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Morphology and Biology of *Porphyrosela minuta* Clarke 1953 (Lepidoptera: Gracillariidae, Lithocolletinae) in Uruguay

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Morfología y Biología de *Porphyrosela minuta* Clarke 1953 (Lepidoptera: Gracillariidae, Lithocolletinae) en Uruguay

RESUMEN - *Porphyrosela minuta* Clarke es un gracilárido que causa daños sobre trébol blanco (*Trifolium repens*) en Uruguay. En este artículo, son descriptos huevo, todos los instares larvarios y pupa. Características morfológicas externas del adulto son igualmente incluidas. Se aporta información sobre la biología en condiciones de laboratorio y campo. Los huevos son depositados simples sobre la epidermis superior de los foliolos. Las larvas forman grandes minas a modo de manchas blancas. La larva pasa por cinco estadios sin permanecer expuesta durante su desarrollo. A 25ºC el ciclo de vida requirió de 16,2 días en completarse, correspondiendo 3,0 a huevos, 8,8 a larva y 4,4 a pupa. Las hembras iniciaron la puesta la primera noche y durante un periodo de oviposición que osciló entre cinco y 12 días depositaron en promedio 71,8 huevos. Es una especie multivoltina que desarrolla de siete a nueve generaciones de noviembre a fines de marzo.

PALABRAS-CLAVE: Insecta, minador de hojas, descripción, desarrollo, reproducción

ABSTRACT - *Porphyrosela minuta* Clarke is a gracillariid that causes damage on white clover (*Trifolium repens*) in Uruguay. In this article, the eggs, all the larval instars, the pupa and the external morphological characteristics of the adult are described. Information about the insect's biology under laboratory and field conditions is presented. The eggs are laid singly on the upper surface of the leaflets. The larvae make extensive mines that appear as white blotches. The larvae develop through five instars and do not remain exposed during their development. At 25ºC the life cycle lasted 16.2 days, made up of 3.0 for eggs, 8.8 for larvae and 4.4 for pupae. Females began laying on the first night, and during an oviposition period that varied between five and 12 days deposited an average of 71.8 eggs. It is a multivoltine species and seven to nine generations occur between November and the end of March.

KEY WORDS: Insect, leaf-miner, description, development, reproduction

The Gracillariids constitute the principal family of plant-mining Lepidoptera (Davis 1987). A list of the neo-tropical species was published by Davis & Miller (1984). Within this group, the sub-family Lithocolletinae is represented in Uruguay by one species known as *Porphyrosela minuta* Clarke (Biezanko *et al*. 1957, 1978). According to Holloway *et al.* (1987), most of the members of this sub-family occur in the Holarctic region, and there are comparatively few south of the tropics. Although the adults of this species are almost unknown because of their very small size and their habits, the larvae are among the insects most commonly found on white clover (*Trifolium repens*) in spring and summer. The larval damage consists of conspicuous white mines on the upper surface of leaves. Like other gracillariids, the larvae appear in two distinct morphological forms. In the early instars the larva is flat, legless, with mouthparts adapted to lacerate cells and ingest the liquid content. In the later instars the body is cylindrical with developed legs and prolegs, a round head and chewing mouthparts. According to Holloway *et al.* (1987) most Lithocolletinae species are host-specific or live on closely related plant species, and host-plant data can be an aid to identification.

P. minuta is widely distributed in the south of Uruguay. Although it is a common species and sometimes relatively abundant, the damage it causes is not important.

This species is not well studied. Clarke (1953) described the adult from the province of Buenos Aires, Argentina. Bourquin (1953) called it *Porphyrosela* sp., and briefly described its stages of development and mentioned some aspects of its biology. Apart from these early studies no other relevant information is known. According to Clarke (1953), it is very similar to *Porphyrosela desmodiella* (Clemens 1859) in North America.

This study was carried out because little is known about

this insect or its habitual presence in our environment. The insect's stages of development are described, and information about its biology is present under laboratory conditions and in the field.

