

Immature stages and natural history of two species of *Forsterinaria* from southeastern Brazil (Lepidoptera: Nymphalidae)

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Abstract: The present paper describes the immature stages and provides data on the population biology of the Neotropical satyrines *Forsterinaria quantius* and *Forsterinaria necys* (Lepidoptera: Nymphalidae). Eggs of *F. quantius* are emerald green and laid singly; those of *F. necys* are bright yellow and oviposition is unknown. In both species, larvae feed on bamboo and pass through four instars. Both species present a predominantly brown pupa, short and smooth, without projections and head horns. Adults presented two peaks of abundance (from April to May and from August to September), and the sex ratio is male-biased in both species. In general, the immature stages of both species are very similar to those of *Forsterinaria pronophila*, the only other species in the genus with known immature stages. Data on immature stages of any additional species of *Forsterinaria* would be extremely important to increase our understanding of morphological variation among the immature stages within the genus.

Resumo: O presente trabalho descreve os estágios imaturos e apresenta dados de biologia populacional dos satiríneos Neotropicais *Forsterinaria quantius* e *Forsterinaria necys* (Lepidoptera: Nymphalidae). Os ovos de *F. quantius* são verde esmeralda e colocados isoladamente; os de *F. necys* são amarelo brilhante e a oviposição não foi observada. As larvas de ambas as espécies se alimentam de bambu e passam por quatro instares. As pupas das duas espécies são predominantemente marrons, curtas e lisas, sem projeções ou espinhos. Os adultos apresentaram dois picos de abundância (de abril a maio e de agosto a setembro), e a razão sexual foi desviada para excesso de machos nas duas espécies. Em geral, os estágios imaturos de ambas as espécies são muito similares aos de *Forsterinaria pronophila*, a única outra espécie no gênero com estágios imaturos conhecidos. Dados de estágios imaturos de quaisquer outras espécies de *Forsterinaria* seriam de extrema importância para uma melhor compreensão dos padrões morfológicos dentro do gênero.

Key words: Atlantic Forest, Euptychiina, *Forsterinaria quantius*, *Forsterinaria necys*, population biology

INTRODUCTION

With over 400 described species, the subtribe Euptychiina is one of the most diverse groups of Satyrinae (Lepidoptera: Nymphalidae) (Lamas 2004; Peña *et al.* 2010; Marín *et al.* 2011). In recent years, combined efforts are tackling taxonomic problems at all levels, from species descriptions to higher classification of the subtribe (e.g., Peña *et al.*, 2010; Matos-Maraví *et al.*, 2013; Barbosa *et al.*, 2015; Nakahara *et al.*, 2015). Nevertheless, knowledge about the immature stages of Euptychiina is still scarce: only about 10% of the described species have any published information on their immature stages (Singer *et al.*, 1983; DeVries, 1987; Murray, 2001; Cosmo *et al.*, 2014 and references therein). In addition, much of the available information comprises limited or incomplete descriptions, and for many genera there is virtually no available information at all, including *Caeruleptychia* Forster, 1964, *Carmina* Ebert, 1998, *Cepheptychia* Forster, 1964, and *Zischkaia* Forster, 1964.

The genus *Forsterinaria* R. Gray, 1973 is a typical example of this lack of information. With 25 described species (Peña & Lamas, 2005; Zubeck *et al.*, 2015) this is one of the seven most species-rich genera of Euptychiina, but immature stages have been described for just a single species, namely *Forsterinaria*

pronophila (A. Butler, 1867) (Freitas & Peña, 2006).

According to Peña & Lamas (2005), *Forsterinaria quantius* (Godart, [1824]) and *Forsterinaria necys* (Godart, [1824]) belong to the “Brazilian group” of *Forsterinaria*, which also includes *Forsterinaria itatiaia* Peña & Lamas, 2005 and, based on molecular data (Peña *et al.* 2010), *Forsterinaria pronophila* (A. Butler, 1867) (all endemic to southeastern South America). However, recent molecular studies showed that *F. quantius* is in fact the sister group of (*F. pronophila* + *F. necys*) + all remaining *Forsterinaria* (Peña *et al.* 2010; Matos-Maraví *et al.*, 2013).

The present paper describes in detail the immature stages of *Forsterinaria quantius* and *Forsterinaria necys*, presenting information on the natural history, behavior and population biology of these two butterfly species.

