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## ENTOMOLOGY

# Notes on the Pemphigine aphid *Pachypappa warshavensis* (Nasonov) (Hemiptera Aphididae) detected on *Populus alba* Linnaeus in Italy

Alberto Bombieri,<sup>1</sup> Michele Ricupero,<sup>2</sup> Daniele Rizza,<sup>1</sup> Franco Picco,<sup>1</sup> Pier Mario Chiarabaglio,<sup>1</sup> Antonio Gugliuzzo,<sup>2</sup> Salvatore Nucifora<sup>2</sup>

<sup>1</sup>CREA, Council for Agricultural Research and Economics, Research Centre for Forestry and Wood, Casale Monferrato; <sup>2</sup>Department of Agriculture, Food and Environment, University of Catania, Italy

## Abstract

Concurrent with the first record of the aphid *Pachypappa warshavensis* (Nasonov, 1894) from mainland Italy in the Emilia-Romagna region, colonies were also identified on ornamental white poplar trees (*Populus alba* L.) in a public park in Casale Monferrato (Piedmont region) during 2025. Species identity was confirmed by morphological features and molecular characterization using a partial *COI* mitochondrial gene. The described identity of the insect provides some distinctive biometric data of the sampled population. Our observations extend the known distribution of the species across various European countries. The aphid, belonging to the tribe Pemphigini within the subfamily Eriosomatinae, is an oligophagous taxon with species of *Populus* as primary hosts. The present study provides an overview of the aphid morphobiological profile to aid its identification, along with an updated list of potential host plants and phytosanitary remarks.

**Key words:** aphid morphology, barcoding, forest pest, insect life cycle, poplar tree.

Correspondence to: Michele Ricupero, Department of Agriculture, Food and Environment, University of Catania, Italy.  
Tel.: +39 095 7147406. E-mail: michele.ricupero@unict.it

