be attributed to the disparity existent between one female and another regarding their physiological and nutritional state, the nutritional state of the host plant leaves (young leaves, under growth have higher level of nitrogen than the mature leaf) [33], and to the abiotic factors, as the temperature. In many insects, the production of eggs is controlled by one or more hormones produced in the *corpora allata*, that control the initial stages of oogenesis and the yolk deposition. Factors such as the temperature can act on these structures, thus affecting the egg production [34].

Subsocial species of the same genus, like *O. tricolorata* [14] and *Omaspides convexicollis* Spaeth, 1909 [35], also present a large number of eggs per cluster (average of 55.1 and 48.8, resp.), if compared to other non-subsocial species such as *Anacassis dubia* Boheman, 1854 with an average

of 9.1 eggs per cluster and *Anacassis languida* Boheman, 1854 with an average of 6.7 eggs per cluster [9, 36]. The female of *Charidotis punctatostriata* Boheman, 1856 produces, annually, an average of 235.5 ± 41 eggs per female [8], a quantity that can be attributed to the high reproductive effort due to the semelparity presented.

The large number of eggs in subsocial species can also be explained by the high reproductive effort, because they spend most of their time investing in the defense of the offspring and in resource allocation, instead of going through various ovipositions. However, the subsociality is one of several adaptations aimed at facing adverse conditions [37]. Unlike the physical protection provided to the eggs by the mother, as in *Acromis sparsa* Boheman, 1854 [38], the non-subsocial Cassidinae can make use of different adaptations, such as the protection of the eggs through an ootheca [39–41] and ootheca and feces [42] or a gelatinous matrix with feces, as in *Hemisphaerota cyanea* Say, 1824 [43], thus making access more difficult for the natural enemies.

Regarding the egg laying site, the ovipositions of *O. pallidipennis* were all deposited on the abaxial surface of *I. alba*, a behavior also present in other subsocial [11, 13, 44, 45] and non-subsocial species [39, 40]. For the species *Gratiana spadicea* Klug, 1829 and *O. tricolorata* this behavioral pattern is related to the temperature [14, 46]. Although it had not been measured, the temperature was also pointed to as a decisive factor of this behavior, because

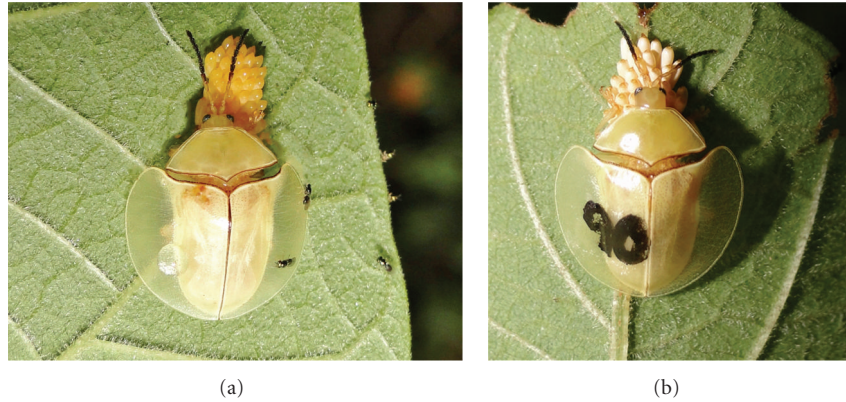


FIGURE 2: *Omaspides pallidipennis* Boheman, 1854 (Chrysomelidae) female (a) on recently laid egg cluster (b) after a few days. A fragment of Atlantic Forest (Floresta Nacional de Passa Quatro, Minas Gerais State, Brazil).

the majority of the host plant leaves were under direct sunlight several hours a day.

The choice of the female for the egg laying site is an important factor for the growth and the survival of their larvae [47]. When ovipositing, the female should consider an appropriate place for the development of the juveniles, thus maximizing their adaptive value. Factors such as the predation risk [47, 48], host plant quality or quantity [33, 49], larval mobility [50], and the intraspecific and interspecific competition [51] should be considered. Of the 170 egg masses observed, 159 allowed to know the oviposition site with certainty. Of these, 116 (73%) were found along the midrib and 43 (27%) in other parts of the leaf blade, no egg masses being placed in the proximal half of the petiole. That preference to oviposit in the distal portions can be explained by the presence of predator ants that constantly visited host plant EFNs. Among them several ants of the genus *Pseudomyrmex* sp. (Formicidae) and *Crematogaster* sp. (Formicidae) preying on eggs and larvae were found. The oviposition preference on the host plant was not altered by the intraspecific competition, not finding more than one egg mass of the species or of other Cassidinae species on the same leaf.

