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Authors: Durante, M. Pilar Mier, Hidalgo, Nicolás Pérez, Martínez-Torres, David, García-Tejero, Sergio, Martínez, Rebeca Peña, et al.

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A NEW GENUS AND SPECIES OF ASTERACEAE-INHABITING APHID (HEMIPTERA: APHIDIDAE) FROM COSTA RICA AND MEXICO

M. Pilar Mier Durante[1,*], Nicolás Pérez Hidalgo[1], David Martínez-Torres[2], Sergio García-Tejero[1], Rebeca Peña Martínez[3] and Juan M. Nieto Nafría[1]
[1]Departamento de Biodiversidad y Gestión Ambiental, Universidad de León, 24071 León, Spain
E-mail: mpmied@unileon.es, nperh@unileon.es, sgart@unileon.es, jmnien@unileon.es.

[2]Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de Valencia, Apdo. Correos 22085, 46071 Valencia (Spain)
E-mail: david.martinez@uv.es.

[3]Prolongación Aldama 188 M1 C55, 16010 México, D.F (Mexico)
E-mail: regecaphis@hotmail.com.

*Corresponding author; E-mail: mpmied@unileon.es

Abstract

Ucrimyzus villalobosi Mier Durante & Pérez Hidalgo **gen. n.**, **sp. n.** (Hemiptera: Aphididae: Macrosiphini) are described from apterous and alate viviparous females collected on species of genera *Bidens*, *Schkuhria*, *Senecio* and *Stevia* (Asteraceae: Asteroideae) in Costa Rica and Mexico. Principal components analysis (PCA) was done to verify that the studied aphids belong to a single species regardless of their geographical origin or host plant. Molecular analyses were carried out on the sequences of a fragment of the mitochondrial gene encoding for cytochrome c oxidase subunit 1 (COI) and of a fragment of the nuclear gene encoding elongation factor 1α (EF1α). The taxonomic discussion takes into account the conclusions of the molecular analyses and the morphologic study compared with other genera of Macrosiphini. The identification keys by Blackman & Eastop (2006) to aphids living on each mentioned plant genus are modified to include the new species.

Key Words: *Ucrimyzus* **gen. n.,** aphids, Macrosiphini, morphology, principal components analysis, cytochrome oxidase 1, elongation factor 1α

Resumen

Se describe *Ucrimyzus villalobosi* Mier Durante & Pérez Hidalgo **gen. n.**, **sp. n.** (Hemiptera: Aphididae: Macrosiphini) a partir de hembras vivíparas ápteras y aladas recogidas en Costa Rica y México sobre plantas de los géneros *Bidens*, *Schkuhria*, *Senecio* y *Stevia* (Asteraceae: Asteroideae). El análisis de componentes principales de los especímenes asegura que son de la misma especie cualquiera que sea su origen geográfico o su planta hospedadora. Se han analizado sendos fragmentos del gen mitocondrial que codifica la subunidad 1 de la citocromo c oxidasa (COI) y del gen nuclear que codifica el factor de elongación 1 α (EF1α). La discusión taxonómica se ha basado en las conclusiones de los análisis moleculares y en el estudio morfológico comparado con géneros de Macrosiphini. Se modifican las claves de identificación de Blackman & Eastop para los pulgones que viven en los mencionados 4 géneros de plantas para incluir en ellas el nuevo género *Ucrimyzus*.

Palabras Clave: *Ucrimyzus* **gen. n.,** pulgones, Macrosiphini, morfología, análisis de componentes principales, citocromo oxidasa I, factor de elongación 1α

Translation provided by the authors.

During expeditions to Costa Rica in 2008, 3 of the authors (M.D., P.H., N.N.) and W. Villalobos (Villalobos Muller et al. 2010) collected apterous and alate viviparous female macrosiphines (Aphididae, Aphidinae, Macrosiphini) on *Bidens pilosa* (Asteraceae, Asteroideae) in the University of Costa Rica main campus. Additional individuals collected on *Bidens, Schkuhria, Senecio* and *Stevia* in several Mexican localities, and kept in the *collection génerale d'aphides of the Muséum national d'Histoire naturelle* (Paris, France), were studied; these specimens had been provisionally identified by the Prof. G. Remaudière as *Hyperomyzus* sp. and marked as possible new species.

Morphological, statistical (PCA) and molecular (genes COI and EF 1α) studies have been carried out and have shown that (i) the studied specimens belong plausibly to a single species independently from their geographical origin or host plant, and (ii) it is a new species, which can not be included in any known Macrosiphini genus, and so, a new genus and its type species are established.