STUDY SITES AND METHODS

Adults and immatures of *F. quantius* and *F. necys* were studied in three different localities in southeastern Brazil: 1) Reserva Biológica Municipal da Serra do Japi, Jundiá (900-1100 m; 23°13'S, 46°57'W), São Paulo; 2) Parque Estadual de Campos do Jordão, Campos do Jordão (1600-1800 m; 22°41'S, 45°27'W), São Paulo; and 3) Serra de São Domingos, Poços de Caldas (1650-1680 m; 21°46'S, 46°34'W), Minas Gerais.

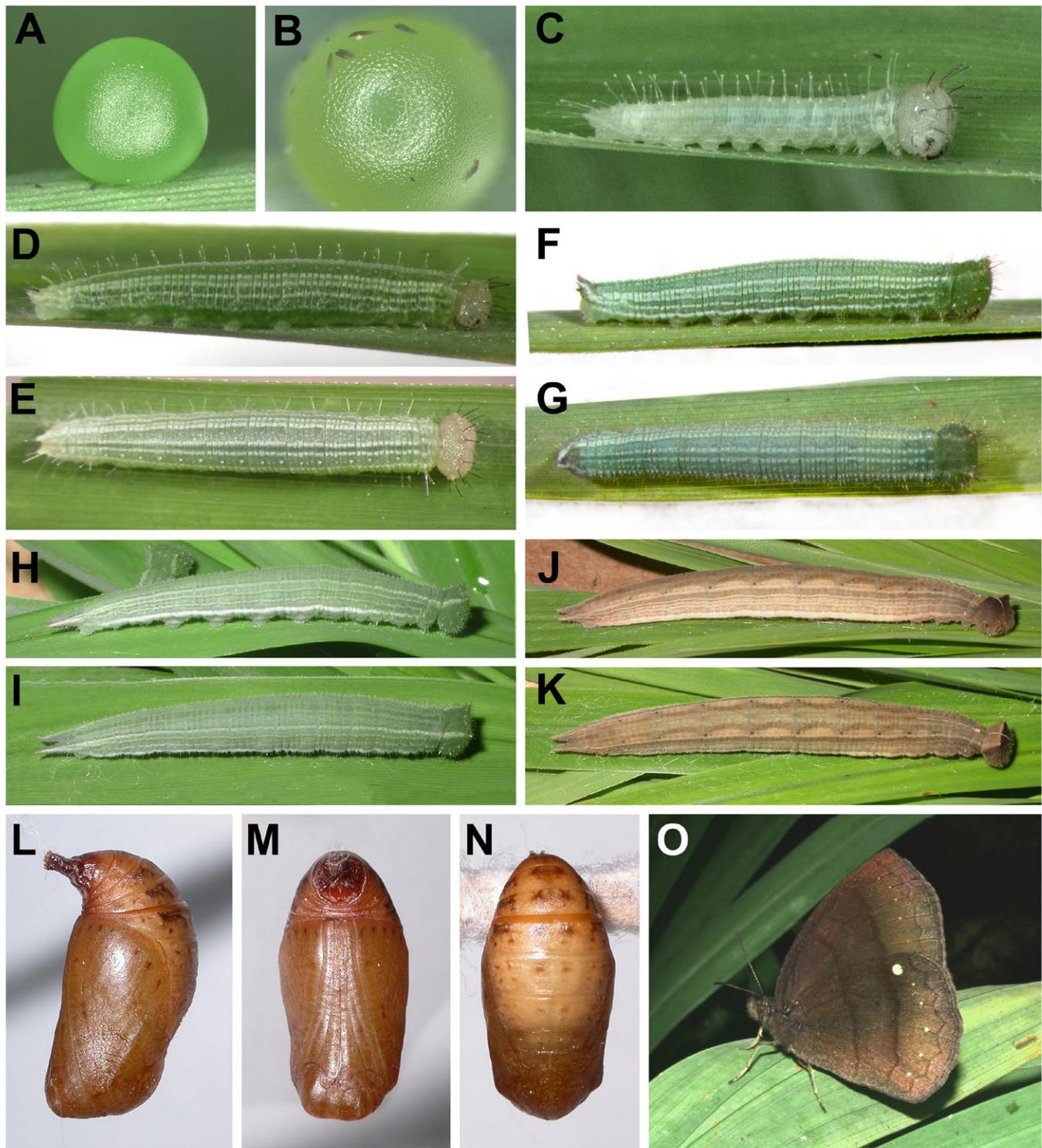


Figure 1. Immature stages and adult of *Forsterinaria quantius*. **A, B**, egg, lateral and dorsal; **C**, early first instar, lateral; **D, E**, late first instar, lateral and dorsal; **F, G**, second instar, lateral and dorsal; **H, I**, third instar, lateral and dorsal; **J, K**, fourth (last) instar, lateral and dorsal; **L, M, N**, pupa, lateral, ventral and dorsal; **O**, adult male from Campos do Jordão, São Paulo.

