Zoological Studies **64:**51 (2025)

Immature Stages of White Belt Owlet Butterfly *Opoptera fruhstorferi* (Röber, 1896) (Lepidoptera: Nymphalidae: Satyrinae) and its Natural Enemy, with a Summary of Brassolini Parasitoids

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(Received 11 March 2025 / Accepted 11 August 2025 / Published -- 2025)
Communicated by Jens Thorvald Høeg

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Opoptera Aurivillius, 1882 belongs to the tribe Brassolini and comprises 10 species. The white belt owlet butterfly *Opoptera fruhstorferi* (Röber, 1896) is endemic to the Atlantic Rainforest, occurring in southeast and south regions of Brazil. Morphological studies, including detailed descriptions of immatures and aspects of the natural history of *Opoptera*, remain poorly documented. This study aims to 1) describe for the first time the morphology of the immature stages of *O. fruhstorferi*; 2) provide a complete view of its life cycle; 3) report tritrophic plant-host-parasitoid interactions; 4) compile records of Brassolini parasitoids from the literature. These data expand the understanding of the natural history of *Opoptera*, a genus that still has several gaps to be filled. This study also contributes to the systematics of Neotropical butterflies.

Keywords: Brassolini, Chaetotaxy, Natural history, Trichogrammatidae, Tritrophic interactions

Citation: Cajé S, de Moura Lima IM, Mielke OHH, Casagrande MM. 2025. Immature stages of white belt owlet butterfly *Opoptera fruhstorferi* (Röber, 1896) (Lepidoptera: Nymphalidae: Satyrinae) and its natural enemy, with a summary of Brassolini parasitoids. Zool Stud **64:**51.

BACKGROUND

Opoptera Aurivillius, 1882 belongs to the tribe Brassolini and comprises 10 species (Cajé et al. 2025a). Amongst these species, the owlet butterfly *Opoptera fruhstorferi* (Röber, 1896) is endemic to the Atlantic Rainforest, where it is recorded in southeast and south regions of Brazil, differing from other species of *Opoptera*, as it is the only species with a white band in the forewings (Cajé et al. 2025a).

Many efforts have been made in morphological studies of immature stages in Lepidoptera (Casagrande and Mielke 2000; Casagrande 2002; Furtado and Campos-Neto 2004; Souza 2006; Neves and Paluch 2016; Heredia and Robbins 2016; Dias et al. 2019; Piovesan et al. 2023; Dresch et al. 2025). These contributions provide valuable data that offer deeper insights into the diversity and biology of the group and can be used to elucidate questions in butterfly systematics, for instance in Nymphalidae (Freitas and Brown 2004; Penz et al. 2013).

For *Opoptera*, detailed descriptions of immatures and aspects of natural history remain poorly documented. There are general descriptions for *Opoptera staudingeri* (Godman & Salvin, 1894) (DeVries 1987), and *Opoptera mexicana* J. Maza & R. G. Maza, 1989 (Wolfe 2017; Cajé et al. 2025a). Hoffmann (1937) provided a brief description for *Opoptera sulcius* (Staudinger, 1887), and after eighty-eight years a more detailed description of the morphology of immatures and host plant this species was provided (Cajé et al. 2025b). The biology of *Opoptera syme* (Hübner, [1821]) has also been documented (Aguiar et al. 2024), and Hoffmann (1936) contributed with descriptions of the egg and first instar larva of *O. fruhstorferi* (treated as *Opsiphanes fruhstorferi*). Although aspects of the life histories of the immatures of six species of *Opoptera* are known, there are no records of associated parasitoids.

So far, there is no detailed description on the immature stages of *O. fruhstorferi*, and this study aims to 1) describe for the first time the morphoplogy of the immature stages of *O. fruhstorferi*; 2) provide a complete of its life cycle; 3) report tritrophic plant-host-parasitoid interactions; 4) compile records of Brassolini parasitoids from the literature.

MATERIALS AND METHODS

Rearing of immatures

Six eggs of *O. fruhstorferi* were collected in April 2023 in São Bento do Sul, Santa Catarina, Brazil (-26.249441, -49.382493) on bamboo leaves and taken to the Laboratório de Estudos de Lepidoptera Neotropical, Universidade Federal do Paraná (UFPR), Curitiba, Paraná, Brazil (-25.449933, -49.233952) for observations and documentation of developmental events throughout their life cycle.

During rearing, the temperature and humidity conditions in the laboratory were uncontrolled. The local climate in Curitiba usually ranged from 12.5 to 22.5°C in temperature and 26 to 100% in humidity (INMET 2023). The eggs were maintained in petri dishes until the larvae hatched.

After hatching, the larvae were transferred to plastic containers wich were cleaned every two days or as needed, and the food plant branches were replaced. Eggs were fixed in 10% Kahle-Dietrich solution and subsequently preserved in 70% alcohol. First instar larvae were killed in boiling water, then fixed and preserved using the same method as the eggs. The head capsules were placed in eppendorf tubes without any solution.

Description of immatures

To describe the immature stages of *O. fruhstorferi*, images of eggs and early instar larvae were captured using an optical stereomicroscope Leica M205 C equipped with a digital camera (Leica DMC4500) and Leica Application Suite (LAS) version 4.12.0 software for focus stacking. From the fourth instar onwards, images were taken using a Sony DSC-HX100V digital camera. The schematic representation was created using Corel Draw 2020. Measurements of eggs and larvae in early stages were obtained using the camera with focus stacking software, while and for larvae from the fourth stage onwards, cephalic capsules, and pupae, measurements were taken using ImageJ software (Schneider et al. 2012). The terminology follows Scoble (1992) for egg; Hinton (1946) Peterson (1962), and Stehr (1987) for chaetotaxy; and García-Barros (1987, 1988) and Harvey (1991) for filiform setae.

The samples used for the Scanning Electron Microscopy (SEM) analyses were prepared at the Laboratório de Estudos de Lepidoptera Neotropical. They were treated with an ethanol series for dehydration (70, 85, and 100%), with 10 minutes of immersion in each concentration, and then left to dry for 24 hours on paper towels in a container filled with a uniform layer of silica gel. The samples were mounted on aluminum stubs using double-sided copper adhesive tape and subsequently taken to the Centro de Microscopia Eletrônica (CME) for gold metallization using a metallizer (Balzers, SCD 050). The micrographs were obtained using a Tescan Vega3 Lmu microscope.

RESULTS

Description of immature stages

Eggs (Figs. 1–3): Diameter: 1.3 mm; Height: 1.1–1.2 mm (*n* = 3). Whitish; spherical; slightly flattened in the micropylar area. Rosette is composed of 11 pentagonal primary petaloid cells. Surrounding the rosette, 51 pentagonal and hexagonal secondary cells, varying in shape and size. Aeropyles, small circular openings, located at the intersections of the ribs, throughout the chorionic surface. Eggs sculptured with 29 longitudinal ribs and over 30 horizontal ridges.

First instar (Figs. 4A–B, 5–8): Duration: 17–18 days; head capsule width: 0.8 mm (*n* = 2). Head without scoli, rounded, and dark brown, with primary and second setae; distal portion of the setae is plumose; six stemmata arranged in a C-shaped pattern; with irregular and interconnected ridges. Body cylindrical; light green; covered with primary translucent setae on chalazae; setae with scale-like protuberances, except for the filiform ones; T1, prothoracic shield with sparse brownish spots; dorsal area with two whitish stripes, and reddish marks between A3 and A4; subdorsal area with a whitish longitudinal stripe, turning yellowish between A3 and A4; supraspiracular area of A8 with reddish spots; and A10 with reddish suranal plate.

