Studies on the immature stages of the planthopper Lepidelphax pistiae (Hemiptera: Delphacidae), a potential biocontrol agent for the aquatic weed Pistia stratiotes (Araceae) from Argentina

Ana M Marino de Remes Lenicov,^{1*} Barbara Defea,¹ José Rusconi² and Guillermo Cabrera Walsh³

¹Facultad de Ciencias Naturales y Museo de La Plata, Division Entomologia, Argentina.
 ²Centro de Estudios Parasitologicos y de Vectores, Argentina.
 ³FuEDEI, Hurlingham, Buenos Aires Argentina.

Abstract Descriptions of the immature stages of the planthopper *Lepidelphax pistiae* Remes Lenicov (Fulgoromorpha), a specialist herbivore of *Pistia stratiotes* L. (Alismatales) from Argentina, are provided for the first time. *P. stratiotes*, or water lettuce, is an important weed in fresh water bodies of the tropics and subtropics. Newly emerged nymphs from eggs collected in the field were reared in rearing chambers. Fifth nymphal instars may be easily recognised by the whitish colour with a distinctive blackish antennal flagellum and ungues, and the relatively long frons and rostrum. Information is provided on nymphal behaviour and the main features of the fifth nymph instar that distinguish it from the two other delphacids that live and feed on plants from central and northern Argentine wetlands.

Key words biology, immature stages, morphology, water lettuce.

INTRODUCTION

Lepidelphax Remes Lenicov was recently described as a new genus with a single species, Lepidelphax pistiae. It is a specialist that feeds and lays eggs on water lettuce, Pistia stratiotes L. (Alismatales: Araceae). This slender and delicate delphacid is easily distinguished from all other Neotropical Delphacini by the following adult features: pale colour, with dark brown to black longitudinal stripes on the face; several brown maculations on the thorax and abdomen; the longlegged, laterally compressed shape, with a narrow and short vertex; and, a small sub-apical areolum continued into a distinctive simple frontal median carina. It also has unique male genital structures: a pygofer with a trilobate ventroposterior margin, a wide and uniformly membranous diaphragm and a ventrocaudally curved aedeagus. Females have a slightly sclerotised subquadrate plate on sternite VIII and basally rounded valvifers VIII (Marino de Remes Lenicov and Cabrera Walsh 2013).

This species feeds and reproduces on *P. stratiotes*, a floating macrophyte native to South America that is an important weed in fresh water bodies of the tropics and subtropics all around the world (Neuenschwander *et al.* 2009). Dense populations of *P. stratiotes* can clog waterways, affecting irrigation, flood control and recreational water uses. They may also block the air–water interface and light penetration, causing a reduction in dissolved oxygen levels, thus making the aquatic habitat less suitable for many aquatic species, including fish. Dense mats can also block animal access to the water and may crowd or

shade out native plants upon which other organisms depend for food or shelter (Howard and Harley 1998). Water lettuce has been controlled through the release of biological control agents in many parts of the plant's exotic distribution to the point where no further intervention (e.g. herbicide applications) has been necessary (Coetzee et al. 2011). However, in other invaded regions, biological control has only been partially successful so additional agents are deemed necessary for successful management (Dray and Center 2002). L. pistiae is found throughout the distribution of P. stratiotes in Argentina. In laboratory tests, this species only survived and reproduced on its host plant and did not survive for more than seven days on test plants of 29 species of the same family, nor on other aquatic plant species that coexist with its host plant (Cabrera Walsh et al. 2014). Furthermore, field tests indicate that this herbivore can cause drastic reductions in the biomass and density of water lettuce. Thus L. pistiae is a natural herbivore of P. stratiotes with promising characteristics as a biocontrol agent because of its host specificity, wide climatic tolerance, high reproductive rate, easy rearing in controlled conditions and compatibility with other biocontrol agents (Cabrera Walsh and Maestro 2014, 2016).

Studies on the nymphs of Fulgoromorpha have advanced considerably in the last two decades, although most of them refer to species of the fauna of North America and Europe (Asche 1985; Wilson and Wheeler 1986, 2005; Wilson and Claridge 1991; Sforza *et al.* 1999; Zenner *et al.* 2005; Szwedo 2007). As for Argentinian species, on the other hand, available morphological or ecological information is scarce. Approximately 32 species of Delphacini are recorded for Argentina, but very few descriptions of nymphs have been published, and those are mostly of agricultural pests, e.g. *Chionomus haywardi*

^{*}marinoremes@gmail.com

(Muir) (Marino de Remes Lenicov and Virla 1996), *Metadelphax propinqua* (Fieber) (as *Toya propinqua*, Marino de Remes Lenicov *et al.* 1997b, 2008), *Dicranotropis fuscoterminata* (Berg) (Marino de Remes Lenicov *et al.* 1997a), *Peregrinus maidis* Ashmead (Marin and Sarmiento 1981; Rioja *et al.* 2006; Marino de Remes Lenicov *et al.* 2008) and *Delphacodes kuscheli* Fennah (Marino de Remes Lenicov *et al.* 2008).

