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Author: De Domenico, Fernando Campos

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FERNANDO CAMPOS DE DOMENICO

Museu de Zoologia da Universidade de São Paulo, Avenida Nazaré, 481 - Ipiranga - São Paulo / SP - Brazil, CEP: 04261-000.
Email: fcdomenico@usp.br

## Abstract

Nymphal instars of *Xyleus discoideus discoideus*, a romaleid grasshopper, are described for the first time. The following characters are recorded for each stage: body length, head width, pronotal length, hind femoral length, number of antennomeres, number of longitudinal eye stripes and the morphology of wing pads and external genitalia. Criteria that can be used to distinguish the instars and sexes are identified. Males and females have a 5-instar cycle under laboratory conditions. Females are larger as adults than males and this is linked to a longer nymphal period in females.

## Key words

*Xyleus discoideus*, nymphal instars, postembryonic development, Romaleidae

## Introduction

Postembryonic studies of grasshoppers are very important for ecological and taxonomic studies (Uvarov 1966). However, almost nothing is known about the nymphal development of Romaleidae (*e.g.*, Zolessi 1963, Turk & Barrera 1976, Sisler 1977, Whitman 1986, Whitman *et al.* 1992, Stauffer *et al.*, 1998). Romaleidae is a very diverse and representative family of the neotropical region, with large to medium-sized species. Some species appear to have increased in abundance attaining pest status, leading to serious injuries to crops (Kevan 1982). In April 2000, 2 grasshopper species, including *Xyleus discoideus*, caused injuries to orange orchards at Maravilha, a municipal district at the extreme west of the state of Santa Catarina (Brazil) (Campos *et al.* 2001). Further studies of the basic biology of *Xyleus discoideus discoideus* (Serville 1831) are therefore recommended.

A complete revision of the genus was recently published by Carbonell (2004), but apart from general observations on its occurrence and habits (*e.g.*, Hayward 1960, C.O.P.R. 1982, Carbonell *op. cit.*), little is known of the biology of *X. discoideus discoideus*. We studied its postembryonic development under laboratory conditions. This paper documents the number and duration of nymphal instars. In addition I describe the morphology of instar wing pads and external genitalia, along with morphometric and meristic characters.

## Materials and Methods

The descriptions and illustrations of the nymphal instars are based on first-generation laboratory-bred individuals. The adults were collected from December 2002 to January 2003, at the Estação Biológica de Boracéia (Brazil: SP, Salesópolis) [lat 23°39′03″ S, long 45°53′36″ W], an Atlantic Forest reserve owned and sustained by the Museu de Zoologia da Universidade de São Paulo. Adults were caged in a windowless 6 × 4-m laboratory room with an internal temperature and humidity-maintenance system. They were reared at 28±2°C, 85±15% RH and 13:11 light:dark photoperiod. The cages were similar to those described by Smith (1952), being of wooden-frame construction and 80 cm wide × 90 cm deep × 80 cm in height. The sides were covered with nylon mesh, while the floor and roof were closed with wood board. A 20-W fluorescent lamp was attached to the center of the roof of each cage. Plastic trays of about 50 × 40 × 10 cm, filled with moist sand, were placed on the floor of each cage and kept there until oviposition was complete. Immediately on hatching each hopper was isolated in a plastic pot (9 cm diameter, 12.5 cm deep) covered with nylon mesh. They were reared at 28±2°C, 85±15% RH and a 13:11 light:dark photoperiod (as for the adults). Light was supplied through 20-W fluorescent lamps fixed above the pots. Fresh lettuce and chard leaves were provided to the hoppers at least 4 times a week. It was not necessary to provide a separate supply of water, because all the water the nymphs needed was supplied by the fresh leaves (Hinks & Erlandson 1994). The pots were cleaned every time the hoppers were fed.

The following data were recorded for each pot: date of hatch, number of moults and date of hopper death. For each developmental stage the following linear measures were taken: head width, pronotal length, body length and hind femoral length. The following were also recorded: number of antennomeres (flagellum segments only) and longitudinal eye-stripes (Fig. 1). Observations were made on the day after each moult.

Measurements (all in mm) were taken with the aid of a micrometric eyepiece; or a caliper rule was used for larger measures that could not be obtained under the stereomicroscope. The nymphs were chilled for 10 min in a freezer to facilitate measuring. The majority of the hoppers appeared totally recovered 20 min after the freezing procedure; however, approximately 50% of hoppers died during development, so some effect of chilling on survival cannot be ruled out.

Besides the morphometric and meristic data, the development of the wing pads and the external genitalia of males (subgenital plate) and females (ovipositor valves) were also analyzed. For this purpose descriptions and illustrations were made with the aid of a camera lucida from animals preserved in 70% alcohol.