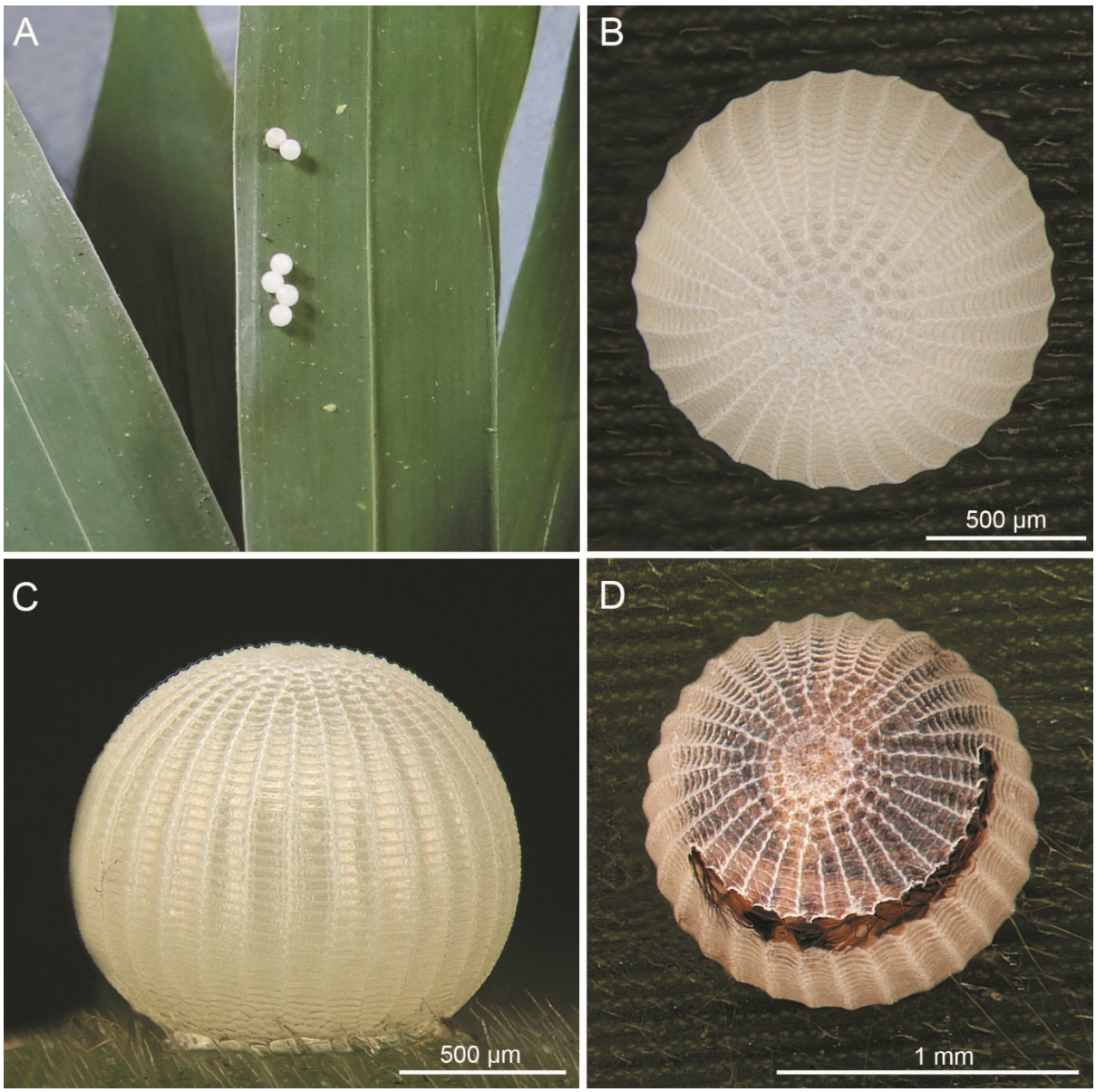

Fig. 1. *Opoptera fruhstorferi*. Eggs: A: Laying eggs on abaxial surface of the host plant leaf; B: Dorsal view; C: Lateral view; D: Dorsal view, showing plumose setae inside the chorion.

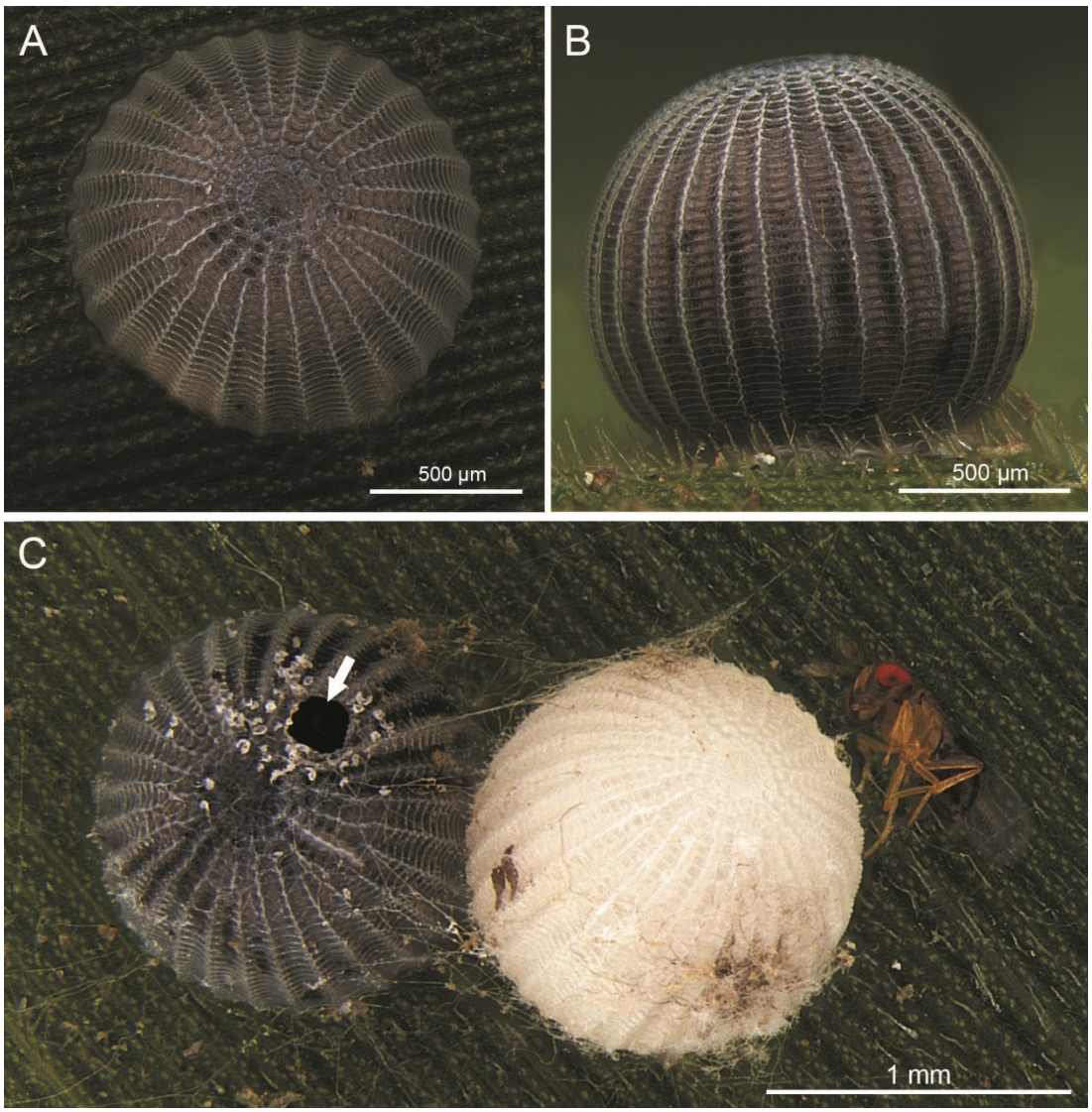

Fig. 2. *Opoptera fruhstorferi*. Parasitized eggs and parasitoid *Trichogramma* sp.: A: Dorsal view; B: Lateral view; C: Egg with the upper opening made by parasitoid (left, white arrow), and a supposedly viable egg with a *Trichogramma* sp. female on top (right).

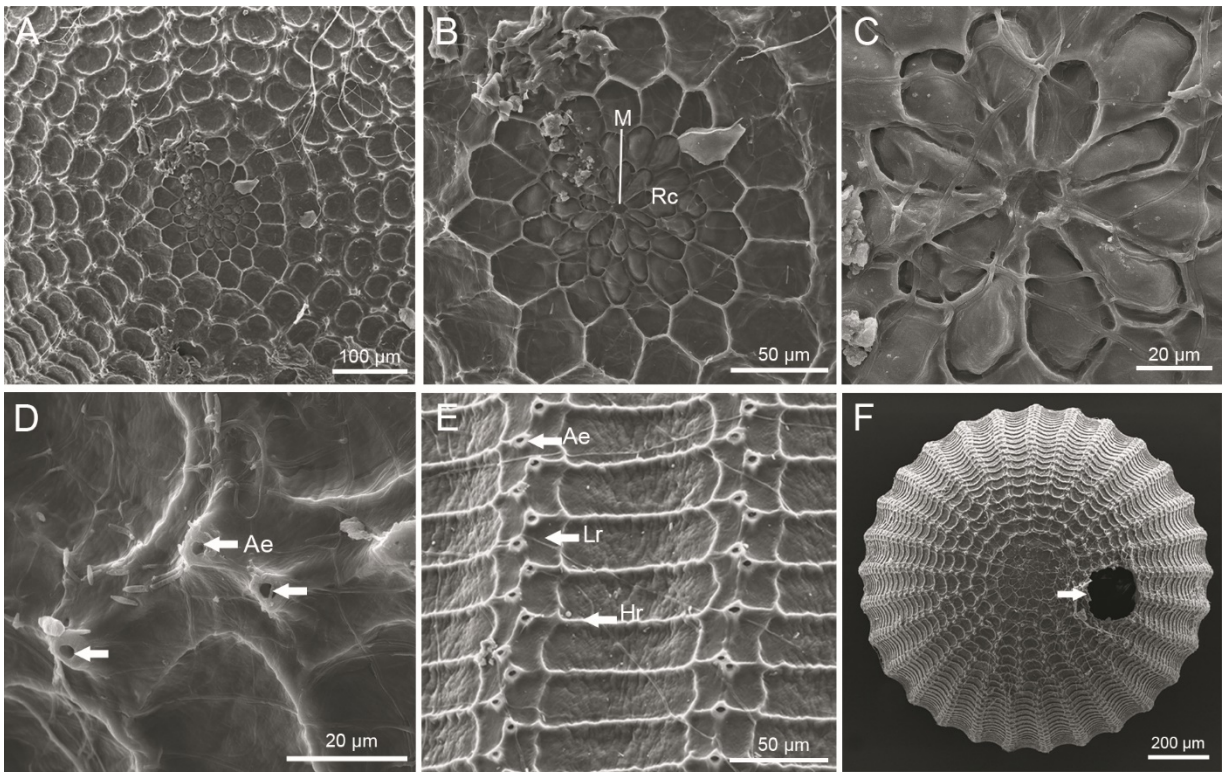

Fig. 3. *Opoptera fruhstorferi*. Scanning electronic micrographs of egg: A–C: Different magnifications of the micropylar area, showing the micropyle opening (M) and rosette cells (Rc); D: Aeropyles (Ae); E: Longitudinal ribs (Lr), horizontal ridges (Hr), aeropyles (Ae); F: Opening of parasitized eggs (white arrow).

