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WAHLGRENIELLA NERVATA (HEMIPTERA: APHIDIDAE), A NEW PEST OF ROSE IN INDIA

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ABSTRACT

First occurrence of the invasive aphid species, *Wahlgreniella nervata* (Gillette) (Hemiptera: Aphididae) is reported from material collected in Bengaluru, India. Apterous and alate viviparous females were found feeding on tender shoots, young leaves and flower buds of rose (Rosaceae). The identity of this aphid species is supported by morphometrics (aptera and alata) corroborated with molecular analysis based on a fragment of the mitochondrial DNA containing the 5' region of the cytochrome c oxidase 1 (mtCOI). Diagnostic characters to differentiate *W. nervata* from other major aphid species viz., *Macrosiphum rosae* (Linnaeus) and *Sitobion rosaeiformis* (Das), on *Rosa* spp., its host plants, distribution and natural enemies are provided. This is a new distributional record of *W. nervata*. The invasive species compendium developed by CAB International, 2013 has listed *W. nervata* as invasive in nature.

Key Words: *Wahlgreniella nervata*, India, rose, mtCOI, phylogenetic tree

RESUMEN

Se re-describe la especie el áfido, *Wahlgreniella nervata* (Gillette) (Hemiptera: Aphididae) de material recolectado en Bengaluru, India. Se encontraron hembras vivíparas ápteros y alados alimentándose de brotes tiernos, hojas jóvenes y capullos de rosa (Rosaceae). La identidad de esta especie de áfido es apoyado por morfometría (aptera y alata) corroborada con el análisis molecular basado en un fragmento del ADN mitocondrial que contiene la región 5 'del citocromo c oxidasa 1 (mtCOI). Se proporcionan claves diagnósticas, descripciones morfológicas, plantas hospederas, distribuciones y enemigos naturales de este áfido. Es un nuevo registro para la fauna de áfidos. El compendio de especies invasoras elaborado por CAB International en 2013 lista *W. nervata* como un invasor potencial natural.

Palabras Clave: *Wahlgreniella nervata*, India, rosa, mtCOI, árbol filogenético

Rose (*Rosa* spp. L; Rosales: Rosaceae), is infested by 55 aphid species (Blackman & Eastop 2000; http://www.aphidsonworldsplants.info/C_HOSTS_Ros_Ryt.htm#Rosa) of which 39 are previously recognized from India (Raychaudhuri 1983; Chakrabarti & Sarkar 2001). *Wahlgreniella nervata* (Gillette 1908) is a spindle-shaped, pale green aphid characterized by elongate swollen siphunculi, apterae without sensoria on third antennal segment, cauda bearing five hairs and well developed lateral frontal tubercles (Heie 1986). It is associated with *Rosa* spp. and other plants, e.g., Ericales: Ericaceae. It can be considered a monotypic species, or divided into 2 subspecies, which present very similar morphological features: *Wahlgreniella nervata nervata* and *W. nervata arbuti* (Davidson, 1910). The nominotypical subspecies is heteroecious holocyclic between *Rosa* spp. and species of Ericaceae (Stroyan 1979); or paramonoecious anholocyclic on *Rosa* spp. (Blackman & Eastop 2006) and it is primarily North American. *Wahlgreniella n. arbuti* is monoecious anholocyclic on Ericaceae (*Arbutus*, *Arctostaphylos* and *Pieris*) as well as Empetraceae (*Empetrum*) species and it is primarily European.

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It has been recorded from several countries (or territories) of Europe: under *W. nervata*: Andorra, Austria, Belgium, Britain Is., Canary Is., Sicily and mainland Spain (http://www.faunaeur.org/full_results.php?id=56351); under *W. n. arbuti* (a subspecies of *W. nervata*): Greece (Tsitsipis et al. 2007), Balearic Is., Corsica, mainland France, mainland Italy, Madeira, Portugal and Sardinia (http://www.faunaeur.org/full_results.php?id=56349); the Americas: Canada (Heie 1994), Colorado, USA (Gillette 1908), Mexico (Heie 1994), Argentina (Nieto Nafria et al. 1994), Brazil (Smith & Cermeli 1979), Chile (Eastop et al. 1997) and Peru (Mallqui & Cobian 2011); and other continents: Turkey (Barjadze et al. 2011), Iran (Rezwani 2001), Israel (Halperin et al. 1988), Burundi, Africa (Blackman & Eastop 2000), including Quetta-Pakistan (Naumann-Etienne & Remaudière 1995). Hitherto this aphid species has not been reported from India.