Apart from L. pistiae, another seven species of Argentinian Delphacidae are known to feed on aquatic or amphibious plants such as Pontederia cordata, Pontederia rotundifolia, Eichhornia crassipes, Eichhornia azurea (Pontederiaceae), Echinodorus grandiflorum (Alismataceae), Limnobium laevigatum (Hydrocharitaceae) and Eryngium sp. (Apiales: Apiaceae) (Sosa et al. 2007; Mariani et al. 2013). However, immature stages of only two delphacid species associated with aquatic plants have been described so far: Megamelus scutellaris Berg (Sosa et al. 2005) and Megamelus bellicus Remes Lenicov and Sosa (Mariani et al. 2007). Both these species are also of interest as biocontrol agents. As these plant species coexist with P. stratiotes throughout its distribution, it is important to know how to differentiate immature stages of species that may potentially co-occur in the same environment.

Besides bioecological interest, information on immature stages also contributes to the unravelling of planthopper phylogenies. A preliminary system of relationships based on nymphal features was proposed by Yang and Fang (1993), Chen and Yang (1995) and Emeljanov (1996, 2002), although there is no general agreement about the phylogeny of fulgoroids in the published literature.

Considering the potential of this planthopper for the control of an important weed, it was deemed necessary to provide full descriptions of the immature stages. This paper presents descriptions and illustrations of the egg and the five nymphal stages of *L. pistiae*. It also highlights the main features, which distinguish the fifth nymphal instars of the three species of delphacids known from aquatic plants in central and northern Argentinian wetlands. A taxonomic key to the species is included.

MATERIALS AND METHODS

Adults and nymphs of *L. pistiae* were periodically collected by aspirating individuals from water lettuce over a 3 year period (2009 to 2011) and used to establish three laboratory colonies. Each colony was started with insects from three extreme locations of the distribution of *P. stratiotes* within Argentina: a northernmost site collection in Pirané, Formosa Province (S 25° 59′ 18″; W 58° 25′ 50″), a westernmost location in Río Hondo, Santiago del Estero Province (S 27° 30′ 29″; W 64° 54′ 09″) and a southernmost site in the lower Delta of the Paraná-Uruguay basin, Buenos Aires Province (S 34° 06′ 33″; W 58° 47′ 18″). Specimens from each colony and from field collections were preserved in 70% EtOH for morphological studies. Others were frozen dry at -18°C, to preserve the original colours. Live

specimens from the lower Delta were also examined to confirm colour patterns.

The description of each stage was based on 24h old nymphs from the laboratory colonies. Specimens were anaesthetised with 95% ethyl ether to preserve colour, cleared in cold 10% KOH solution and fixed in Faure liquid for microscopic examination and illustration. Eggs were obtained by dissecting a gravid female.

The fifth instars (presumptive brachypters and macropters) are described in detail; only major differences are highlighted for earlier stages. Interspecific differences among fifth instars were based on the presumptive brachypters. Measurements (median and range) were taken from anaesthetised specimens and are given in mm. Dimensions are expressed as L.: total body length (from the tip of vertex to the tip of abdomen), W.: maximum body width (across the widest part of the mesothorax) and t.l.: thoracic length (from the anterior margin of the pronotum to the posterior margin of the metanotum along the midline). Other measurements are relative. Morphological terminology follows Vilbaste (1968). In regard to the terms vertex and frons, we follow the generalised custom in current literature. However, these structures should not be interpreted as homologous to those in other insect groups, as pointed out by Anufriev and Emeljanov in 1988, who proposed the terms metope and coryphe instead of frons and vertex, respectively (see Emeljanov 2002).

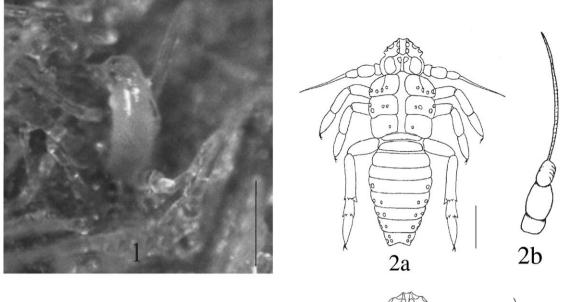
Drawings were made with a Leitz-Westzlar microscope with a camera lucida and from photographs using a RRID 18 HD digital camera adapted to this microscope.