A variance analysis was made to verify if the 4 morphometric variables showed significant differences between males and females for each nymphal instar. For this analysis, ANOVA and Mann-Whitney tests were used as appropriate (following tests of normality and of homogeneity of sample variance) (Zar 1999). A multivariate
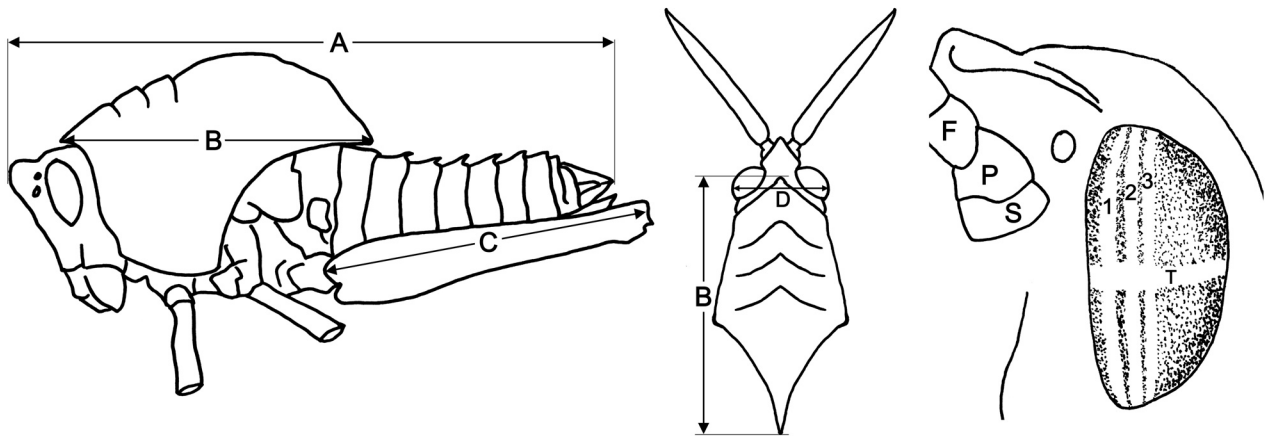
**Fig. 1.** Morphometrical and meristical characters. 1, lateral view of the whole individual (antennae and part of the legs suppressed); 2, dorsal view of the head and pronotum; 3, head detail in lateral view. A, total length; B, pronotum length; C, hind femoral length; D, head width; F, flagellum; P, pedicel; S, scape; T, transversal eye stripe; (1, 2, 3), longitudinal eye stripes.

discriminant analysis (Wilks' Lambda test with 'Forward Selection' strategy) was made to determine the set of variables which allows better discrimination of the instars within each sex than when the variables are employed alone.

## Results

A total of 122 nymphs were hatched and reared (72 males, 50 females). Of these, 36 males and 24 females grew to the adult stage. All the nymphs that attained the adult stage had 5 instars. According to Uvarov (1966), most species in the more basal orthopteran groups (as are Romaleidae) and those with large-size adults, pass through 5 or more instars. This is true for all romaleid species studied so far in regard to postembryonic development. In *Taeniopoda eques* (Burmeister 1838) males and females complete development in 5 instars (Whitman 1986, Whitman *et al.* 1992). Whitman *et al.* (*op. cit.*) have also studied the development of *Romalea microptera* (Beauvois 1805), which completes development in 5 instars (both sexes). *Cromacris speciosa* (Thunberg 1824) and *Coryacris angustipennis* (Bruner 1900) both develop under a 6-instar sequence, males and females (respectively, Turk & Barrera 1976, Zolessi 1963).

There is clear sexual size dimorphism in the adults of *X. discoideus discoideus*, with females being larger. However, females and males show the same number of instars. According to Uvarov (1966), "in many species with notable sexual size dimorphism, the larger female normally has 1 instar more than the male". Apparently, this is not valid for the Romaleidae. All romaleid species studied so far with regard to postembryonic development (see above) show sexual size dimorphism, the male being smaller. In all these species females complete development with the same number of instars as males. Nevertheless, females take more time developing than males (Zolessi 1963, Turk & Barrera 1976, Whitman 1986, Whitman *et al.* 1992). The same occurred with *X. discoideus discoideus*. Females developed in approximately 68 d and males in 56 (Table 1). This difference occurred mainly in the 4th and 5th instars. Females took 16 and 25 d to complete the 4th and 5th instars respectively, compared to 13 and 18 d for males.

The number of antennomeres differed between successive instars (Table 1), but there was no perceptible difference in this character between the sexes.

At the first instar, nymphs of both sexes show a single longitudinal eye stripe and the wing pads are undeveloped. Also, the

dorsal pronotal crest is undeveloped. The posterior margin of the pronotum covers only the mesonotum, the metanotum being free (Fig. 2, 1st). The general coloration of the body is bright green (Fig. 3) and the posterior tibiae have a dark brown spot at their distal edges. A bright brown longitudinal median band marks the body of the hoppers dorsally, from the anterior pronotum edge to the end of the abdomen (posterior margin of the 9th tergite), being