During a regular survey, apterous and alate viviparous females of an unknown aphid colony were observed infesting rose at Bengaluru, India, i.e., at the homestead garden of Sanjay Nagar, Dec 2011 by Sunil Joshi, and in an experimental field of the Indian Institute of Horticultural Research, Mar 2012 by D. Lokeshwari (Fig. 1). Aphids were found on the leaves, tender shoots and young flower buds of cultivated *Rosa* sp. The aphids excreted honeydew and left their exuviae on the leaves and flower buds. Natural enemies of common occurrence on other species of aphids on rose, viz., *Betasyrphus linga* Ghorpade (Diptera: Syrphidae), *Ischiodon scutellaris* F. (Diptera: Syrphidae) and *Cheilomenes sexmaculata* F. (Coleoptera: Coccinellidae) were observed to feed on these aphids. No serious damage was observed as the infestation level was kept low by the natural enemies.

Live aphids along with the plant material were transferred to the laboratory. Specimens were collected and preserved in 70% ethanol at -20 °C. Morphological identifications were carried out prior to molecular studies (Blackman & Eastop 2000); examinations showed that the aphid characteristics matched those of *W. nervata*. The viviparous apterous females were deposited as microscopic slide-mounted specimens at National Bureau of Agriculturally Important Insects, Bengaluru. Literature searches indicated that this is the first report of the occurrence of *W. nervata* in India and this species is a new inclusion for Indian aphid fauna.

Diagnostic characters for identifying *W. nervata* (Gillette), *Macrosiphum rosae* (Linnaeus) and *Sitobion rosaeiformis* (Das) are given in Fig. 2, Fig. 3 and Fig. 4 respectively. The pictured specimen was stained during the slide mounting process to facilitate illustration of important characters.

Over the centuries, identification of insect pests has remained a challenging task (Joshi & Lokeshwari 2013). Accurate and rapid identification of invasive aphid species like *W. nervata* is important in order to evolve effective management practices, develop biological control

Fig. 1. Late instar nymph (left) and apterous viviparous female (right) of *Wahlgreniella nervata* (Gillette) on rose.

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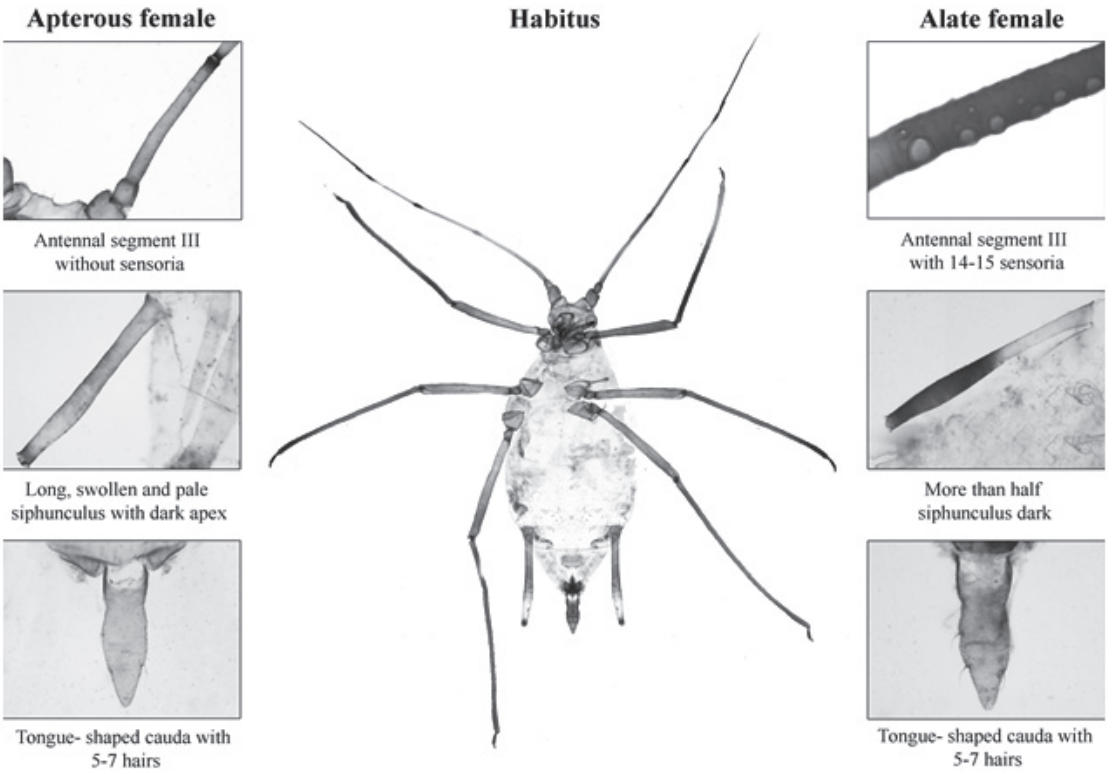