Material and Methods

Ten samples collected in 1 Costa Rican and 6 Mexican localities have been studied (see "Types" section), considering a sample as the group of specimens collected on the same species of plant in a locality on a specific date. Specimens were preserved in microscopic slides with a water-soluble mounting medium (Nieto Nafría & Mier Durante 1998). Aphids for molecular analyses were preserved in 96% ethanol until processing; these individuals were caught together with the holotype on the same plant specimen.

Morphological measurements were made according to Nieto Nafría & Mier Durante (1998). In the description, measurements are lengths except when indicated otherwise as width or diameter.

The comparative morphological study was conducted on species (i) whose apterae possess swollen siphunculi and have been recorded in North America or (ii) are known to feed on Asteroideae species over the World. Specimens belonging to diverse genera presumably related with the new one and also specimens of the most part of American species currently classified *Neonasonovia* Hille Ris Lambers, 1949 (subgenus of *Hyperomyzus* Börner 1933), in the collections of the *Muséum national d'Histoire naturelle* (Paris, France) and the *Universidad de León* (Leon, Spain) were examined. Several taxonomic works, mainly Blackman & Eastop (2006), Blackman (2010), Foottit et al. (1993), Heie (1992, 1994, 1995), Miyazaki (1971), and Nieto Nafría et al. (in press), were consulted.

A Leica DC digital camera with IM 1000 version 1.10 software was used for the photomicrographs.

The variables used in the PCA (principal components analysis) are the metric and meristic characters mentioned in the description of 39 apterous viviparous females; all samples are represented. Variables were standardized to zero mean and unit standard deviation prior to the analysis to give the same weight to all of them.

Molecular phylogenetic analyses were done (i) on a fragment of the mitochondrial DNA containing the 5' region of the cytochrome c oxidase 1 (COI) and (ii) on a fragment of the nuclear gene coding for elongation factor-1 alpha (EF1α) of 3 specimens of the Costa Rican sample.

DNA extraction was done following the HotSHOT (Hot Sodium HidrOxide and Tris) method (Truett et al. 2000).

PCR amplifications of each mentioned fragment were done on 3 µL of the extracted DNA. A 710 bp fragment of the 5' region of the COI gene was amplified using primers LCO1490 and HCO2198 described by Folmer et al. (1994). PCR conditions for COI amplification were as follows: 94 °C for 1 min, 35 cycles of 94 °C for 30 s, 48 °C for 1 min, and 68 °C for 1 min; a final extension step of 7 min at 68 °C was included after cycling. Amplification of the EF1α fragment was done using 2 consecutive PCR reactions with primers Efs175 (Moran et al. 1999) and Efr1 (5'GTGTGGCAATSCAANACNGGAGT3') in the first reaction and then primers Efs175 and Efr2 (5"TTGGAAATTTGACCNGGGTGRTT3') in the second semi-nested reaction. PCR conditions used in the first reaction were 94 °C for 1 min; 40 cycles of 94 °C for 30 s, 50 °C for 1 min and 68 °C for 1.5 min; a final extension step of 7 min at 68 °C was included after cycling. The semi-nested PCR was done similarly but using 52 °C for the annealing step and 1 µL of the first PCR product.

PCR products were purified by ammonium precipitation and reconstituted in 10 mL of LTE buffer (10 mM Tris, 0.1 mM EDTA). Direct sequencing of the amplified fragments was done on both directions using PCR primers (Efr2 was used as reverse primer for sequencing the EF1α fragment). Sequencing was conducted using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) following manufacturer's instructions, and samples were loaded into an ABI 3700 automated sequencer.

Chromatograms were revised and sequences assembled using the Staden package v1.6.0 (Bonfield et al. 1995). Multiple alignments were done with Clustal X v1.81 (Larkin et al. 2007) with gap opening and gap extension penalties of 10.0 and 0.2, respectively.

Molecular phylogenetic analyses were conducted using MEGA 5 (Tamura et al. 2011).

Results and Discussion

Ucrimyzus villalobosi Mier Durante and Pérez Hidalgo, **gen. n., sp. n.**

The combined description of the new species and new genus is made under article 13.4 of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature, 1999).

