ALICE CASIRAGHI ^a - DAVID MARTÍNEZ-TORRES ^a - NICOLÁS PÉREZ HIDALGO ^aCONFIRMATION OF THE PRESENCE OF *PACHYPAPPA WARSHAVENSIS* (NASONOV, 1894) [HEMPITERA APHIDIDAE] IN THE IBERIAN PENINSULA^a*Instituto de Biología Integrativa de Sistemas (I²SysBio). Universidad de Valencia - CSIC. Centro Mixto Universidad de Valencia-CSIC (Paterna, Valencia). Spain.*

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Casiraghi A., Martínez-Torres D., Pérez Hidalgo N. – Confirmation of the presence of *Pachypappa warshavensis* (Nasonov, 1894) [Hempitera Aphididae] in the Iberian Peninsula.

The aphid *Pachypappa warshavensis* (Nasonov) (Aphididae: Eriosomatinae: Pemphigini) on its primary host *Populus alba* L. is properly confirmed in the Iberian Peninsula. The species was initially cited as *Asiphum varsoviensis* (Mordvilko, 1835) in Spain, but the absence of specimens in the entomological collections, as well as the alleged limited distribution to the countries of Northern and Eastern Europe, made it removed from the list of the Ibero-Balearic aphids. *P. warshavensis* is a Palaearctic species that is widely distributed in Europe (Belarus, Germany, Hungary, Poland, Romania, Serbia, Spain, Sweden and Ukraine) and Central Asia (Kazakhstan). The identification based on morphological and molecular characters (COI) has been confirmed and the fundatrix and its winged fundatrigenia are described. In addition, phylogenetic relationships based on COI sequences of *P. warshavensis* with other *Pachypappa* species available in the databases are presented.

KEY WORDS: Aphids, COI, *Populus alba*, faunistic studies, Eriosomatinae, Pemphigini.

INTRODUCTION

The genus *Pachypappa* Koch 1856 corresponds to a little aphid taxon from the Pemphigini tribe (Hemiptera: Aphididae: Eriosomatinae), that host alternates between species of *Populus* L. (the primary host) and different secondary hosts, not always known, but frequently being different species of *Picea* Dietrich (five species) or *Salix* L. (two species) (PIKE *et al.*, 2012; BLACKMAN and EASTOP, 2019).

Pachypappa groups 13 species with Holarctic distribution (FAVRET, 2019). Of these, three are Nearctic (Canada and United States), and the rest are widely distributed in the Palaearctic region, from Britain to Japan, or only in a part of it (BLACKMAN and EASTOP, 2019).

Currently, six species are present in several countries of Northern and Central Europe: *P. marsupialis* Koch 1856, *P. myrtilli* Börner 1950 (only in Germany), *P. populi* (Linnaeus 1758), *P. tremulae* (Linnaeus 1761), *P. vesicalis* Koch 1856 and *P. warshavensis* (Nasonov 1894) (NIETO NAFRÍA *et al.*, 2011; BLACKMAN and EASTOP, 2019). The genus is characterized by large fundatrices without waxy plates and alatae spring migrants always having wax plates and forewings generally with a once-branched medial vein (BLACKMAN and EASTOP, 2019).

Like most species of Pemphigini and other Eriosomatinae, their full (holocyclic) life cycles are rather complex and their ability to develop paracycles on subterranean parts of their secondary hosts makes their taxonomy problematic in some species. However, nowadays some of these taxonomic problems can be solved using molecular studies (PIKE *et al.*, 2012).

In early-June 2018 typical pseudogalls made by *Pachypappa* aphid colonies were found on several white poplars in the city of León (Fig. I). A detailed study of the fundatrix, and alatae viviparous females revealed that they

belonged to the aphid genus *Pachypappa*, and more specifically to *Pachypappa warshavensis* (Nasonov), a species that was mentioned for the first time in the Iberian Peninsula by GÓMEZ-MENOR and NIETO (1977) as *Asiphum varsoviensis* (Mordvilko, 1835), currently synonym of *P. warshavensis* (Nasonov, 1894). However, the absence of specimens of this sample in the entomological collections that could confirm it, the null results after the efforts made for its location, as well as its limited distribution to the countries of northern and eastern Europe, made think to PÉREZ HIDALGO and NIETO NAFRÍA (2003a) that this species was not present in Spain and that it could be an identification error.