Fig. 2. Diagnostic characters for identification of *W. nervata* (Gillette) collected on rose.

programs and detect invasive quarantine pest species introduced into countries along with agricultural products at the port of entry. Molecular identification comes in handy at this juncture and aids in quick identification of insect pests. Hence, in this study morphological identification corroborated with molecular methods to identify *W. nervata* from other major aphid species on rose are provided along with its host plants, distribution and natural enemies.

For molecular identification, total genomic DNA was extracted from single individuals using CTAB method (Stewart et al. 1993) and PCR was carried out in a thermal cycler (Eppendorf, New York, USA) with the following cycling parameters; 94 °C for 3 min as initial denaturation followed by 35 cycles of 94 °C for 30 s, 47 °C for 45 s, 72 °C for 45 s and 72 °C for 20 min as final extension using primers specific to mtCOI, viz., LCO-1490F; 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3'and HCO-2198R; 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3'(Hebert et al. 2004). PCR was performed in 25 µL total reaction volume containing 10 picomoles of each primer, 1.5 mM $MgCl_2$, 0.25 mM of each dNTP and 0.5 U of Taq DNA polymerase (Thermo scientific, USA). The amplified products were resolved in 1.0% agarose gel, stained with ethidium bromide (10 µg/mL) and visualized in a gel documentation system *(*Syngene, USA).

The PCR amplified fragments were eluted using Nucleospin® Extract II according to the manufacturer's protocol *(*Machery-Nagel, Germany) and ligated into the general purpose-cloning vector, InsT/Aclone; transformation was carried out according to manufacture's protocol (Fermentas, Germany) and blue/white selection was done. All the white colonies (with insert) were maintained on LBA containing ampicillin (100 mg/mL), incubated at 37 °C overnight and stored at 4 °C. Plasmids were isolated from the overnight culture of 5 randomly selected positive colonies cultured in LB broth using GeneJET™ Plasmid Miniprep Kit (Fermentas, Germany) according to manufacturer's protocol. Sequencing was carried out in an automated sequencer (ABI Prism® 3730 XL DNA Analyzer; Applied Biosystems, USA) using M13 universal primers both in forward and reverse directions.

The gene mtCOI was successfully sequenced from an individual of *W. nervata*. Comparison of the triplicate sequences showed no evidence of mismatch, indicated there were no sequencing errors. Homology search was carried out us-

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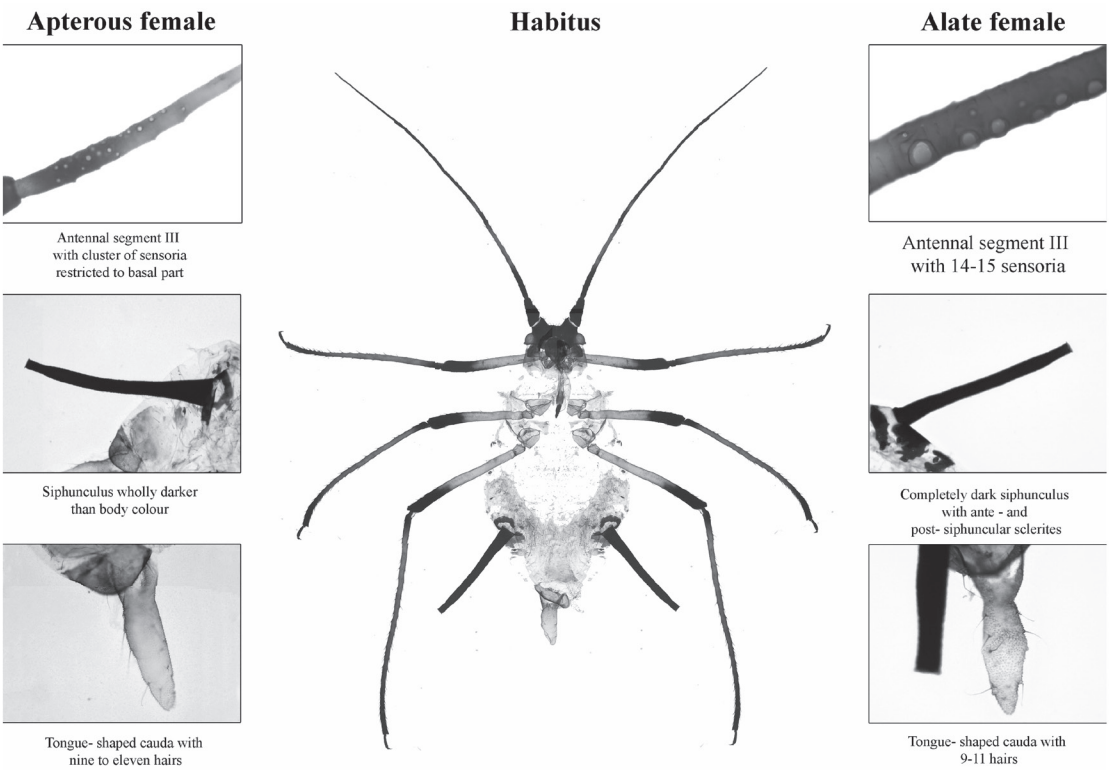