For *F. quantius*, fertile eggs were obtained from five medium-age, wild-captured females (two from Poços de Caldas and three from Serra do Japi) confined in plastic bags (capacity 3 L) warmed by a 40W bulb from 09:00 to 17:00 and provided with leaves of several species of bamboos and grasses. Females typically started ovipositing after 3-4 hours in the first two days, after being manually fed with a solution of 1:3 honey/

water, laying eggs everywhere including leaves, stems and in the plastic bag. For *F. necys* the method described above did not work, and after four unsuccessful attempts, eggs were obtained by gently pressing the abdomen of wild-captured females. Larvae were reared in plastic containers cleaned daily, with fresh plant material provided every two or three days (following Freitas 2007). Data were recorded on behavior and

development time for all stages. Dry head capsules and pupal cases were retained in glass vials. Immature stages were fixed in Kahle-Dietrich solution (Triplehorn & Johnson, 2005) when the number of specimens was sufficient. Voucher specimens of the immature stages were deposited in the Museu de Zoologia "Adão José Cardoso" (ZUEC-AVLF), Universidade Estadual de Campinas, Campinas, São Paulo, Brazil. Measurements were taken and general aspects of morphology were observed using a Leica®MZ7.5 stereomicroscope equipped with a micrometric scale. Egg size is presented as height and diameter, and head capsule size is the distance between the most external ocelli (as in Freitas, 2007). Terminology for early stages descriptions followed Garcia-Barros & Martín (1995) for eggs and Stehr (1987) for larvae and pupae.

Adult ecology was also studied through a four-year trap study in Serra do Japi, Jundiá, São Paulo. Fifty traps were placed in five transects at two heights: 25 in the understory (1.5 m above ground) and 25 in the canopy (8 - 18 m above ground, beneath tree crowns). Traps were installed in groups of ten per transect at alternating heights to avoid the interference of canopy traps on understory traps (following Ribeiro & Freitas, 2012). A standard mixture of mashed banana with sugar cane juice, fermented for at least 48 h, was used as attractant. The bait was placed inside the traps in plastic pots with a perforated cover. The traps were checked every 24 h, and the baits were replaced at each visit. All traps were kept open simultaneously in the field for periods of five days. Butterflies were sampled monthly from October 2011 to September 2015. All butterflies were marked with an individual number on the ventral surface of the hind wings and released unharmed. For further details about the methods, see Freitas *et al.* (2014).

RESULTS

Forsterinaria quantius

Description of immature stages

The following descriptions and measurements are based on material reared from one female from Serra do Japi, Jundiá, São Paulo (material reared from Poços de Caldas, Minas Gerais, gave similar results).

Egg (Figs. 1A,B). Emerald-green and round, with a somewhat rough chorion. Height 1.14 - 1.20 mm; diameter 1.20 - 1.30 mm; duration 3 - 5 days (n = 15).

First instar (Figs. 1C,D,E). Head capsule width 0.86 - 0.96 mm (mean = 0.92 mm; SD = 0.04 mm; n = 6); head scoli 0.12 - 0.14 mm (mean = 0.13 mm; SD = 0.01 mm; n = 6). Head light greenish yellow, with enlarged chalazae, bearing a pair of short scoli on vertex, each with two long narrow black setae. Third stemma larger than other stemmata. Body light green, with white longitudinal stripes, turning dark green after first meal; caudal filaments very short. Legs and prolegs light green. Setae light green and short; all dorsal and subdorsal setae are clubbed at tip. Maximum length 9 mm. Duration 6 - 10 days (n = 12). Body chaetotaxy is presented in Fig. 2.