Materials and Methods

The observations were made from 2004 to 2006 in parks and rural areas near the city of Montevideo. Follow up in the field was done through fortnightly observations in the ground cover in the areas where the studies was made. Damaged leaves were collected and kept in the laboratory with the petioles immersed in water (see below). In order to study the morphology and determine the number of instars, larvae of different ages were collected and fixed in a solution of nine parts ethyl alcohol (80%) and one of glacial acetic acid for 48h, and then transferred to ethyl alcohol (80%) and glycerine (Holloway *et al*. 1987). Some specimens were treated with KOH (10%) and then prepared on laminas, in line with the procedure proposed by Stehr (1987) for small lepidopteran larvae. The pupae were fixed in ethyl alcohol (80%). For the setal nomenclature of the larvae we followed the system established by Hinton (1946). The damage was studied by fixing white clover leaflets with mines in FAA, and then cutting them with microtome and coloring them with Toluidine blue.

Laboratory rearing started with damaged white clover leaves. These were collected in spring and placed in test tubes (100 mm long, 15 mm diameter) with the petioles immersed in water. Once larval development was complete, the pupae were extracted and placed on absorbent paper in plastic receptacles (55 mm diameter, 14 mm deep). When the adults emerged we formed pairs, and these were placed in glass receptacles (180 mm long, 100 mm diameter) with the upper and lower ends closed with petri dishes. Because this species is so small, the adults were sexed by observation of the position of the genital aperture in the remains of the pupal exuviae through a light microscope. The adults were fed on honey. As a substrate for oviposition, three white clover leaves were used, whose petioles were immersed in water in a small receptacle. The leaves were taken out every day so the number of eggs deposited could be counted, and these were placed individually in test tubes containing water. They were observed daily to measure the duration of embryo and larval development. The duration of the larval development was measured from the time each larva emerged to the day it pupated. The data were obtained from the first generation reared in the laboratory.

Rearing was carried out at the temperature of 25 ± 1ºC, with humidity at 70 ± 10% and a photoperiod of 16h of light. To maintain relative humidity closed glass boxes were used, which contained a saturated solution of sodium chloride (Winston & Bates 1960). The measurements were made with an ocular micrometer and averages are expressed as the mean ± standard errors. The duration of the different stages, the fertility of the females and the longevity of the adults are also expressed as the average of the observations ± standard error.

Results and Discussion

Description

Egg. Flat, oval, with membranous corion and finely ornamented with small holes. When it is found on the surface of the plant it is greenish white with glassy-iridescent corion. Shortly before emergence the body of the already-formed larva can be seen, with the cephalic capsule easily visible. After emergence the corion shrinks to a small brilliant white lamina from which the mine begins. Length: 0.31 ± 0.0025 mm, width: 0.22 ± 0.0009 mm (n = 21).

Larva. *First instar* (Fig. 1A, 1B). Cephalic capsule width: 0.12 ± 0.0016 mm (n = 31). Body length: 0.46 ± 0.0041 mm (n = 24). Body semi transparent and slightly yellow. Cephalic capsule wider than long, and markedly flat at its front end so it has a wedge-shaped lateral profile. Stemmata absent. Labrum without a pronounced medium opening. Mandibles flat with a conspicuous tooth at the distal end. Thoracic segments, particularly the prothorax and mesothorax, wider than the head. Thoracic legs absent. Abdomen with 10 segments; the last narrower than the others. Prolegs absent. Setae absent or inconspicuous.

Second instar. Cephalic capsule width: 0.17 ± 0.0015 mm (n = 47). Body length: 0.98 ± 0.0163 mm (n = 54). Head similar to the first instar, with the labrum clearly visible and slightly bilobed. Prothoracic shield wide, rectangular, slightly thickened and dark brown. Second and third thoracic