**Table 1.** Number of antennomeres and duration of nymphal instars for *X. discoideus discoideus* males and females.

| MALES | | | | | | |
|---|---|---|---|---|---|---|
| | Instar | 1 | 2 | 3 | 4 | 5 |
| Number of antenna articles | Mean | 11.2 | 13.4 | 14.6 | 17.3 | 19.4 |
| | Standard deviation | 0.5 | 0.7 | 1 | 1.1 | 0.9 |
| | Sample size | 40 | 34 | 29 | 30 | 30 |
| | Maximum | 12 | 14 | 17 | 19 | 21 |
| | Minimum | 10 | 11 | 13 | 15 | 17 |
| Length of each instar (days) | Mean | 8.2 | 7.5 | 10 | 13 | 17.9 |
| | Standard deviation | 1.7 | 1.5 | 2.4 | 3.6 | 3.2 |
| | Sample size | 72 | 55 | 41 | 37 | 34 |
| | Maximum | 13 | 13 | 16 | 23 | 26 |
| | Minimum | 6 | 5 | 7 | 8 | 12 |
| FEMALES | | | | | | |
| | Instar | 1 | 2 | 3 | 4 | 5 |
| Number of antenna articles | Mean | 11.1 | 13.6 | 15.2 | 18.2 | 19.6 |
| | Standard deviation | 0.3 | 0.6 | 0.8 | 0.9 | 1 |
| | Sample size | 31 | 30 | 32 | 33 | 24 |
| | Maximum | 12 | 14 | 17 | 20 | 21 |
| | Minimum | 11 | 12 | 14 | 16 | 17 |
| Length of each instar (days) | Mean | 8.8 | 8.1 | 10.5 | 15.7 | 24.8 |
| | Standard deviation | 2 | 1.2 | 1.9 | 2.7 | 6.1 |
| | Sample size | 49 | 37 | 32 | 29 | 24 |
| | Maximum | 14 | 11 | 16 | 22 | 41 |
| | Minimum | 7 | 6 | 7 | 11 | 16 |
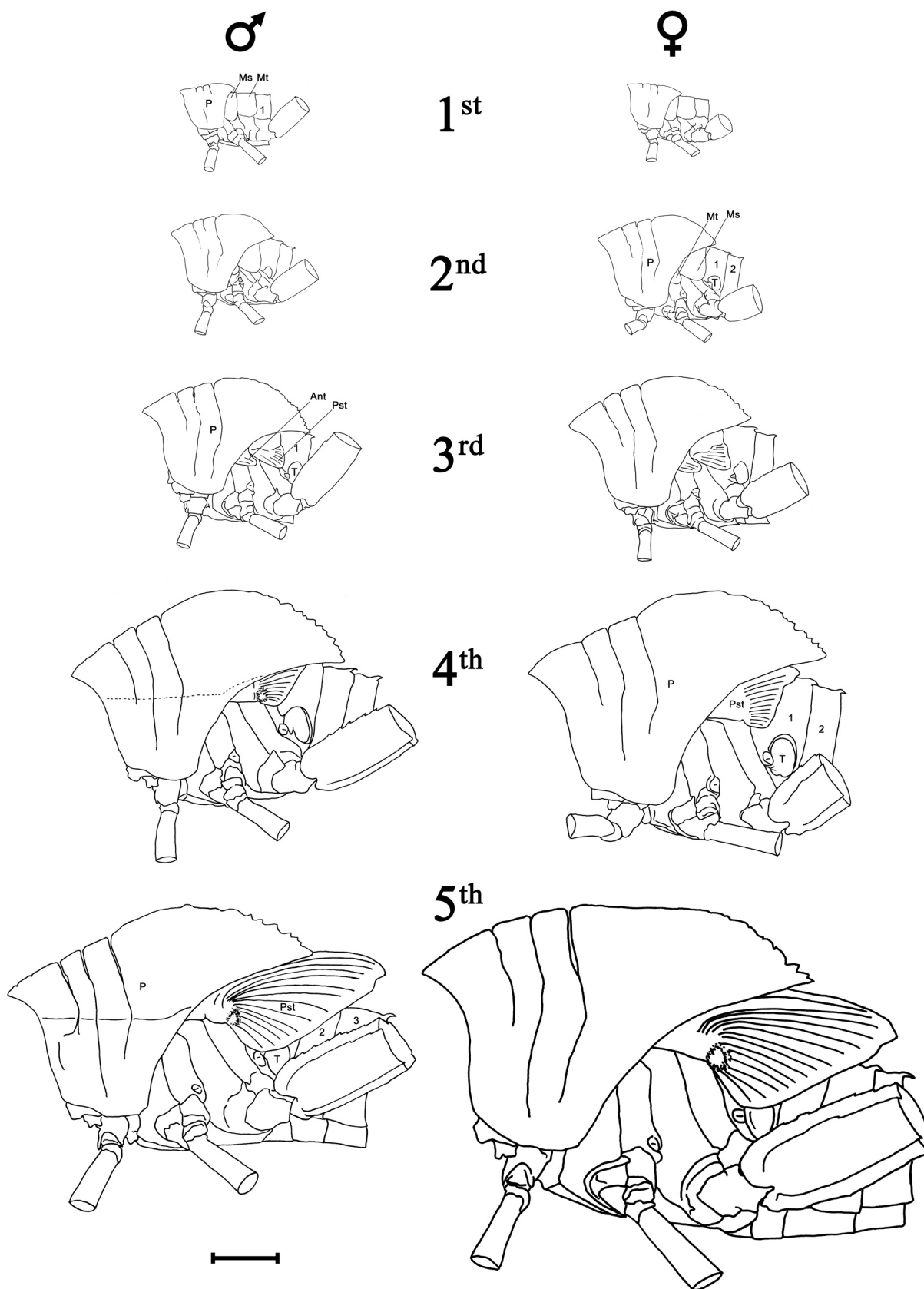
**Fig. 2**. Lateral view of male and female *X. discoideus discoideus* thorax (part of the legs suppressed). 1st-5th, first to fifth instar; (1, 2, 3), first, second and third abdominal segments; Ant, anterior wing pad; Pst, posterior wing pad; Ms, mesonotum; Mt, metanotum; P, pronotum; T, tympanum. Scale line represents 2.5 mm and applies throughout.