Second instar (Figs. 1F,G). Head capsule width 1.30 - 1.42 mm (mean = 1.37 mm; SD = 0.04 mm; n = 7); head scoli 0.24 - 0.30 mm (mean = 0.28 mm; SD = 0.02 mm; n = 7). Head dark green with two short scoli on vertex. Body green, striped longitudinally with white; caudal filaments short. Legs and prolegs light green. Maximum length 13 mm. Duration 5 - 7 days (n = 12).

Third instar (Figs. 1H,I). Head capsule width 2.00 - 2.10 mm (mean = 2.04 mm; SD = 0.05 mm; n = 6); head scoli 0.36 - 0.40 mm (mean = 0.38 mm; SD = 0.02 mm; n = 6). Head green, with two diverging very short scoli on vertex. Body green with several longitudinal white stripes; caudal filaments short and diverging. Legs and prolegs light green. Maximum length 23 mm. Duration 7 - 8 days (n = 9).

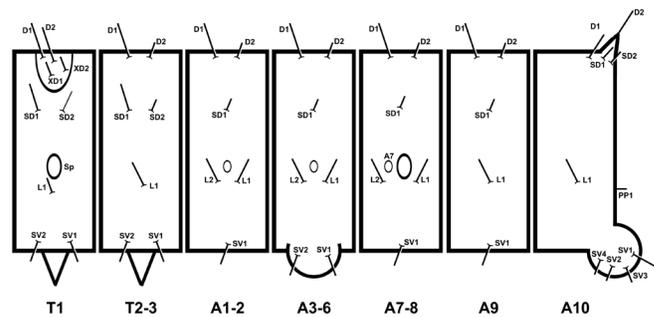


Figure 2. Larval chaetotaxy of first instar of *Forsterinaria quantius* based on material from Jundiá, SP (body diagram in lateral view). For chaetotaxy abbreviations see Stehr (1987) and Murray (2001).

Fourth (last) instar (Figs. 1J,K). Head capsule width 2.92 - 3.08 mm (mean = 2.98 mm; SD = 0.06 mm; n = 5); head scoli 0.40 - 0.61 mm (mean = 0.49 mm; SD = 0.09 mm; n = 5). Head brown, with two diverging, short scoli on vertex and a conspicuous thin white dorsal median stripe extending through frons. Body brown with a faint pattern of longitudinal stripes; a pair of subdorsal scalloped stripes; caudal filaments short. Legs and prolegs light brown. Maximum length 36 mm. Duration 10 - 14 days (n = 6).

Pupa (Figs. 1L,M,N). Short and smooth; mostly light brown with darker areas; short rounded ocular caps; cremaster dark reddish; dorsal abdomen smooth without projections. Total length 11.0 - 11.5 mm (n = 5). Duration 11 - 12 days (n = 4).

Behavior and natural history: Adults (Fig. 1O) are common in a variety of habitats in the Atlantic Forest, especially from 600 to 1800 m. Oviposition behavior was observed in Serra do Japi, São Paulo; at 14:00 hrs a female was observed fluttering among a dense patch of bamboos (Bambusae), apparently testing several leaves. After some time the female was observed laying solitary eggs on mature leaves. Eggs were also laid singly in the laboratory. In the laboratory, larvae easily accepted the cultivated garden bamboo *Bambusa gracilis* hort. Ex Rivière & C. Rivière (Poaceae). Larvae are solitary in all instars and did not present cannibalistic behavior, with several larvae of different instars being reared together in the same container.

Adult population biology: In total, 289 individuals were captured over four years of sampling, 132 in the understory (83 males, 46 females and three with sex not recorded) and 157 in the canopy (83 males, 72 females and two with sex not recorded). Distribution among strata did not differ from 1:1 for both sexes combined ($X^2 = 2.16$, $P = 0.14$; $DF = 1$), but females were marginally more abundant in the canopy considering the distributions of both sexes in the two strata ($X^2 = 3.38$, $P = 0.07$; $DF = 1$). Recaptures were recorded for 11 individuals (ca. 4% recapture success), seven males (six in canopy and one in understory) and four females (three in the canopy and one in understory). Adults typically presented two peaks of abundance, the first from April to May and the second from August to September. The exception was the very dry year of 2014, when the mid wet season was relatively dry and adults were uniformly low in abundance from January to October (Fig. 4). The sex could be attributed to 284 of the captured individuals, totaling 166 males and 118 females, a clearly male biased sex ratio (1.41:1, $X^2 = 8.11$, $P = 0.004$; $DF = 1$).