The oviposition peaks occurred during the months of November and February, not observing any new egg masses, in December, January, and April. The average of incubation period of the eggs was 19.2 ± 1.4 days ($n = 31$ offspring) for the first cycle and 16.7 ± 1.4 days ($n = 71$ offspring) for the second cycle (Table 1). The incubation time differed significantly in the two cycles ($U = 239.00$; $P < 0.0001$). Characteristics such as abiotic factor variations can explain such difference. In *Metriona elatior* Klug, 1829 the average incubation time of the eggs is lower at 30°C (5.6 days) than at 20°C (11.3 days) [52]. Another factor to be considered is the quality and the quantity of the host plant that can alter nutrient acquisition, thus interfering in the production of eggs [53]. However, more research is necessary to explain these characteristics.

During the biological cycles, three females oviposited twice during the same cycle. In all those cases their first

TABLE 1: Duration of the developmental immature stages of *Omaspides pallidipennis* Boheman, 1854 (Chrysomelidae), for the first and second cycle in a fragment of Atlantic Forest (Floresta Nacional de Passa Quatro, Minas Gerais State, Brazil).

| | First cycle | Second cycle |
|------------|-----------------------------|-----------------------------|
| | Mean \pm SD | Mean \pm SD |
| Egg | 19.2 ± 1.4 ($n = 31$) | 16.7 ± 1.4 ($n = 71$) |
| Larvae | 26.0 ± 1.5 ($n = 19$) | 27.0 ± 2.4 ($n = 35$) |
| Pupae | 8.7 ± 0.8 ($n = 20$) | 10.2 ± 1.5 ($n = 30$) |
| Total time | 54.3 ± 9.0 | 54.4 ± 8.7 |

oviposition had been preyed upon. The time spent between one oviposition and the other varied from 1 to 19 days.

3.2.2. Larvae. The larvae of *O. pallidipennis* are light yellow, presenting a slightly dorsal-ventrally flat body. There are nine pairs of lateral scoli and a caudal furca (Figure 1(b)) where the exuvial-fecal shield is attached [18] (Figure 1(c)). In some species of Cassidinae *s.str.*, this structure works as physical protection against dissection and predation [54, 55]. A chemical defense function, through compounds that are present in this attachment, is evidenced, also, in other species [56–58]. *Eurypedus nigrosignatus* Boheman, 1854 (Cassidinae: Physonotini) obtains those chemical compounds from its host plant *Cordia curassavica* (Jacques) Roemer and Schultes [59]. Studies evidence that these structures have been shown to be efficient against some natural enemies, but not against others. In *Cassida rubiginosa* Müller, 1776 the exuvial-fecal shield was effective against *Formica exsectoides*, Forel 1886 (Hymenoptera: Formicidae) [54] but not against *Polistes dominulus* Christ, 1791 (Hymenoptera: Vespidae) [60]. The fecal shield was also not effective for *Chelymophra reimoseri* Spaeth, 1928 against *Polistes* sp. and *Piaya cayana* Linnaeus, 1766 (Cuculiformes: Coccyzidae) [61]. However, in *H. cyanea*, the fecal attachment was efficient against the coccinellid *Cycloneda sanguinea* Linnaeus, 1763 and the hemipteran *Stiretrus anchorago* Fabricius, 1775 but not



FIGURE 3: Leaf with signs of herbivory caused by *Omaspides pallidipennis* Boheman, 1854 (Chrysomelidae) in first stages. A fragment of Atlantic Forest (Floresta Nacional de Passa Quatro, Minas Gerais State, Brazil).

against *Calleida viridipennis* Say, 1823 (Coleoptera: Carabidae) [43].

In relation to the scoli, Eisner et al. [54] found evidences in *C. rubiginosa* that they act in the defense, because when they are touched, the larvae respond by quickly raising their fecal attachment.

Most of the Cassidinae larvae seem to have five development stages, like *O. pallidipennis*, *O. tricolorata* [14], *Cassida obtusata* Boheman, 1854 [62], and *M. elatior* [10]. However, some species present wide variation in the larval stages [3], arriving in *Chelobasis perplexa* Baly, 1858 (Hispininae s.str.) at eight development stages. That determination of the number of stages can be made through the measurement of the cephalic capsule [9, 63] or by counting the accumulated exuviae in the exuvial-fecal shield [14].