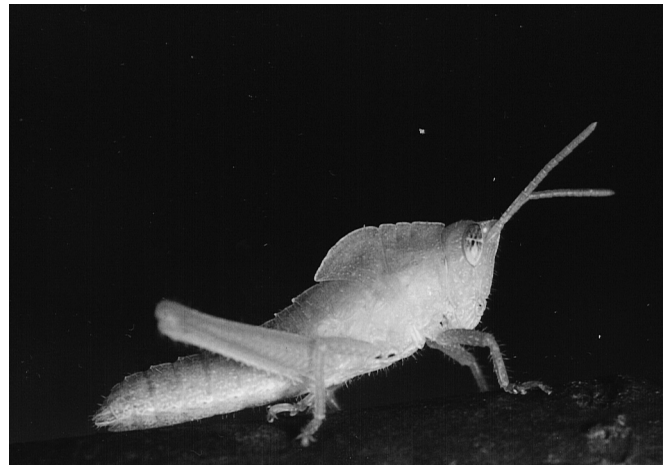**Fig. 3.** *X. discoideus discoideus* 1st instar male nymph.



**Fig. 4.** *X. discoideus discoideus* 2nd instar male nymph.



**Fig. 5.** *X. discoideus discoideus* 3rd instar male nymph.



**Fig. 6.** *X. discoideus discoideus* 4th instar male nymph.



**Fig. 7**. *X. discoideus discoideus* 5th instar male nymph.

approximately 1/5 the width of the dorsum.

Second instar hoppers have 2 longitudinal eye stripes. The wing pads begin to differentiate. An expansion of the posterior ventral margins of the meso- and metanotum is noticeable. The pronotum already has a small crest that projects over all of the metanotum, reaching half the length of the first abdominal segment (Fig. 2, 2nd). The nymphs have basically the body coloration of the previous instar (Fig. 4), but some exhibit more yellowish or brown tones. In most individuals the spots in the posterior tibiae are no longer present.

If present, they are clearer and relatively smaller than those found in the first instar. The bright brown longitudinal dorsal band can still be present.

In the 3rd instar the nymphs have 3 longitudinal eye stripes. The wing pads are already developed; however it is not possible to find clear differences between males and females. The same is true for the pronotum. The pronotum is longer, covers practically all the first abdominal segment, and has a very evident crest (Fig. 2, 3rd). Body coloration begins to diversify: some nymphs keep the bright green coloration (Fig. 5) and there are others with a paler, more yellowish or grayish-brown coloration. This latter is similar to the adults' coloration. The bright brown longitudinal dorsal band is not present anymore.

The 4th instar nymphs possess 4 longitudinal eye stripes. In both males and females reversal of the wing pads occurs, so that the costal margin assumes a dorsal position and the hind wing pads are placed outside the forewing pads. The course of alar development in this species follows the rule stipulated by Uvarov (1966) that "the reversal precedes the final molt by 2 instars". In all the romaleid species studied with regard to postembryonic development (see above), the reversal occurs at the molt preceding the penultimate instar. This seems to be a valid rule for the Romeleidae in general.

The posterior edge of the posterior wing pads of 4th instar *X. discoideus discoideus* nymphs, reaches to a transverse line between the posterior edges of the tympana. These are not yet completely covered. The pronotum is already well developed, with a noticeable