Type species of *Ucrimyzus* **gen. n.** *Ucrimyzus villalobosi* **sp. n.**

Types of *Ucrimyzus villalobosi* **sp. n.**

HOLOTYPE: Apterous viviparous female number 4 of sample CRI-344: COSTA RICA, San Jose province, San Pedro de Montes de Oca, campus

de la Universidad de Costa Rica (1214 m), *Bidens pilosa*, 1-III-2008, Pérez Hidalgo *leg.*, *colección zoológica de la Universidad de León* [*CZULE*], Leon (Spain). Paratypes: 42 alate [al.] and 65 apterous [ap.] viviparous females (*CZULE*, *Muséum national d'Histoire naturelle*, Paris, France, and *Natural History Museum*, London, U. K.): 3 al. and 17 ap. caught at same time as the holotype; 2 al. and 2 ap., MEXICO, Aguas Calientes state, Pabellon de Arteaga (1904 m), *Schkuhria anthemoidea*, 7-IX-1981, Adame *leg.*; 4 al. and 2 ap., MEXICO, Durango state, El Salto (ouest) (2600 m), *Bidens* sp., 18-X-1980, Remaudière & Peña Martínez *leg.*; 1 al., MEXICO, Mexico state, Chapingo, *Bidens pilosa* (2250 m), 18-IX-1979, Remaudière *leg.*; 4 al. and 2 ap., MEXICO, Guanajuato state, San Miguel de Allende (2000 m), 8-X-1980, Remaudière & Peña Martínez *leg.*; 1 ap., MEXICO, Durango state, Durango (10 km west) (2200 m), *Senecio* sp., 17-X-1980, Remaudière & Peña Martínez *leg.*; 1 ap., MEXICO, Durango state, Durango (10 km west) (2200 m), *Stevia* sp., 17-X-1980, Remaudière & Peña Martínez *leg.*; 2 al. and 11 ap., MEXICO, Queretaro state, Caderyta (2010 m), *Schkuhria virgata*, 7-X-1980; 26 al. and 29 ap., MEXICO, Veracruz state, Cumbres de Maltrata (ouest Santa Rosa) (1750 m), *Bidens* sp., 28-IV-1979, Remaudière *leg*..

Etymologies and Genus Name Gender

Ucrimyzus is a noun formed with "UCR" and "*myzus*" plus the thematic vowel "i" to facilitate pronunciation. "UCR" is the acronym of *Universidad de Costa Rica*, the former University in Costa Rica, and "*myzus*" forms part of the name of many aphid genera that present more or less close similarities to *Myzus*. The gender of *Ucrimyzus* is masculine following the gender of *Myzus*. The specific name *villalobosi* is a noun in apposition in case genitive, in gratitude to William Villalobos Muller (University of Costa Rica) for his work improving the Costa Rican aphid fauna knowledge.

Apterous Viviparous Females. Fig. 1. Based on 66 Specimens.

Color in life dull pale green, with yellowish appendages and siphunculi. Body 1.65-2.45 mm including cauda.

Head. Pale-brown; cuticular ornamentation reduced to fine and scattered striae. Frontal margin undulated and frontal tubercles rounded; lateral tubercles divergent and low, but bigger than the medial. Dorsal setae with rounded apex, 15-17 µm and 0.42-0.70 times the basal diameter of the antennal segment III [henceforth D]; ventral setae pointed and similar in size. Ventral margin of the antennal socket with a striate protuberance. Antennae are 1.75-2.62 mm and 0.93-1.28 times the body length. Antennal segments I and II pale brown and delicately rugous on the inner margin. Antennal segment III 0.41-0.70 mm and 1.1-2.1 times IV, pale brown (on a proximal portion) to dark brown, with scales (ventrally on paler portion) and striae, blunt setae 7-15 µm and 0.2-0.6 times D, and 1-20 secondary sensoria with double-lined and non-ciliate margin placed in a ventral line of the basal third of the segment. Antennal segments IV to VI brown to dark brown and imbricated; IV, 0.25-0.50 mm; V, 0.23-0.37 mm; processus terminalis of VI 0.55-0.82 mm, 0.9-1.7 times antennal segment III and 5.3-8.2 times base, which is 0.09-0.12 mm. Rostrum as pigmented as head, smooth and reaching the hind coxae. Ultimate rostral segment triangular with straight margins, 0.12-0.14 mm, 1.8-2.9 times its basal diameter, 1.1-1.4 times base of the antennal segment VI and 1.1-1.3 times second tarsal segment of the hind legs; with 3-6 accessory setae, which are 12-20 µm and 0.50-0.66 times D.

Thorax. Membranous, very pale and with pale spiracular and inconspicuous intersegmental sclerites. Prothorax with 2 spinal and 2 pairs of marginal setae, similar to other dorsal setae in shape and size; spiracular apertures rounded and only slightly wider than those on the abdomen. Coxae, trochanters pale brown, femora basad pale brown and darkening to the apex, tibiae brown to dark brown, tarsi dark brown. Femur and tibia of the hind legs 0.58-0.81 and 0.97-1.50 mm, respectively. Second tarsal segment of hind legs 0.10-0.12 mm. Setae on femora scattered, blunt and short, those on hind legs are 12-18 µm and 0.41-0.60 times D. Setae on basal part of tibiae like the femoral ones, others are pointed and longer than those (20-25 µm and 0.61-1.0 times D). First tarsal segments with 3 setae.

