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ORIGINAL PAPER

Identifying cryptic species of *Planococcus* infesting vineyards to improve control efforts

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Abstract

Mealybugs (Hemiptera: Coccomorpha: Pseudococcidae) constitute important agricultural pests that often require control measures. Different mealybug taxa might, however, react differently to natural enemies and pesticides so that appropriate control measures against mealybugs rely heavily on the correct species identification. The mealybug *Planococcus ficus* (Signoret) is the most damaging scale insect infesting vineyards worldwide. Despite its economic impact, the taxonomic status of this mealybug species is still unclear, and recent studies suggest the possibility that *P. ficus* from eastern (i.e., Egypt) and western (i.e., France) Mediterranean regions may correspond in fact to two distinct species. The purpose of this work was to deepen our current knowledge of putative *P. ficus* from eastern Mediterranean using molecular tools and morphological analysis and test for the existence of cryptic species within *P. ficus*. Mealybug samples were collected from Egyptian vineyards to better characterize the genetic diversity and analyze the population structure of putative *P. ficus* along the eastern Mediterranean. We also estimated the phylogenetic relationships among the *P. ficus* complex haplotypes in different vineyard regions worldwide and analyzed the morphological characters of the different clades obtained. Morphological and molecular analyses confirmed the existence of two species: *P. ficus* (Signoret) *s.str.* and *P. vitis* (Niedielski), a species that was previously synonymized as *P. ficus*. These results have direct implications for pest management and could explain the lack of success in previous implementations of biological control programs against this pest in several vineyard regions.

Keywords Cryptic species · Coccoidea · Vine mealybug · Vineyard

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Key messages

The phylogenetic and morphological relationship among *P. ficus* populations were investigated.

Morphological and molecular analyses suggest the existence of two species: *P. ficus* and *P. vitis*.

Genetic structure suggests Egypt may be part of the species native range.

Existence of different species is discussed with respect to regional control measures.

Introduction

Mealybug (Hemiptera: Pseudococcidae) infestations cause important economic losses to grape producers worldwide (Daane et al. 2012). The mealybug *Planococcus ficus* (Signoret) is arguably the most damaging scale insect infesting vineyards (Walton et al. 2004; Bettiga 2013; Daane et al. 2018a; Cocco et al. 2021). *Planococcus ficus* causes both cosmetic and physiological damage and can be especially harmful due to its high fecundity and short developmental time (Daane et al. 2012). It is also known to play a key role in the transmission of viruses that reduce vineyard crop yields and quality (Cabaleiro et al. 2007; Bertin et al. 2010; Almeida et al. 2013). Furthermore, *P. ficus* can be found on the grape throughout the year, feeding in protected areas (e.g., on the roots), where it has some refuge from natural enemies and contact chemical applications (Gutierrez et al. 2008; Prabhaker et al. 2012). Accurate mealybug identification is important to optimize control measures, determining the presence of susceptible stages to time the release of natural enemies or insecticide applications (Millar et al. 2002), or using species-specific pheromones for mating disruption (Walton et al. 2006; Sharon et al. 2016; Franco et al. 2022). Integrated pest management strategies vary in efficiency depending on the specific targeted pest; therefore, proper species identification can be critical, particularly for biological control programs where selecting the appropriate natural enemies is key to optimizing biological control (Beltrà et al. 2015; Malausa et al. 2016).

Planococcus ficus has been reported as the main mealybug species infesting vineyards in many regions, including California, Egypt, Israel, Italy, South Africa, Turkey, and Uruguay (Walton and Pringle 2004; Abd-Rabou et al. 2012; Sharon et al. 2016; Daane et al. 2018b; Pacheco da Silva et al. 2020; Cocco et al. 2021). Worldwide, several parasitoid species have been associated with *P. ficus* (Trjapitzin and Trjapitzin 2002; Daane et al. 2004; Walton and Pringle 2004; Fallahzadeh et al. 2011; Japoshvili et al. 2018; Pacheco da Silva et al. 2021), but none have provided adequate control. This might be due to temperature development constraints (Daane et al. 2004) or refuges on the vine where the mealybug is unavailable to the parasitoid (Daane et al. 2006; Gutierrez et al. 2008). Parasitoid performance might also be impacted by mealybug species or geographic populations. For example, *Pseudococcus viburni* (Signoret) is controlled in New Zealand by the encyrtid *Acerophagus maculipennis* Signoret (Charles 2011), whereas in Chile and Brazil, *P. viburni* is controlled by *Acerophagus flavidulus* Brèthes (Ripa and Rojas 1990; Pacheco da Silva et al. 2021), with each encyrtid species not being effective against *P. viburni* in the other region. It is unclear how these parasitoid species exhibit a level of host discrimination that may differentiate between geographic populations of *P. viburni*, but microsatellite markers later confirmed genetic differentiation between *P. viburni* populations from Chile and France (Correa et al. 2014). This illustrates the importance of accurate mealybug identification in the development of management strategies (Daane et al. 2012). Still, significant phenotypic plasticity makes morphological study of *Planococcus* species particularly challenging and *P. ficus* has been confused with related species, such as *Planococcus citri* (Risso) (Saccaggi et al. 2008; Correa et al. 2015). Moreover, some diagnostic characters are highly variable and might change depending on temperature conditions (Cox 1983; García Morales et al. 2016), complicating taxonomic identification even further. Therefore, there is a need to improve identification methods before biological control techniques can be implemented in the field (Demontis et al. 2007; Malausa et al. 2011).

Originally described in southern France, *P. ficus* populations are commonly found on fig trees, pomegranates, and grapevines throughout the Mediterranean Basin (De Lotto 1975). Ezzat and McConnell (1956) assigned the name *Planococcus vitis* (Niedielski) to an apparently new form occurring in countries of the eastern Mediterranean, but the criteria adopted by the authors to discriminate species were inconclusive and later authors synonymized *P. ficus* and *P. vitis* (Cox and Ben-Dov 1986). Recent DNA barcoding, however, suggests significant intra-specific variation within *P. ficus* populations (Park et al. 2011; Daane et al. 2018b) and, therefore, the validity of an independent eastern Mediterranean clade remains unsolved. As Daane et al. (2018b) suggest, the question of whether the Mediterranean and Middle Eastern groups of *P. ficus* represent cryptic species cannot be determined until further studies include morphological evaluations of *P. ficus* along with molecular analyses to characterize the genetic variation within the Middle Eastern group.

The purpose of this work was to deepen our current knowledge of putative *P. ficus* from eastern Mediterranean

using molecular tools and morphological analysis and test for the existence of cryptic species within *P. ficus*. The genetic diversity of putative *P. ficus* along the eastern Mediterranean was characterized using mealybug samples from Egyptian vineyards. Morphological traits and genetic markers were compared among different geographic populations and our results confirmed that *P. ficus* is composed of two cryptic species. These findings have direct implications for *P. ficus* management and could shed light on inconsistent results from previous biological control programs targeting this pest.

Materials and methods

Sampling, DNA extraction, PCR´s, and sequencing

A total of 233 mealybugs were collected from 23 localities distributed along the 6 main vineyard regions in Egypt: Marsa Matrouh (MM), West of Nobaria (WN), Elbostan (EB), Menouf (MF), Elkhatatba (EK), and Giza (GZ) (Fig. 1; Table 1). Mealybugs were stored in 95% ethanol and at −20 °C for later study. Genomic DNA was extracted using the DNeasy Tissue Kit (QIAGEN, Hilden, Germany), following the method of Malausa et al. (2011). Polymerase Chain Reactions (PCR) were performed to amplify a fragment of the subunit I cytochrome oxidase (COI) gene using the primer pair: C-LCOn-F AYAATATAATRATTA CWWTWCATGC and C-LCOn-R TTTWCCATTTAAWGT TATTATTC, and following the PCR conditions reported by Abd-Rabou et al. (2012). Quality of PCR products was checked by electrophoresis in 2% agarose gels. Positive PCR products were sent for bidirectional sequencing to Genoscreen (Lille, France) and the consensus sequences generated were checked with Seqscape v2.7 (Applied Biosystems). Sequences presenting nucleotide differences were considered as different haplotypes. Reference sequences for each haplotype were deposited in Genbank under accession numbers OM362525–OM362543.

Genetic diversity and population genetic analyses within Egyptian samples

Genetic diversity indices such as number of haplotype (K), number of polymorphic sites (N), haplotype diversity (Hd) and nucleotide diversity (π) were estimated for each sampled site using the software DnaSP v6.10 (Librado and Rozas 2009). Genealogical relationships between haplotypes were inferred using the median-joining network algorithm as implemented in the software POPART (Leigh and Bryant 2015). Pairwise genetic differentiation among sampling sites was estimated measuring F_{ST} values and its significance was obtained based on 1,000 permutation tests implemented in Arlequin v3.5 (Excoffier and Lischer 2010). The distribution of genetic variance at different geographical levels was also estimated by an analysis of molecular variance (AMOVA) using Arlequin. To quantify the correlations and relationship between genetic differentiation and geographical distance, a Mantel test was performed using the Adegenet R package with the function mantel.randtest (Jombart 2008).

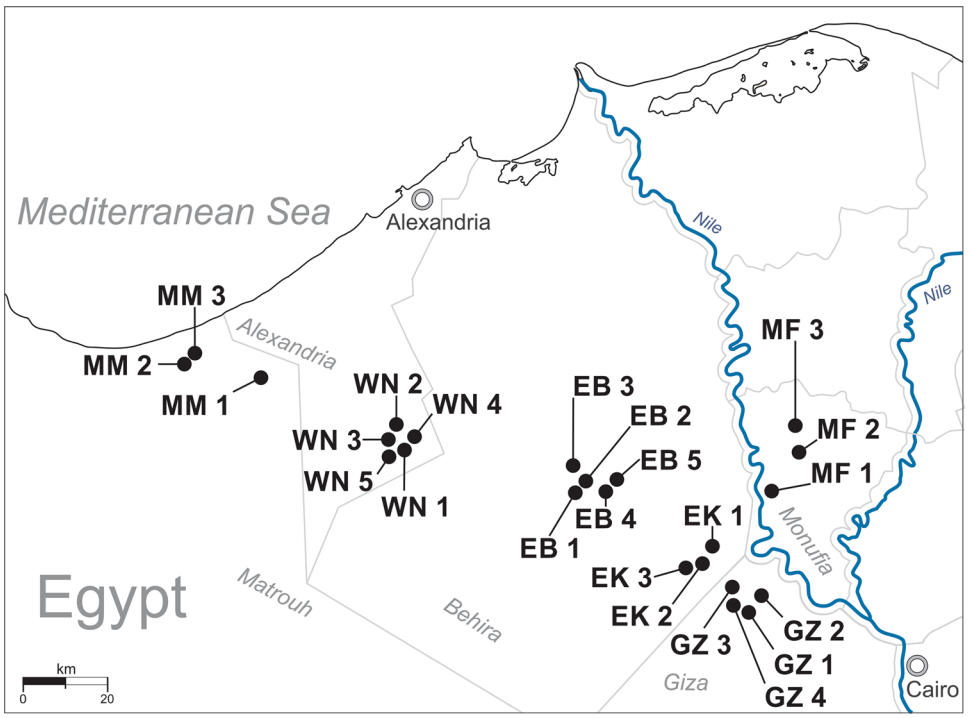

Fig. 1 Sampling localities of putative *Planococcus ficus* populations. Mealybugs were collected from 23 localities distributed along the 6 main vineyard regions in Egypt namely, Marsa Matrouh (MM), West of Nobaria (WN), Elbostan (EB), Menouf (MF), Elkhatatba (EK), and Giza (GZ)

Table 1 Sampling sites and genetic diversity information of each sampling site: Number of polymorphic sites (N), number of haplotypes (K), haplotype diversity (Hd), number of haplotypes (K), number of polymorphic sites (S), and the number of nucleotide differences between any two sequences (π) for each population

Governorate	Region	Pop. Code	Latitude	Longitude	N	K	Hd	π
Matrouh	Marsa Matrouh	MM1	N 30° 45′ 21"	E 29°29′ 52"	9	1	0.0000	0.0000
		MM2	N 30° 47′ 38"	E 29°17′ 51"	10	2	0.2000	0.0017
		MM3	N 30° 48′ 17"	E 29°18′ 51"	10	2	0.5333	0.0045
Alexandria	West of Nobaria	WN1	N 30°37′24"	E 30° 1′ 33"	13	3	0.2949	0.0023
		WN2	N 30°38′26"	E 29°59′38"	13	2	0.2821	0.0004
		WN3	N 30°37′23"	E 29°59′23"	11	5	0.7636	0.0049
		WN4	N 30°38′18"	E 30°02′30"	9	2	0.3889	0.0033
		WN5	N 30°36′32"	E 29°59′57"	9	2	0.3889	0.0006
Buhayra	Elbostan	EB1	N 30°30′02"	E 30°21′33"	12	2	0.4849	0.0027
		EB2	N 30°31′06"	E 30°22′35"	12	2	0.4849	0.0041
		EB3	N 30°31′54"	E 30°22′01"	14	2	0.4396	0.0037
		EB4	N 30°30′19"	E 30°26′18"	14	4	0.6484	0.0043
		EB5	N 30°30′46"	E30°26′52"	12	3	0.6212	0.0065
Monufia	Menouf	MF1	N 30°29′ 13"	E 30° 51′ 34"	12	2	0.4849	0.0048
		MF2	N 30° 35′ 51"	E 30° 57′ 40"	13	2	0.4615	0.0033
		MF3	N 30° 39′ 36"	E 30°57′ 02"	13	2	0.4615	0.0026
Behira	Elkhatatba	EK1	N 30° 22′ 34"	E 30°42′ 29"	12	3	0.6212	0.0029
		EK2	N 30° 20′ 01"	E 30°41′ 13"	11	1	0.0000	0.0000
		EK3	N 30° 19′ 10"	E 30°38′ 45"	10	2	0.4667	0.0013
Giza	Giza	GZ1	N 30° 14′ 32"	E 30° 43′ 15"	4	1	0.0000	0.0000
		GZ2	N 30° 15′ 29"	E 30° 47′ 7"	4	1	0.0000	0.0000
		GZ3	N 30° 16′ 14"	E 30° 42′ 11"	3	2	0.6667	0.0038
		GZ4	N 30° 15′ 5"	E 30° 42′ 12"	3	2	0.6667	0.0047

This function compares the matrices of pairwise estimates of genetic differentiation (F_{ST} values) with the matrix of geographic distances to test for the presence of isolation by distance. Finally, the effect of spatial barriers in determining the genetic structure of *Planococcus* populations from Egypt was further evaluated using pairwise F_{ST} values and visualized with the software BARRIER v2.2 (Manni et al. 2004). This method links a matrix of geographical coordinates with their corresponding distance matrix (F_{ST}) and applies the Monmonier's maximum distance algorithm to identify 'barriers' to gene flow among sites, namely the zones where differences between pairs of sites are the largest.

Phylogenetic relationships among putative *Planococcus ficus* haplotypes worldwide

To establish the phylogenetic relationship among putative *P. ficus* populations collected in Egypt and those from other countries, COI sequences obtained by our team and others in previous studies were downloaded from the public database Genbank. Sequences belonged to samples collected from Egypt (Abd-Rabou et al. 2012), France (Malausa et al. 2011), USA, and South Africa (Saccaggi et al. 2008; Park et al. 2011). The complete dataset was aligned using the software MAFFT with default parameters (Katoh and Standley 2013). Conserved blocks of sequence from the alignment were identified by using Gblocks server with default settings (Talavera and Castresana 2007). Selection of the most suitable nucleotide substitution model was performed according to the BIC criterion as implemented in MEGA v7 (Kumar et al. 2016). Maximum Likelihood phylogenetic trees were inferred using MEGA v7. *Planococcus citri* and *P. minor* were selected as outgroup taxa because they are valid sister species and *P. citri* is the closest species to *P. ficus* (Rung et al. 2008). Support values for each node were evaluated with 2,000 bootstrap replicates over nucleotide positions. The aligned sequences and selected evolutionary model were used to obtain estimates of evolutionary divergence over sequence pairs (i.e., genetic distances) within each of the main groups identified from the phylogenetic trees, and to calculate net divergences between groups. The number of base substitutions per site and the corresponding standard error estimates were obtained using MEGA v7.

Morphological assessment and description of *Planococcus ficus* complex

Adult females of putative *P. ficus* from Egypt, France, Italy, North America, Turkey, and Uruguay were slide-mounted for morphological analysis according to described procedures for mealybugs (Kosztarab and Kozár 1988; Williams and Granara de Willink 1992) and examined under a

phase-contrast compound microscope. A total of 15 adult females were prepared and measured from Egypt (West of Nobaria, Elbostan, Menof, ShbinElkom, Tala, ElKhatatba, and Rowysat), eight from France (Limoux), ten from Italy (Alghero and Badesi), 19 from USA (Fresno, San Luis Obispo and Napa), 20 from Turkey (Adana and Mersin) and 13 from Uruguay (Las Piedras, Juanicó and Montevideo). These individuals were identified as *P. ficus* as they were presenting most of the diagnosis criteria for this species (Cox 1989). A morphological re-analysis of multiple slide preparations previously identified as *P. ficus* was performed by using specimens from Egypt, France and North American populations (Malausa et al. 2011; Abd-Rabou et al. 2012; Daane et al. 2018b). Mealybug morphological vouchers are stored in: Anses, Laboratoire de la Santé des Végétaux, Montferrier-sur-Lez, France; Çukurova University, Adana, Turkey; and Facultad de Ciencias Entomological Collection, Montevideo, Uruguay.

Results

Genetic diversity and population genetic structure analyses within Egypt

The aligned COI sequences from Egyptian populations presented 20 polymorphic sites that resulted in 19 different haplotypes. All mutation sites corresponded to synonymous mutations. The number of haplotypes (K) per population varied from one to five, haplotype (Hd) and nucleotide (π) diversity varied from 0 (MM1, EK2, GZ1, and GZ2) to 0.7636 (WN3) and from 0 (MM1, EK2, GZ1, and GZ2) to 0.0065 (EB5) respectively (Table 1). The three most abundant haplotypes represent more than half of the total mealybugs sampled, with relative frequencies of 18.03% (H1), 21.46% (H2), and 12.02% (H4) respectively (Fig. 2A). Six haplotypes were shared between at least two regions (H1-2, H4-5, H8, and H10), while 12 of them are private (H3, H6-7, H9, H11-19). The region with more private haplotypes was Menouf, with four private haplotypes, whereas the Marsa Matrouh region presented none. The median-joining network showed a close relationship among haplotypes (Fig. 2A) with at most 13 mutational steps separating two haplotypes (H12 and H13). The high number of connections presented by H1 in addition to its high frequency and interior location within the network suggests that it is an ancestral haplotype. Estimates of genetic differentiation between sampling sites (F_{ST}) were statistically significant in most population pairs (Supp Table 1). The Mantel test showed no significant correlation between geographic distances and pairwise F_{ST} values ($r = 0.128$, $P = 0.169$), which indicates no pattern of isolation by distance. Interestingly, the non-hierarchical AMOVA showed more substantial differentiation among populations within the same geographical region ($F_{SC} = 0.502$) than among regions ($F_{CT} = 0.073$) (Supp Table 2). In agreement with this, the BARRIER analyses showed that genetic differentiation is highest between samples from Elkhatatba and those from Giza and Menouf (Fig. 2B).

Phylogenetic relationships and genetic distances between COI haplotypes

After including Genbank sequences into our dataset, a global alignment of 872 base pairs was obtained. The model selection approach revealed Tamura 3-parameter (T92) as the most likely DNA substitution model (BIC = 6035.86; lnL = −1855.14), with a larger proportion of transitions over transversions (ratio of 2.65). The estimated AT bias was significantly high (83%), which is consistent with previous estimates obtained from mitochondrial sequences of mealybug species. Two main groups of haplotypes were identified through ML analyses, one formed by the species pair *P. citri* and *P. minor*, and a second clade formed by all the putative *P. ficus* samples (Fig. 3A). The *P. ficus* clade further split into two well-supported clades: one clade formed by Egyptian and USA samples and another formed by mealybugs collected in France and South Africa. Interestingly, genetic divergence estimates gave further support to the separation of two clades within *P. ficus*, with net evolutionary divergence between the pair of sister species *P. citri* / *P. minor* (0.020 ± 0.006) being smaller than distances observed between the two *P. ficus* clades (0.029 ± 0.007) (Fig. 3B). Both estimates were about three times smaller than divergences between species from different pairs (e.g., *P. citri* / *P. ficus* $= 0.062 \pm 0.011$) and more than twice as large as divergences within clades, ranging from 0.001 ± 0.0005 in *P. minor* to 0.009 ± 0.002 in the *P. ficus* clade including samples from California and the eastern Mediterranean.

Morphological analyses of *Planococcus ficus* samples from groups I and II

The distinctive morphological features of *P. ficus* according to Cox and Ben-Dov (1986) are: (i) the absence or low numbers of oral collar tubular ducts on the ventral margins of the head and thorax (almost absent near cerarii 6 and 8), (ii) the slender cerarian conical becoming stout flagellate on the head, (iii) high dorsal rim tubular ducts, (iv) multiple multilocular disc pores on the thorax, particularly behind the fore coxae, and finally (v) the occasional presence of translucent pores on the hind femora, depending on developmental stage. After a thorough evaluation of the morphological traits present in putative *P. ficus* from Egypt, Italy, France, North America, Turkey, and Uruguay, the character that can be used to distinguish specimens from Group I (Eastern Mediterranean and California) and Group II (other

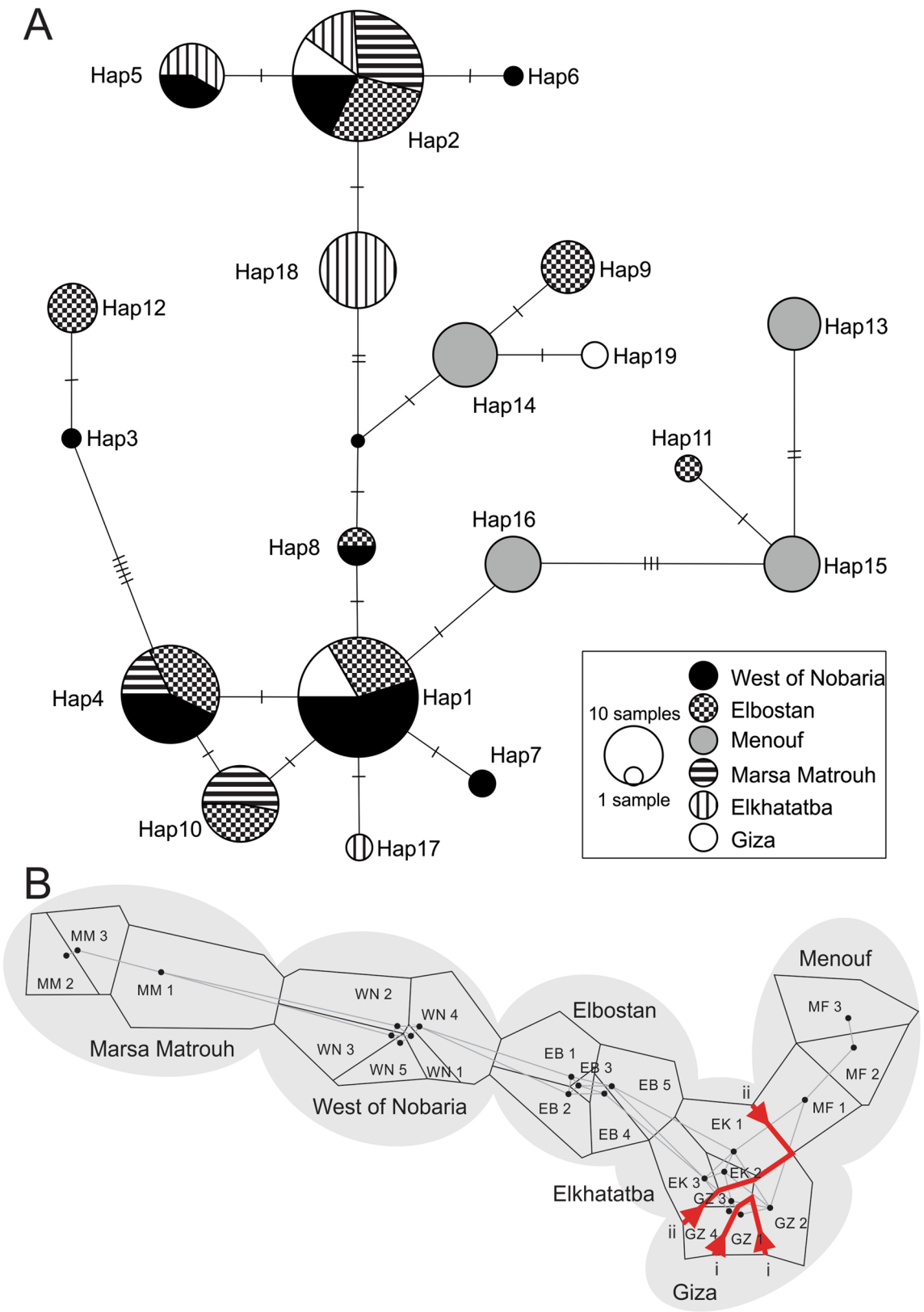

Fig. 2 Haplotype distribution and population structure in *Planococcus* populations from Egypt. A) Median-joining network showing the genetic distance and geographic distribution of the 19 haplotypes found in the present study. B) Main genetic barriers (i and ii, highlighted in red) obtained from the Monmonier's maximum distance algorithm as implemented in BARRIER

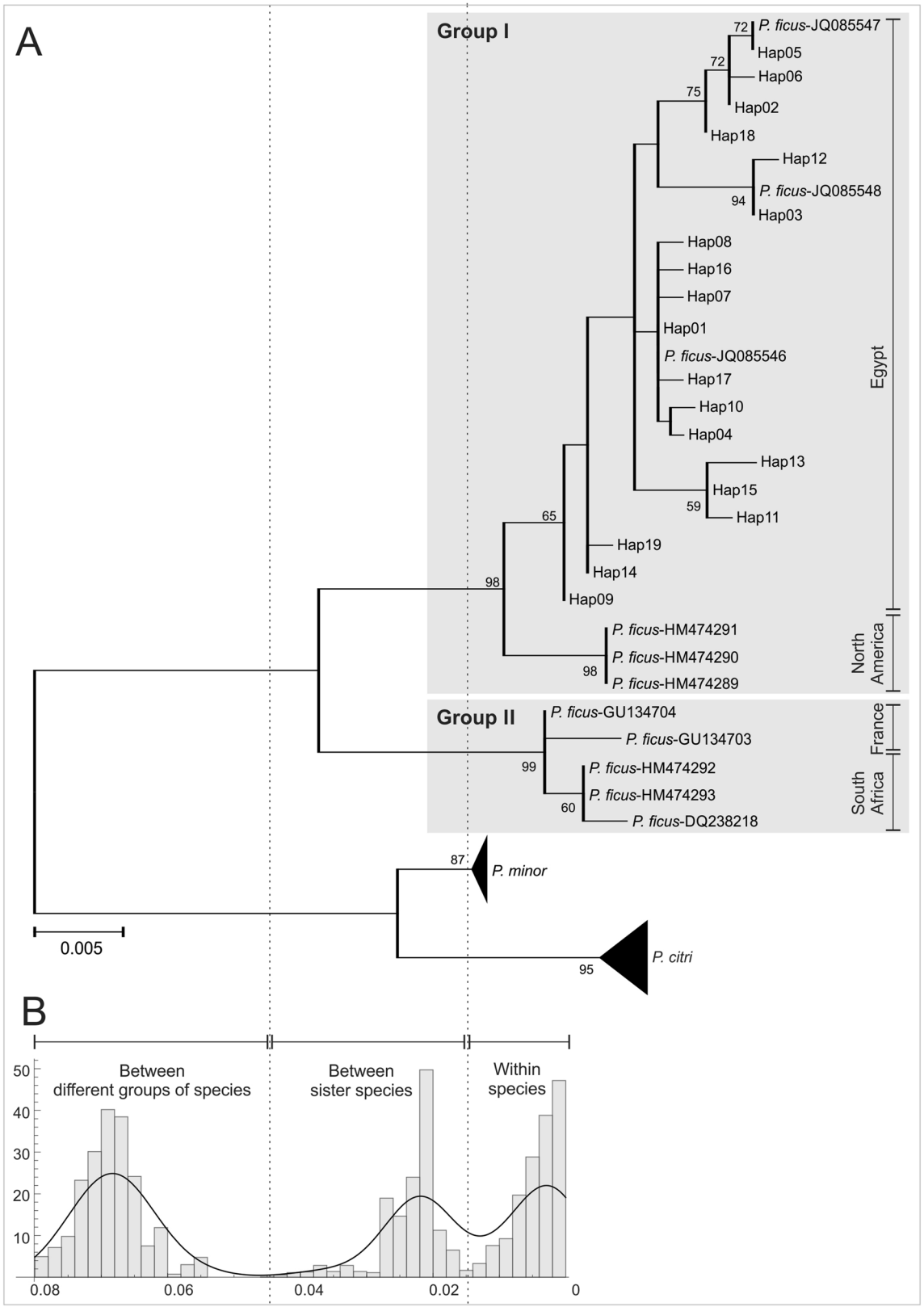

Fig. 3 Neighbor-Joining tree and histogram of genetic distances. **A** Phylogenetic tree revealing the genetic relationships between the 19 haplotypes found in this work and data from previous studies. **B** Genetic distance observed (i) Within species, (ii) Between species, and (iii) Between different groups of species. Note the presence of the so-called "Barcoding gap"

countries) is mainly the number of multilocular pores near the fore coxae and on the whole ventral thorax. Multilocular pore numbers varied from 0 to 4 near fore coxae and 0 to 6 across ventral thorax in the Egyptian and North American individuals, while it ranged from 4 to 8 near fore coxae and 12 to 32 across ventral thorax in individuals from the other evaluated countries. This character has been also used to distinguish other *Planococcus* species, as *P. citri* and *P. minor*, with *P. citri* presenting 0 to 6 and *P. minor* 7 to 12 multilocular pores behind front coxae, which most probably was a mixed population (Cox 1989). Given these morphological differences, together with the DNA results and independent evidence (see Discussion), we propose here to resurrect the name *P. vitis* for the Group I species as described by Ezzat and McConnell (1956). The species can be found on vines and figs, like *P. ficus*, and it is distributed, at least, from the eastern Mediterranean from the Nile Delta in Egypt to Israel.

Planococcus ficus (Signoret) (Fig. 4) (Adapted from Cox 1989 and Ezzat and McConnell 1956).

Adult female description: body oval, 1.4–3.2 mm long, 0, 8–2.2 mm wide. Eyes situated on margins, each 15–25 µm wide. Antenna with 8 segments, 400–445 µm long, with 5 fleshy setae on distal segment, each seta 21.5–37.5 µm long; apical segment about 93 µm long, 25.0–32.5 µm wide, with apical setae each 30.0–32.5 µm long. Clypeolabral shield 175–205 µm long, 160–190 µm wide. Labium 3-segmented, 95–140 µm long, 112–133 µm wide. Anterior spiracles each 50 µm long, 30 µm wide across atrium; posterior spiracles each 55 µm long, 38 µm wide. Circulus quadrate, 90–107 µm long, 115–150 µm wide. Legs well-developed; elongate, posterior leg segment lengths: coxa 165–190 µm, trochanter + femur 235–355 µm, tibia + tarsus 275–395 µm, claw about 30 µm. Ratio of length of tibia + tarsus to trochanter + femur 1.07–1.17:1; ratio of length of tibia to tarsus 2.20–2.30:1; ratio of length of trochanter + femur to greatest width of femur 3.45–3.53:1; translucent pores on hind coxae and tibiae, and often also on hind femora; tibia with 41–70 translucent pores. Tarsal digitules hair-like, each 40 µm long. Claw without a denticle, digitules capitate, each about 30.0–32.5 µm long. Both pairs of ostioles present, with inner edges of ostioles moderately sclerotized; anterior ostioles each with a total for both lips of 8–18 trilocular pores and 1–3 setae. Posterior ostioles each with a total for both lips of 10–23 trilocular pores and 1–4 setae. Anal ring about 84 µm wide, with 6 setae, each seta about 133 µm long. Anal lobe bar distinct, extending to base of apical seta; bar setae about 64 µm long.

Dorsum. Derm membranous, with 18 pairs of cerarii around body margin, each cerarius containing 2 cerarian setae, slenderer towards the anterior of the body, and 4–7 associated trilocular pores. Each anal lobe cerarius set on a small, moderately sclerotized area and containing 2 enlarged setae each 20.0–23.0 µm long, and about 20 associated trilocular pores and 2–3 auxiliary setae, 10–25 µm long. Dorsal setae flagellate, each 3.75–20.00 µm long, scattered throughout the dorsum, longest seta on abdominal segment VI or VII, 23–50 µm long. Trilocular pores, each 3.75–5.00 µm in diameter, scattered. Minute discoidal pores, of two sizes; larger pores, if apparent, larger than trilocular pores, present in groups of 1–3 pores on mid-line of some thoracic and abdominal segments, each about 2.5 µm in diameter; smaller pores scattered over dorsum, each about 2.5 µm in diameter. Oral collar tubular ducts often with distinct rims, usually occurring singly adjacent to some or most cerarii, each about 10 µm long and 5 µm wide. Multilocular disc pores absent.

Venter. Setae flagellate, each 12.5–147.0 µm long, longest setae located medially on head. Apical seta on anal lobe 240 µm long. Multilocular disc pores, each 6.2–7.5 µm in diameter, present around vulva, in single rows across posterior edges of abdominal segments III-VII or II-VII and across anterior edges of segments V-VII; in marginal groups on posterior abdominal segments, usually as far forward as segment II; often present in groups of up to 12 pores associated with fore coxae (4–12); and sparsely scattered over median areas of the thorax (9–32 on the whole ventral thorax); number on each abdominal segment as follows: segments I, II, III 1–4, IV 32–37, V 32–55, VI 39–47, VII 33–48, VIII + IX 17–31. Trilocular pores scattered, each about 3.75 µm in diameter. Minute discoidal pores scattered. Oral collar tubular ducts of 2 sizes: (i) larger ducts, each about 10.00 µm long and 3.75 µm wide, present as segmental clusters on margin of abdominal segments II-VIII, in rows across median areas of abdominal segments, as follows: segment I with 12–38, II 10–23, III 22–34, IV 30–46, V 30–58, VI 49–80, VII 60–110, VIII + IX 52–122, scattered over median area of thorax, a single pore sometimes present on head, and up to 3 pores sometimes present on each margin of the thoracic segments; and (ii) smaller ducts, each 7 µm long and about 3 µm wide, present in bands across abdominal segments, as follows: segment V with 18–32, VI 13–27, VII 61–95.

Planococcus vitis (Niedielski) (Fig. 5) (Adapted from Ezzat and McConnell 1956)

Adult female description: body oval, 1.4–3.0 mm long (average 2.3 mm), 0.8–2.0 mm wide (average 1.5 mm). Eyes situated on margins, each 15–27 µm wide. Antenna with 8 segments, 400–445 µm long, with 5 fleshy setae on distal segment, each seta 21.5–37.5 µm long; apical segment 81–96 µm long, 25.0–32.5 µm wide, with apical setae each 30.0–32.5 µm long. Clypeolabral shield 175–205 µm long, 160–190 µm wide. Labium 3-segmented, 150 µm long, 95 µm wide. Anterior spiracles each 60 µm long, 35 µm wide across the atrium; posterior spiracles each 65 µm long, 45 µm wide. Circulus oval to rectangular, 140 µm long, 90 µm wide. Legs well-developed; elongate, posterior leg segment

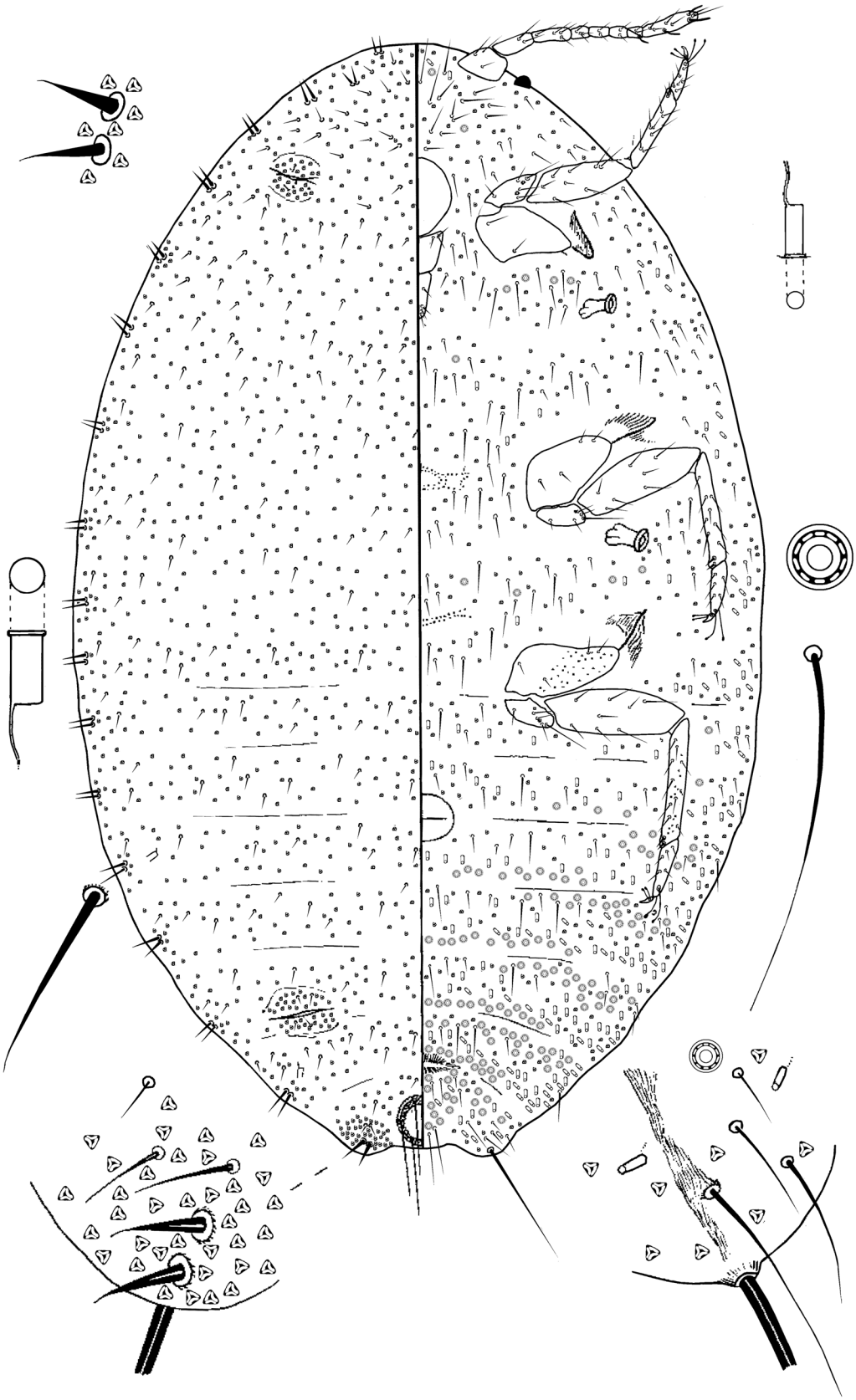

Fig. 4 Adult female of *Planococcus ficus* (Signoret) (Adapted from Cox 1989 and Ezzat and McConnell 1956)

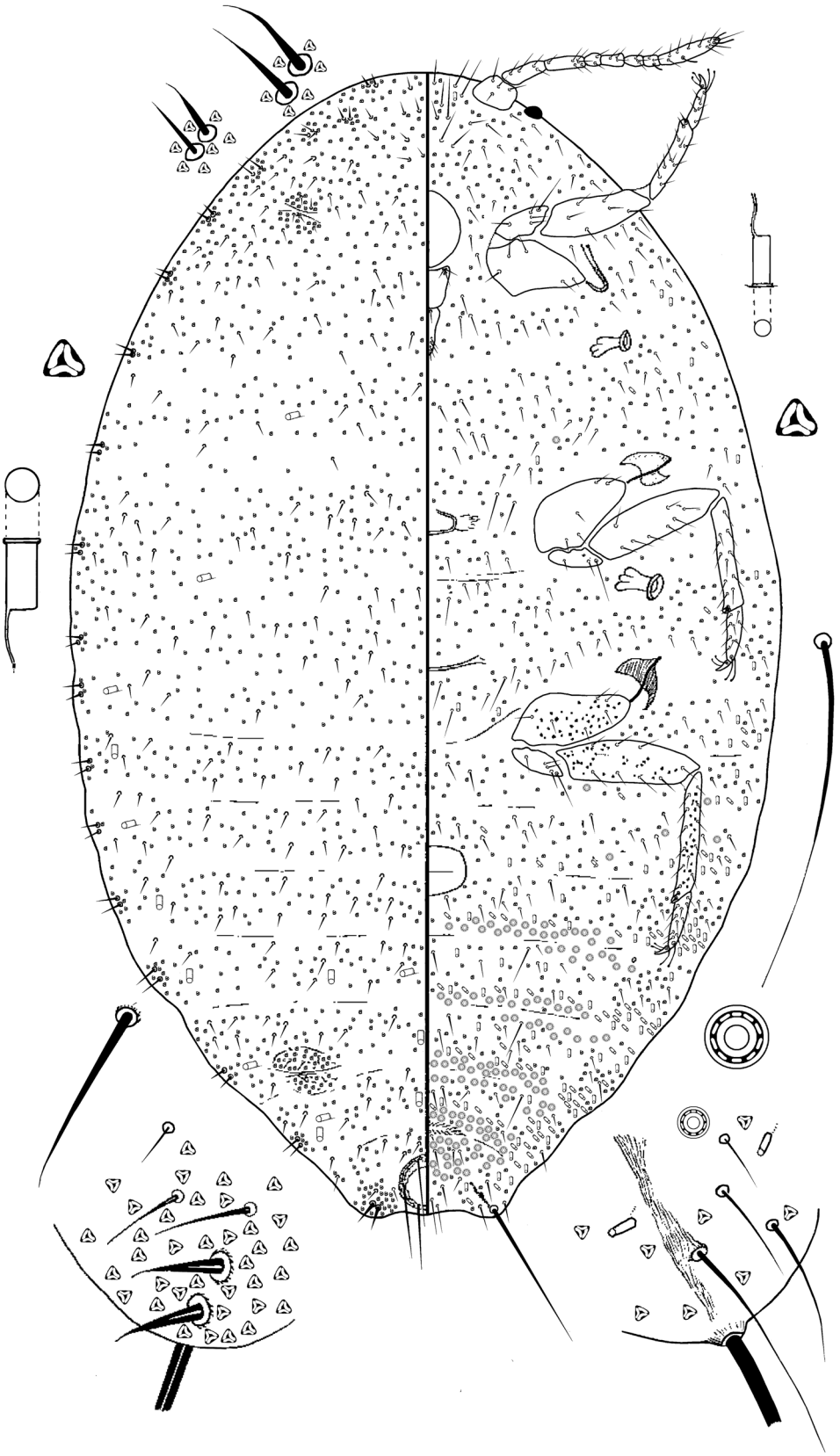

Fig. 5 Adult female of *Planococcus vitis* (Niedielski) (Adapted from Ezzat and McConnell 1956)

lengths: coxa 165–190 µm, trochanter + femur 235–355 µm, tibia + tarsus 275–395 µm, claw about 36 µm. Ratio of length of tibia + tarsus to trochanter + femur 1.07–1.17:1; ratio of length of tibia to tarsus 2.20–2.30:1; ratio of length of trochanter + femur to greatest width of femur 3.45–3.53:1; translucent pores on hind coxae, femora and tibiae; tibia with 41–70 µm translucent pores. Tarsal digitules hair-like, each 50 µm long. Claw without a denticle, digitules capitate, each about 35 µm long. Both pairs of ostioles present; anterior ostioles each with a total for both lips of 17 trilocular pores and 3 setae. Posterior ostioles each with a total for both lips of 20 trilocular pores and 3 setae. Anal ring about 90 µm wide, with 6 setae, each seta about 130 µm long. Anal lobe bar narrow, heavily sclerotized; extending to the apical seta. Apical setae long; on average about 250 µm long, bar setae about 64 µm long.

Dorsum. Derm membranous, with 18 pairs of cerarii around body margin, each cerarius containing 2 cerarian setae, slenderer towards the anterior of the body and 7–12 associated trilocular pores. Each anal lobe cerarius set on a small, moderately sclerotized area and containing 2 enlarged setae each 25.0–30.0 µm long, and about 25 associated trilocular pores and 4 auxiliary setae, 10–25 µm long. Dorsal setae short and stout, each 3.75–20.00 µm long, scattered throughout the dorsum, longest seta on abdominal segment VI or VII, 23–50 µm long. Trilocular pores, each 3.75–5.00 µm in diameter, scattered. Minute discoidal pores, of two sizes; larger pores, if apparent, larger than trilocular pores, present in groups of 1–3 pores on mid-line of some thoracic and abdominal segments, each about 2.5 µm in diameter; smaller pores scattered over dorsum, each about 2.5 µm in diameter. Oral collar tubular ducts often have distinct rims, usually occurring singly adjacent to some or most cerarii, each about 10 µm long and 5 µm wide. Multilocular disc pores absent.

Venter. Setae flagellate, each 12.5–147.0 µm long, longest setae located medially on head. