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ORIGINAL ARTICLE

Description of the immature stages of *Delphacodes kuscheli* Fennah (Hemiptera: Delphacidae), vector of "Mal de Río Cuarto virus" on maize in Argentina

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Delphacodes kuscheli Fennah is the most efficient vector of Mal de Río Cuarto virus (MRCV) maize disease in Argentina, which is caused by a persistently transmitted fijivirus. *Delphacodes kuscheli* is abundant in a wide area of Central Argentina (32–35°S), affecting oat, wheat and maize crops, and weeds. All five immature instars of *D. kuscheli* are described, keyed and illustrated. A key is provided to highlight the main features for identification of the fifth-instar nymphs of three other Delphacid species on maize. Adults of *D. kuscheli* were reared under controlled conditions in individual glass cages containing oat plants. The description of each instar was based on 1-day-old nymphs. Coloration pattern, shape of frons, length of the rostrum segments, and length, shape and number of spur teeth are useful features for identification of the fifth nymphal instars of these species.

Keywords: Argentina; *Delphacodes kuscheli*; immature stages; key; maize disease vector; morphology

Introduction

Delphacodes kuscheli Fennah, 1955 (we use the original name of this delphacid species as originally proposed by Fennah; however, it should be noted that its taxonomic position is still under revision), is the most important vector of Mal de Río Cuarto virus (MRCV) on maize (Remes Lenicov et al., 1985), a disease that has been affecting maize (*Zea mays* L.) production in the major agriculturally productive areas of Argentina since 1967 (Lenardón, 1987). The pathogen is a fijivirus—now considered as distinct from Maize rough dwarf fijivirus—which occurs in the Mediterranean region (Rodríguez Pardina et al., 1998). The characteristic symptoms are the appearance of galls or "enations" in leaves, shortening of the stem internodes, poorly developed root system and general stunting. It is considered as the most important viral disease affecting this crop in northern and central provinces of Argentina. In addition, it can also attack wheat (*Triticum aestivum* L.), sorghum (*Sorghum vulgare* L.), millet (*Panicum miliaceum* L.), oat (*Avena sativa* L.) and several wild grass that grow in maize agroecosystems, particularly Poaceae and Cyperaceae (Laguna et al., 2000). According to field surveys, maize traders and growers, the worst epidemic in the country happened during the 1996–1997 agricultural year and yield losses were estimated at US$120 million (Lenardón et al., 1999). This disease has also been reported in the neighbouring countries of Brazil and Uruguay (Rodríguez Pardina et al., 1998). Although MRCV-resistant maize strains are being used, they are not always effective due to the high densities attained by the vector populations under certain environmental conditions.

Delphacodes kuscheli was originally described by Fennah in 1955 on only one brachypterous male specimen from Juan Fernández Island, Chile; Remes Lenicov & Tesón (1978) mentioned it from Argentina and described the female allotype, the macropterous forms as well as the suspected host plants and the geographical distribution. Because of the high densities that its populations reach in meadland—particularly between 32 and 35°S—it is considered native to central Argentina; toward the North it occurs in restricted areas, especially at higher elevations (cultivated sites more than 1500 m) (Virla & Remes Lenicov, 2000). Although the species develops population outbreaks in oat during spring, it also breeds on wheat, barley (*Hordeum vulgare* L.) and several annual and perennial weeds, with up to four generations occurring during summer (until late April to May) (Dagoberto et al., 1985; Remes Lenicov & Virla, 1999). Most of these host plant species are known reservoirs of MRCV (March et al., 1997; Laguna et al., 2002). *Delphacodes kuscheli* does not

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successfully breed on maize. However, virus transmission occurs when the infected macropterous adults migrate to feed on juvenile maize plants due to the senescence or harvest of oat, which is their most important winter host (Remes Lenicov et al., 1986). The MRCV is also transmitted by immature stages (Arneodo et al., 2002). Diverse aspects of the biology of *D. kuscheli* are known, including its life cycle on different hosts (Remes Lenicov et al., 1991b; Virla & Remes Lenicov, 1991; Maragliano & Virla, 1992), feeding behaviour (Brentassi & Remes Lenicov, 1997, 2005, 2006; Brentassi & Maldonado, 2002), plant resistance (Costamagna et al., 2005), reproductive behaviour (Costamagna, 1998; Brentassi & Remes Lenicov, 1999), ecology (Grilli & Gorla, 1997) and natural enemies (De Santis et al., 1988; Remes Lenicov & Tesón, 1990; Remes Lenicov et al., 1991a; Olmi & Virla, 1993; Triapitsyn, 1997; Virla & Olmi, 1998; Liljesthron & Virla, 2004). However, the morphology of the immature stages is still unknown.