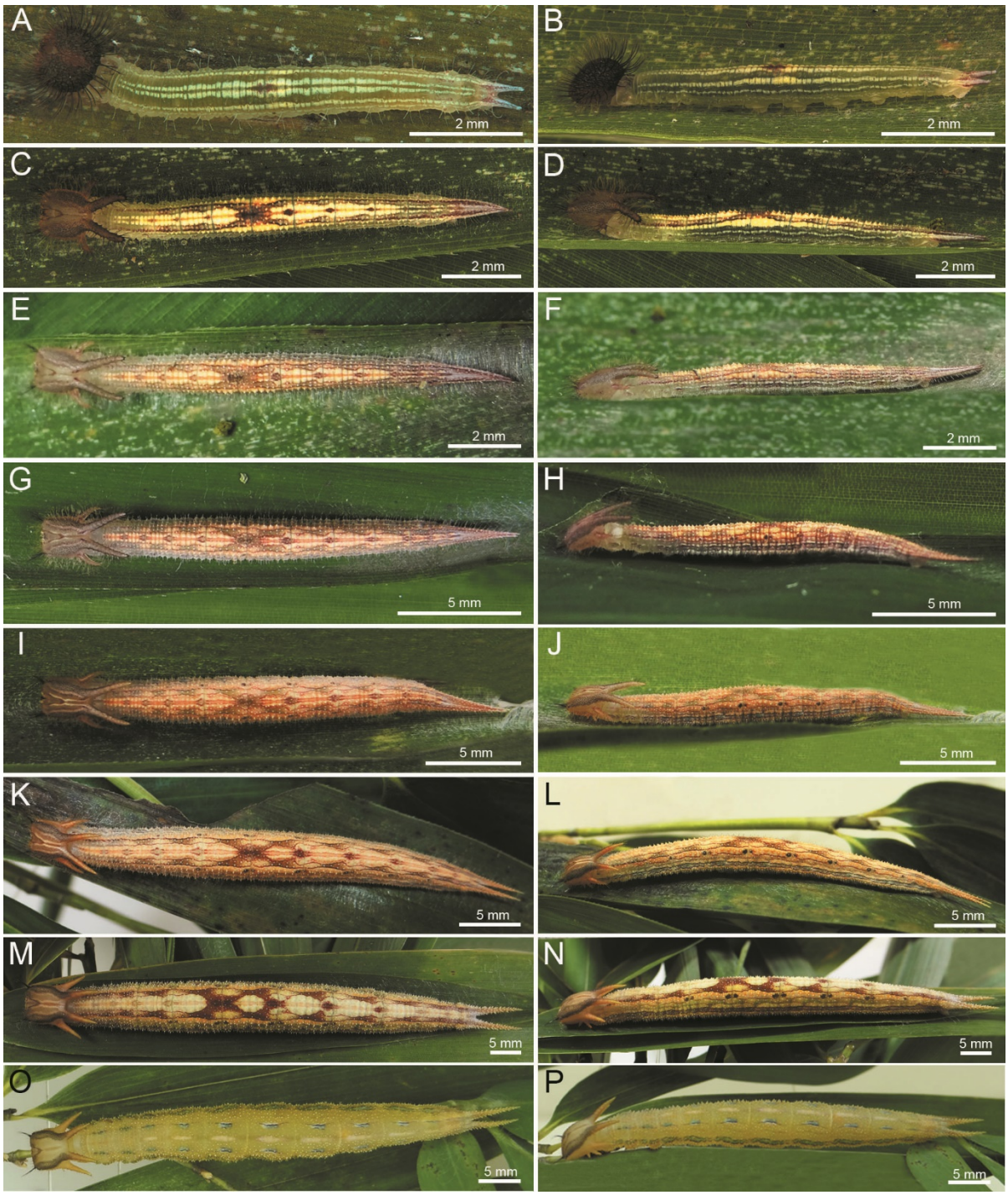

Fig. 4. *Opoptera fruhstorferi*. Larva: A–B: First instar, dorsal and latera views; C–D: Second instar, dorsal and lateral views; E–F: Third instar, dorsal and lateral views; G–H. Fourth instar, dorsal and lateral views; I–J: Fifth instar, dorsal and lateral views; K–L: Sixth instar, dorsal and lateral views; M–N: Seventh instar, dorsal and lateral views; O–P: Prepupa, dorsal and lateral views.

Chaetotaxy: Head capsule (Fig. 5). Plumose setae; groups of setae adfrontal (AF1, AF2); frontal (F1); clypeal (C1, C2); labrum (L1, L2, M1 and M2); and the pore (Fa). Body (Figs. 6–8). T1, prothoracic plate with one pair of setae in anterior margin (XD2), two pairs of dorsal (D1, D2) setae anterior and posterior, respectively, and one pore (P); one pair of subdorsal (SD1); two pairs of filiform (FA, FP) arising from chalazae; FA in the subspiracular area; and FP subraspiracular;

one pair of lateral (L2); two pairs of subventral (SV1, SV2), and ventral (V1), and proprioceptor setae. T2 and T3, with one parallel pairs of dorsal (D1, D2); one pair of lateral (L2); one pair of subdorsal (SD1); one pair of filiform (F); two pairs of subventral (SV1, SV2), and ventral (V1), and proprioceptor setae (PP). A1, A2, A7 and A8, with two pairs of dorsal (D1, D2) anterior and posterior, respectively; one pair of subdorsal (SD1); two pairs of lateral (L1, L2); two pairs of subventral (SV1, SV2); ventral (V1); and proprioceptor setae (PP). A3–A6, with a pair of dorsal (D1, D2); one pair of subdorsal (SD1); two pairs of lateral (L1, L2); two pairs of subventral (SV1, SV2); ventral (V1); and proprioceptor setae (PP). Prolegs with uniordinal and penellipse crochets. A9, with one parallel pairs of dorsal (D1, D2); one pair of filiform (F); one pair of lateral (L3); one pair of subventral (SV1); ventral (V1); and proprioceptor setae (PP). A10, with two pairs of dorsal (D1, D2), anterior and posterior, respectively; two pairs of subdorsal (SD1, SD2); one pair of subventral (SV1); ventral (V1); one pair of paraproctal (PP).

Second instar (Fig. 4C–D): Duration: 23–31 days; head capsule width: 1–1.3 mm (n = 2); Head rectangular; with primary and secondary setae; reddish-brown; with three pairs of scoli: dorsal pair longer than the others, and dark brown; dorsolateral pair with half the length of the dorsal pair with a dark brown distal portion; lateral pair, the smallest, and ligth brown; dorsal and dorsolateral pair with their apex directed posteriorly; epicranium with two reddish-brown lateral spots, extending to the base of the dorsal scoli; with six stemmata; with a tuft of dark brown setae near stemmata I-IV, forming a conspicuos “mustache” (as described by Young and Muyshondt 1975); with two lateral bands from the anterior margin of the occipital foramen to the labrum. Body with simple, primary and secondary setae on chalazae; dorsal area with yellowish line extending to the subdorsal area, with brownish spots, and a reddish-brown mark between A3 and A4 similar to a spider positioned on the hub of its web, as described for *O. sulcius* (Cajé et al. 2025b); subdorsal areas with a narrow reddish stripe extending from the thorax to the suranal plate; supraspiracular area has a narrow whitish stripe.