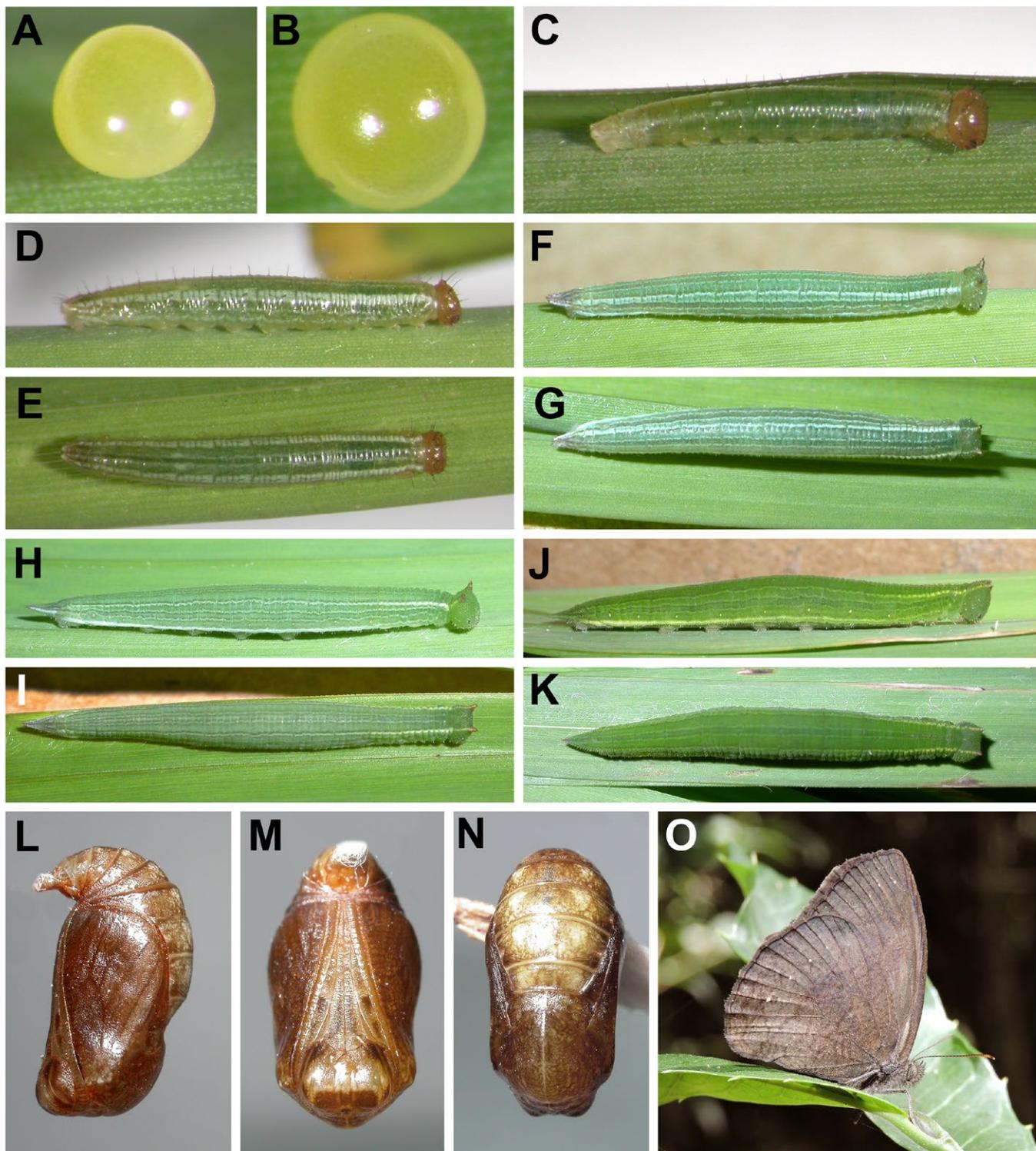


Figure 3. Immature stages and adult of *Forsterinaria necys*. **A, B.** egg, lateral and dorsal; **C.** early first instar, lateral; **D, E.** late first instar, lateral and dorsal; **F, G.** second instar, lateral and dorsal; **H, I.** third instar, lateral and dorsal; **J, K.** fourth (last) instar, lateral and dorsal; **L, M, N.** pupa, lateral, ventral and dorsal; **O.** adult male from Serra do Japi, Jundiá, São Paulo.