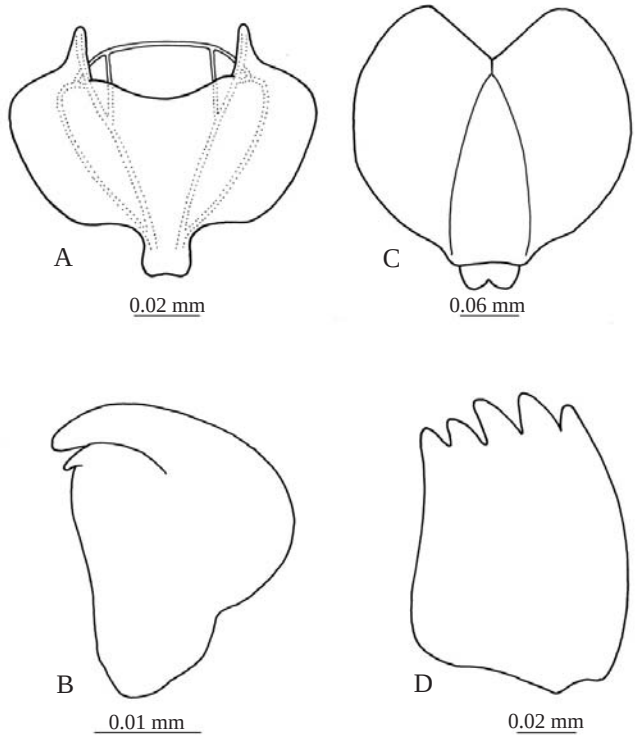

Fig. 1. *P. minuta.* A, cephalic capsule in the first instar; B, mandible in the first instar; C, cephalic capsule in the fourth instar; D, mandible in the fourth instar.

segments with a dark brown transverse dorsal stripe. The first seven abdominal segments with a dark brown transverse dorsal stripe, similar to those found on the thorax.

Third instar. Cephalic capsule width: 0.24 ± 0.0023 mm (n = 64). Body length: 2.08 ± 0.0227 mm (n = 28). Thoracic and abdominal segments (except on the dorsal side of the prothorax) with a dark brown transverse stripe in the dorsal and ventral regions. The ventral bands smaller and less pronounced than the dorsal ones.

Fourth instar (Fig. 1C, 1D). Cephalic capsule width: 0.30 ± 0.0031 mm (n = 25). Body length: 2.65 ± 0.0737 mm (n = 34). The cephalic capsule is translucent and light brown. Body greenish yellow and the prothoracic shield light brown. Cephalic capsule round with the frontoclypeus lengthened, short epicranial suture and three well-differentiated stemmata present on each side. Labrum bilobed, the spinneret developed and the labial palpi well differentiated. Mandibles of chewing type with five teeth; maxillae present. Thoracic legs developed with a robust, curved tarsal nail. Prolegs present on the third to the fifth and the last segments. Biordinal crochets in a circle on the first three pairs and uniordinal in a band on the posterior pair. In most specimens the dorsal and ventral stripes on the thoracic and abdominal segments disappear. Spiracles circular and not evident.

Fifth instar (Figs. 2, 3). Cephalic capsule width: 0.35 ± 0.0024 mm (n = 30). Body length: 3.52 ± 0.0401 mm (n = 14). Similar to the fourth instar. Chaetotaxy. Thorax. SD1 developed and SD2 absent on segment 1; two SD setae present on segments 2 and 3; XD2 very near SD1; group L bisetose in the three segments; group SV absent. Abdomen. Group D bisetose in the first eight segments; SD1 absent and SD2 developed; group L unisetose on segments 1 to 9;

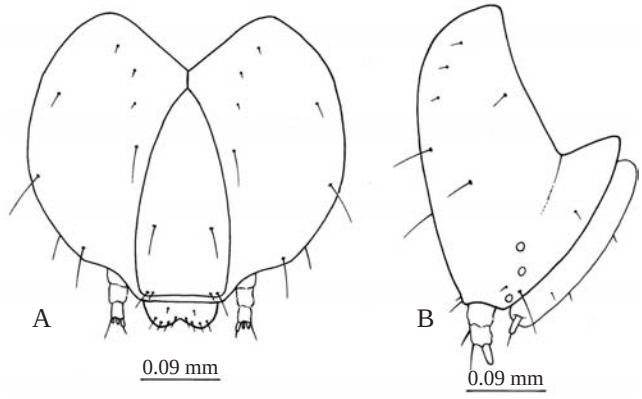

Fig. 2. *P. minuta*, cephalic capsule in the fifth instar. A, frontal view; B, lateral view.