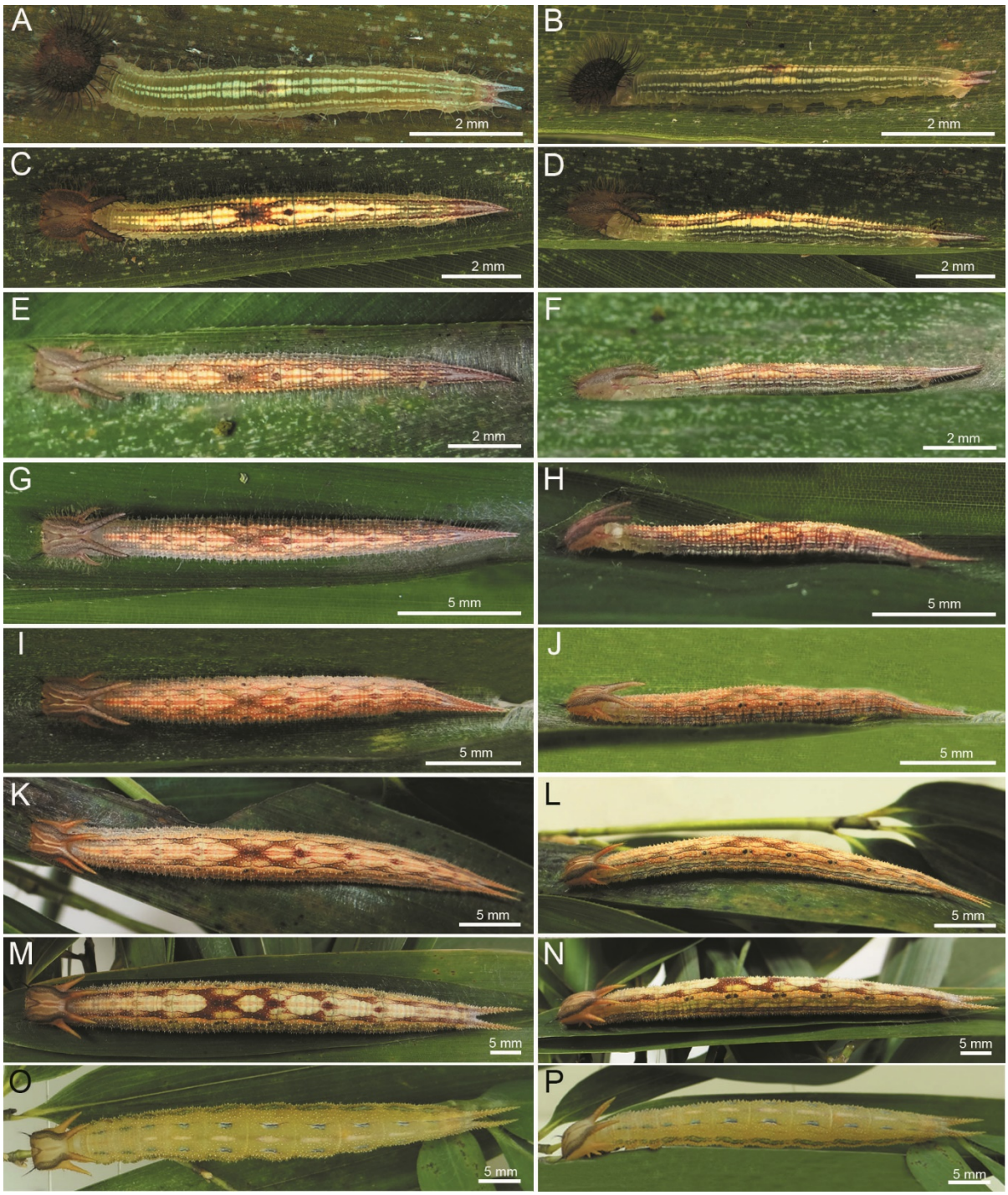

Fig. 5. *Opoptera fruhstorferi*. Head capsule of first instar: A: Head capsule with plumose setae; B: Groups of setae adfrontal = AF1, AF2; frontal = F1; clypeal = C1, C2; labrum = L1, L2, M1 and M2, frontal view; C: Detail of the stemmata 1, 2, 3, 4; D: Genal area with six stemmata 1–6, and mandible (md), antenna (an), maxillary palpus (mxp) and maxilla.

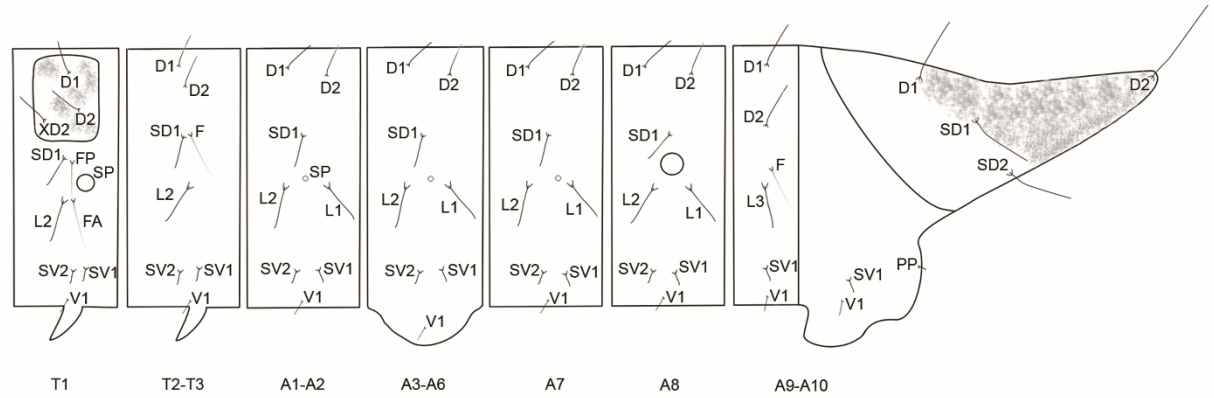

Fig. 6. *Opoptera fruhstorferi*. Body chaetotaxy of first instar larva, schematic representation of thorax and abdomen, lateral view. Thorax (T1-T3), and abdomen (A1-A10), with the setae groups: XD = anterior margin setae; D = dorsal; SD = subdorsal; F = filiform; FP = posterior filiform; FA = anterior filiform; L = lateral; SV =subventral; V = ventral.

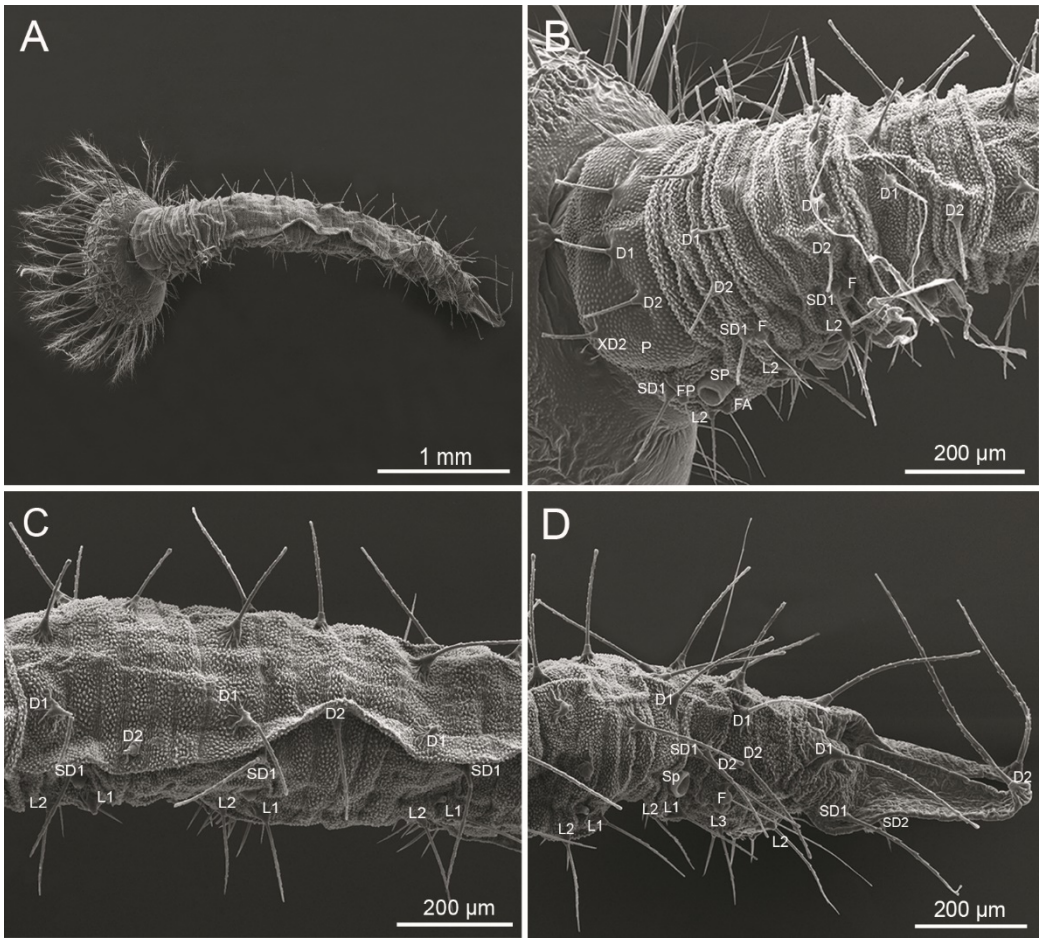

Fig. 7. *Opoptera fruhstorferi*. Scanning electronic micrographs of first instar: A: General view; B: Distribution of setae on thorax, dorsal view; C–D: Distribution of setae on A1–A3, and A8–A10 segments.

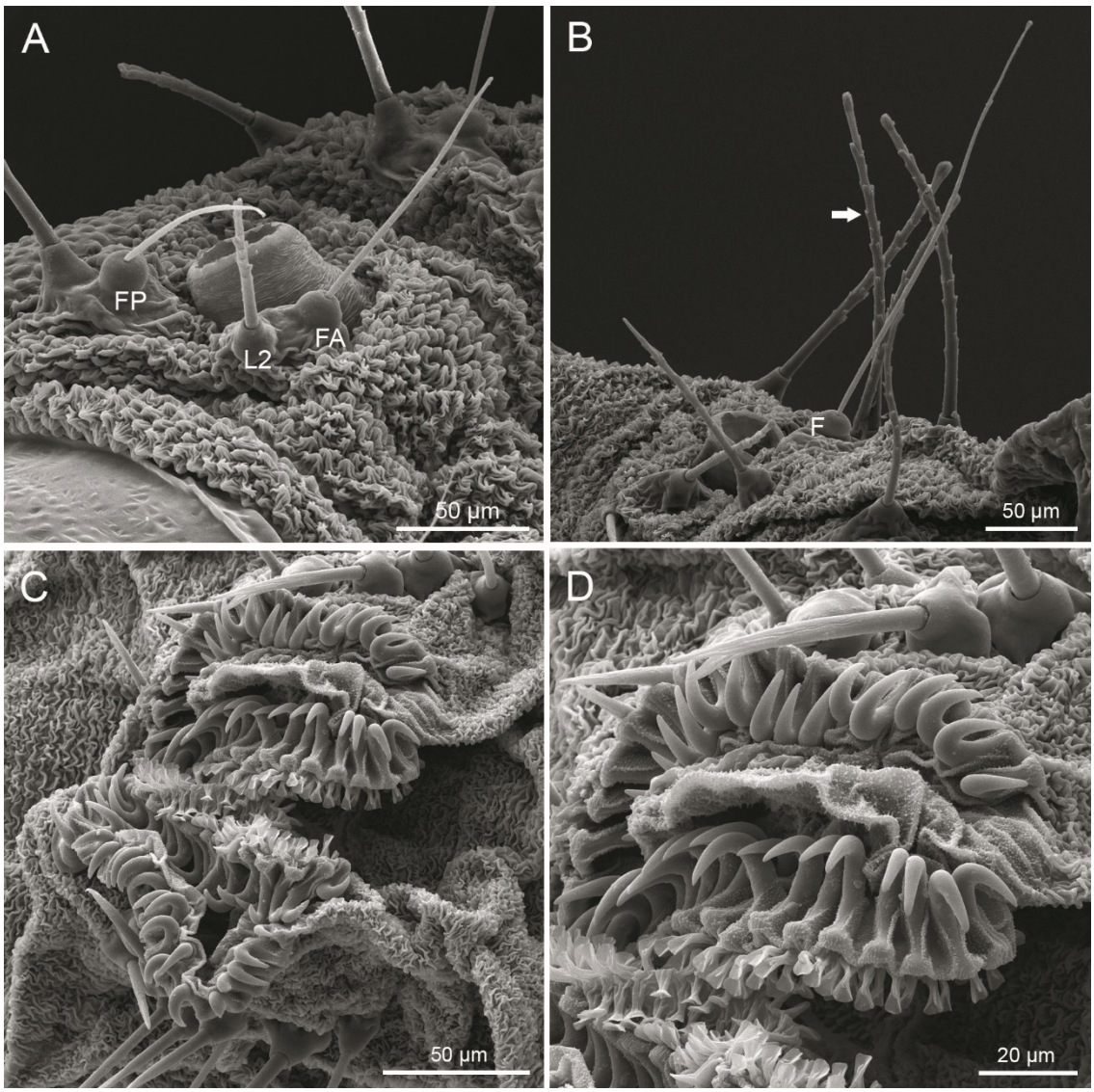

Fig. 8. *Opoptera fruhstorferi*. Scanning electronic micrographs of first instar: A:Filiform (FP, FA), and lateral (L2) setae on T1 segment; B: Filiform seta on segment A9, and showing the setae with scale-like projections (white arrow); C–D: Prolegs with uniordinal and penellipse crochets on A6 segment.

Third instar (Fig. 4E–F): Duration: 41–46 days; head capsule width: 1.3 mm (n = 2). Similar to the previous instar. However, head with four pairs of scoli. Body with a supraspiracular area with reddish stripes; in the subspiracular area, adjacent to the whitish stripe, a reddish-brown stripe begins to appear; abdomen with intersegmental areas between A1 and A4 with dark brown spots.

Fourth instar (Fig. 4G–H): Duration: 39–44 days; head capsule width: 1.5–1.6 mm (n = 2). Similar to the previous instar, but body with the dorsal area with greenish spots.

Fifth instar (Fig. 4I–J): Duration: 27–35 days; head capsule width: 2–2.2 mm (n = 2). Similar to the previous instar, but head with scoli with a yellowish apex and more pointed than in the previous instar. Body, also similar to the fourth instar, but the supraspiracular area with dark brown spost between A1 and A6.

Sixth instar (Fig. 4K–L): Duration: 20–25 days; head capsule width: 3 mm (n = 2). Similar to the previous instar. Body, dorsal area with brown spots instead of green, and the supraspiracular area with more evident brown spots.

Seventh instar (Fig. 4M–N): Duration: 27–32 days (n = 2); head capsule width: 4.6 mm (n = 1). Head and body similar to the sixth instar, but the dorsal orange midline faded; and the subspiracular area with a light green region. Prepupa (Fig. 4O–P) duration 3–14 days; body yellowish, with a faded color pattern of spots and stripes, and subspiracular area with green stripes.

Pupa (Fig. 9): Duration: 16–17 days (n = 2). Predominantly yellowish, with several discontinuous and not well defined brown longitudinal lines; dorsal and lateral areas with greenish spots, while ventrally with brown spots. Head with a pair of projections, with brown spots extending laterally to the alar theca; eyes elliptical; labrum triangular; antenna and proboscis of the same length, extending to A4. Thorax, alar theca with a well defined costal margin, and with a brown spot laterally. Abdominal segments, fifth to eighth with lateral ellipsoidal spiracles, each highlighted by a greenish stripe; cremaster yellowish, with bumps surrounding the anal opening slit.

Fig. 9. *Opoptera fruhstorferi*. Pupa: A: Ventral view; B: Lateral view; C: Dorsal view.

Natural history

The female oviposited on the abaxial leaf surface (Fig. 1A) of an unidentified species of bamboo. However, under laboratory conditions, *Phyllostachys aurea* Carrière ex Rivière & C.

Rivière (Poaceae) was used as a food plant for rearing the larvae. This bamboo was chosen due to its availability and previous records of other bamboo species as host plants for *Opoptera* (Silva et al. 1968; Zikán and Zikán 1968; Cubero 1985; DeVries 1987; Wolfe 2017). The eggs are whitish during the first days after oviposition, but in just a few days, they develop pinkish tripes. Close to hatching, the plumose setae become visible inside the egg (Fig. 1D).

At the moment of hatching, the larvae cut the chorion circularly near the micropylar area forming a hinged cap. After hatching, they eat the chorion (Fig. 1D). Larvae then begin feeding on the leaf of *P. aurea*, with feeding activity occurring during the evening. When not feeding, they rest on either the adaxial or abaxial leaf surface. The two viable larvae kept in the same rearing container did not exhibit "face to face" behavior, as observed in *O. sulcius* (Cajé et al. 2025b), nor did they exhibit cannibalism or gregarious behavior. During the moulting period, larvae deposit a greater quantity of silk on the adaxial region of the leaf where they rest and molt. Under laboratory conditions, pupation occurred on the branches of the plant. One male and one female *O. fruhstorferi* emerged (Fig. 10). The 3º instar is the longest, representing 18,41% of the total development time (Fig. 11). The duration of the postembryonic period was approximately 233,5 days, indicating that *O. fruhstorferi* has a univoltine life cycle—that is, a single generation per year (Table 1). These butterflies are diurnal (Hoffmann 1936), with flight activity observed from November to April (Cajé et al. 2025a).

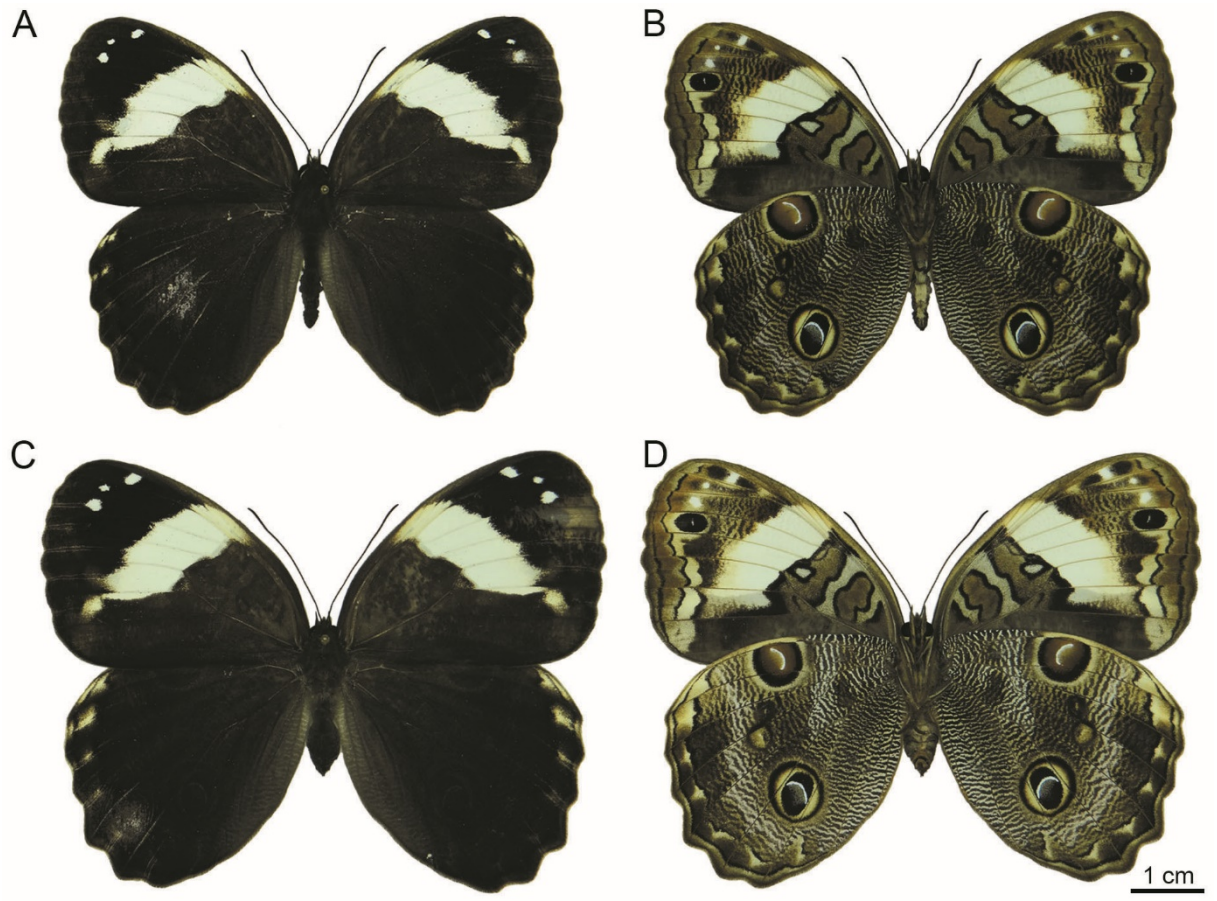

Fig. 10. *Opoptera fruhstorferi*. A–B: Male, dorsal and lateral views; C–D: Female, dorsal and ventral views.

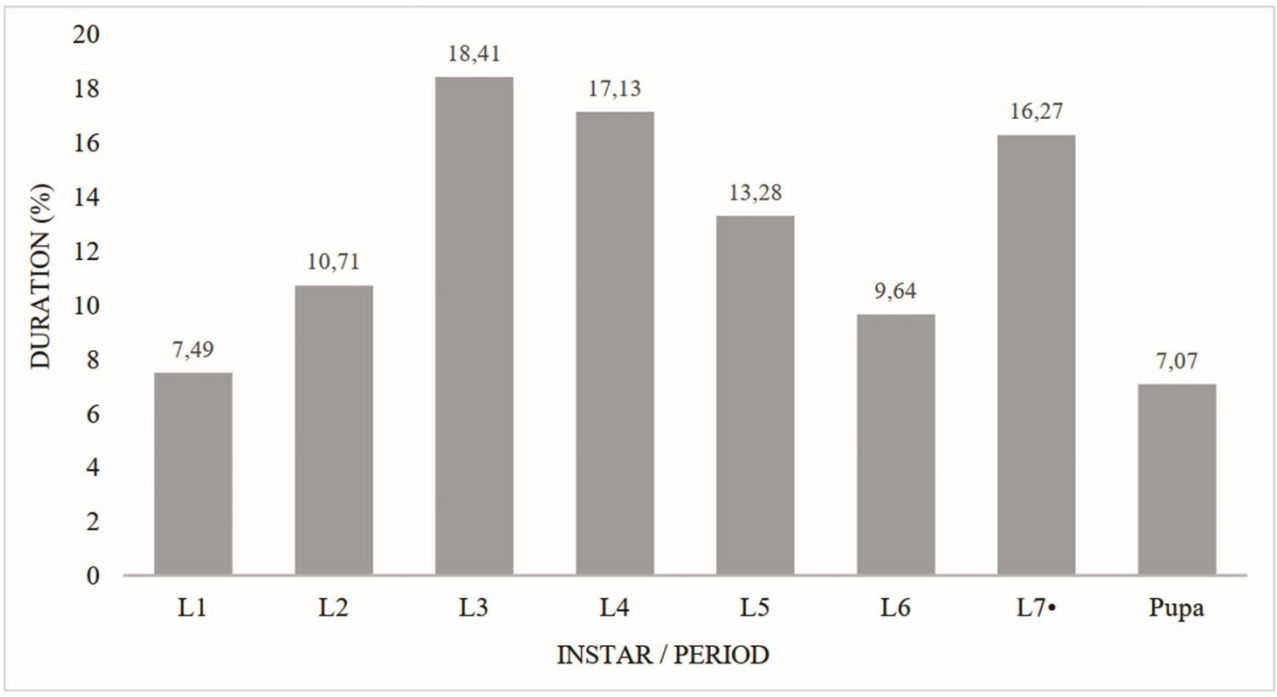

Fig. 11. Histogram of the percentage of time use throughout the preimaginal period of *Opoptera fruhstorferi*. • = 7th instar with values corresponding to the active and prepupal periods.

Table 1. The use of time throughout the preimaginal period, as well as the duration of each instar.

Instar/Period	Duration (days)		Mean duration
	Specimen 1	Specimen 2	
1st instar	17	18	17,5
2nd instar	23	27	25,0
3rd instar	40	46	43
4th instar	44	36	40
5th instar	27	35	31
6th instar	20	25	22,5
7th instar (active)	32	27	29,5
7th instar (prepupa)	3	14	8,5
7th instar (total)	35	41	38
Larval total active	203	214	208,5
Larval total active + prepupa	206	228	217
Pupal	17	16	16,5
Inactive period•• (prepupa + pupa)	20	30	25
Postembryonic period	223	244	233,5

••stop feeding.

One of the 6 collected eggs was parasitized (16.7% parasitoidism ratio). This egg had the same size as the non-parasitized eggs. In terms of color pattern, the parasitized egg turned dark (Fig. 2A–C). Five days after the larvae hatched, two female of *Trichogramma* sp. (Hymenoptera: Trichogrammatidae) parasitoids egressed. This parasitoid makes a circular opening in the dorsal region, near the micropylar area (Figs. 2C, 3F). After egression, one female came into contact with an egg (Fig. 2C). Apparently, this egg was viable, but no *O. fruhstorferi* larvae hatched, nor were any parasitoids egressed.

Due to the low number of larvae reared (two from six eggs) and uncontrolled conditions (*e.g.*, differences in climate between the collection and rearing sites), certain interpretations (*e.g.*, duration, parasitism ratio) must be treated with caution.

DISCUSSION

The head capsule of the first instar of *O. fruhstorferi* is plumose, as in *O. syme*, *O. sulcius* (Aguiar et al. 2024; Cajé et al. 2025b), and other Brassolini (Casagrande 2002; Freitas et al. 2002; Greeney et al. 2009; Penz et al. 2013). The first instar of *O. fruhstorferi* has sparse brownish spots on the prothoracic plate, unlike *O. syme* and *O. sulcius* which have a uniform dark brown spot (Aguiar et al. 2024; Cajé et al. 2025b). The body of the first instar of *O. fruhstorferi* is covered by setae with scale-like protuberances along their length (Fig. 8B), similar to *O. sulcius* (Cajé et al.

2025b), but this feature is not described for *O. syme* (Aguiar et al. 2024). During seta development, the trichogen cell secrets the scale-like protuberances, which are sometimes innervated and serve as sensory organs in addition to other potential functions (Kristensen and Simonsen 2003). These surface patterns are very similar to those found in other Nymphalidae, such as *Heliconius sara apseudes* (Hübner, [1813]) (Barão et al. 2015).

The filiform setae in *O. fruhstorferi* have the same pattern as those in *O. sulcius* (Cajé et al. 2025b), but this pattern is not described for *O. syme* (Aguiar et al. 2024). In other Brassolini, such as *Selenophanes cassiope guarany* Casagrande, 1992, the seta descried as SD1 in segments T1, T2, T3 and A9 (Shirai et al. 2016) seems to correspond to the pattern of the filiform observed in *O. fruhstorferi*; in *Bia actorion* (Linnaeus, 1763) the seta SD1 on thorax and A9, and L2 on thorax are mentioned as filiform (Freitas et al. 2002); and *Blepolenis bassus* (C. Felder & R. Felder, 1867) has the filiform on T1, T2, T3, A3-A7, and A9 (Piovesan et al. 2023). The first illustration of this setae was made by García-Barros (1987, 1988), but was Harvey (1991) who used for the first time the position of filiform setae in the classification of Nymphalidae; and characterized it as thin and flexible compared to other setae. Despite the efforts, there are still confusions in the descriptions of these setae in Brassolini.