**Table 2.** Morphometric values for the nymphal instars of *X. discoideus discoideus* males and females.

| MALES | | | | | | |
|---|---|---|---|---|---|---|
| | Instar | 1 | 2 | 3 | 4 | 5 |
| Head width (mm) | Mean | 1.69 | 2.17 | 2.73 | 3.39 | 4.28 |
| | Standard deviation | 0.05 | 0.1 | 0.06 | 0.13 | 0.85 |
| | Sample size | 40 | 34 | 29 | 30 | 30 |
| | Maximum | 1.78 | 2.64 | 2.84 | 3.68 | 8.7 |
| | Minimum | 1.54 | 2.02 | 2.63 | 3.16 | 3.84 |
| Pronotal length (mm) | Mean | 2.31 | 4.52 | 7.79 | 11.03 | 13.19 |
| | Standard deviation | 0.11 | 0.32 | 0.37 | 1.17 | 1.05 |
| | Sample size | 40 | 34 | 29 | 30 | 30 |
| | Maximum | 2.52 | 4.97 | 8.61 | 12.39 | 14.56 |
| | Minimum | 2.04 | 3.72 | 6.89 | 5.66 | 8.86 |
| Body length (mm) | Mean | 9.44 | 12.63 | 16.52 | 21.68 | 28.23 |
| | Standard deviation | 0.8 | 1.25 | 0.82 | 1.02 | 1.38 |
| | Sample size | 40 | 33 | 29 | 30 | 30 |
| | Maximum | 11.3 | 14.86 | 18.88 | 23.6 | 30.4 |
| | Minimum | 8.04 | 10 | 15.2 | 19.3 | 25 |
| Hind femur length (mm) | Mean | 5.13 | 6.53 | 8.68 | 11.62 | 15.26 |
| | Standard deviation | 0.34 | 0.62 | 0.27 | 0.47 | 0.45 |
| | Sample size | 40 | 34 | 29 | 30 | 30 |
| | Maximum | 5.9 | 7.42 | 9.1 | 12.28 | 16.16 |
| | Minimum | 4.67 | 3.71 | 7.95 | 10.56 | 14.4 |
| FEMALES | | | | | | |
| | Instar | 1 | 2 | 3 | 4 | 5 |
| Head width (mm) | Mean | 1.72 | 2.18 | 2.94 | 3.74 | 4.77 |
| | Standard deviation | 0.04 | 0.38 | 0.09 | 0.16 | 0.26 |
| | Sample size | 31 | 30 | 32 | 33 | 25 |
| | Maximum | 1.81 | 2.41 | 3.12 | 4.1 | 5.25 |
| | Minimum | 1.61 | 0.23 | 2.72 | 3.28 | 4.28 |
| Pronotal length (mm) | Mean | 2.33 | 4.73 | 8.54 | 12.82 | 16.14 |
| | Standard deviation | 0.13 | 0.23 | 0.44 | 0.96 | 1.23 |
| | Sample size | 31 | 30 | 32 | 33 | 25 |
| | Maximum | 2.6 | 5.08 | 9.92 | 15.52 | 18.56 |
| | Minimum | 2.08 | 4.24 | 7.3 | 10 | 14.4 |
| Body length (mm) | Mean | 9.34 | 13.51 | 18.5 | 24.25 | 32.92 |
| | Standard deviation | 0.92 | 1.24 | 1.23 | 2.02 | 1.99 |
| | Sample size | 31 | 30 | 32 | 33 | 25 |
| | Maximum | 11.52 | 17 | 22.4 | 28.2 | 37.1 |
| | Minimum | 6.25 | 11.41 | 15.84 | 18.88 | 29 |
| Hind femur length (mm) | Mean | 5.06 | 7.08 | 9.45 | 13.05 | 18.32 |
| | Standard deviation | 0.34 | 1.34 | 0.52 | 0.77 | 0.94 |
| | Sample size | 31 | 30 | 32 | 33 | 24 |
| | Maximum | 5.74 | 14 | 10.4 | 14.24 | 21 |
| | Minimum | 4.26 | 5.9 | 7.62 | 9.67 | 16.8 |

serrated posterior edge. At least one half of the 2nd abdominal segment is dorsally covered by the pronotum (Fig. 2, 4th). The nymphs present a variety of colorations and clear spots may be present on the sides of the pronotum (Fig. 6).

In the 5th instar, the last before the hoppers become adults, they have 5 longitudinal eye stripes. The reversed wing pads are already well developed and cover the first 3 abdominal segments and part of the tympana. The pronotum, with deep ridges and particularly evident segments, is much larger, but presents few differences in comparison with the previous instar, partially covering the 2nd abdominal segment (Fig. 2, 5th). The hoppers show a great diversity of color (Figs 7, 8), with scattered lateral spots on the pronotum, metanotum and abdomen.

*External genitalia morphology.*— (Fig. 9) Males and females can be distinguished from the 1st to the 5th instar, based on the morphol-

ogy of the external genitalia. First-instar males have the subgenital plate as an indented expansion of the 9th sternite, reaching the base of the paraproct. At the 2nd instar the subgenital plate reaches approximately to the middle of the paraprocts. It is narrowed and still presents an indented apex. At the 3rd instar, the subgenital apex is no longer indented. It reaches almost to the apex of the paraprocts. There is a clear subdivision between the 9th sternite and the subgenital plate. The 4th-instar subgenital plate extends until the apex of the paraprocts, completely covering them. At the 5th instar the apex of the subgenital plate surpasses the paraprocts, the plate resembling that of the adults.

First-instar females have the anterior valves of the ovipositor as a slender and small plate, located between the 8th and 9th sternite, with a slight median re-entrance on its posterior border. The posterior valves are represented by 2 small triangular expansions of the 9th sternite. They do not reach the middle of the paraprocts.