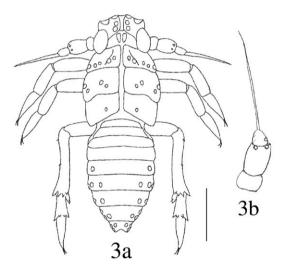
RESULTS

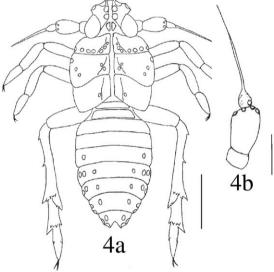
Key to the instars of L. pistiae

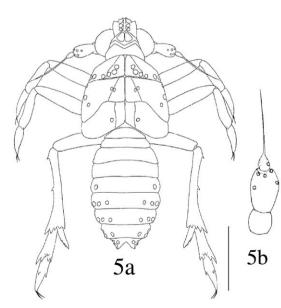
- Metatibial spur less 2 times length of longest apical spines of the metatibia. Antennal pedicel without sensorial pits (Figs 2a,b).....first instar

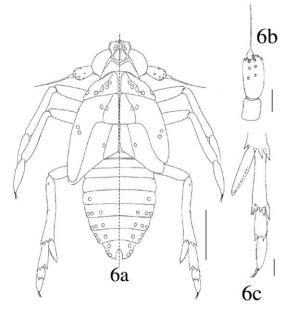
- **3** Metatibial spur without marginal teeth. Metatarsomere 1 with apical transverse row of four spines (Figs 3a,b)second instar
- Metatibial spur with two or three marginal teeth.
 Metatarsomere 1 with apical transverse row of five spines (Figs 4a,b).....third instar
- **4** Metatibial spur with five marginal teeth. Fore wing pads covering half of hind wing pad laterally (Figs 5a,b)fourth instar
- Metatibial spur with 10–13 marginal teeth. Fore wing pads extending to apex of hind wing pads, overlapping the third urotergite (macropterous form) (Figs 6a left side, b, c) or











Figs 1–6. Lepidelphax pistiae, immature stages.

L. pistiae Remes Lenicov, 2013

(Figs 1-7)

Description of immature stages

Specimens examined. ARGENTINA: Formosa, Herradura (S 26° 30′ 59″; W 58° 16′ 54″), 10 nymphs V, 10 nymph IV, 10 nymphs III, 10, nymphs II and 10 nymphs I; 11-II and 1-XII-2012, on *P. stratiotes*; Buenos Aires, Vicente Lopez (S 34°29′ 32.63″; W 58°28′42.53″), 4 nymphs V, 5 nymph IV, 3 nymph III, 3 nymphs II and 3 nymphs I; 2-IV-2015, on *P. stratiotes*.

Diagnosis. The nymphal instar of *L. pistiae* can be reliably separated from the other two delphacid species that live in similar environments by its generally uniformly whitish unmarked colour, slender body and especially by the relatively long rostrum and ungues. These last two traits are a perfect match for the dense mat of woolly trichomes that cover the leaf surface of its host plant.

Eggs. (Fig. 1) (n=10). Length: 1.40 (±0.01), width: 0.50 (±0.01).

Eggs milky white, ellipsoidal. Chorion translucent.

First instar. (Figs 2a,b) (*n*=5). L: 0.80 (0.74–0.84); W: 0.24 (0.22–0.25); t. 1.: 0.22 (0.20–0.24).

Body uniformly whitish translucent, with a distinctive blackish antennal flagellum.

Vertex subtriangular, length sub-equal to width (1.2:1); lateral and submedian carinae of vertex extend onto frons. Frons with lateral margin slightly convex and carinate, about $2 \times longer$ than wide in middle line, regularly wider towards the frontoclypeal suture; lateral margins carinate and paralleled by sub median carina which are prominent at base and regularly evanescent towards apex; each laterofrons below the eyes $1.5 \times$ wider than interfrons; with 13 sensorial pits on each sides: nine pits on area between submedian and lateral carinae (six visible in ventral view, three in dorsal aspect) and four pits between lateral carinae and eyes. Antennal pedicel sub-cylindrical ca. $2-2.5 \times$ the length of scape, without pits; base of flagellum bulbous, ³/₄ pedicel length (Fig. 2b). Rostrum reaching fifth urosternite, apical segment $0.6 \times$ longer than subapical.

Thoracic nota divided into three pairs of plates by longitudinal mid-dorsal line. Pronotal plates subtrapezoidal, lateral carinae divergent and slightly convex towards posterior margin. Each plate with seven pits extending from near middorsal line posterolaterally to lateral margin (two pits in line next to lateral carinae on posterior half of segment and five pits extending laterally from the external side of carina along the posterior border of plate, one proximal to it and four most lateral not visible in dorsal view). Mesonotum as long as metanotum. Mesonotal plate with four pits, two median on disk and two next to lateral margin. Metatibiae without lateral spines, with apical transverse row of three stiff spines; tiny spur, approximately one fourth length of metatarsomere 1, with apical spine. Metatarsomere 1 shorter than metarsomere 2 (0.5:1) with four apical spines.