Other planthopper species, *Delphacodes haywardi* Muir, 1929 and *Toya propinqua* (Fieber, 1866), have been recently reported as vectors (Presello et al., 1997; Velázquez et al., 2001, 2003). These species, as well as *Dicranotropis fuscoterminata* (Berg, 1879), are widely distributed in central Argentina. All of them coexist with *D. kuscheli* within the endemic MRCV area (Remes Lenicov et al., 1999). Some morphological and bioecological aspects of these species, such as development stages and some demographic parameters, have been studied previously (Remes Lenicov & Virla, 1996; Remes Lenicov et al., 1997a, 1997b).

In this contribution the egg and nymphal instars of *D. kuscheli* are described and illustrated, and a key for identification of the fifth nymphal instars of the four species associated to maize agroecosystems is provided.

Material and methods

In the three-year period 1999–2001, adults and nymphs of *D. kuscheli* were periodically collected by sweep net in oat in Córdoba, Argentina, and used to establish a laboratory population. Several generations were reared in cages ($61 \times 61 \times 61$ cm) on oat, under controlled conditions. Plants were added weekly or when required.

All instars of nymphs were examined 24 h after moulting. Specimens were killed with 95% ethyl ether to record their coloration, cleared in cold 10% KOH solution, fixed in Faure liquid and finally prepared for microscopic examination and illustration. Scanning electron microscopy was used to illustrate some cephalic sensorial structures and leg base characters. For this purpose, nymphs were cleaned by soaking in chloroform (3 min) and washing twice in 70% ethanol. Afterwards they were dehydrated in increasing concentrations of ethanol, dried by the critical-point technique and coated with a 65/70 µm gold-palladium film.

A detailed description of the first instar is given, whereas only major differences from previous instars are highlighted in the later stages. Specimens used for the descriptions were the macropters, this form was the most abundant morphotype in nature. Twenty specimens of each stage were measured, and the data are given in millimetres. The dimensions are expressed as: L, total length from tip of vertex to apex of abdomen; W, width across the widest part of body; HL, head length from tip of vertex to anterior margin of pronotum; HW, head width between outer margins of eyes; EW, vertex width between inner margins of eyes in dorsal view; FW, frons width across widest part. Pit arrangement follows Vilbaste (1968). Drawings were made with a stereoscopic microscope with a camera lucida. Average is expressed as mean $\pm$ SE.

Series of six specimens per instar were deposited in the collections of the Museo de La Plata.

Results

Eggs (Figure 1)

The postembryonic stage measurements are given in Table I.