At the 2nd instar, the anterior valves are projected as small triangles until reaching the base of the posterior ones. The anterior valves are contiguous in their median portion, but not at the base and apex. Posterior valves are longer, but do not surpass the middle of the paraprocts. The internal valves are already recognizable.

At the 3rd instar, anterior valves are in contact from their base to their apex. They surpass the base of the posterior valves. Posterior valves are longer and acute, reaching the middle of the paraprocts. The internal valves can be recognized between the anterior and posterior ones.

Fourth-instar anterior valves are sharpened and longer, completely covering the internal valves, and partially covering the posterior ones. Posterior valves are longer, surpassing the middle of the paraprocts. The egg guide can be recognized at the center of the posterior border of the 8th sternite.

At the 5th nymphal instar, the ovipositor is very similar to that of the adults. Anterior valves, amply sclerotised and with many folds,

extend approximately until the apex of the paraprocts. Posterior valves, also well sclerotised, surpass the paraprocts. The egg guide is completely developed as a finger-like median process of the posterior border of the 8th sternite.

*Morphometry.*—Averages of the morphometric variables are presented in Table 2 and the results of the variance analysis in Table 3. In Tables 4 and 5 are the results of the discriminant multivariate analysis for males and females respectively.

For the variance analysis, all 4 morphometric variables (head width, pronotum length, body length and hind femoral length) present a significant difference ($p < 0.05$) between males and females from the 2nd to the 5th instar. In the 1st instar, only head width results in a significant difference between the sexes. In the comparison of body length of males and females at the 4th instar, a rejection of homogeneity between the analyzed values occurred. Therefore, a Mann-Whitney test was applied (Table 3), and the result was a significant sex-based difference in body length.

The multivariate analysis indicates that the best way to discriminate the instars of males is to employ all 4 morphometric variables in a set (pronotum length + femur length + body length + head width) (Table 4.). For the females the analysis shows that the combination that best discriminates the instars is pronotum length + head width + femur length (Table 5).

## Discussion and conclusions

Almost all characters assessed were useful in characterizing the instar and/or the sex of *X. discoideus discoideus* nymphs.

The 'longitudinal eye stripes' provide a rather practical and reliable instar-characterizing parameter, since on each nymphal moult a new eye stripe is added. As the stripes are, in most cases, easily visualized with the aid of a stereomicroscope, they may be the
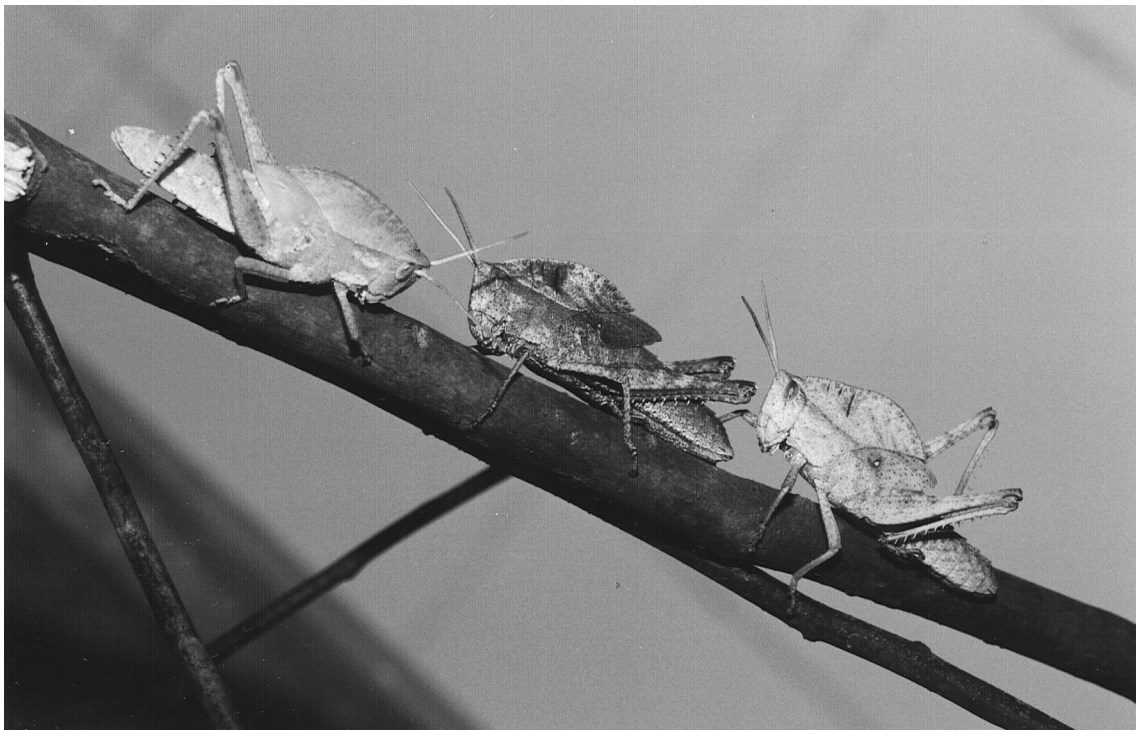


**Fig. 8.** Three *X. discoideus discoideus* 5th instar female nymphs bred under the same alimentary, temperature, humidity and photoperiod conditions, showing a great variation in body coloration.