Abdomen with nine apparent segments, widest across segment 5. Tergite 5 with one pit and tergites 6–8 each with three pits on either side of midline. Segment 9 surrounding anus with three pits on each side, two subapical and one dorsal median.

Second instar. (Figs 3a,b) (*n*=5). L: 0.96 (0.80–1.00); W: 0.30 (0.28–0.30); t. l.: 0.26 (0.25–0.30).

Colour pattern similar to former instars.

Antennal pedicel sub-cylindrical ca. $2 \times$ the length of scape, with two small pits on apical half; flagellum whip-like distally, bulbous at base one half length of pedicel (Fig. 3b). Rostrum overlapping fifth urosternite, apical segment 0.7 × longer than subapical. Mesonotum longer than metanotum (0.7:1); posterior margin of mesonotum shallowly excavated. Wing-pads undeveloped. Metatibiae with the two lateral spines barely developed and a transverse row of four stiff spines; spur reaching near one half the length of metatarsomere 1; with one apical tooth and a short subapical hair on external surface. Metatarsomere 1 shorter than metatarsomere 2 (0.6:1).

Third instar. (Figs 4a,b) (*n*=4). L: 1.23 (1.20–1.26); W: 043 (0.40–0.50); t. 1.: 0.33 (0.31–0.36).

Colour pattern similar to former instars.

Antennal pedicel sub-cylindrical ca. 4 × the length of scape, with four apical sensory pits; flagellum with bulbous portion ca. one-third length of pedicel (Fig. 4b). Rostrum reaching sixth urosternite, apical segment $0.7 \times \text{longer}$ than sub apical.

Mesonotal plate with the two discal pits on both sides of the lateral carinae. Fore wing pads short, each covering one fourth of metanotal segment laterally, with three pits, one on costal area and the others on middle half. Metanotum median length as long as mesonotum, widely excavated on posterior margin. Metatrochanter subcylindrical, with row of nine to ten interlocking flattened folds on posteromedial aspect. Metatibiae with apical transverse row of five stiff spines; spur half the length of metatarsomere 1, with one apical tooth and two or three marginal teeth. Metatarsomere 1 with apical transverse row of five spines. Apical tarsomere with two spines (one each side) in middle of tarsomere.

Fourth instar. (Figs 5a,b) (*n*=10). L: 1.50 (1.25–1.70); W: 0.60 (0.40–0.70); t. 1: 0.45 (0.40–0.52).

Colour pattern similar to former instar.

Frons longer than wide (1.5: 1). Antennal pedicel sub cylindrical ca. $2 \times$ the length of scape, with about five pits on the apical half (Fig. 5b). Rostrum reaching fifth urosternite, apical segment $0.6 \times$ longer than sub apical.

Fore wing pads short, each covering approximately one half of hind wing pad laterally with three pits, one on costal area and two next to subcostal carina. Metanotal median length subequal to mesonotum; hind wing pad subtriangular, reaching the base of first abdominal segment. Metatibia with apical transverse row of six stiff spines decreasing in size towards plantar surface; spur two third the length of metatarsomere 1, with one apical tooth and five marginal teeth. Metatarsi with two tarsomeres: tarsomere 1 with apical transverse row of six spines, apical tarsomere partially subdivided in middle, with two distinctive ventrolateral spines in middle of plantar surface. Metatarsomere 1 as long as the apical.

Fifth instar. (Figs 6a–c; 7). Macropterous form (Fig. 6a left side) (n=9). L: 1.9 (1.85–2.00); W: 1.06 (0.95–1.2); t. 1.: 0.70 (0.63–0.74). Brachypterous form (Figs 6a right side, 7) (n=10). L: 1.70 (1.6–2.0); W: 0.80 (0.65–0.95); t. 1: 0.55 (0.50–0.63).

Colour pattern similar to former instars but slightly yellowish on thoracic nota. A distinctive brownish area corresponding to the endoskeletal system of the metathoracic segment is clearly observed in cleared specimens.

Form elongate, lightly compressed, widest across mesothoracic wing pads. Head protruding beyond anterior margin of eyes, ca. one fourth of the eyes length, posterior margin obscured by anterior margin of pronotum.