Abdomen. Membranous, with small and unpigmented intersegmental and spiracular sclerites, pale but rugous postsiphuncular sclerites, and sometimes marginal sclerites on segments VII and VIII also pale and rugous or with spinules. Spiracular apertures subcircular to reniform. Papillae absent. Dorsal setae on anterior segments blunt, 8-10 per segment, 7-12 µm and 0.2-0.4 times D. Ventral setae pointed, more numerous and 12-30 µm. Abdominal segment VIII with 4-6 setae, 8-20 µm and 0.2-0.8 times D. Siphunculi asymmetrically swollen (outer edge is almost straight), with broad base and well-defined preapical incision and flange; 0.37-0.50 mm and 0.18-0.25 times body length; 4.7-5.3 times diameter at base (0.075-0.100 mm), 6.8-11.5 times diameter of peduncle at middle (0.03-0.07 mm) and 5.0-8.3 times greatest diameter of swollen part (0.06-0.09 mm); pigmented like femora and darkening apically; mostly smooth, with striae on incision and few striae or wrinkles on peduncle. Genital plate pale, with 2 discal and 6-12 posterior setae, all of them with pointed apex. Anal plate

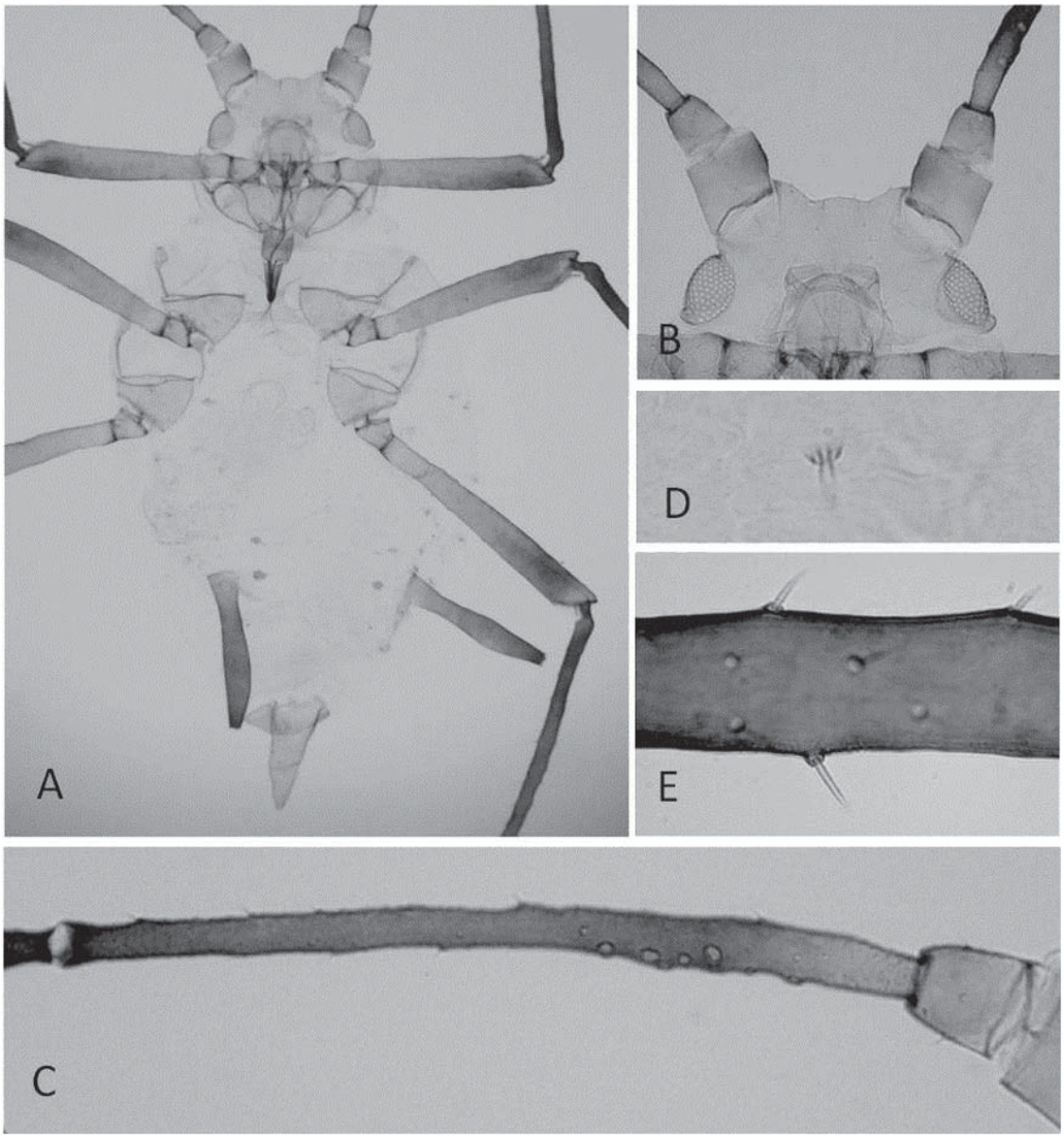

Fig. 1. Apterous viviparous female: A, habitus; B, head; C, III antennal segment; D, dorsal seta on III abdominal segment; E, setae on hind tibiae.