Our records, therefore confirm definitively the presence of this species of Pemphigini in the Iberian Peninsula.

MATERIALS AND METHODS

MATERIAL COLLECTED

Aphids were collected on 4-vi-2018 from various plants of *Populus alba* L. located between coordinates 42.610522, -5.590680 and 42.605037, -5.586273, in a green area on the left bank of the Bernesga river, along a path in the city of León. Half of the sampled aphids were put in 100% ethanol for molecular analysis and the other half in 70% ethanol solution for morphological studies. The aphids (sample references: 1850 and 1779) and the DNA were deposited in the aphidological collection of the Institute for Integrative Systems Biology (Mixt Center of University of Valencia and CSIC).

MORPHOLOGICAL STUDY

Subsequently, the individuals in 70% ethanol were used to mount microscope slides following the methodology

Fig. 1 – Several colonies of *Pachypappa warshavensis* (Nasonov) on *Populus alba*.

described by NIETO NAFRÍA and MIER DURANTE (1998). Identification at the species-level was carried out using quantitative and qualitative characters, following the identification keys of HEIE (1980) and BLACKMAN and EASTOP (2019). A camera lucida fitted to the microscope was used for the drawings and the microphotographs were taken with a Leica DC digital camera with IM 1000 version 1.10 software.

DNA EXTRACTION AND PCR AMPLIFICATION

The specimens conserved in 100% ethanol were used in DNA extraction following the HotSHOT method (Hot Sodium Hydroxide and Tris) (TRUETT *et al.*, 2000). In a second step, a 710 bp fragment of the 5' region of the mitochondrial cytochrome c oxidase subunit 1 (COI) was amplified using primers LCO1490 and HCO2198 (FOLMER *et al.*, 1994). 3 µl of the extracted DNA was used for every amplification. PCR conditions 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.

SEQUENCING AND ANALYSIS OF DNA SEQUENCES

PCR products were purified by ammonium precipitation and reconstituted in 10 µL of LTE buffer (10mM Tris, 0.1mM EDTA). Sequencing of the amplified fragments was done using PCR primers and conducted using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) following the manufacturer's instructions.

Samples were loaded onto an ABI 3700 automated sequencer. Sequences corresponding to each sample were assembled using Staden package v2.0.0 (STADEN *et al.*, 1998). Multiple alignments were carried out with Clustal X v1.81 (THOMPSON *et al.*, 2002) with gap opening and gap extension penalties of, respectively, 10.0 and 0.2, and then manually revised. MEGA7 (KUMAR *et al.*, 2016) was used to align sequences and to do the phylogenetic analysis. The Neighbor-joining algorithm on Kimura 2-parameters distances was used to build a phylogenetic tree including several *Pachypappa* sequences available at NCBI (accession numbers MG508635.1, MF830952.1, KR029847.1 and GU679671.1) along with the sequence of *P. warshavensis* here reported. Node support was obtained after 500 bootstrap replicates. The sequence of the species *Mordwilkoja vagabunda* (Walsh 1863) was used as outgroup (accession number KR043679.1).

RESULTS

MORPHOLOGICAL DATA

The analysis of qualitative and quantitative (metric and meristic) characters of the specimens available (one fundatrix and hundreds of fundatrigeniae viviparous females) confirmed that they belong to the genus *Pachypappa*, because the fundatrix lacks waxy plates and the fundatrigeniae viviparous females have oval primary rhinaria surrounded by distinct rings of short hairs and small

siphuncular pores, with a diameter shorter than 2.3 times the articular diameter of antennal segment III (HEIE, 1980). The association with *Populus alba* and the presence of secondary sensoria with thick sclerotic rims on the distal half of the antennal segment III (Fig. II, 1), among other characters, identified it as *P. warshavensis* (Nasonov) (HEIE, 1980; BLACKMAN and EASTOP, 2019).