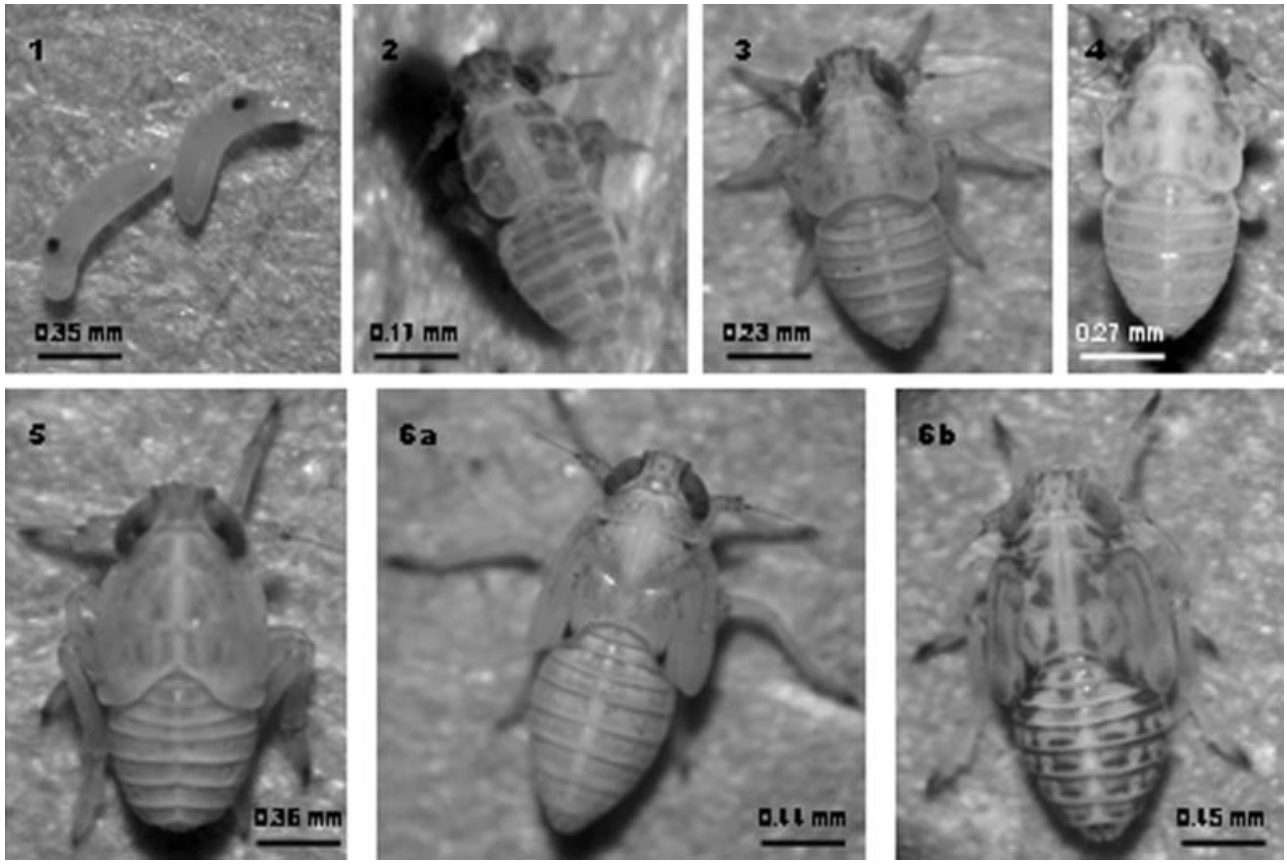
Curved, with cephalic end sharp and posteriorly rounded; ventral surface slightly concave, dorsal surface convex. Milky white when laid, turning yellowish before hatching. Chorion translucent, smooth.

First-instar nymph (Figure 2)

Ground colour light yellowish, yellow (darker) on abdomen; uniformly brownish grey on frons, clypeus, rostrum and antennae except segment I, the tergal plates in thorax and abdomen and legs. Eyes red.

Body elongated, subcylindrical, widest across mesothorax. Vertex broader than long (1.2:1), depressed medially; posterior margin straight, subtriangular, narrowing anteriorly with two pairs of longitudinal carinae which extend on to frons. Frons broader than clypeus, oval, convex in profile; lateral margin slightly convex and carinate, median carinae strongly carinate converging toward apical margin. Clypeus depressed, narrowing distally, without evident carina.

Rostrum three-segmented, extending beyond mesocoxae, segment I almost completely hidden by



Figures 1–6. *Delphacodes kuscheli*, immature stages. (1) Egg. (2) First instar. (3) Second instar. (4) Third instar. (5) Fourth instar. (6a, b), Fifth instar.

anteclypeus, segments II and III subequal. Antennae three-segmented, segment I short; segment II subcylindrical, slightly longer than wide, without sensory pits; segment III bulbous basally bearing a plaque-organ, ending in a short bristle-like extension reaching the mesothorax laterally (Figure 12).