Fig. 3. Diagnostic characters for identification of *Macrosiphum rosae* (Linnaeus) collected on rose.

ing BLAST (http://www.ncbi.nlm.nih.gov). Both the sequence and collection data of *W. nervata* were entered in National Center for Biotechnology Information (NCBI), GenBank under the accession number KF285590. Sequence generated in the present study along with other major aphid species on rose, viz., *Ericaphis wakibae* (Hottes), *Metopolophium dirhodum* (Walker), *Macrosiphum impatientis* (Williams), *Sitobion rosivorum* (Zhang), *Macrosiphum rosae* (Linnaeus) and *Pentalonia nigronervosa* (Coquerel) (as outgroup) (Retrieved from NCBI) were aligned using Clustal W program in BioEdit V7. 0. Phylogenetic analysis of aligned sequences was done using MEGA. V5. 0. (Tamura et al. 2011). The method of neighbor-joining (NJ) with the Kimura two-parameter model (Kimura 1980) was utilized to build the phylogenetic tree. To assess the robustness of the tree, 1000 bootstrap replicates were selected.

The phylogenetic tree demonstrated genetic distinction of the species with bootstrap values greater than 70% (Fig. 5). Rose aphids formed 2 major groups; *W. nervata* and *E. wakibae* clustered to form Group I, while *M. dirhodum, M. impatientis, S. rosivorum* and *M. rosae* clustered to form Group II. Pairwise alignment of mtCOI sequence of *W. nervata* collected from India showed 1.37-1.98% variation (mean divergence of 1.68%, SE 0.043%) corresponding to an average of 11 base changes with those collected from USA and Canada (available at NCBI database) indicating it is not a single cosmopolitan species (Davidson 1910) and requires further investigation to ensure the same. The sequence divergence between the genus *Wahlgreniella* and others, viz., *Ericaphis*, *Metopolophium, Macrosiphum* and *Sitobion* ranged 3.49-5.77% (mean divergence of 4.63%, SE 0.163%) indicating *W. nervata* is more closely related to *E. wakibae* (96.5% homology) than to *M. dirhodum* (95%), *M. impatientis* (94.7%), *S. rosivorum* (94.4%) and *M. rosae* (94.2%). In addition, the study showed that mtCOI sequences are consistent among species and is able to differentiate the species well; thus it proves to be a useful tool for identification of aphids.

It was also observed that *B. linga*, *I. scutellaris* and *C. sexmaculata* as predators of *W. nervata* manage this aphid in field sites at Bengaluru, India. Although this species has been considered to be of invasive nature (CAB Intl. 2013), it has not assumed serious proportions at the locations involved in the present study. Also the aphid is