Soon after eclosion, the larvae begin to feed around the egg mass, moving towards the distal end of the leaf. In all of the larval stages feeding on the borders of the leaf towards the petiole was always observed. In the first stages, “they scraped” the parts between the ribbing, leaving the leaf with lacy aspect (Figure 3). Starting from the third stage, they fed on the whole leaf (primary and secondary ribs and petiole), changing to another leaf only when the previous was totally eaten. The larvae feed from the abaxial surface, as well as the adaxial surface, always joining after the feeding in cycloaexy, a form of gregariousness [64]. The larval gregariousness provides some advantages to the initial stage larvae, such as ease of feeding, economic use of restricted resource and group protection against their natural enemies [65, 66] thus not having interference of the intraspecific competition, as already mentioned, in the choice of the egg laying site for the female. During the whole developmental period of the juveniles, the female was only observed just feeding when the offspring were in the larval stage. At the end of the fifth stage, the larvae moved via the plant stem and were positioned in a clustered, imbricated manner, fastening the end portion of the abdomen to the branch, to then pupate (Figure 4).

The larval stage is the longest juvenile stage. For the first cycle, the larval development was 26.0 ± 1.5 days ($n = 19$ offspring), counted from eclosion to reaching the pupal



FIGURE 4: Imbricated pupae of *Omaspides pallidipennis* Boheman, 1854 in stem of its host plant *Ipomoea alba* L. (Convolvulaceae). A fragment of Atlantic Forest (Floresta Nacional de Passa Quatro, Minas Gerais State, Brazil).

stage. In the second cycle the duration was 27.0 ± 2.4 days ($n = 35$ offspring; Table 1). The n sample corresponds to the group of larvae that reached the pupal stage. The time of larval development among the two cycles did not show significant difference (t -test, $P = 0.0555$; $df = 50.69$).

During the research, offsprings were seen with number of visibly smaller individuals. It can be considered another factor, besides the predation. Because the *O. pallidipennis* host plant was under constant sunlight exposure, it is possible that death by dehydration had occurred. Gandolfo et al. [52] reared *M. elatior* under different temperatures (20°C , 25°C , and 30°C) and their juveniles had faster development at higher temperatures. However, at 30°C the larvae suffered damage, not reaching the pupal stage. Frieiro-Costa and Vasconcellos-Neto [14] suggest that the larvae of *O. tricolorata* exposed to high temperatures can dehydrate and die.

3.2.3. Pupae. Soon after reaching the pupal stage they presented yellowish coloration, becoming yellowish brown with dispersed dark patches on the body after a period of 24 hours (Figures 1(d) and 4). As in the *A. languida* [36] species *O. pallidipennis* did not retain the exuvial-fecal shield at pupation. However, there are Cassidinae species that keep the exuvial-fecal attachment [67] or only the exuviae [68].

The pupal stage was the shortest of the development stages. In the first cycle, the duration was 8.7 ± 0.8 days ($n = 20$ offspring), presenting an average of 10.2 ± 1.5 days ($n = 30$ offspring) for the following cycle (Table 1). The difference in the time of development between the cycles was highly significant (t -test, $P < 0.0001$; $df = 45.97$), a reason that can be attributed here, as well as in the incubation period, to the variation of the abiotic factors. In the duration of the pupal development time, the larval stage group individuals that reached the subsequent stage were used as a basis. The prepupal period was not considered due to the short duration of that stage, which did not allow precise verification.

Of 43 studied groups, 35 pupated on the stem, and seven of these pupated on plants other than the host, which were

support for *I. alba*. The eight groups remained pupated on the abaxial leaf surface. Of the groups, 19 were found pupated in areas under sunlight and the others in shaded locations. When the pupas stayed under direct sunlight, they protruded out, probably to increase the air circulation among them. High temperatures can hinder or impede the development of juvenile stages [52].

3.3. Adults. The adults are gregarious and they show no apparent sexual dimorphism. Upon emergence, the elytra and pronotum were a translucent yellow color, becoming straw-yellow after total sclerotization, that occurred in approximately seven days. During this period the female stayed close to juveniles on the abaxial leaf surface of the host plant. In *H. cyanea*, the adult, when emerging, was under its exuvial-fecal shield until total sclerotization of the elytra [43]. Recently emerged adults were not found mating.

Juveniles feeding started after about seven days. The adults started feeding from the edges of the *I. alba* leaf or preexisting holes in the leaf blade.

This paper explains the importance of observational studies in the field to understand the biology and ecology of the species. Subsocial Cassidinae provide excellent study material, because they are easily observed since they remain restricted to the development site of the juveniles throughout their development. However, further research should be conducted to further elucidate the relationship between subsocial or non-subsocial Cassidinae and their host plants.

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