## Introduction

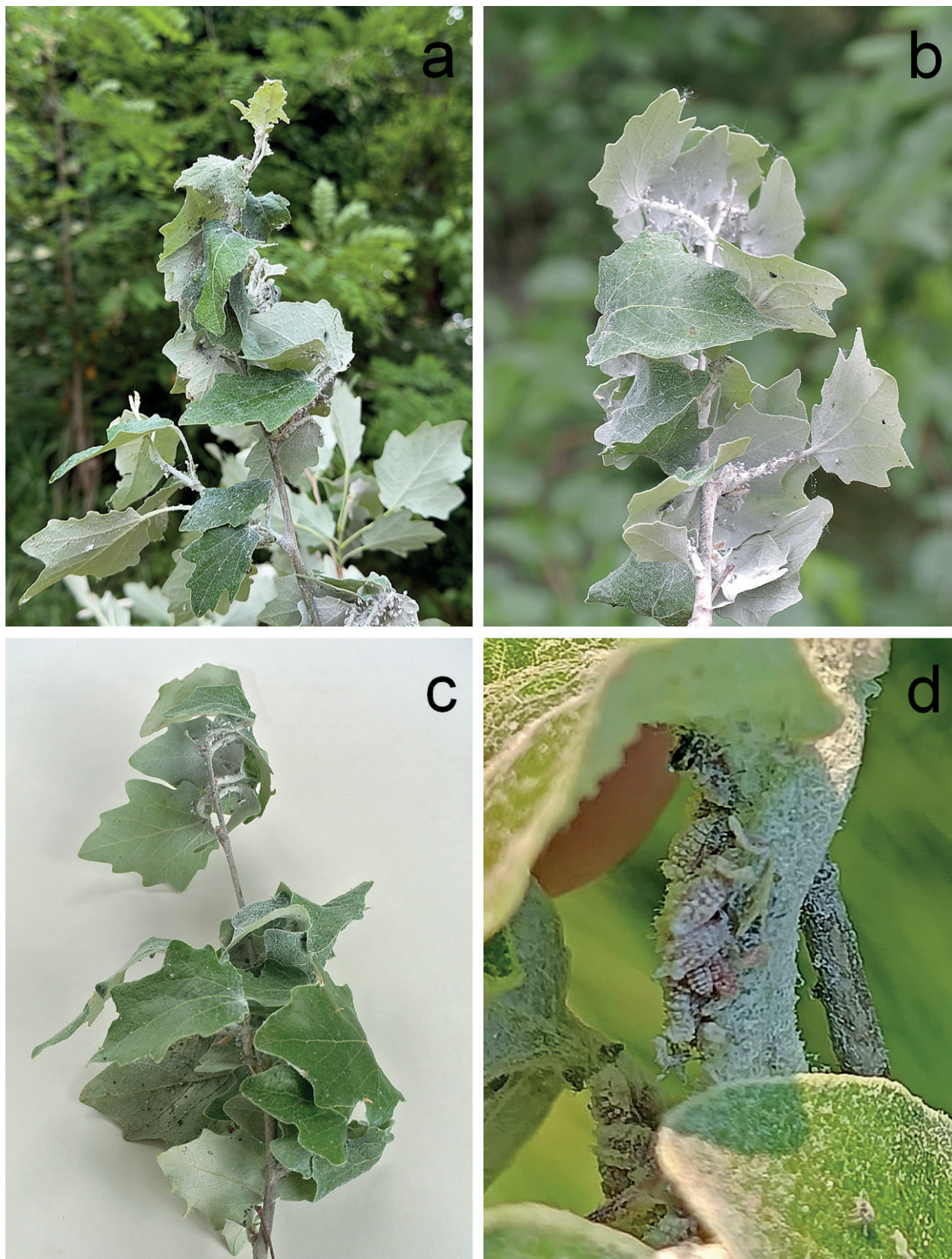
Insect pests can represent one of the most critical threats to forest health and stability worldwide, often driving substantial ecological and economic losses across diverse ecosystems (Jactel *et al.*, 2021; Brockerhoff *et al.*, 2023). The exponential increase in global trade and climate change are providing unprecedented pathways for the introduction and establishment of forest pests in new environments, where they can rapidly spread and cause serious damage to susceptible hosts (Liebhold *et al.*, 2023; Nahrung *et al.*, 2023).

In late spring 2025, the presence of a small number of leaf-nests (pseudogalls) likely caused by a Pemphigine aphid infestation, was detected on some cultivated white poplar trees in a public park, named 'Parco della Cittadella', in Casale Monferrato (Piedmont region) in NW Italy (45°07'44" N, 8°27'41" E, and 45°07'43" N, 8°27'42" E) (Figure 1).

Colonies of the aphid, belonging to the *Pachypappa* genus of the Pemphigine systematic group, were thus collected for further observations. Several alate specimens were obtained from these colonies, a sample of which was subsequently sent to Prof. S.

Barbagallo (University of Catania, Italy), who identified them as alate viviparous emigrants of *Pachypappa warshavensis* (Nasonov, 1894) (Aphididae, Eriosomatinae, Pemphigini). Subsequent molecular analysis confirmed the aphid identity.

*P. warshavensis* is a Eurasian aphid species already recorded in several European countries. During the review process of this article, a short report on the first record of this species from mainland Italy was published (Casiraghi *et al.*, 2025). Therefore, the presence of *P. warshavensis* in Italy is corroborated by the simultaneous detection of colonies on *Populus alba* in two Italian regions (Emilia-Romagna and Piedmont). Despite its currently sporadic presence in the investigated location, *P. warshavensis* can pose a potential threat to poplar and other host trees, as known for related species (Danielsson, 1990; Carter and Danielsson, 1993). Its feeding activity leads to the formation of characteristic leaf-nests or pseudogalls, where leaves are tightly curled and stunted. The species exhibits a holocyclic life cycle with host alternation, but key aspects remain to be fully understood, particularly the identity of secondary hosts, which is often unconfirmed in literature, as for other species of *Pachypappa*, though it is hypothesized to be some species of willows (*Salix* spp.) or even species of *Populus*. (Szelegiewicz, 1982; Favret *et al.*, 2026). The aphid holocycle involves, on species of *Populus*, the fundatrix