and cauda pale brown, paler than siphunculi. Cauda lanceolate, 0.21-0.34 mm, 1.6-2.6 times its basal width, 0.60-0.74 times the siphunculus, and carrying 5-7 fine, curved and pointed setae.

Alate Viviparous Females. Fig. 2. Based on 42 Specimens.

Identical to apterae in qualitative, metric and meristic features, including the ranges of these last two, except the following ones: those being properly alatae (e.g. pterothorax), slightly more pigmented legs and sometimes antennae, pale brown marginal and sometimes spiracular abdominal sclerites, and 25-43 scattered secondary sensoria on antennal segment IV, 5-19 aligned ones on segment IV and 0-2 on segment V. The wing venation is not noticeably.

Bionomics. Aphids of this species form dense colonies on the stems of plants of genera *Bidens*, *Schkuhria*, *Senecio* and *Stevia,* and perhaps of

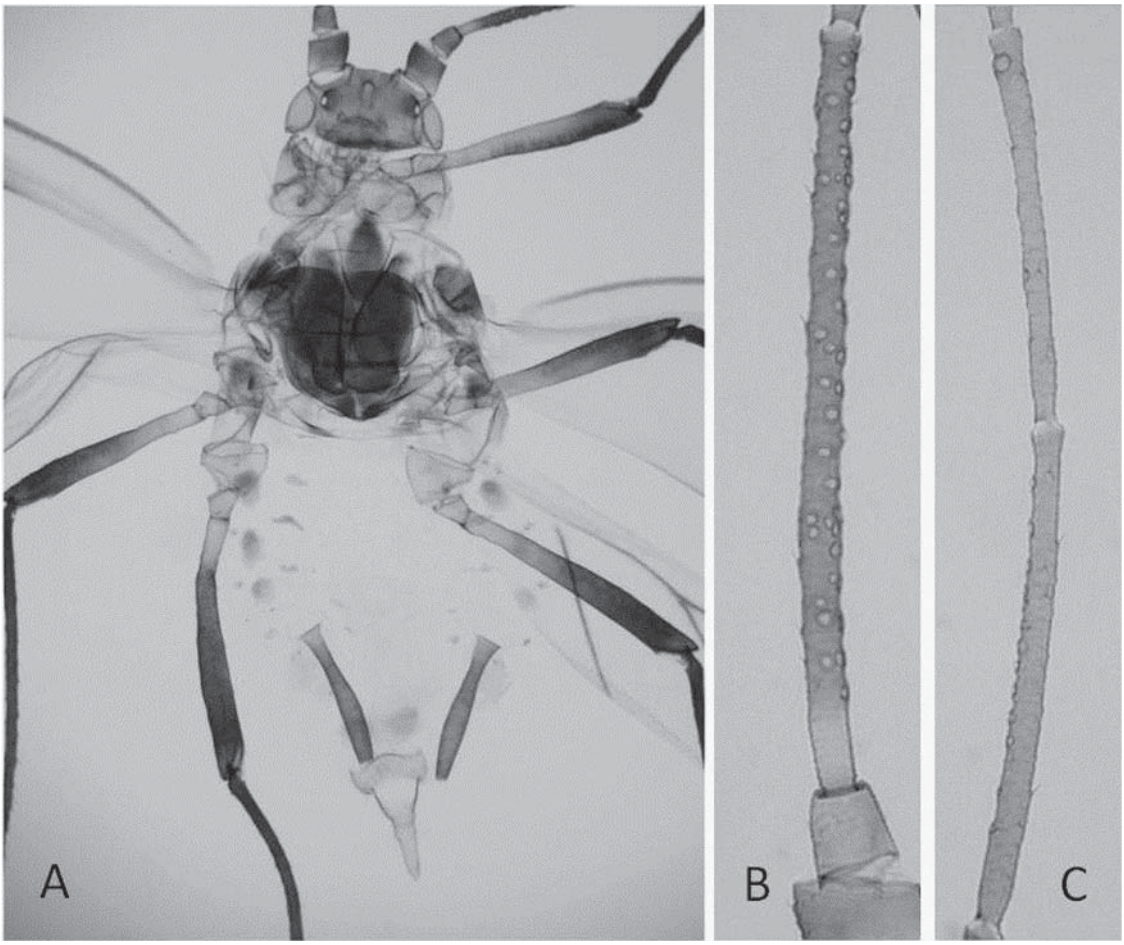

Fig. 2. Alate viviparous female: A, habitus; B, III antennal segment; C, IV and V antennal segments.

other species belonging to subfamily Asteroideae (Asteraceae). Nothing is known regarding host alternation or lack thereof.