The only one **fundatrix** caught is large (4.34 mm) but with short appendages (antenna of 0.6 mm and 0.14 times the body length and hind tibia 0.62 mm and 0.14 times the body length). In life, it is reddish-brown and lacks waxy plates and siphunculi. The antenna (Figs. II, 2; III, 2) has five segments without secondary sensoria and the antennal segment III is 0.64 times the rest of antennal flagellum and the terminal process is 0.09 times the part basal of the last antennal segment (*D*). The rostrum is around 0.77 mm and the apical rostral segment is 0.19 mm, 1.32 times its basal width and 0.9 times the segment of the hind tarsus. The cauda is rounded and short (0.09 mm and 0.38 times its width). The anal plate has a large number of setae (around 35) and the genital plate have 14 on the anterior part and 25 on posterior margin (Fig. III, 3). The setae on the vertex is around 0.09 mm and 2.73 times the articular diameter of antennal segment III. The spinal setae on abdominal segment III is 0.036 mm and the marginal of the same segment is 0.042 mm (1.09 and 1.27 times *D*, respectively) and the setae on abdominal segment VIII is 0.093 mm and 2.82 times *D*.

The **alate fundatrigenia females** (measurements based on 20 specimens) are large (2.9-3.84 mm), with wings of 3.69 mm and 2.88 times its maximal width and with the medial vein variable: unbranched (14% of 100 studied specimens) (Fig. IV, 1) or with a fork, short (7%) (Fig. IV, 2) or quite big (75%) (Fig. IV, 3), even different medial vein phenotypes can appear in the same individual. Colour in life

Fig. II – Antenna of the fundatrigenia alata (1), with detail of secondary sensoria on antennal segment III and primary sensoria of the last antennal segment, and antenna of the fundatrix (2) of *Pachypappa warshavensis* (Nasonov).

Fig. III – Fundatrix of *Pachypappa warshavensis* (Nasonov): habitus (1) and detail of the anterior (2) and posterior (3) part of the body.

Fig. IV – Forewings of several fundatrigeniae alatae of *Pachypappa warshavensis* (Nasonov) with medial vein variable: unbranched (1), with a short (2) or big (3) fork (the arrows indicate the branches of the medial vein).

is reddish-brown (see also http://influentialpoints.com/Gallery/Pachypappa_aphids.htm) and in slide, they have brown head, thorax, legs, spiracular sclerites and cauda, anal and genital plates and spinal sclerotization on abdominal segments IV to VIII (Fig. V).

The **antennae** are 1.05-1.17 mm, and 0.30-0.37 times the body length (Fig. V, 1). The antennal segment III is 0.31-0.36 mm and have 3 to 7 secondary sensoria located in 68% of the distal part of the segment (Fig. II, 1), the IV is 0.16-0.19 mm and have from 1 to 4 secondary sensoria, the V is 0.18-0.23 mm and have until 3 secondary sensoria, and the VI is 0.22-0.26 mm and without secondary sensoria; the terminal process is 0.13-0.20 times the basal part of the last segment and the diameter of the primary sensoria on antennal segment VI is 0.02-0.05 mm. The antennal segment III have 5 to 12 setae that are 1.22-2.14 times the articular diameter of the same article (D). The rostrum is 0.69-0.77 mm and its apical rostral segment is 0.17-0.20 mm, 1.51-2.56 times its basal width and 0.59-0.68 times the segment of the hind tarsus; and have two accessory hairs. The siphunculi pores are very small, around 0.04-0.05 mm and 0.60-0.89 times the diameter in the middle of the hind tibia. The cauda is rounded and short (1.1-2.10 mm and 0.20-0.41 times its width) and have 16 to 20 setae, some of which are longer and thicker (Fig. V, 3). The anal plate shows a variable number of setae, from 28 to 48, while the genital plate has around 18-27 (with 6 to 11 on anterior half), with the setae of anterior part slightly longer and

Fig. V – Fundatrigenia alata of *Pachypappa warshavensis* (Nasonov): habitus (1), abdomen (2) and dorsal (3) and ventral (4) view of the posterior part of the body.