Vertex subtriangular, length sub-equal to width (1.2:1); lateral carinae of vertex extend onto frons, submedian carinae attaining basal margin of vertex laterally extending onto frons as prominent submedian carinae. V-shaped transverse carina between medial and submedial carinae prominent, delimiting three shallow depressions: one, as wide as long, just anterior to transverse carina and two big basal compartments, each one as wide at middle length as its greatest length. Frons flattened and uniformly wide, about $2 \times \text{longer than wide in middle line, regularly}$ wider towards the frontoclypeal suture; lateral margins carinate and paralleled by sub median carina which are prominent at base and regularly evanescent towards apex; each laterofrons below the eyes $1.5 \times$ wider than interfrons. Area between submedian and lateral carinae with nine pits on each side (six visible in ventral view, three in dorsal aspect – five upper pairs and the lower ones lining the submedian carina, median pair next to lateral carinae), between lateral carinae and eyes with four pits on each side. Anteclypeus longer than wide at base (1.7:1), not carinated along its length; frontoclypeal suture slightly convex. Antennae with scape short, ring-like; pedicel slender, 2.5 × as long as wide, and $2 \times$ the scape length, with about 10–11 pits: four on apex and six arranged in two rows on the apical half; flagellum whip-like distally, bulbous at base, approximately one sixth the length of pedicel (Fig. 6b). Rostrum three-segmented, exceeding seventh urosternite, segment I obscured by postclypeus; apical segment $0.9 \times \text{longer than subapical.}$

Thoracic nota divided by longitudinal mid-dorsal line into three pairs of plates. Pronotal plates strongly projected anteriorly one third of its length in midline, laterals convex; each with long distinct lateral carina slightly convex towards posterior margin. Each plate with seven pits extending from near middorsal line

liaceous, a little shorter than metatarsomere I, with one apical tooth and 10 to 13 marginal teeth. Pro- and mesotarsi with two tarsomeres, tarsomere 1 wedge-shaped; tarsomere 2 subconical, curved, with pair of relatively long apical claws and median membranous pulvillus. Metatarsi with three tarsomeres; tarsomere 1 cylindrical, as long as tarsomere 2 plus 3, with apical transverse row of seven spines on plantar surface; tarsomere 2 cylindrical, one half the length of tarsomere 1, with apical transverse row of four spines on plantar surface; tarsomere 3, similar to apical tarsomere of other legs. Abdomen with nine apparent segments, slightly flattened dorsoventrally, widest across segment 5. Segment 9 surrounding anus with three evident pits on each side, two subapical and one dorsal median, in both sexes. Female with two pairs of prolonged structures - the rudiment of the ovipositor - that are visible as outgrowths from the posterior margin of sternites 8 and 9. Males with the sternite 9 blunt and slightly bilobed posteriorly in ventral view, without rudiment of the genital structures (Figs 7a,b).



posterolaterally to lateral margin (two pits in line next to lateral

carinae on posterior half of segment and five pits extending later-

ally from the external side of carina along the posterior border of

plate, one proximal to it and four most lateral not visible in dorsal

view) (Fig. 6a). Mesonotal median length $1.2 \times$ the pronotum,

with distinctive lateral carina, slightly sinuate, originating on an-

terior margin in median one fourth and extending

posterolaterally reaching posterior border of plate; with five pits:

two median on disk, on both sides of carinae, and three on wing

pad; fore wing pads elongate, extending to apex of hind wing

pad in macropterous (Fig. 6a, left side) or covering lateral

approximately two third of hind wing pad in brachypterous (Fig. 6a, right side), with one pit, on costal area and two near

middle, next to subcostal carina. Metanotal disc with median

length slightly shorter than mesonotum, weak lateral carina orig-

inating on anterior margin in median one fourth and terminating

on posterior margin, one pit just lateral to carina; hind wing pads

broadly lobate, extending laterally almost reaching to anterior

margin fourth abdominal tergites in macropter and third tergite

in brachypter (Fig. 6a, left and right side, respectively). Pro-

and mesocoxae slender, posteromedially directed; metacoxae fused to sternum. Metatrochanter subcylindrical, with row of

12-14 interlocking flattened folds on posteromedial aspect.

Metatibiae (Fig. 6c) each with two lateral spines on the basal half

of shaft, apical transverse row of five stiff spines - not or weakly

pigmented - grouped two on the inner side and three on the outer

side of plantar surface, and flattened slender spur, moderately fo-

Figs 7. Lepidelphax pistiae; genital segment of fifth instar nymphs.

Biology

L. pistiae insert its eggs in the plant tissue of either side of the leaf. Nymphs are sedentary and usually found on the lower surface of the leaves. Younger instars, first and second, live amidst the dense layer of leaf trichomes of the host plant and feed on the surface. The last instars and adults are found outside the trichome layer, and they reach the leaf surface using their unusually long rostrums. They tend to move to the underside of the leaves or hop to the water surface when disturbed. Adults are normally found higher up on the plant and tend to fly or jump when disturbed.