Forsterinaria necys

Description of immature stages

The following descriptions and measurements are based on material reared from two females from Serra do Japi, Jundiá, São Paulo.

Egg (Figs. 3A,B). Bright yellow and round, with a somewhat rough chorion. Height 0.94 - 0.98 mm; diameter 0.98 - 1.02 mm (n = 5); duration 7 days (n = 2).

First instar (Figs. 3C,D,E). Head capsule width 0.68 - 0.72 mm (n = 2); head scoli 0.10 (n = 2). Head light brown, with enlarged chalazae, bearing a pair of short scoli on vertex, each with two long narrow black setae. Third stemma larger than other stemmata. Body light green, with two broad white longitudinal stripes, turning dark green after first meal; caudal filaments very short. Legs and prolegs light green. Setae black and short; not clubbed at tip. Maximum length 6.5 mm. Duration 4 - 5 days (n = 2).

Second instar (Figs. 3F,G). Head capsule width 1.08 - 1.10 mm (n = 2);

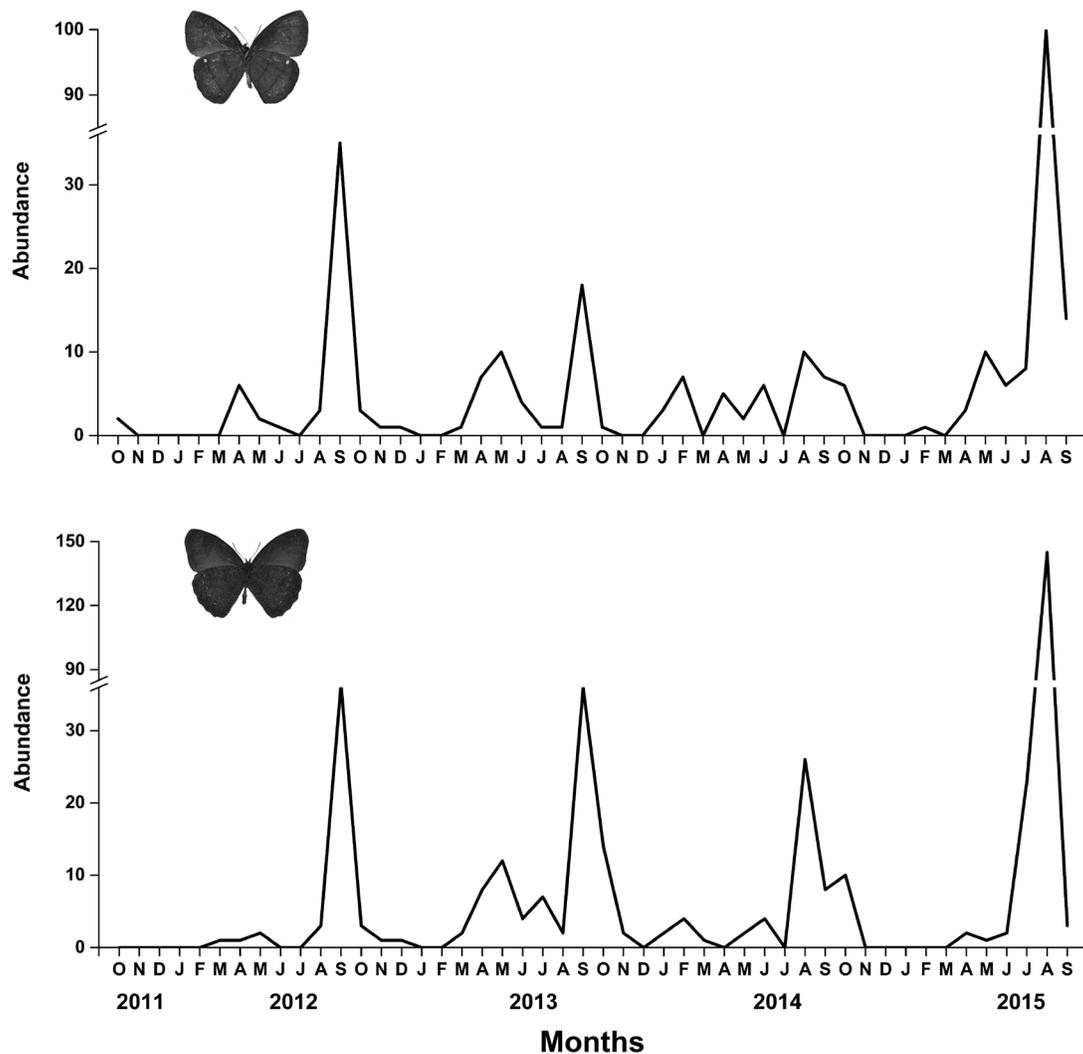


Figure 4. Monthly number of individuals of *Forsterinaria quantius* (above) and *Forsterinaria necys* (below) recorded in fruit-baited traps in Serra do Japi, Jundiá, São Paulo State, from October 2011 to September 2015.