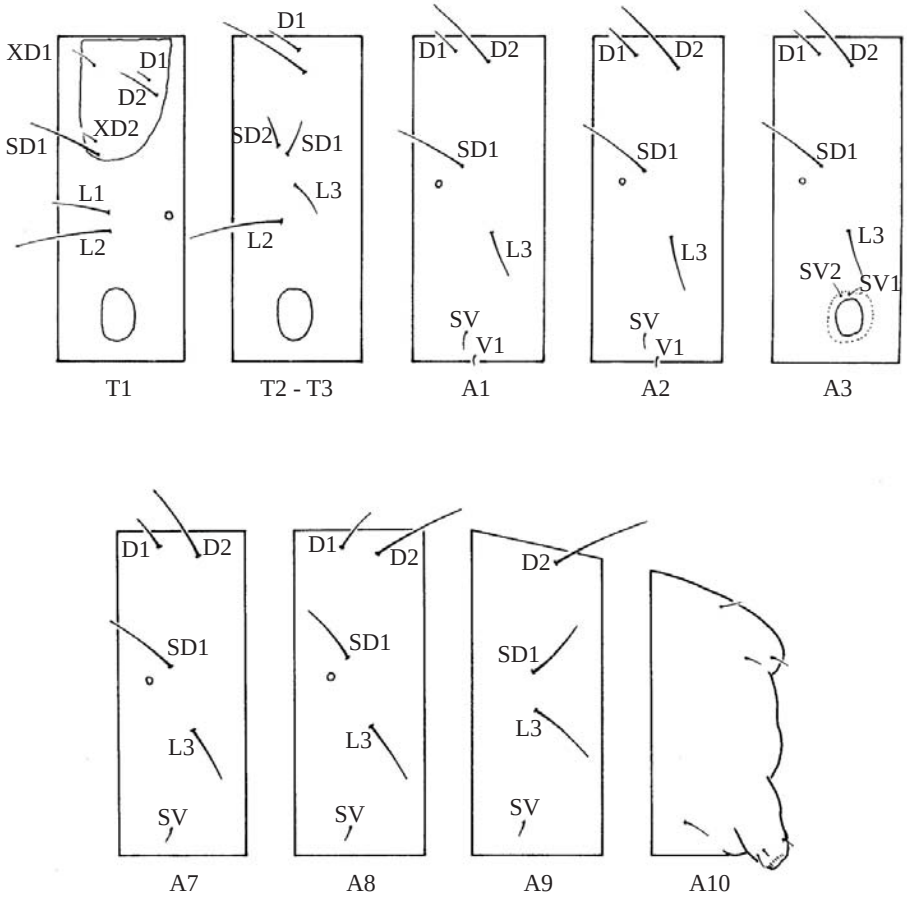

Fig. 3. *P. minuta.* Setal map of the thorax and abdomen.

group SV unisetose on all segments except the third to the fifth where it is bisetose.

The main characteristics of the chaetotaxy of the larva are that SD2 are absent from the prothorax and the SV group on the three thoracic segments. According to Davis (1987), in gracillariids the SV group is bisetose on the first thoracic segment and uni- or bisetose on the other two. Likewise, other authors show the presence of these setae on the thorax in illustrations (Peterson 1948, Mackay 1972). However, on the 40 *P. minuta* larvae studied none showed setae belonging to this group, which seems to be a constant characteristic of the species.

Pupa (Fig. 4)**.** Thin and sub-cylindrical. Brown, with a lighter colored abdomen and a slightly darker cephalic process. The general coloring of the pupa becomes darker close to the adult emergence. Maximum length of the female: 2.60 ± 0.0134 mm (n = 20), and of the male: 2.26 ± 0.0141 mm (n = 18). Cephalic process well developed, like robust spines, and ventrally arched. Apex of the antennae extended beyond the apex of the wings. Apexes of the antennae and of the metathoracic feet coincide at the same height. Distance between the apexes of the mesothoracic and metathoracic legs approximately one and a half times the distance between the apexes of the prothoracic and mesothoracic feet. Abdominal segments with abundant spinulas; rounded spiracles. Cremaster reduced, consists of four little hooks, two ventral and two dorsal, the latter above the former. The sexes can be distinguished by the disposition of the genital opening.