Key to species of Delphacidae species associated with aquatic plants from Argentina (fifth instar nymphs)

- Medium size ca 2.5–4mm. Body yellowish heavily marked in brown. Rostrum short, reaching or exceeding mesocoxae. Spur broad with strong black tipped teeth irregularly sized

The main features that contribute to the accurate identification of the fifth instars for each species are as follows:

L. pistiae (Fig. 8). Overall uniformly whitish translucent, yellowish on thoracic nota with a distinctive blackish antennal

flagellum and ungues. Rostrum long, exceeding seventh urosternite with apical segment nearly twice the length of subapical segment. Meta-tibial spur slender and moderately foliaceous, a little shorter than metatarsomere, $1.4 \times$ longer than broad at base, with 13 marginal fine teeth of regular size.

Brachypterous form: (*n*=10). L: 1.70±0.02; W: 0.80±0.06; t. 1.: 0.55±0.01.

M. bellicus (Fig. 9). Relatively larger. Heavily marked in brown, with only one lighter stripe on the frontoclypeal suture and some longitudinal pale marks on the meso- and metanota. Rostrum short, reaching mesocoxae, with apical and subapical segment subequal. Meta-tibial spur broad, foliaceous, as long as metatarsomere, $1.4 \times$ longer than broad at base, with 11–14 marginal black-tipped teeth of varying size.

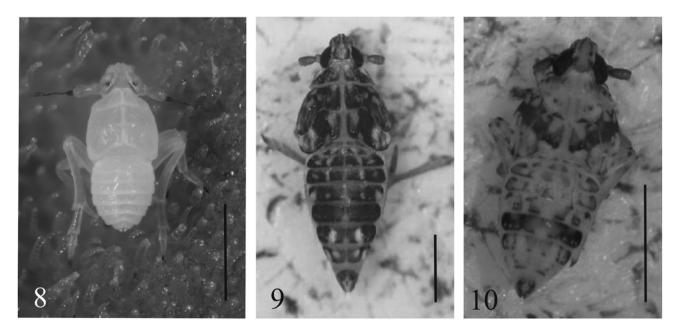
Brachypterous form (the only known morphotype for this species): (n=10). L: 3.50 ± 0.04 ; W: 1.00 ± 0.06 ; t.l.: 1.20 ± 0.05 .

M. scutellaris (Fig. 10). Colour pattern distinguishable by two transverse stripes, a dark broad stripe on frons below the eyes, and a whitish one on frontoclypeal margin, a V-like brown spot on the mesonotum and metanotum and conspicuous annular dark brown stripes on legs. Rostrum short, extending beyond the mesocoxae, with apical and subapical segment subequal. Meta-tibial spur foliaceous, $5 \times$ longer than broad at base, shorter than metatarsomere 1, with five to eight marginal strong black tipped teeth of varying size.

Brachypterous form: (*n*=10). L: 2.37±0.09; W: 1.07±0.06; t. 1.: 0.87±0.01.

DISCUSSION

The general habitus of the immature stages of *L. pistiae* is similar to the adult, but the wings are undeveloped and the body covered



Figs 8–10. Habitus of fifth instar nymphs of delphacids associated with aquatic plants in Argentina.

with numerous sensory pits. The general colour of the nymphs is uniformly whitish, differing from the adults that have dark stripes and maculation.

L. pistiae nymphs differ from other delphacines mainly in the smaller size, and comparatively very long frons, rostrum and ungues. Only small differences were found in chaetotaxy and the number and position of the sensory pits, which are arranged in a regular pattern and with high bilateral symmetry as is typical for Delphacidae (Vilbaste 1968; Wilson and Claridge 1991). The legs also have a pattern of metatarsomere division common to all delphacines. Fourth instar nymphs have a partial subdivision of the terminal metatarsomere with two distinctive spines near the middle on the plantar surface, while the fifth instar nymphs have three well-defined metatarsomeres with terminal teeth. Wilson and Wheeler (1986) stated that the three metatarsomeres with an apical transverse row of four spines on the plantar surface represent a plesiomorphic condition within the delphacids.

It is well known that the rudiments of the external genitalia in delphacids appear in the third instar nymphs (Kathirithamby 1981). However, Vilbaste (1968) and Wilson and Wheeler (1986) indicated that the sexes can only be differentiated reliably from the fifth instar in the presence of the prolonged genital rudiments of the ovipositor. This diagnosis also applies to *L. pistiae* nymphs.

The life history and diagnostic descriptions of the immature stages of an insect species are significant additions to biological knowledge. This information has heightened importance and economic relevance when it helps to identify and discriminate species with the potential to affect human activities and the environment from those that do not.