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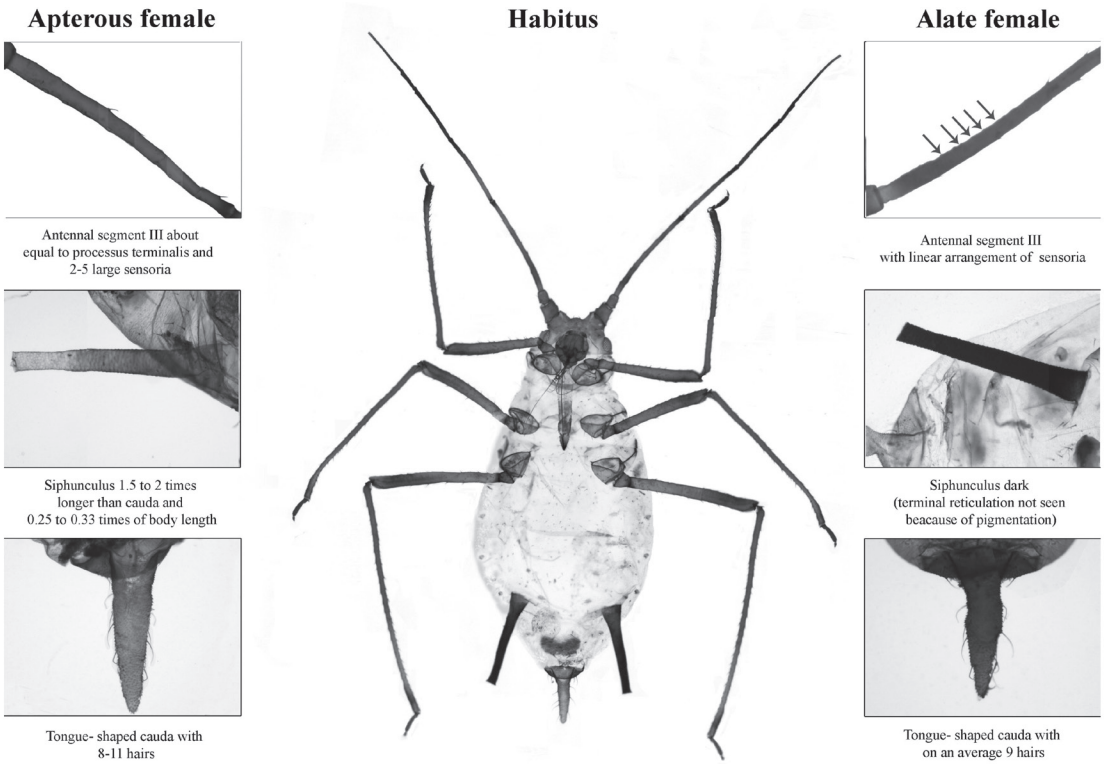

Fig. 4. Diagnostic characters for identification of *Sitobion rosaeiformis* (Das) collected on rose.

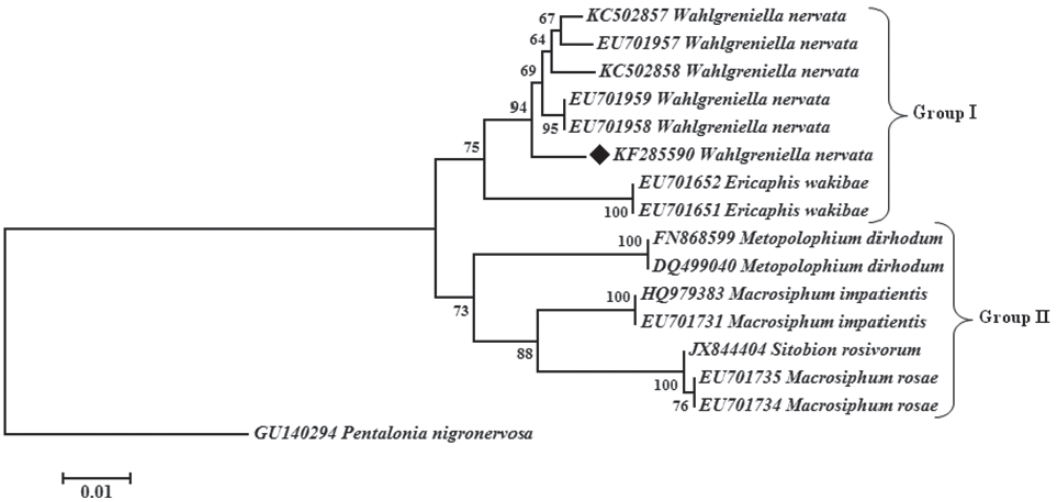

Fig 5. Neighbor joining tree of *W. nervata* with other major rose aphids for partial sequences of CO-I with bootstrap support (1000 replicates). Bootstrap values greater than 60% are indicated above the branches. *Pentolonia nigronervosa* was used as outgroup.

not known to transmit any viral diseases, and thus it is of less economic significance. However, our study will aid in proper quarantine measures from the view point of biosecurity.

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