**Figure 1.** Different aspect of pseudogalls produced by *P. warshavensis* on shoots of *P. alba* (a-c) with a detail on a group of alate emigrant nymphs on a leaf petiole (d).

and a second generation with winged forms (alate viviparous females) from which alienicolae morphs develop on roots of the secondary host during summer, and which sexuparae migrate back to the primary host in autumn. These then give rise to the sexual generation, in which amphigonice females lay their overwintering eggs.

The discovery of *P. warshavensis* in Italy provides crucial data on its geographical distribution. Here, brief notes on its identification, together with a list of host plants, are presented. Moreover, we provide considerations on the aphid life cycle and its phytosanitary relevance. Further studies are needed to fully clarify its life cycle and ecological interactions in the newly invaded area.

## Materials and Methods

### Morphological identification

Specimens of *P. warshavensis* (i.e., alate viviparous females and their nymphs) used for this report and collected in the previously indicated locality on May 22, 2025, were temporarily stored in 70% ethanol and then clarified and mounted on permanent microscopic slides, using Canada balsam as a medium. We followed the standard technique currently recommended for aphids and other soft-body insects (Favret *et al.*, 2026). Field photographs of infested poplar shoots were taken using a Nikon D100 Camera. Microscopic pictures of the aphid morphological structures were taken using a Zeiss Axiolab 5 microscope, equipped with a microscopy Zeiss Axiocam 208 color camera, and a software ZENcore version 3.11.106.0500 Carl Zeiss microscopy GmbH; the same equipment was also used to record biometric data of the studied population from a selection of 20 specimens mounted on slides (as indicated above).

### Molecular characterization

Molecular characterization of collected aphids was performed by sequencing the amplified mitochondrial cytochrome C oxidase subunit I (*mtCOI*) gene fragment as follows. DNA was isolated from several specimens of *P. warshavensis* using the E.Z.N.A.<sup>®</sup> Tissue DNA Kit (Omega Bio-tek, Inc., Norcross, GA, USA). A destructive DNA extraction protocol was followed to allow morphological identification of the processed specimens. Samples of positive DNA control and a negative control without DNA were also included in the extrac-

tion. Universal Folmer's primer pairs LCO1490 (5'-GGTCAA-CAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTT CAGGGTGACCAAAAAATCA-3') were used to amplify the expected ≈630 bp of the *mtCOI* target region (Folmer *et al.*, 1994). Polymerase chain reaction (PCR) was performed according to the protocol proposed by Ricupero *et al.* (2021). Reactions were performed in 20 μL volumes with 0.85X FailSafe™ PCR 2X PreMix F (Lucigen Corporation, Middleton, WI, USA), 0.5 μM of each primer 10 μM, 1.5 U Taq DNA Polymerase 5U (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA), and 2 μL DNA template. Cycling conditions were as follows: 96°C for 5 min, 35 cycles at 96°C for 45 s, 45°C for 1 min, 72°C for 1 min, followed by a final cycle at 72°C for 10 min. Reactions and cycling conditions were carried out in Applied Biosystems™ MiniAmp™ Plus Thermal Cycler (Thermo Fisher Scientific, Waltham, USA). PCR products were first checked by electrophoresis using a 1% agarose gel, then they were shipped to BMR Genomics (Padova, Italy) for purification and sequencing using Sanger's method. The coding regions were manually checked for errors, trimmed for low quality in Unipro UGENE version 1.26.1. Resulting FASTA files were aligned to reference sequences from the National Center for Biotechnology Information (NCBI) GenBank<sup>®</sup> using the Basic Local Alignment Search Tool (BLAST) sequence analysis tool for species identification. Sequences were deposited in GenBank under accession number: PX452162.