Geographical Distribution

Collection data from Costa Rica and high altitude localities in Central Mexico indicate that the species inhabits a great part of Mesoamerica, from Costa Rica to the Mexican state of Durango.

Taxonomic Discussion

Principal Components Analysis. PCA summarizes multivariate data in a reduced number of dimensions which are linear combinations of the original variables.

The first 2 axes of the PCA represent 42.6% of the total variance (Fig. 3). Individuals from different Costa Rican and Mexican localities and caught on diverse host plants (belonging to genera *Bidens*, *Schkuhria*, *Senecio* and *Stevia*) are

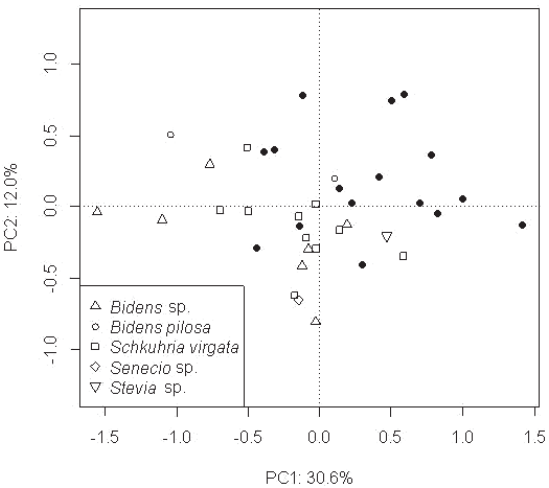

Fig. 3. Plot of Principal Components Analysis (PCA) of apterous viviparous females of *Ucrymyzus villalobosi* **sp. n.** from Costa Rica (●) and Mexico (○) recorded on different host-plant species.

shown intermixed with each other. This suggests that all individuals belong to a single species no matter their procendence or host plant. Remarkably, some individuals from Costa Rica, which were bigger than the others, scored high on the first axis, which was dominated by antennae, leg, siphunculus and cauda variables.

Morphological Discussion

Ucrimyzus **gen. n.,** *Hyperomyzus* Börner, 1933, *Neoamphorophora* Mason, 1924 and *Utamphorophora* Knowlton, 1946 are the only American genera that belong to Macrosiphini (Aphididae, Aphidinae) with the following features: (i) asymmetrically swollen siphunculi without a reticulated apical section; (ii) smooth head; (iii) frons with divergent lateral tubercles and shallow sinus; (iv) ventral margin of the antennal socket with a protuberance and (v) pointed or rounded dorsal setae, never clavate like those of *Cryptomyzus* Oestlund, 1923; and (vi) short setae on appendages, never long and erected like those in *Decorosiphon* Börner, 1939.

Neoamphorophora and *Utamphorophora* differ conspicuously from *Ucrimyzus* **gen. n.** The *Neoamphorophora* apterous viviparous females have protruding frontomedial tubercle and a dorsoabdominal patch, and the alatae have dorsoabdominal cross bands. In *Utamphorophora* the ultimate rostral segment is short-triangular (characteristic shape of grass-inhabiting aphids) with only 2 (infrequently 3 or 4) accessory setae.

Only *Hyperomyzus*, including subgenus *Neonasonovia*, presents features resembling those of the new genus: appearance and color of living apterae, smooth swollen siphunculi, cephalic cuticular ornamentation, frontal profile, secondary sensoria on several antennal segments of apterae. However, the prothoracic spiracular apertures are typically bigger than the abdominal ones in the subgenera of *Hyperomyzus*.

Many species of Macrosiphini live on plant species belonging to Asteraceae, including species of *Bidens*, *Schkuhria*, *Senecio* and *Stevia*. They are included in the keys (one per host plant genus) by Blackman & Eastop (2006). Since the characteristics exhibited by *U. villalobosi* **sp. n.** differ from those of the other Macrosiphini species, the identification keys were modified to include the new species (see annex 1).