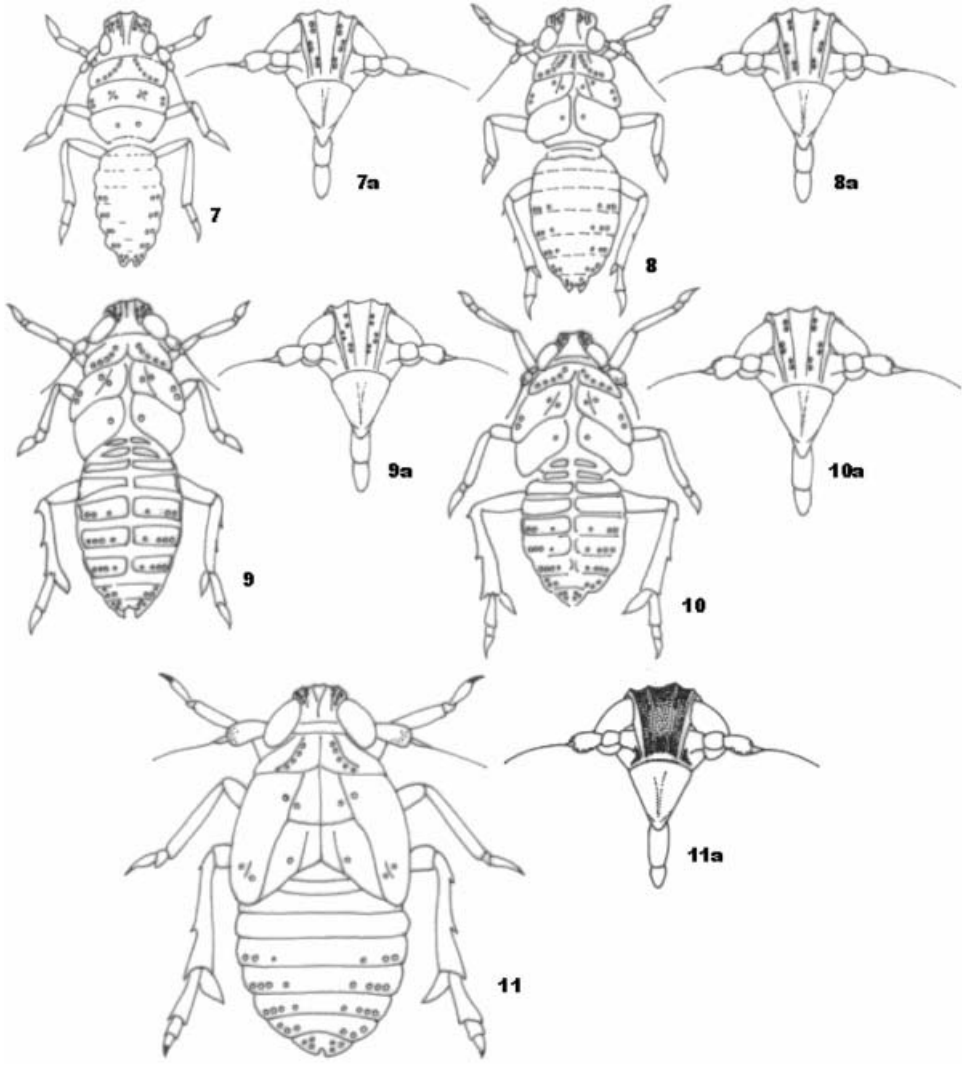
Thoracic nota divided into three pairs of plates by longitudinal mid-dorsal line. Notal plates subtrapezoidal, pronotal and mesonotal plates with prominent and divergent lateral carina. Legs subcylindrical, metatrochanter bearing 9–10 cuticular folds medially; metatibiae unarmed laterally, bearing apical row of four short black-tipped spines on plantar surface, spike-like spur undifferentiated, quite similar to the largest metatibial spine; protarsi and mesotarsi with divisions between tarsomeres obscure, metatarsomeres equal in length, metatarsomere I bearing apical row of four black-tipped spines, with external longest; tarsomeres II of all legs subconical, slightly curved, with pair of small black claws and long pulvilli apically (Figure 17).

Abdomen nine-segmented, subcylindrical, widest in segments III–V. The first two tergites rather small and appearing as short trapezoidal sclerites in dorsal view. Hind tergites trapezoidal cross-plates bearing sensorial pits from fifth segment onward.

Table I. Measurements (mean ± SE) of the postembryonic stages of *Delphacodes kuscheli* Fennah.