thicker (Fig. V, 4). The hind legs have femora of 0.32-0.45 mm and tibiae of 0.48-0.57 mm and 0.09-0.14 and 0.14-0.17 times the body length, respectively; and the setae of hind tibia are 0.05-0.08 mm and 2.00-2.35 times *D*. Formula tarsal 2.2.2. The wax plates are absent on the head and present on the prothorax and metathorax (a spinal pair and another marginal), mesothorax (one pair spinal) and on all abdominal segments (a spinal pair on abdominal segments I to VIII and a marginal pair on the segments I to VII, that in the latter can be divided) (Fig. V, 2). The body setae are pointed and large: on frons are 0.05-0.09 mm (2.25-3.43 times *D*), the spinal setae on the abdominal segment III are 0.11-0.15 mm (3.89-6.71 times *D*) and the marginal of the same segment are 0.10-0.13 mm (3.56-6.00 times *D*), while the ventral ones are more delicate and a little shorter (0.08-0.15 mm and 3.25-6.25 times *D*) and the setae on the abdominal segment VIII (4 to 10) are 0.18-0.21 mm and 6.67-8.71 times *D*. Nine specimens were dissected to count the embryos, finding a range between 105-269, with a mean value of 200 embryos.

MOLECULAR DATA

The phylogenetic tree including other *Pachypappa* species built using molecular data corresponding to a partial sequence of the mtDNA gene encoding the subunit I of the cytochrome oxidase (COI) confirmed that the specimens belong to the *Pachypappa* genus (Fig. VI).

BIOLOGY AND DISTRIBUTION

Pachypappa warshavensis shows mainly *P. alba* as primary host, although there are also records on *P. canescens*, *P. euphratica* and *P. pruinosa* (SHAPOSHNIKOV, 1964; CARTER and DANIELSSON, 1993) and there is a record on the Nearctic *Populus tremuloides* Michx. (POLJAKOVIĆ-PAJNIK, 2006) in Serbia, that must be confirmed. According to SZELEGIEWICZ (1982), apterae recorded on roots of *Salix caprea* L. could be assigned to this species. Also, CARTER and DANIELSSON (1993) tentatively associate *P. warshavensis* to adult apterae and alatoid nymphs found around roots of *Populus canescens* in Wales (in October) and on fine roots in England (in June), but these both relations need experimental transfers and molecular studies.

Pachypappa warshavensis is a Palearctic species that is

widely distributed in Europe (Belarus, Germany, Hungary, Poland, Romania, Serbia, Sweden and Ukraine) and more recently was recorded in Central Asia (Kazakhstan) (HOLMAN, 2009; KADYRBEKOV, 2011). Several authors (GÓMEZ-MENOR and NIETO, 1977) and later HEIE (1980) mentioned *P. warshavensis* in Spain but PÉREZ HIDALGO and NIETO NAFRÍA (2003a,b) not included this species in the review of the Iberian Eriosomatinae because they did not find specimens to corroborate this record in any aphidological collection. Our record confirms the presence of this species in the Northwest of Spain (province of León) and allows us to think that it is also present in the province of Madrid according GÓMEZ-MENOR and NIETO (1977).

DISCUSSION

The species of genus *Pachypappa* present complex cycles with up to six very different morphs (fundatrix, fundatrigenia alata, virginogenia aptera, sexupara, ovipara and male). As a result, not all morphs have been correctly described for every species and some of them are not even known (PIKE *et al.*, 2011; BLACKMAN and EASTOP, 2019). In the case of *P. warshavensis* there are descriptions of fundatrix and fundatrigenia alata (HEIE, 1980) made with few specimens and characters (SZELEGIEWICZ, 1982), which sometimes generates problems when identifying the known forms of the different species.

Our morphological identification was carried out using the keys for fundatrix of HEIE (1980) and the keys to identify alatae of HEIE (1980) and BLACKMAN and EASTOP (2019). In these last keys, they use the medial vein parameter (once-branched or unbranched vein) to separate the genus *Gootiella* Tullgren and to discriminate *Pachypappa* at a specific level. In particular, the last operation results problematic, because the polymorphism of the medial vein (Fig. V), which can have a big branch, a small branch or be unbranched and also because every individual can show different polymorphism at the same time, one on each forewing (see description of fundatrigenia alata).

Also in the identification, we even considered the description of *P. warshavensis* of HEIE (1980) and we

Fig. VI – Neighbour-joining phylogenetic tree (COI) from sequences of species of *Pachypappa*.

noticed that our fundatrix is slightly bigger (4.34 mm) than the fundatrix presented by him (4 mm). But, in any case, the number of the hairs can clearly separate the fundatrix of *P. warshavensis* (Fig. II) of the fundatrix of *P. tremulae* (see figure A in plate I in STROYAN, 1975).

Other problems showed up with the molecular analysis because sequences of *Pachypappa warshavensis* are not present in NCBI and we did not find specific matches using BLAST.

Although the species of the subfamily Eriosomatinae are among the first species of aphids mentioned in the Iberian Peninsula (PÉREZ HIDALGO and NIETO NAFRÍA, 2003b), some of them are difficult to locate although they live on very abundant and frequent plants, such as different species of *Populus*, or because they form colonies in roots of their secondary hosts.

Currently, the lack of aphidologists and faunistic studies means that there are large gaps in the known distribution of many species. However, the recent fondness for photography has helped in meeting and citing some species of aphids, apparently scarce, by naturalists or photography enthusiasts, as is the case of *Plocamaphis flocculosa* (Weed) a boreal species detected recently in the Iberian Peninsula (PÉREZ HIDALGO et al., 2016).

The known distribution of *Pachypappa* species and their chorology suggested aphidologists that their presence in territories of the Iberian Peninsula was unlikely since they were assumed to be boreal species (PÉREZ HIDALGO and NIETO NAFRÍA, 2003a).

Pachypappa warshavensis is widely distributed in Europe on *Populus alba* and probably also in the Iberian Peninsula. For this reason, we consider appropriate and useful the contribution that citizen science can give (WHEELER and VALDECASAS, 2010). Allowing naturalists to search and document the presence of organisms and interacting with experts to obtain information without excessive effort, may thus establish a beneficial symbiosis for all players in this game of knowledge enrichment (HAND, 2010; GURA, 2013).

Pachypappa species are recorded as important pests for both deciduous (as *Populus*) or evergreen (as *Picea*) forest trees (BARJADZE and JAPOSHVILI 2007). In particular, spruces can suffer from aphids attacks: for example, damages to spruce seedlings, some very severe, have been documented in nurseries in Canada (LAVALLÉE, 1987). Targeted plants appear severely affected, with gradually yellowing of needles and sometimes even needles falling. The genus can even affect the Norway spruce, grown for Christmas market (ALFORD, 1991). In Europe, *Pachypappa vesicalis* is considered as pest (WATT et al., 1997) and its presence is well established in spruce plantations in Britain, on trees of all ages (CARTER and DANIELSSON, 1991). *P. warshavensis* is recorded as pest of aspen with *P. marsupialis* in Georgian urban habitats (BARJADZE and JAPOSHVILI, 2007), but the commercial interest in *Populus* spp. is not relevant.

At the moment the genus *Pachypappa* is under control and causes very little damage (SUTHERLAND and VAN EERDEN, 1980; WONG, 1982; EL-SAYED, 2019) but we lack information about this aphid group as disease vector for plants and at the same time we know little about its natural predators and pathogens that can be used in its biological control. Experiments were carried out only for another species of Pemphigini, for example, *Pemphigus betae* Doane, and never with *Pachypappa* (LAVALLÉE, 1987; SWAN and PAPP, 1972; HARPER, 1961). More investigations are required to understand the possible risks for the agroforest business.

ACKNOWLEDGEMENTS

The authors are very grateful to the reviewers of the manuscript, to Barbara Osiadacz (Poznań University of Life Sciences, Poznań, Poland) and Andrey Stekolshchikov (Zoological Institute of Russian Academy of Science, Saint Petersburg, Russia) for facilitating the publications of N.V. Nasonov, A. Mordvilko and H. Szelegiewicz about *Pachypappa warshavensis* and to Francesca Maria Gatti (Civica Scuola Interpreti e Traduttori Altiero Spinelli, Milan, Italy) for the translation of articles in Russian. The investigation was conducted in the context of Project CGL2015-68188-P, funded by “Ministerio de Economía, Industria y Competitividad” of Spain (MIMECO).

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120 - Blank Page