## Results and Discussion

### Morphological features

The aphid morphs so far known and described in the literature are the fundatrix and the alate viviparous female (emigrant morph). A short description of the former is given by Heie (1980) and, in more recent years, by Casiraghi *et al.* (2019), who also give a detailed description and illustrations of the above-named alate morph (as alate fundatrigenia). For further color illustrations on the aphid infestation on White Poplar, see also "InfluentialPoints" (2025).

The alate emigrant is currently the only morph available to us and, to facilitate its identification, brief morphological data are here reported (Table 1), mostly based on local population specimens, unless otherwise indicated. Its body is 2.80-3.20 mm in length and

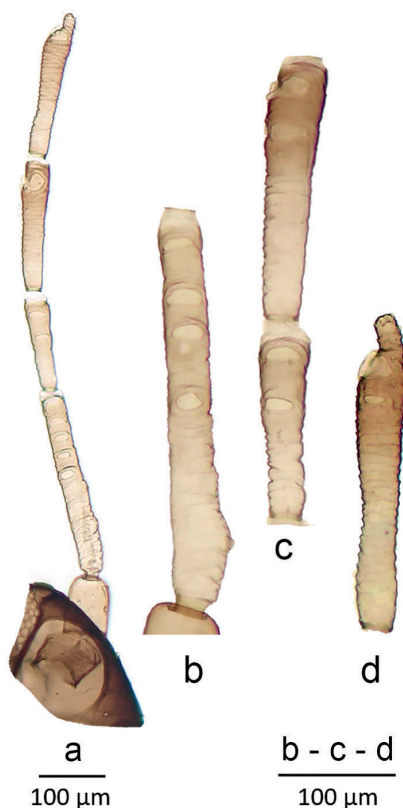
**Table 1.** Biometric data of *Pachyappia warshavensis*, alate viviparous emigrant. From a representative selection of specimens studied. All specimens collected on *Populus alba*, May 22, 2025, at Casale Monferrato (Italy).

Spec. no.	Body length	Ant. length	Antennal segments length				No. sec. rhinaria				Last r. segm.		II h. t. length	No. subg. pl. setae	No. caud. setae
			III	IV	V	VI	III	IV	V	VI	length	suppl. se.			
1	2.85	1.06	0.30	0.15	0.20	0.19+0.03	4/5	2/2	1/1	1/1	0.15	2	0.24	7 + 12	11
2	2.80	1.10	0.32	0.18	0.20	0.20+0.03	6/5	3/2	0/2	0/0	0.16	0 ?	0.25	9 + 11	11
3	2.89	1.02	0.29	0.14	0.20	0.20+0.02	4/6	2/1	1/1	0/0	0.15	2	0.24	7 + 13	13
4	2.98	1.08	0.32	0.13	0.22	0.22+0.04	6/5	2/-	1/3	1/-	0.16	0	0.26	7 + 9	14
5	2.90	1.02	0.30	0.16	0.17	0.20+0.03	5/5	2/1	1/0	0/0	0.15	1	0.24	10 + 11	14
6	2.95	1.03	0.27	0.14	0.20	0.20+0.03	5/-	2/2	1/1	0/0	0.15	1	0.23	11 + 11	16
7	2.84	1.04	0.30	0.14	0.21	0.19+0.03	4/5	2/1	1/1	0/-	0.14	0 ?	0.25	10 + 12	13
8	2.84	1.02	0.30	0.14	0.19	0.21+0.03	6/5	2/2	1/0	0/0	0.16	2	0.23	11 + 14	12
9	2.99	1.14	0.34	0.15	0.22	0.21+0.03	5/6	1/-	0/-	0/-	0.16	1	0.26	-	12
10	3.13	1.08	0.29	0.16	0.21	0.22+0.03	6/5	2/3	0/0	0/0	0.15	0	0.23	9 + 13	11
11	2.77	1.05	0.30	0.14	0.20	0.21+0.03	5/5	2/3	1/0	0/0	0.15	0	0.23	10 + 14	13
12	3.15	1.05	0.30	0.15	0.20	0.20+0.03	5/-	2/-	1/-	0/-	0.14	2	0.24	9 + 11	14