Postembryonic stage	L	W	HL	HW	EW	FW
Egg	0.80 ± 0.02	0.19 ± 0.01	–	–	–	–
First-instar nymph	0.66 ± 0.016	0.28 ± 0.04	0.13 ± 0.006	0.21 ± 0.0085	0.12 ± 0.004	0.18 ± 0.004
Second-instar nymph	0.86 ± 0.023	0.38 ± 0.01	0.13 ± 0.003	0.29 ± 0.005	0.13 ± 0.002	0.22 ± 0.004
Third-instar nymph	1.07 ± 0.05	0.52 ± 0.02	0.15 ± 0.09	0.39 ± 0.01	0.16 ± 0.08	0.27 ± 0.09
Fourth-instar nymph	1.45 ± 0.04	0.67 ± 0.02	0.19 ± 0.09	0.52 ± 0.02	0.18 ± 0.06	0.35 ± 0.003
Fifth-instar nymph	1.83 ± 0.05	0.95 ± 0.02	0.21 ± 0.01	0.65 ± 0.008	0.2 ± 0.003	0.44 ± 0.02

L, total length; W, width of body; HL, head length; HW, head width; EW, vertex width; FW, frons width.



Figures 7–11. Arrangement of the sensorial pits of *Delphacodes kuscheli* nymphal instars. (7) First instar, dorsal view; (a) face. (8) Second instar, dorsal view; (a) face. (9) Third instar, dorsal view; (a) face. (10) Fourth instar, dorsal view; (a) face. (11) Fifth instar, dorsal view; (a) face.

Arrangement of pits (Figure 7, 7a)

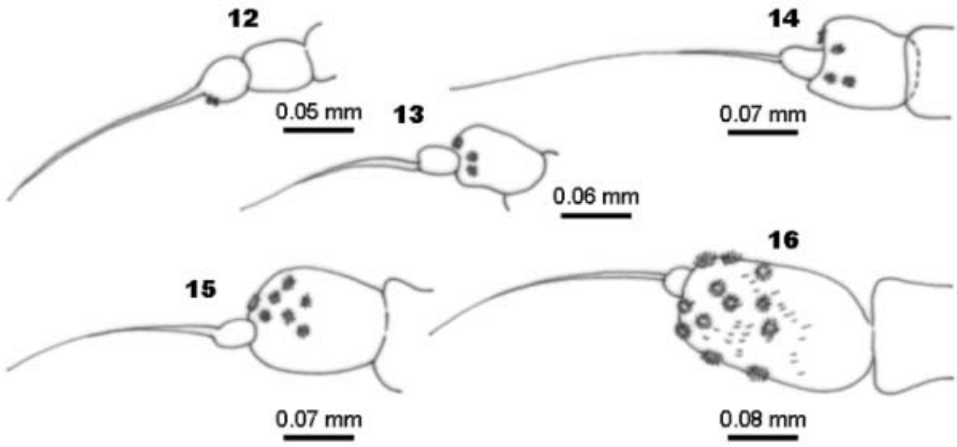
Head: with 11 sensorial pits on both sides; three pairs of sensory pits—upper, median and lower, respectively—on the laterofrons; one dorsal pit at the lateral keel and one pair at the medial keel. One pair of pits near the anterior corner of each eye. Thorax: 12 on pronotum, six on each lateral plate, bordering the lateral keel; eight on mesonotum, four on each plate—one median pair on either side of the oblique longitudinal carinae and one pair on lateral margin; two on metanotum, one near middle on each plate. Abdomen: tergum of segments V–VIII: 2; IX: 3.

Second-instar nymph (Figure 3)

Similar coloration pattern but tergal plates of thorax and abdomen not uniformly coloured along the body. Femur, tibiae and tarsi of legs I and II darker than those of leg III. Clypeus with darker longitudinal areas on either side of midline.

Rostrum segment II slightly longer than III. Antennal segment II longer than wide (1.5:1) bearing two or three sensorial pits on dorsal aspect near apex; length of segment III similar to early instar, bulbous portion ca. one-half length of pedicel (Figure 13).