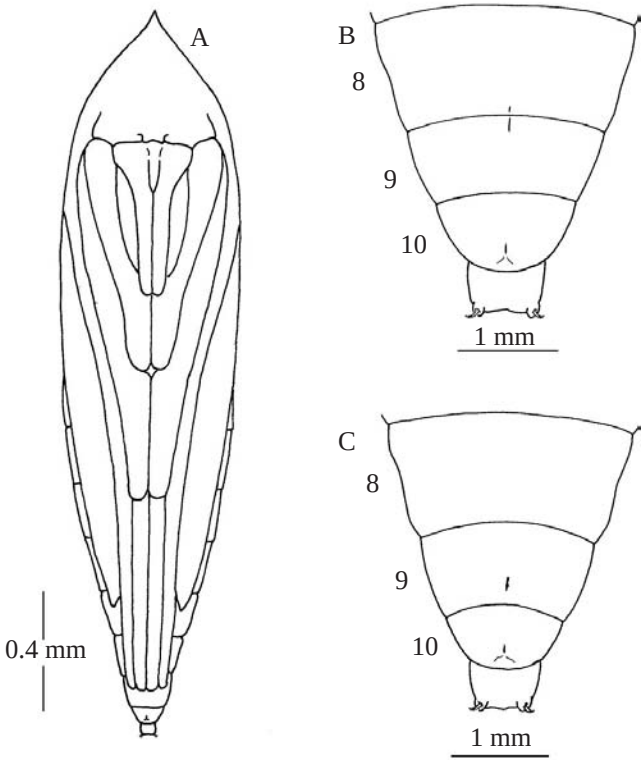

Fig. 4. Pupa of *P. minuta*. A, ventral view; B, terminal abdomen segments of the female; C, terminal abdomen segments of the male.

Adult. Wing expanse: 4 mm to 6 mm. Maximum length: 2.2 mm to 2.7 mm. Head with erect brown hair-scales on the vertex; forehead with silvery white flat scales. Eyes developed, hemispherical and dark. Antennae slightly shorter than the front wings, black and with the last eight or nine segments white. Proboscis developed and without scales. Labial palpi developed, with three segments, black, and with the third segment pointed; diminutive maxillary palpi. Thorax with silvery white flat scales on the dorsal side, tegula with the same coloring. Lanceolate fore wings 1.9 mm to 2.2 mm in length. With orange brown metallic highlights. The first third present an oblique silvery white transverse band with a line of black scales on both sides. There is another similar band running obliquely in the opposite direction in the centre of the wing. The two bands are farther apart on the anterior margin than on the inner margin. Near the apex of the wing there are two silvery white patches with black scales at the edges, one on the anterior margin and the other, which is not so apical, on the inner margin. Hind wings lanceolate, very narrow, and 1.5 mm to 1.7 mm in length. Covered with elongated brown scales and provided with a fringe of long hair all around the edges. Long legs, mainly metathoracic legs. Dark coloring, coxae, trochanters and femurs with silver highlights, and the rest with copper highlights. In many specimens the distal ends of the metathoracic tibia are silvery. Tibial spurs: 0-2-4. Abdomen with copper highlights, and the fourth, fifth and seventh ventral segments silvery white.

Biology

Egg. The eggs are adhering tightly to the upper surface of the white clover leaves. They are laid singly, they are never found together or very near each other, and nor are they found on the underside of the leaf or elsewhere on the plant. The number of mines per leaflet varies. Of 149 white clover leaves with incipient damage collected in the spring of 2005, 60.9% of the leaflets had mines. The number of incipient mines per leaflet varied from one to four, with an average of 1.5. There was one exceptional leaflet on which seven incipient mines were found.

Under controlled conditions in the laboratory the duration of development varied between three and four days, with a mean of 3.0 ± 0.01 (n = 334). Bourquin (1953) reported that this stage did not exceed four days.