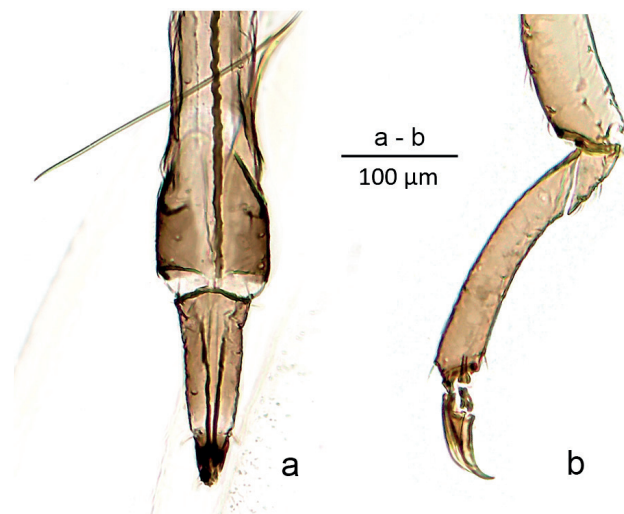
Spec. no., specimen number; Ant. length, antennal length; No. sec. rhinaria, number of secondary rhinaria (of right and left antennae); Last r. segm., last rostral segment; suppl. se., supplementary setae; II h. t., second hind tarsomer; No. subg. pl. setae, number of subgenital plate setae (discal and posterior); No. caud. setae, number of caudal setae; ?, uncertain data. The length of the sixth segment is divided into two parts: the basal part and the processus terminalis.

orange-brownish in color, covered by a dense whitish wax secretion in life. *Antennae* (Figure 2) 6-segmented, 0.32-0.40 of body length and having a short processus terminalis, as usual in Pemphigine aphids, being 0.12-0.18 of the basal part of the VI segment. Antennal segment III slightly imbricated and bearing 4-6 transversally oval secondary rhinaria, which are usually confined to its distal half or slightly more extended (up to 0.62-0.65%). Antennal segments IV-VI with transverse rows of fine denticles progressively more imbricated compared to the III segment. Joint IV and V bear as well, on their distal part, 1-3 and 0-3 secondary rhinaria respectively; while these latter are usually absent on the VI segment, except for two specimens out of the 20 examined, which show a distal secondary rhinarium, just near the primary one on the same segment. All secondary rhinaria have a rather thick peripheral border, which is finely fimbriated, whereas the primary rhinaria on the V and VI joints are more or less roundish in shape or occasionally lobed and are shortly ciliated. *Rostrum* 0.74-0.86 mm long and just passing at rest, the middle coxae. Its ultimate segment (Figure 3) has a conical shape with straight lateral margins and a subdistal ventral pale area and bears 0-2 supplementary hairs; it is 0.14-0.17 mm in length or 2.55-3.00 times its basal width and 0.55-0.77 (more frequently 0.58-0.66) the length of the II hind tarsomer (including the unguiferus). *Forewing* having a hyaline and hairless membrane with the median vein sometimes unbranched [up to 14% of specimens, as quoted by Casiraghi *et al.* (2019)] or more frequently once forked, with its two distal arms of variable length, but shorter than the common basal one (for wing figures see: Szelegiewicz, 1982; Casiraghi *et al.*, 2019). *Siphuncular pores* hardly visible and located just anteriorly the VI

pair of stigmal plates. *Abdomen* (Figure 4) with a full set of wax gland plates along the marginal and spinal lines of its urites (Heie, 1980). *Cauda* largely rounded, about 4-5 times its length and bearing 10-14 hairs of variable length. *Genital plate* having 7-11 hairs on its anterior half and an additional 9-14 hairs along its posterior margin.