Metatrochanter bearing nine cuticular folds medially. Metatibiae bearing two small lateral black-tipped spines, one near base, the other beyond mid-length, bearing apical row of four short black-tipped spines on plantar surface, spur slightly larger than largest metatibial spine; bearing apical row of four short black-tipped spines on plantar surface, spur twice the length of longest spine, bearing one apical tooth and one external subapical hair. Tarsi



Figures 12–16. Antennae of *Delphacodes kuscheli* nymphal instars. (12) First instar. (13) Second instar. (14) Third instar. (15) Fourth instar. (16) Fifth instar.

with visible divisions between tarsomeres. Metatarsomere I bearing apical row of four black-tipped ventral spines. Metatarsomere I and II subequal in length (Figure 18).

Arrangement of pits (Figure 8, 8a)

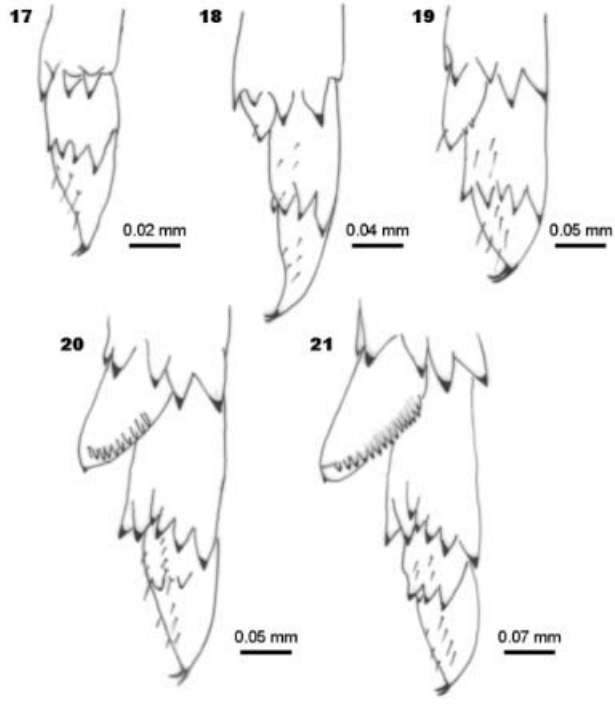
Head and thorax as the first-instar nymph; abdominal segments V–VIII: one+two pits on segment VIII with different arrangement; IX with three caudal pits.

Third-instar nymph (Figure 4)

Ground colour yellow; vertex yellowish, brown marked on vertex between carinae. Frons uniformly dark except along the median and lateral carinae and the lower margin, which are whitish; clypeus with two darker longitudinal submedian areas. Thorax and abdomen with irregular brown spots; lighter on dorsum. Legs darker on tarsi I and II and apical region of III.

Frons oval, lateral margin strongly convex and carinate in profile; lateral areas wider than medial area. Parallel median carinae strongly carinate, continuous with lateral margin of vertex and not reaching apical margin. Clypeus convex, narrowing distally, with a weak medial carina, obscure on base. Rostrum with subapical segment slightly longer than apical (1.3:1). Antennal segment II longer than wide (1.5:1) bearing four apical sensory pits; flagellum with bulbous portion ca. one-third length of pedicel (Figure 14).

Mesonotal wingpads slightly lobate, covering one-fifth of metanotal wingpad laterally. Metanotal wingpads extending laterally, not covering tergite III. Metatrochanter bearing 9–10 cuticular folds on internal margin. Metatibiae bearing apical row of five black-tipped ventral spines; spur subtriangular, as long as two-thirds of metatarsomere I, bearing one apical and two or three marginal teeth. Metatarsomere I bearing apical row of five black-tipped ventral spines. Metatarsomere II slightly longer than I (Figure 19).



Figures 17–21. Metatibial spur and metatarsi of *Delphacodes kuscheli*. (17) First instar. (18) Second instar. (19) Third instar. (20) Fourth instar. (21) Fifth instar.

Arrangement of pits (Figure 9, 9a)

Similar pattern to former instar, with two additional pits on head, close to the anterior corner of each eye, one pit on each lateral margin of pronotum and one on urotergites VI and VII (one+three).

Fourth-instar nymph (Figure 5)

Ground colour yellowish with similar coloration pattern. Legs with black apical tarsi.