head scoli 0.28 - 0.30 mm (n = 2). Head light green with two short scoli on vertex. Body green, striped longitudinally with white; caudal filaments short. Legs and prolegs light green. Maximum length 12 mm. Duration 5 days (n = 2).

Third instar (Figs. 3H,I). Head capsule width 1.60 - 1.64 mm (n = 2); head scoli 0.40 (n = 2). Head green, with two diverging short scoli on vertex, these are green with reddish tips. Body green with several longitudinal white stripes; caudal filaments short and not diverging, giving the impression of a single pointed process. Legs and prolegs light green. Maximum length 16 mm. Duration 5 - 6 days (n = 2).

Fourth (last) instar (Figs. 3J,K). Head capsule width 2.46 - 2.50 mm (n = 2); head scoli 0.50 - 0.52 mm (n = 2). Head green, with two diverging, short scoli on vertex, these green with reddish tips. Body green with several thin yellow longitudinal stripes; caudal filaments short and converging, giving the impression of a single pointed process. Legs and prolegs green. Maximum length 33 mm. Duration 11 - 12 days (n = 2).

Pupa (Figs. 3L,M,N). Short and smooth; mostly light brown with darker areas; short rounded ocular caps; cremaster dark reddish; dorsal abdomen smooth without projections. Total length 10.0 mm (n = 2). Duration 14 - 15 days (n = 2).

Behavior and natural history: Adults (Fig. 3O) are common in a variety of habitats in the Atlantic forest, especially from 600 to 2000 m. Oviposition behavior was never observed. In the laboratory, larvae easily accepted the cultivated garden bamboo *Bambusa gracilis* hort. Ex Rivière & C. Rivière (Poaceae).

Adult population biology: In total, 370 individuals were captured over four years of sampling, 78 in the understory (46 males and 32 females) and 292 in the canopy (169 males, 117 females and six with sex not recorded). Distribution among strata was clearly biased towards canopy for both sexes combined ($X^2 = 123.7$, $P < 0.0001$; $DF = 1$) and also considering the distributions of both sexes in the two strata ($X^2 = 0.0$, $P = 0.98$; $DF = 1$). Recaptures were recorded for 20 individuals (ca. 5% recapture success), 12 males (nine in canopy and three in understory) and eight females (six in the canopy and two in understory). Similar to *F. quantius*, adults typically presented

two peaks of abundance, the first from April to May and the second from August to September (Fig. 4). The sex could be attributed to 364 of the captured individuals, totaling 215 males and 149 females, a clearly male biased sex ratio (1.44:1, $X^2 = 11.9$, $P = 0.0007$; $DF = 1$).

DISCUSSION

Freitas & Peña (2006) previously discussed the overall similarities between the immature stages of *F. quantius* and *F. pronophila*. In general, immature stages of both species are similar in the shape of the first and last instars, which present very short scoli on the head capsule, and in the general shape of the pupae, which are short and stubby, with short, rounded ocular caps. The main differences are the egg color (green in *F. quantius* and yellow in *F. pronophila* and *F. necys*) and the general coloration of last instar larvae, which are predominantly brown in *F. quantius* and predominantly green in *F. pronophila* and *F. necys*. Although the above similarities between *F. pronophila* and *F. necys* could be indicative of phylogenetic relationships between the two species, there is yet no evidence that these character states represent synapomorphies, and a broad phylogenetic study is needed to polarize characters. Nonetheless, data on immature stages of any additional species of *Forsterinaria*, especially Andean species, would be extremely important to improve understanding of the morphological variation within the genus. Given the apparent ease of obtaining eggs in the laboratory (see Methods section), we invite any person interested in butterfly immature stages to make efforts to unveil the life cycles of other species of *Forsterinaria* using such methods.

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