In the second instar, head characters are also informative and can be used in systematics of group, such as the number and position of the scoli. In *O. fruhstorferi* from third instar onward, the larvae have four pairs of scoli, a characteristic shared with *O. syme* (Aguiar et al. 2024). This differs from *O. sulcius* which has four pairs of scoli starting from the second instar (Cajé et al. 2025b). Other Brassolini, such as *Caligo teucer teucer* (Linnaeus, 1758) (Souza et al. 2006), *Opsiphanes quiteria meridionalis* (Neves and Paluch 2016), and *Opsiphanes cassiae cassiae* (Linnaeus, 1758) (treated as *Opsiphanes cassiae crameri* C. Felder & R. Felder, 1862 by Orlandin et al. 2020) (Piovesan et al. 2022) also have four pairs of scoli.

The pupae of *O. fruhstorferi* (Fig. 9) have the general coloration similar to dry bamboo, consistent with the pattern described by DeVries (1987) for *O. staudingeri*; *O. syme* (Aguiar et al. 2024); and *O. sulcius* (Cajé et al. 2025b). However, the pupa of *O. fruhstorferi* can be distinguished from those species by the lateral stripe on abdomen and dorsal area with greenish spots.

In the field, the species of the host plant was not identified. However, *P. aurea* was offered to larvae of *O. fruhstorferi*, and they were able to complete their life cycle by feeding on this plant. Although the host plant for *Opoptera* is still poorly documented, there are records for *Opoptera aorsa* (Godart, [1824]) (Zikán and Zikán 1968; Silva et al. 1968), *O. mexicana* (treated as *Opoptera staudingeri mexicana* Wolfe 2017), *O. staudingeri* (DeVries 1987; Cubero 1985), *O. sulcius* (Hoffmann 1937; Cajé et al. 2025b), and *O. syme* (Zikán and Zikán 1968; Silva et al. 1968; Aguiar et al. 2024), with most of these records associated with Poaceae. However, Hoffmann (1936)

obtained eggs of *O. fruhstorferi* (treated as *Opsiphanes fruhstorferi*), which were laid on a thorn palm leaf, an Arecaceae. Similarly, Hoffmann (1937) mentions this plant for *O. sulcius*. Thus far, these are the only records of *Opoptera* associated with Arecaceae.

Parasitoidism is widely documented in several insect orders, such as Coleoptera, Diptera, Hymenoptera, Lepidoptera, Neuroptera, Strepsiptera and Trichoptera (Labandeira and Li 2021), with the majority of parasitoids belonging to the orders Hymenoptera and Diptera (Eggleton and Belshaw 1992). Amongst the Brassolini, previous records of parasitoids Hymenoptera—Braconidae, Chalcididae, Encyrtidae, Eupelmidae, Eulophidae, Scelionidae, Trichogrammatidae—(Waterston 1923; Cleare and Squire 1934; Lepesme 1947; Costa Lima 1950; Mariconi and Zamith 1954; Muesebeck 1958; Malo and Willis 1961; Harrison 1963; Silva et al. 1968; Guagliumi 1972; Condie 1976 in Canet 1986; Ruszczyk and Ribeiro 1998; Zhang et al. 2005; Marcicano et al. 2007; Margaría et al. 2007; Santos 2009; Greeney et al. 2011; Salgado-Neto 2013; Shirai et al. 2016; Salgado-Neto et al. 2019; Salgado-Neto et al. 2021; Lara-López et al. 2023; Figueroa et al. 2024), and Diptera—Tachinidae, Phoridae, Sarcophagidae—(Travassos Filho and Carrera 1941; Piza and Zamith 1944; Gonzaga and Lordello 1952; Mariconi and Zamith 1954; Malo and Willis 1961; Harrison 1963; Silva et al. 1968; Condie 1976 in Canet 1986; Laverde and Borja 1998; Calvo 2004; Marcicano et al. 2009; Santos 2009; Greeney et al. 2011; Salgado-Neto 2011); these parasitoids are associated with different developmental stages, including egg, larvae, larval-pupal, pupae, and larvipupal parasitoids of *Brassolis* Fabricius, 1807, *Caligo* Hübner, [1819], *Eryphanis* Boisduval, 1870, *Opsiphanes* Doubleday, [1849], and *Selenophanes* Staudinger, 1887 (Table 2). However, some records do not clearly indicate whether the parasitoid egressed from the larva or the pupa, nor do they specify the deposition of parasitoid vouchers in biological collections.

Egg parasitoids are largely used in biological control, primarily targeting Lepidoptera (Querino 2012; Dudczak et al. 2017; Queiroz et al. 2020; Fortes et al. 2023). Amongst these parasitoids, Trichogrammatidae comprises nearly 1.000 species, which are usually solitary or gregarious primary endoparasitoids of eggs (Melo 2024). Some of these species may have a wide range and potentially impact non-target species (Bueno et al. 2024). So far the only record of these tiny wasps for Brassolini is *Xenufens* sp. near *ruskini* Girault associated with *Caligo eurilochus* (Cramer, 1775) (Malo and Willis 1961). While parasitoids have never been documented for *Opoptera*, this finding represents the first evidence of *Trichogramma* sp. parasitizing the egg of this butterfly.

Table 2. Records of parasitoids (Hymenoptera, Diptera) associated with Brassolini

Host / Species	Parasitoid		Host stage	Data source
	Hymenoptera	Diptera		
Brassolis astyra Godart, [1824]	*Conura* (= *Spilochalcis*) *morleyi* (Ashmead, 1904) (Chalcididae)		Larval	Costa Lima (1950)
		Xanthozona melanopyga (Wiedmann, 1830) (Tachinidae)	Larvipupal	Travassos Filho and Carrera (1941); Piza and Zamith (1944); Salgado-Neto (2011)
Brassolis astyra astyra Godart, [1824]	*Anastatus reduvii* (Howard, 1880) (Eupelmidae)		Larval	Silva et al. (1968)
	Apanteles marquesi (Brèthes, 1924) (Braconidae)		Larval	Silva et al. (1968)
	Aracnophaga hirtibasis Gahan, 1943 (Eupelmidae)		Larval	Silva et al. (1968)
	Brachymeria incerta (Cresson, 1865) (Chalcididae)		Larval	Silva et al. (1968)
	Brachymeria ovata (Say, 1824) (Chalcididae)		Larval	Silva et al. (1968)
	Conura (= *Spilochalcis*) *morleyi*		Larval	Silva et al. (1968)
		Hemimasipoda sp. (Tachinidae)	Larval	Silva et al. (1968)
		Lespesia haywardi (Blanchard, 1942) (Tachinidae)	Larval	Silva et al. (1968)
	Tetrastichus sp. (Eulophidae)		Larval	Silva et al. (1968)
		Xanthozona melanopyga	Larvipupal	Silva et al. (1968)
Brassolis isthmia H. Bates, 1864	*Anastatus reduvii*		Egg	Waterston (1923); Mariconi and Zamith (1954)
Brassolis sophorae (Linnaeus, 1758)	*Anastatus reduvii*		Egg	Mariconi and Zamith (1954)
		Belvosia sp. (Tachinidae)	Pupa	Gonzaga and Lordello (1952)
	Brachymeria annulata (Fabricius, 1793) (Chalcididae)		not mentioned	Waterston (1923)
	Brachymeria incerta		Pupal	Waterston (1923)
	Conura (= *Spilochalcis*) *morleyi*		Pupal	Waterston (1923); Ruszczyk and Ribeiro (1998)
	Telenomus sp. (Scelionidae)		Egg	Costa Lima (1950); Lepesme (1947) in Mariconi and Zamith (1954)
	Telenomus nigrocoxalis Ashmead, 1894 (Scelionidae)		Egg	Cleare and Squire (1934) in Mariconi and Zamith (1954)
		Xanthozona melanopyga	Larval	Piza and Zamith (1944)
			Pupal	Gonzaga and Lordello (1952)
		Winthemia pinguis (Fabricius, 1805) (Tachinidae)	Larvipupal	Mariconi and Zamith (1954)
Brassolis sophorae sophorae (Linnaeus, 1758)	*Anastatus reduvii*		Larval	Silva et al. (1968)
		Argoravinia sp. (Sarcophagidae)	Larval	Silva et al. (1968)
	Brachymeria annulata (Fabricius, 1793)		Larval	Silva et al. (1968)
	Brachymeria incerta		Larval	Silva et al. (1968)
	Brachymeria ovata		Larval	Silva et al. (1968)
		Belvosia bicincta Robineau-Desvoidy, 1830 (Tachinidae)	Larval	Silva et al. (1968)
	Conura (= *Spilochalcis*) *morleyi*		Larval	Silva et al. (1968)
		Hemimasipoda sp.	Larval	Silva et al. (1968)
	Tetrastichus sp.		Larva	Silva et al. (1968)
		Xanthozona melanopyga	Larval	Silva et al. (1968)
		Winthemia erythrura (Meigen, 1838)	Larval	Silva et al. (1968)
		Winthemia pinguis	Larval	Silva et al. (1968)
Brassolis sophorae ardens Stichel, 1903		*Xanthozona melanopyga*	Larval	Silva et al. (1968)
Brassolis sophorae laurentii Stichel, 1925	*Conura morleyi*		Pupal	Marcicano et al. (2007)
		Winthemia analis (Macquart, 1846) (Tachinidae)	Larvipupal	Marcicano et al. (2009)
Brassolis sophorae luridus Stichel, 1902		*Xanthozona melanopyga*	Larval	Silva et al. (1968)
Brassolis sp.	*Anastatus reduvii*		Egg	Waterston (1923); Mariconi and Zamith (1954)