Frons longer than wide (1.5:1). Rostrum subapical segment longer than apical (1.4:1). Antennal segment II with five to seven sensorial pits, bulbous portion of flagellum ca. one-quarter length of pedicel (Figure 15).

Mesonotal wingpads distinctly lobate, extending more than one-third of length of midline, covering laterally more than half of the metanotal ones; metanotal wingpads extending to first abdominal segment. Metatrochanter bearing 13–15 cuticular folds medially. Metatibiae bearing five apical spines; spur length more than two-thirds of metatarsomere I, subtriangular in profile, external margin straight, internal margin rounded with one apical tooth and a row of seven to nine small submarginal teeth; tarsomere I of metatarsi bearing an apical row of five or six black-tipped ventral spines; tarsomere II with two to three weakly developed black-tipped ventral spines near middle of partially subdivided tarsomere (Figure 20).

Arrangement of pits (Figure 10, 10a)

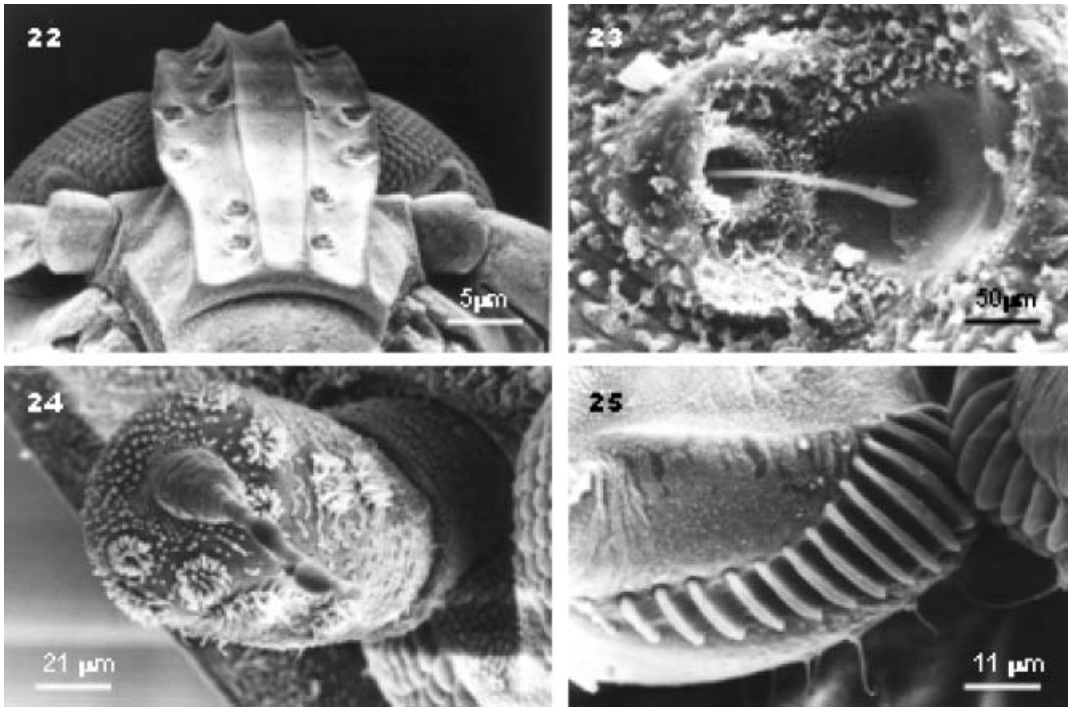
Pattern similar to former instar.

Fifth-instar nymph (Figure 6a, b)

Ground colour brown yellowish. Coloration pattern similar to former instar, brownish areas more defined along body, lighter on the midline and around the pits. Male darker than female.

Head considerably longer than previous instars. Frons twice as long as wide, parallel submedian carinae. Rostrum subapical segment longer than apical (1.6:1). Antennal segment II with 10–12 sensorial pits, bulbous portion of flagellum ca. one-sixth length of pedicel (Figures 16, 24).