In this work, we described comprehensively details of the anatomy and biology of a potential biocontrol agent for an aquatic weed. These will provide other workers with the information necessary to identify and assess the impact of this delphacid. A few elements of the biology of this species remain to be studied, such as parasitoids and other natural enemies that may affect its population levels. These may be relevant because if L. pistiae is susceptible to attack by generalist predators or parasitoids, its effectiveness as a biocontrol agent may be compromised. For example, macropterous and brachypterous forms have been found in the field hosting Dryinidae larvae (unpublished data), but the parasitoid prevalence and the relative susceptibility of L. pistiae nymphs to attacks are unknown. Other aspects of the field biology of L. pistiae are currently under study by us, such as overwintering stages in the field, population growth and dispersion, female fertility and egg viability. These data could be relevant in the future if this species is eventually deemed suitable for release to control P. stratiotes in any of the areas where this aquatic plant is a weed.

ACKNOWLEDGEMENTS

We want to thank to Dr Alejandro Sosa (CONICET – FUEDEI) who provided us the photos of nymphs of *Megamelus* species and Dr Arnaldo Maciá (CIC: Scientific Research Committee of the Province of Buenos Aires, Argentina) for technical assistance

with the illustrations. We would like to formally thank to the reviewers for thoughtful and constructive comments that have greatly improved this paper. This work was partially supported by Fundación para el Estudio de Especies Invasivas (FUEDEI), Hurlingham, Buenos Aires, Argentina, La Plata National University (UNLP: grant N° 730) and the National Research Council (CONICET). B.D and JM.R. are Postgraduate CONICET fellows.

REFERENCES

- Anufriev GA & Emeljanov AF. 1988. Suborder Cicadinea (Auchenorrhyncha). In: Keys to the Insects of the Far East of the USSR, Vol. 2 (ed. PA Ler), pp. 12–495. Nauka, Leningrad Russia.
- Asche M. 1985. Zur phylogenie der Delphacidae Leach, 1815 (Homoptera Cicadina Fulgoromorpha). Marburger Entomologische Publikationen 2, 1–910.
- Cabrera Walsh G & Maestro M. 2014. Evaluation of intraguild interactions between two species of insect herbivores on *Pistia stratiotes. Biological Control* 76, 74–78.
- Cabrera Walsh G & Maestro M. 2016. Impact of introduced native herbivores on a *Pistia stratiotes* infestation close to the Paraná Delta in Argentina. *Biocontrol Science and Technology* 26, 35–46.
- Cabrera Walsh G, Maestro M, Sosa A & Tipping PW. 2014. Specificity of Lepidelphax pistiae (Hemiptera: Delphacidae) to Pistia stratiotes (Araceae). Biocontrol Science and Technology 24, 485–488.
- Chen S & Yang CT. 1995. The metatarsi of the Fulgoroidea (Homoptera: Auchenorrhyncha). *Chinese Journal of Entomology* 15, 257–269.
- Coetzee JA, Hill MP, Byrne MJ & Bownes A. 2011. A review of the biological control programmes on *Eichhornia crassipes* (C.Mart.) Solms (Pontederiaceae), *Salvinia molesta* D.S.Mitch. (Salviniaceae), *Pistia stratiotes* L. (Araceae), *Myriophyllum aquaticum* (Vell.) Verdc. (Haloragaceae) and *Azolla filiculoides* Lam. (Azollaceae) in South Africa. *African Entomology* **19** (sp), 451–468.
- Dray FA & Center TD. 2002. Chapter 5: Waterlettuce. In: *Biological Control of Invasive Plants in the Eastern United States*, (eds RG Van Driesche, B Blossey & M Hoddle), Publication FHTET-2002–04, pp. 65–78. USDA Forest Service, Morgantown, West Virginia.
- Emeljanov AF. 1996. On the question of the classification and phylogeny of the Delphacidae (Homoptera, Cicadina), with reference to larval characters. *Entomological Review* 75, 134–150.
- Emeljanov AF. 2002. Contribution to classification and phylogeny of the family Cixiidae (Hemiptera, Fulgoromorpha). Denisia 04, Zugleich Kataloge des OÖ. Landesmuseums, Neue Folge 176, 103–112. Biologiezentrum Linz/Austria.
- Howard GW & Harley KLS. 1998. How do floating aquatic weeds affect wetland conservation and development? How can these effects be minimized? Wetland Ecology and Management 5, 215–225.
- Kathirithamby J. 1981. Common Auchenorrhyncha (Homoptera) in rice fields in Southeast Asia. *Bulletin Ministry of Agriculture, Malaysia* 155, 1–36.
- Mariani R, Sosa A & Marino de Remes Lenicov AM. 2007. Megamelus bellicus Remes Lenicov & Sosa (Hemiptera: Delphacidae) immature stages and biology. Revista de la Sociedad Entomológica Argentina (RSEA) 66, 189–196.
- Mariani R, Sosa AJ & Marino de Remes Lenicov AM. 2013. A new species of Megamelus from Argentina and the reassignment of Stenocranus maculipes (Berg) (Hemiptera: Delphacidae). Revista de la Sociedad Entomológica Argentina (RSEA) 72, 169–178.
- Marin RL & Sarmiento J. 1981. Biología y secuencia estacional de Peregrinus maidis (Ashmead) (Homoptera-Araeopidae). Revista Peruana de Entomología 24, 107–111.
- Marino de Remes Lenicov AM, Brentassi ME & Toledo A. 2008. Description of the immature stages of *Delphacodes kuscheli* Fennah (Hemiptera: Delphacidae), vector of "Mal de Río Cuarto virus" on maize in Argentina. *Studies on Neotropical fauna and Environment SNFE* **43**, 25–33.