Molecular Results and Discussion

A 710 bp DNA fragment containing a portion of the mitochondrial COI gene was amplified through PCR from the 3 individual aphids analyzed. After removing primer sequences, useful sequences consisted of 658 nucleotides. Sequences for the 3 sampled aphids were identical so a single sequence was finally deposited in Genbank (accession number KC110893). The online identification engine available at the Barcode of Life Data Systems (BOLD) (Ratnasingham & Hebert 2007) using the COI species database, failed to find any record corresponding to any identified species that matched our sequence. After a BLASTN search against the non-redundant nucleotide database at the NCBI, sequences from different aphid genera of the tribe Macrosiphini, such as *Aulacorthum*, *Acyrthosiphon, Hyperomyzus, Macrosiphum, Nasonovia* and *Uroleucon* were most similar to our sequence (94-96% identical). Since the COI sequence from *U. villalobosi* **sp. n.** did not match any sequence available at the DNA barcode reference library, we must conclude that it corresponds to a species not represented in the database, which currently hosts sequences from near 300 species from 70 genera within the subfamily Aphidinae. Moreover, we were also unable to assign the new species to any previously described genus based on the COI sequence as both strict tree-based and best close match assignment criteria (Wilson et al. 2011) were not fulfilled. Phylogenetic reconstructions using a set of available sequences from different Macrosiphini representatives were not informative about the phylogenetic affiliation of *U. villalobosi* **sp. n.**, which grouped with *Delphiniobium hanla*, although with very low support, since they differed at 3.8% of the aligned positions (results not shown). This lack of resolution of COI for phylogeny inference is not surprising and it may be a weak phylogenetic signal compared with other markers as previously reported (Wilson 2010).

For the elongation factor-1 alpha gene fragment (EF1α) we obtained an identical sequence of 910 bp from the 3 analyzed individuals that was deposited in Genbank with accession number KC110894. Using sequences available for EF1α at NCBI for different Macrosiphini species, a Maximum Likelihood tree, which included the sequence obtained for our species, was built (Fig. 4). Contrary to COI, phylogenetic analysis using EF1α did not support the grouping of *U. villalobosi* **sp. n.** with the only *Delphiniobium* sequence available in GenBank (excluded from the final analysis because of its shorter length), but rather it grouped within a highly supported clade formed by 3 groups of sequences. One group contained sequences from species of genera such as *Acyrthosiphon*, *Aulacorthum*, *Macrosiphum*, *Metopolophium* and *Sitobion*. A second group included sequences from *Nasonovia*, *Hyperomyzus* and *Obtusicauda*. A third group contained sequences from *Uroleucon* and *Macrosiphoniella* (see Fig. 4). *U. villalobosi* **sp. n.** occupied a somewhat intermediate position between the first 2 groups of this clade, grouping, albeit with low support, with either of them depending on the precise parameters or the algorithm used in phylogenetic inference.