Wingpads lobate. Mesonotal wingpads extending to, or almost to, apex of metanotal wingpads laterally; metanotal wingpads almost reaching fifth abdominal segment. Metatrochanter bearing 13–15 cuticular folds medially as in fourth nymphal instar (Figure 25). Spur slightly shorter than metatarsomere, with 13–18 submarginal thin black-tipped teeth lined on their convex margin on the inner surface. Metatarsi three-segmented, tarsomere I bearing ventral apical row of six or



Figures 22–25. Scanning electron micrographs of *Delphacodes kuscheli* fifth instar. (22) Frontal view illustrating position of sensorial pits. (23) Detail of a frontal sensorial pit. (24) Antennae illustrating position of sensorial pits on segment II. (25) Detail of metatrochanteral cuticular folds.

seven black-tipped spines, tarsomere II bearing four ventral black-tipped spines, tarsomere II and III subequal (Figure 21).

Arrangement of pits (Figures 11, 11a, 22, 23)

Similar to previous instar.

Key to nymphal instars of *Delphacodes kuscheli*

1. Metatarsi three-segmented; if two-segmented, then with three weakly developed black-tipped ventral spines in the middle of tarsomere (Figures 20, 21) 2
– Metatarsi two-segmented (Figures 17–19) . 3
2. Metatibial spur with more than 10 submarginal teeth (Figure 21); mesonotal wing-pads laterally overlapping the metanotal wingpads (Figure 6) . . . . . Fifth-instar nymph
– Metatibial spur with seven to nine submarginal teeth (Figure 20). Mesonotal wingpads extending beyond half length of metanotal wingpads . . . . . Fourth-instar nymph
3. Metatibia with two lateral spines on shaft. Metatibial spur more than 2 × length of longest apical spine (Figures 18–21); antennal pedicel with several sensorial pits (Figures 13–16) . 4
– Metatibia without lateral spines on shaft. Metatibial spur less than 2 × length of longest apical spines (Figure 17); antennal pedicel without sensorial pits (Figure 12) . . . . . First-instar nymph
4. Metatibial spur without marginal teeth. Metatarsomere I with apical transverse row of four spines (Figure 18) . . . . Second-instar nymph
– Metatibial spur with two or three submarginal teeth. Metatarsomere I with apical transverse row of five spines (Figure 19) . . . . . Third-instar nymph

Remarks

The nymphs of the species included in this study are morphologically very similar at their earlier instars, but they can be reliably separated at the fifth nymphal instar. We wish to highlight some features that contribute to the precise identification of immature instars of these species. *Delphacodes haywardi* Muir nymphs have whitish ground colour and the head coloration pattern—black spot on frons flanked by round white areas below the eyes (Remes Lenicov & Virla, 1996). *Dicranotropis fuscoterminata* (Berg) nymphs also have dark marks in all instars but the ground colour is darker and the pattern on the body is different (Remes Lenicov et al., 1997a). *Toya propinqua* Fieber nymphs is yellow uniform coloration, except for the brown intercarinal areas of frons (Remes Lenicov et al., 1997b). Apart from the colour differences mentioned above, other noteworthy differences are the shape of the frons, length of the rostrum segments, and length, shape and number of spur teeth.

Key to species of fifth-instar nymphs of Delphacidae on maize in Argentina

1. Ground colour uniformly yellow, with brown pigment particularly on frons between carinae. Subapical and apical rostrum segment lengths subequal . . . . . *Toya propinqua*
– Ground colour yellow or whitish, heavily spotted with dark brown on head and dorsal surface of thorax and abdomen. Subapical rostrum segment longer than apical one 2
2. Rostrum reaching mesocoxa; frons with well-defined protruding whitish area on lower margin (Figure 11a) . . . . . *Delphacodes kuscheli*
– Rostrum reaching the metacoxa; frons without protruding whitish area on lower margin; face with other pattern coloration. 3
3. Ground colour whitish, head with black spot on frons flanked by conspicuous rounded white areas below the eyes. Metatibial spur as long as metatarsomere I, with 19 sharp prominent marginal teeth . . . . . *Delphacodes haywardi*
– Ground colour yellowish, with fuscous marks on frons, clypeus and dorsal surface of thorax and abdomen, especially around the sensorial pits and ventrolateral wax pore. Metatibial spur shorter than metarsomere I (0.7:1), with 12–15 sharp marginal teeth . . . . . *Dicranotropis fuscoterminata*

Acknowledgments

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