- Marino de Remes Lenicov AM & Cabrera Walsh G. 2013. A new genus and species of Delphacini associated to hydrophytic plants in Argentina (Hemiptera, Fulgoromorpha, Delphacidae). *Florida Entomologist* 96, 1350–1358.
- Marino de Remes Lenicov AM, Mariani R & Costamagna A. 1997a. Aspectos morfológicos y bioecológicos de *Dicranotropis fuscoterminata* (Berg) sobre cultivos de maíz (Insecta: Homoptera: Delphacidae). *Neotropica* 43, 7–14.
- Marino de Remes Lenicov AM & Virla E. 1996. Descripción de los estados inmaduros de Delphacodes haywardi y notas acerca de su comportamiento en condiciones de laboratorio (Homoptera: Delphacidae). Revista de la Sociedad Entomológica Argentina (RSEA) 55, 165–174.
- Marino de Remes Lenicov AM, Virla E & Varela G. 1997b. Descripción de los estados inmaduros de *Toya propinqua* (Fieber) y notas acerca de su comportamiento en condiciones experimentales (Insecta: Homoptera: Delphacidae). *Neotropica* 43, 85–91.
- Neuenschwander P, Julien MH, Center TD & Hill MP. 2009. 17: Pistia stratiotes L. (Araceae). In: Biological Control of Tropical Weeds Using Arthropods (eds R Muniappan, GVP Reddy & A Raman), pp. 332–352. Cambridge University Press, UK.
- Rioja TC, Vargas HE & Bobadilla DE. 2006. Biología y enemigos naturales de *Peregrinus maidis* (Ashmead) (Hemiptera: Delphacidae) en el Valle de Azapa. *IDESIA* 24, 41–48. Chile.
- Sforza R, Bourgoin T, Wilson S & Boudon-Padieu E. 1999. Field observations, laboratory rearing and descriptions of inmatures of the planthopper Hyalesthes Obsoletus (Hemiptera: Cixiidae). *European Journal of Entomology* **96**, 409–418.
- Sosa AJ, Marino de Remes Lenicov AM & Mariani R. 2007. Species of Megamelus (Hemiptera-Delphacidae) associated with Pontedereaceae in South America. Annals of the Entomological Society of America 100, 798–809.

- Sosa AJ, Marino de Remes Lenicov AM, Mariani R & Cordo H. 2005. Life history of *Megamelus scutellaris* Berg with description of immature stages (Hemiptera, Delphacidae). *Annals of the Entomological Society* of America 98, 66–72.
- Szwedo J. 2007. Nymphs of a new family Neazoniidae fam. n. (Hemiptera: Fulgoromorpha: Fulgoroidea) from the Lower Cretaceous Lebanese amber. *African Invertebrates* 48, 127–143.
- Vilbaste J. 1968. Preliminary key for the identification of the nymphs of North European Homoptera Cicadina. I. Delphacidae. Annales Entomologici Fennici 34, 65–74.
- Wilson MR & Claridge MR. 1991. Handbook for the Identification of Leafhoppers and Planthoppers of Rice, pp 142. CAB International, Oxon, UK.
- Wilson S & Wheeler AG Jr. 1986. Pentagramma longistylata (Homoptera: Delphacidae): descriptions of immature stages. Journal of the New York Entomological Society 94, 126–133.
- Wilson S & Wheeler AG Jr. 2005. An African grass, *Eragrostis curvula* (Poacea), planted in the Southern United States recruits rarely collected native planthoppers (Hemiptera: Fulgoroidea:Dyctyopharidae, Fulgoridae). *Journal of the New York Entomological Society* 113, 174–204.
- Yang CT & Fang SJ. 1993. Phylogeny of Fulgoromorpha nymphs, first results. In: Proceeding of the 8th Auchenorrhyncha Congress, Delphi, Greece, 9-13 August 1993, (eds S Drosopoulos, PV Petrakis, MF Claridge, PWF de Virijer), pp. 25–26.
- Zenner G, Stöckmann M & Niedringhaus R. 2005. Preliminary key to the nymphs of the families and subfamilies of the German Auchenorrhyncha fauna (Hemiptera, Fulgoromorpha et Cicadomorpha). *Beiträge zur Zikadenkunde* **8**, 59–78.

Accepted for publication 16 August 2016.