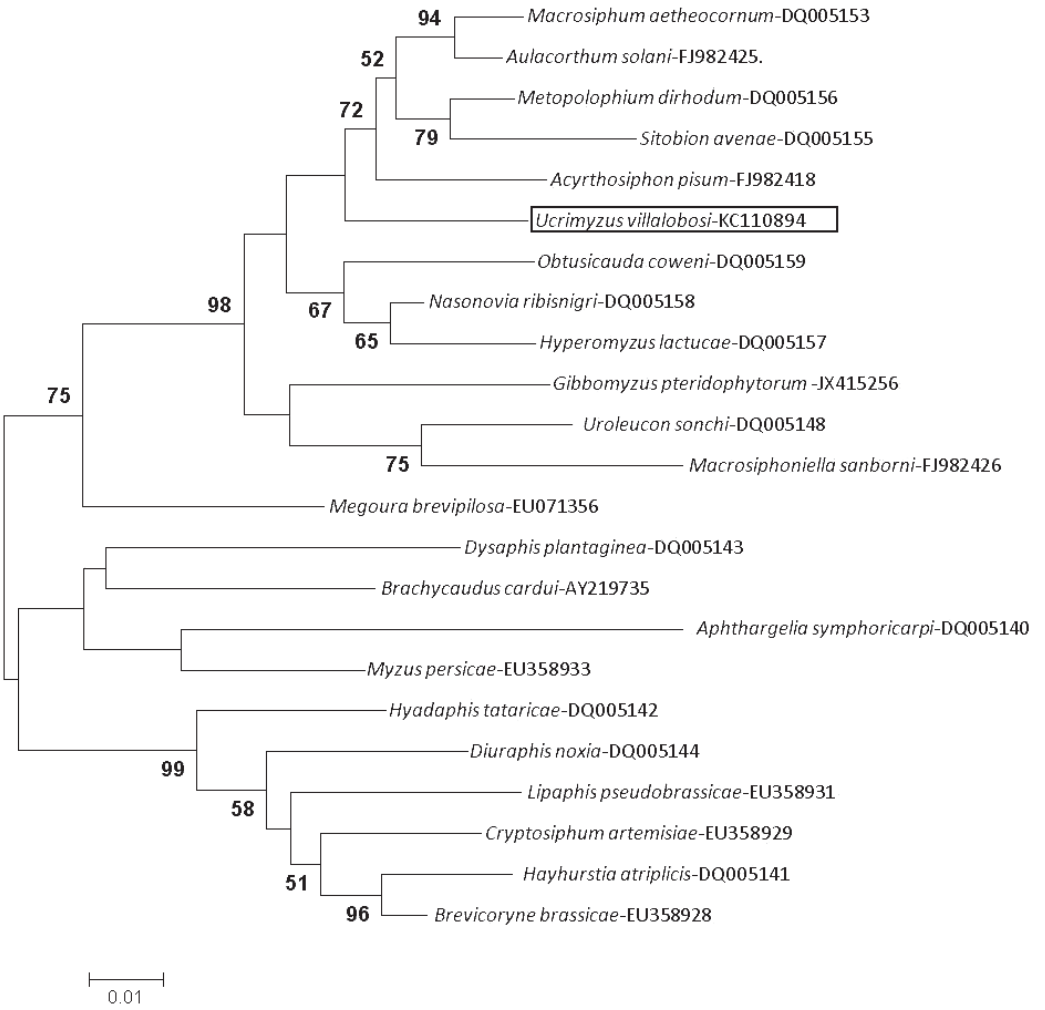

Fig. 4. Maximum Likelihood tree obtained for EF1α sequences from different Macrosophini species including *Ucrimyzus villalobosi* **sp. n.** (highlighted). Sequences from aphid species other than *U. villalobosi* were obtained from the NCBI database and their accession numbers are indicated. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.4096)). The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*], 47.0010% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated leaving a total of 816 positions in the final dataset. Percentage bootstrap support values obtained after 200 replicates are indicated above branches when higher than 50%.

Interestingly, another new species sampled from the same region (Nieto Nafría et al. in press), also grouped within this clade but closer to the third group described above.

Acknowledgments

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ANNEX 1

MODIFICATION OF THE KEY TO APHIDS ON *BIDENS* FOR ADDITION OF *UCRIMYZUS VILLALOBOSI*

10. [without modifications] 11

— [without modifications] 16B

16B. SIPH swollen. Inner faces of ANT tubercles smooth and divergent . . . . *Ucrimyzus villalobosi*

— SIPH tapering, cylindrical or swollen; but **if** swollen, inner faces of ANT tubercles convergent and wrinkled or spinulose 17

KEY TO APHIDS ON *SCHKUHRIA*, FOR ADDITION OF *UCRIMYZUS VILLALOBOSI*

— SIPH cylindrical or tapering with distal polygonal reticulation . . . . . *Uroleucon compositae*

— SIPH swollen without distal polygonal reticulation, only 3 rows of imperfect cells at the most . . . . . *Ucrimyzus villalobosi*

MODIFICATION OF THE KEY TO APHIDS ON *SENECIO* (INCL. *KLEINIA*) FOR ADDITION OF *UCRIMYZUS VILLALOBOSI*

45. [without modification] . . . . . *Indomasonaphis anaphalidis*

— Cauda with 6-10 hairs. ANT III with 1-30 rhinaria. Dorsal hairs short and blunt, shorter than ANT BD III 45B

45B. Prothoracic spiracular apertures no much larger than those on abdomen. Hairs on ABD TERG 8 are 8-20 µm long. Secondary rhinaria only present on ANT III, 1-20. ANT PT/BASE 5.3-8.2. Alatae without dorsoabdominal patch . . . . . *Ucrimyzus villalobosi*

— Prothoracic spiracular apertures much larger than those on abdomen, which are reniform. Hairs on ABB TERG 8 are 8-50 µm, but **if** less than 20, secondary rhinaria distributed on ANT III 11-29, IV (0)1-16, V 0-(9), and ANT PT/BASE 4.3-5.6. Alatae with dorsoabdominal imperfect patch 46

MODIFICATION OF THE KEY TO APHIDS ON *STEVIA* FOR ADDITION OF *UCRIMYZUS VILLALOBOSI*

0. SIPH swollen and darker than cauda. ABD TERG 1 and 7 without marginal tubercles (MTu) 1

— [second proposition of the couplet without number, without modification] . . . . *Aphis solitaria*

1. Tergum with an extensive dark sclerotic shield and transversal sclerotized bands . . . . . *Hyperomyzus niger*

— Tergum pale, without sclerites . . . . . *Ucrimyzus villalobosi*