**Table 3**. Results of ANOVA and Mann-Whitney tests for analysis of the morphometric variables in the nymphal instars between *Xyleus discoideus discoideus* males and females.

| Instar | Morphometrical variable | ANOVA | | Mann-Whitney | | n | |
|--------|------------------------|-------|-------|------|---------|-------|---------|
| | | F | p | U | p | males | females |
| 1 | Head width | 7.419 | 0.008 | | | 40 | 31 |
| | Pronotal length | 0.741 | 0.392 | | | 40 | 31 |
| | Body length | 0.197 | 0.658 | | | 40 | 31 |
| | Hind femur length | 0.575 | 0.451 | | | 40 | 31 |
| 2 | Head width | 13.231 | 0.001 | | | 34 | 30 |
| | Pronotal length | 9.023 | 0.004 | | | 34 | 30 |
| | Body length | 8.018 | 0.006 | | | 33 | 30 |
| | Hind femur length | 5.582 | 0.021 | | | 34 | 30 |
| 3 | Head width | 114.079 | < 0.001 | | | 29 | 32 |
| | Pronotal length | 52.367 | < 0.001 | | | 29 | 32 |
| | Body length | 55.801 | < 0.001 | | | 29 | 32 |
| | Hind femur length | 48.809 | < 0.001 | | | 29 | 32 |
| 4 | Head width | 88.904 | < 0.001 | | | 30 | 33 |
| | Pronotal length | 30.994 | < 0.001 | | | 30 | 33 |
| | Body length | - | - | 97 | < 0.001 | 30 | 33 |
| | Hind femur length | 68.886 | < 0.001 | | | 30 | 33 |
| 5 | Head width | 15.302 | < 0.001 | | | 30 | 25 |
| | Pronotal length | 79.525 | < 0.001 | | | 30 | 25 |
| | Body length | 100.64 | < 0.001 | | | 30 | 25 |
| | Hind femur length | 283.285 | < 0.001 | | | 30 | 24 |

most practical way to quickly assess in which instar the specimen is. However, in some cases their visualization may be difficult, and after the insect´s death the eyes quickly become dark: dried preserved specimens do not retain the stripes. When the grasshoppers are kept in 70% alcohol, the eye stripes are lost gradually. Becker & Ferreira (1995) examined 133 late-instar nymphs (5th, 6th and 7th) of *Rhammatocerus conspersus* (Bruner 1904), kept for 2 y in 70% alcohol, and only 58% exhibited conspicuous stripes; in another instance 57 early-instar nymphs were analyzed: none retained the stripes after 2 y.

The 'wing pads' and 'external genitalia morphology', when analyzed together, supplied rather safe data with which to determine the sex and instar of the hopper. The external genitalia morphology shows clear differences between males and females as early as the first nymphal instar.

'General color of the body' does not supply very accurate data for either sex or instar recognition, due to the variety of colors from the second instar on, and to the absence of striking color differences between males and females and among sucessive instars.

Even though body length showed significant differences between males and females from the 2nd instar on, it is not a very reliable parameter, since during each instar there is an increase in body length due to the expansion of the intersegmental membranes. This can introduce experimental error unless the precaution is taken of always measuring the hoppers the day after each moult. More sclerotised parts of the body (pronotum, femur, head) do not present such growth (Uvarov 1966).

'Head width' was the most consistent morphometric character separating male and female instars: only this character showed a significant difference between males and females for each of the 5 nymphal instars. However, all 4 morphometric variables are very important in the characterization of the instars: when analyzed in a set they readily separate each one of the 5 male nymphal instars; and when all 3 variables, except body length, are analysed in a set, it is possible to readily discriminate all 5 of the female nymphal instars as well.

It is important to point out that all the analyzed individuals in this experiment were bred under artificial and controlled condi-

**Table 4.** Discriminant multvariate analysis results for comparision of morphometric variables between nymphal instars of *X. discoideus discoideus* males. Number of variables in model: 4; grouping: 5 instars (males). Wilks' Lambda: 0.002. $F(16, 465) = 169.85$, $p < 0.0001$.

| | Wilks' Lambda | F | p |
|--------|---------------|-------|-----|
| | | (4, 152) | |
| Head width | 0.003 | 7.569 | < 0.001 |
| Pronotal length | 0.013 | 140.514 | < 0.001 |
| Body length | 0.003 | 8.119 | < 0.001 |
| Hind femur length | 0.004 | 21.384 | < 0.001 |

**Table 5.** Discriminant multvariate analysis results for comparision of morphometric variables between nymphal instars of *X. discoideus discoideus* females. Number of variables in model: 4; grouping: 5 instars (females). Wilks' Lambda: 0.00102. $F(16, 428) = 228.65$, $p < 0.0001$.