**Figure 2.** Antennal structure of *Pachypappa warshavensis* (alate viviparous emigrant): (a) general antennal shape; (b-d) antennal segments III-VI at a higher magnification (note the presence here of a small secondary rhinaria on sixth segment).



**Figure 3.** Distal parts of rostrum (a) and hind tibia with tarsum (b) of *Pachypappa warshavensis*, alate viviparous emigrant.



**Figure 4.** Distal part of abdomen (a) and genital plate (b) at higher magnification of *Pachypappa warshavensis*, alate viviparous emigrants.

## Comparative morphological differences from other similar aphid species

Among the five known European species of *Pachypappa* living on species of *Populus* (four of which are at present also recorded to Italy), the most similar to *P. warshavensis* is *P. tremulae* (L.), having *Populus tremula* as the primary host plant. This latter species can be separated as emigrant alate viviparous female from the former, by the shorter last rostral segment (being 0.45-0.55 the length of the II hind tarsomer), which usually is without supplementary hairs (though rarely 1-3 hairs can be present); in addition the subgenital plate of *P. tremulae* bears comparatively a higher number of setae (21-32) along its posterior margin (Stroyan, 1975).

Looking at the remaining extra-European species, the Nearctic *P. rosettei* (Maxon), living in North America on *Populus tremuloides*, is also morphologically similar to *P. warshavensis*. That aphid species can be distinguished from the latter for the ratio of last rostral joint to the second hind tarsomer (which is again 0.45-0.55 as in *P. tremulae*), and because the same segment usually bears up to four supplementary hairs, in addition to a few other differences.

Furthermore, it is perhaps useful to consider that another species, *P. vesicalis* Kock, is also known to colonize the main primary host plants of *P. warshavensis* (i.e., *P. alba* and *P. × canescens*). That species can be distinguished from the latter one by several morphological features, such as the presence of setae on the fore-wing membrane, the more developed siphuncular pores, the more haired cauda and other additional differences (Heie, 1980; Favret *et al.*, 2026); the pseudogalls produced on those plants by the two aphid species have also a different shape (Carter and Danielsson, 1993).

Further species of *Pachypappa* recorded on species of *Populus*, as well as other taxa belonging to closely related genera, such as *Pachypappella lactea* (Tullgren) and *Gootiella tremulae* Tullgren, both infesting *P. tremula*, differ from *P. warshavensis* for several morphological features, as shown in the identification keys on alate viviparous females reported by Favret *et al.* (2026), and the accurate morphological descriptions given by Heie (1980).

## Molecular inferences

Morphological identification of *P. warshavensis* was confirmed by amplifying a *mtCOI* fragment from genomic DNA, followed by direct sequencing and BLAST analysis. The obtained sequences were aligned with reference sequences from NCBI and compared with publicly available GenBank data, showing 100% identity and an E-value=0 with *P. warshavensis* isolates from Spain (MK936318.1). The same accession can be useful in determining the specific identity of alien and amphigonic forms if later found in the habitat where the aphid was discovered.

## Life cycle, host plants and current distribution

*P. warshavensis* is undoubtedly a holocyclic aphid species, because its life cycle begins annually with a fundatrix, which gives rise to a consistent generation of alate viviparous females; the latter leave the poplar host plants in search of the secondary host plant(s). Thus, the aphid primary host plants are mainly represented by *P. alba* L. and *P. × canescens* (Aiton) Sm., a stabilized hybrid taxon, the latter originating from *P. alba* × *P. tremula*, where it produces leaf-nests similar to those of *P. tremulae* (Heie, 1980). Also, *P.*

*euphratica* Oliver and *P. pruinosa* Schrenk are reported as primary hosts of the same aphid (Favret *et al.*, 2026). Other records on primary host plants of the same aphid concern *P. × canadensis* Moench; this latter is a well-known hybrid taxon of forestry economic importance, derived from *P. nigra* × *P. deltoides*, and therefore unlikely to really serve as a good host plant for the relevant aphid species. Another host plant reported for the same aphid (Shaposhnikov, 1964, as *Asiphum varsoviensis* Mordv.) is *P. × hybrida*, which is indeed inconsistent in terms of botanical taxon, as following the two different authors who proposed this binomial name, it is cited as a synonym for *P. × canescens* or even *P. alba* (POWO, 2025).