		Belvosia wiiliamsi (Aldrich, 1928)	Larvipupal or pupal	Salgado-Neto (2011)
	Telenomus nigrocoxalis		Egg	Waterston (1923)
Caligo atreus (Kollar, 1850)		*Winthemia* sp.	Larval	Calvo (2004)
Caligo brasiliensis brasiliensis (C. Felder, 1862)		*Hemimasipoda* sp.	Larval	Silva et al. (1968)
	Telenomus californicus Ashmead, 1893 (Scelionidae)		Egg	Margaría et al. (2007)
Caligo eurilochus (Cramer, 1775)	*Apanteles opsiphanis* Schrottky 1909 (Braconidae)		Larval	Malo and Willis (1961)
		Achaetoneura sp. (Tachinidae)	Larvipupal	Malo and Willis (1961)
		Megaselia sp. (Phoridae)	Larval	Malo and Willis (1961)
		Megaselia sp. (Phoridae)	Pupal	Malo and Willis (1961)
		Sarcophaga (*Sarcodexia*) *lambens* (Wiedemann) (Sarcophagidae)	Larvipupal	Malo and Willis (1961)
		Sarcophaga sp. (Sarcophagidae)	Larvipupal	Malo and Willis (1961)
	Xenufens near *ruskini* Girault, 1915 (Trichogrammatidae)		Egg	Malo and Willis (1961)
	Xenufens sp.		Egg	Harrison (1963)
		Winthemia sp.	Larvipupal	Malo and Willis (1961)
Caligo illioneus (Cramer, 1775)		Unidentified	Pupa	Laverde and Borja (1998)
		Unidentified	Pupal	Santos (2009)
Caligo illioneus illioneus (Cramer, 1775)	*Brachymeria annulata*		Not mentioned	Waterston (1923)
	Unidentified Eulophidae		Egg	Santos (2009)
	Conura maculata (Fabricius, 1758) (Chalcididae)		Pupal	Santos (2009)
Caligo illioneus praxsiodus Fruhstorfer, 1912	*Brachymeria* sp. (Chalcididae)		Pupal	Guagliumi (1972)
	Conura sp. (Chalcididae)		Pupal	Guagliumi (1972)
Caligo telamonius memnon (C. Felder & R. Felder, 1867)	Unidentified Eupelmidae		Egg	Canet (1986)
	Ooencyrtus sp. (Encyrtidae)		Egg	Condie (1976) in Canet (1986); Harrison (1963)
	Ooencyrtus papilionis Ashmead, 1905 (Encyrtidae)		Egg	Zhang et al. (2005)
	Xenufens ruskini		Egg	Harrison (1963)
		Winthemia pinguis	Larval	Condie (1976) in Canet (1986)
		Sarcophaga lambens	Pupal	Harrison (1963)
Eryphanis greeneyi Penz & DeVries, 2008		*Winthemia* sp. near *analis* Macquart	Larval	Greeney et al. (2011)
	Protapanteles eryphanidis Whitfield, 2011 (Braconidae)		Larval	Greeney et al. (2011)
Opoptera fruhstorferi (Röber, 1896)	*Trichogramma* sp. (Trichogrammatidae)		Egg	This study
Opsiphanes fabricii fabricii (Boisduval, 1870)	*Conura maculata*		Pupal	Figueroa et al. (2024)
	Cotesia invirae Salgado-Neto & Whitfield, 2019 (Braconidae)		Larval	Lara-López et al. (2023)
Opsiphanes cassiae cassiae (Linnaeus, 1758)[a]	*Apanteles opsiphanis*		Larval	Waterston (1923); Muesebeck (1958)
	Horismenus opsiphanis (Schrottky, 1909)(Eulophidae)		Larval	Waterston (1923)
Opsiphanes invirae (Hübner, [1808])	*Conura* (= *Spilochalcis*) *morleyi*		Not mentioned	Waterston (1923)
	Cotesia invirae		Larval	Salgado-Neto et al. (2019)
Opsiphanes invirae invirae (Hübner, [1808])[b]	*Cotesia cassina* Salgado-Neto, Vásquez & Whitfield, 2021 (Braconidae)		Larval	Salgado-Neto et al. (2021)
Opsiphanes invirae amplificatus Stichel, 1904	*Cotesia alius* (Muesebeck, 1958) (Braconidae)		Larval	Salgado-Neto (2013)
Opsiphanes cassiae tamarindi C. Felder & R. Felder, 1861[c]	*Ooencyrtus submetallicus* (Howard, 1897) (Encyrtidae)		Egg	Harrison (1963)
Selenophanes cassiope guarany Casagrande, 1992	*Anastatus* sp. (Eupelmidae)		Egg	Shirai et al. (2016)
	Galeopsomyia sp. (Eulophidae)		Egg	Shirai et al. (2016)

[a]cited as *Opsiphanes crameri* C. Felder & R. Felder, 1862, but currently is synonymous with *Opsiphanes cassiae cassiae* (Linnaeus, 1758); [b]cited as *Opsiphanes cassina* C. Felder & R. Felder, 1862, now synonymous with *Opsiphanes invirae invirae* (Hübner, [1808]); [c]cited as *Opsiphanes tamarindi* C. Felder & R. Felder, 1861, currently is synonymous with *Opsiphanes cassiae tamarindi* C. Felder & R. Felder, 1861 (Piovesan et al. 2022).

CONCLUSIONS

This study provides a detailed description of the morpholgy of the immature stages and the complete life cycle of *O. fruhstorferi*. In addition, it reports tritrophic plant-host-parasitoid interactions, documents a new alternative host plant, and, for the first time, records *Trichogramma* in eggs of *O. fruhstorferi*, documenting this microhymenopteran for Brassolini. It also provides a compilation of Brassolini parasitoid records. These data expand the understanding of the natural history of *Opoptera*, a genus that still has several gaps to be filled, and represent an important contribution to the systematics of Neotropical butterflies.

Acknowledgments: We are grateful to Ivo Rank for providing adults and eggs of *Opoptera fruhstorferi*. We extend our thanks to Marília Locatelli Corrêa Ferreira from the Herbarium UPCB, Departamento de Botânica, Universidade Federal do Paraná, for her assistance in preparing the exsiccate and depositing it in the herbarium. We also thank Regina Tomoko Shirasuna for identifying the plant. Our gratitude goes to Fábio Sampaio and Marcelo Maciel from the Laboratório de Controle Integrado de Insetos for their help with the slide-mounting techniques. We are grateful to Tamara Takahashi from the Laboratório de Biologia de Insetos USP/Esalq for identifying the parasitoid, and to Rosângela Borges Freitas and Flávia Priscila Souza Afonso from the Centro de Microscopia Eletrônica (CME), Universidade Federal do Paraná (UFPR) for capturing the images. We thank editor Jens Thorvald Høeg and two anonymous reviewers for their valuable comments, suggestions, and corrections, which improved this manuscript. SC (162126/2021-2) and MMC (310277/2021-3) thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the respective fellowships granted.

Author's contribution: SC: conceptualization, data curation, investigation, methodology, writing-original draft preparation, reviewing and editing. IMML: writing-original draft preparation and reviewing. OHHM: data curation, supervision and reviewing. MMC: project administration, data curation, supervision, writing-original draft preparation and reviewing.

Competing interests: The authors declare that is no conflict of interest regarding the publication of this paper.

Availability of data and material: Voucher specimens of were deposited in the Coleção Entomológica Padre Jesus Santigo Moure, Departamento de Zoologia, Universidade Federal do Paraná (DZUP), Curitiba, Paraná, Brazil, under the codes DZ 57.790 (female), 57.791 (male), and immatures in the Coleção de Imaturos de Lepidoptera, DZUPIL 0189, together with the slide-mounted parasitoid specimens. The plant exsiccata was deposited in the Herbarium UPCB, affiliated with the Departamento de Botânica, Universidade Federal do Paraná, under code UPCB0065915, and available online at JBRJ (2025).

Consent for publication: Not applicable.

Ethics approval consent to participate: Not applicable.

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