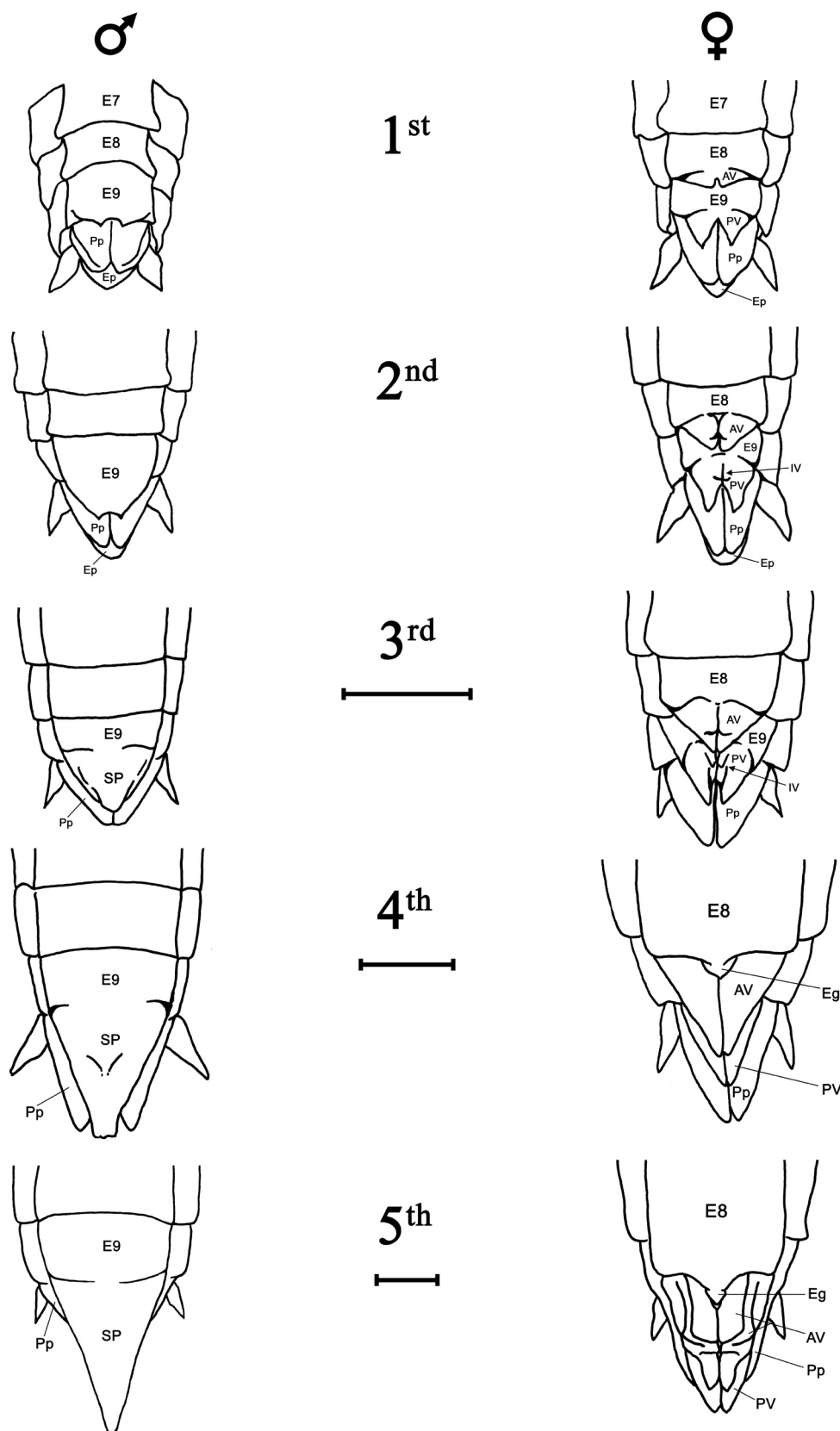| | Wilks' Lambda | F | p |
|--------|---------------|-------|-----|
| | | (4, 140) | |
| Head width | 0.002 | 48.289 | < 0.001 |
| Pronotal length | 0.009 | 276.297 | < 0.001 |
| Body length | 0.001 | 2.176 | < 0.075 |
| Hind femur length | 0.001 | 8.524 | < 0.001 |

**Fig. 9.** Ventral view of the terminal segments of male and female *X. discoideus discoideus* abdomen. 1st-5th, first to fifth instar; AV, anterior valves; Eg, egg-guide; Ep, epiproct; IV, internal valves; Pp, paraprocts; PV, posterior valves; S, sternite; SP, subgenital plate. All scale lines represent 1.0 mm; that on the third line refers to the first, second and third instars; that on the fourth line refers to the fourth instar; that on the fifth line refers to the fifth instar.

tions and more studies are necessary to obtain comparable results for specimens under natural conditions.

## Acknowledgments

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