Larva. The larvae of many gracillariids remain exposed in the later instars of their development, when they acquire the typical caterpillar shape with chewing mouthparts (Réal & Balachowsky 1966, Davis 1987). *P. minuta* larvae, on the other hand, complete their development inside the mine. They feed on sap during the first three instars, and ingest solid particles in the last two. In order to emerge, the larvae perforate the egg on the surface that is in contact with the vegetal tissue and penetrate directly into it. At first the mine is tiny, but after a few days it takes the form of an irregular white blotch that is approximately 7 mm to 9 mm wide and 10 mm to 12 mm long (Fig. 5). This occurs mainly in the first three instars, which is the sap-feeding stage. The larvae progress through the vegetal tissue on the edge of the mine, pressing with the cephalic capsule while the mandibles,

which move horizontally, lacerate the cell walls. In a cross section of leaflets with mines the larvae feed on the palisade parenchyma under the epidermis and in most cases do not reach the spongy parenchyma. During the first three instars larval excrement is liquid and after a time it appears as small dark stains adhering to the vegetal tissue. When there is more than one mine on the same leaflet these usually join up and form a large white blotch that can cover almost the whole surface of the leaflet.

In the later instars the larvae can usually be found in areas that have been mined previously, and they feed by going deeper into the mesophyll, but they spare the veins and also the lower epidermis, which remains as a semi-transparent membrane. Now the damage is visible from the outside, on the underside of the leaflets, appearing as small white blotches in the areas where only the epidermis remains. Solid excrement, which is dark green at first and black later on, accumulates inside the mine.

The duration of larval development seems to show very little variation. Under laboratory conditions larval development in spring lasted from eight to nine days with a mean of 8.8 ± 0.04 (n = 141). Only two larvae in the sample required 10 days to pupate. These data are in line with the findings of Bourquin (1953), who reported larval duration of eight days.

Pupa. The pupa is found inside the mine. At the end of its development the larvae line the mine with silk threads, which causes the surface of the leaflet to curve, usually around the central vein, and the upper epidermis shrinks (Bourquin 1953). In this way a chamber is formed inside the mine and it is here that the pupa is lodged. The pupa remains free inside the mine and does not make a cocoon. It uses the cephalic process to break the epidermis, usually on one of the sides, and it is partially exposed when the adult emerges. Under laboratory conditions this state lasted four to five days, with a mean of 4.4 ± 0.06 (n = 69).

Adult. At rest the adults remain with the body horizontal, supported on the three pairs of legs, the metathoraxes extended below the abdomen, with the antenna pointing backwards resting on the wings and often inclined slightly downwards. In movement the insects walk quickly and their antennae are active. They are nocturnal, but they do exhibit a certain amount of activity during the day. Under laboratory conditions they even feed, and one pair were observed to copulate in the middle of the morning. Females were never observed to lay eggs during the day. In the laboratory, females copulated on the day that they emerge and then begin to oviposit a little later. Only two females out of 11 began laying eggs on the second day. The females lived from six to 14 days

Fig. 5. *P. minuta*. Blotches on upper leaf surface of white clover.

and each deposited between 33 and 113 eggs, with a mean of 71.8 ± 7.84. Oviposition usually took place on consecutive days, and most of the eggs were deposited in the first three or four days. The maximum number of eggs deposited by a female in one day was 27. The oviposition period varied between five and 12 days, with a mean of 7.1 ± 0.74.

Voltinism. *P. minuta* is a multivoltine species that develops continually throughout the year. In winter, populations are considerably reduced because of the climatic conditions. They mainly remain in the larval state, but due to the low temperatures development takes considerably longer. From the middle of November onwards populations show a marked increase. At that time an abundance of incipient mines can be seen. The activity of the insect continues through the summer and populations are relatively high, but they start to decline at the end of March. Specimens in different stages of development can be found at the same time, and also mines of different sizes. Since the life cycle is so short (16.2 ± 0.07 days from the egg to the emergence of the adult) we can deduce that between seven and nine generations succeed each other from the middle of November to the end of March or start of April.

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