Secondary host plants of the aphid, following field observations by several researchers (Szelegiewicz, 1982; Danielsson, 1990; Carter and Danielsson, 1993; Favret *et al.*, 2026), have been recorded as species of *Salix* (*S. caprea*, *S. silesiaca*) or *Populus* × *canescens*, on whose roots (or nearby roots) apterous alienicolae and (on one occasion) alate sexuparae attributed to *P. warshavensis* were collected. However, the latter two morphs have apparently not been described in the literature and thus remain unknown.

An attempt was made by us in early June 2025 to tentatively transfer the aphid infestation to the roots of three young potted white poplar plants, kept in a greenhouse, inside a light-net cage, together with several alate emigrants, developing from the aphid nymphs in leaf-nests collected from infested shoots on white poplar. However, no positive results were obtained. Also, on June 27, no signs of aphid presence were observed on the poplars where they were first detected, as the insect had likely moved to the roots of an unknown plant(s) for the next phase of its life cycle. On August 6, another on-site survey was made to search for aphid presence on white poplar roots, but nothing was found.

The distribution of the aphid has been recorded so far from several European countries, from Sweden to Spain and eastward to Belarus, Poland, Ukraine, Turkey, as well as in Central Asia (Kazakhstan) (Kadyrbekov, 2011), in addition to the record for Italy. For more detailed information, see Casiraghi *et al.* (2019) and Favret *et al.* (2026).

## Phytosanitary remarks

The European white poplar, *Populus alba* L., is a dioecious tree with a wide natural distribution in river valleys all over Europe, Central Asia, and North Africa. It is a fast-growing pioneer tree characterized by its strikingly white leaves (Caudullo and de Rigo, 2016). It is a riparian species occurring in Europe, both in linear formations along rivers and as isolated trees. It is typical of azonal vegetation. In fact, Italy is located in all regions from sea level up to low mountain sites (Isebrands and Richardson, 2014). It is cultivated for biomass energy production, renaturation projects, and as an ornamental tree.

Many aphid species are recorded as sap-feeding insects, both on white and the grey poplars plant taxa; among these, the most common and sometimes harmful, both in Italy and other European countries, are *Chaitophorus populialbae* (Boyer de Fonscolombe) and *C. populeti* (Panzer), while several other aphids are generally much less common or even sporadic, thus being moderately harmful or not at all dangerous.

The aphid *P. warshavensis* is potentially a rather harmful species, consistent with other congeneric species either on their primary or secondary host plants (Barjadze and Japoshvili, 2007; Carter and Danielsson, 1991; Casiraghi *et al.*, 2019), though its presence in Italy appears so far to be of moderate economic relevance.

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Contributions: Alberto Bombieri, Daniele Rizza, Salvatore Nucifora: conceptualization. Alberto Bombieri, Michele Ricupero, Salvatore Nucifora: methodology. Alberto Bombieri, Daniele Rizza, Franco Picco, Pier Mario Chiarabaglio: field investigation, field sampling, aphid breeding and photos. Salvatore Nucifora: microscope photographs and figures editing, morphological studies. Michele Ricupero: molecular studies, funding acquisition. All authors: resources, writing—original draft preparation, writing—review and editing.

Conflict of interest: the authors declare that they have no competing interests, and all authors confirm accuracy.

Ethics approval: this research did not require ethical approval.

Availability of data and materials: the data that support the findings of this study are available from the corresponding author upon reasonable request.

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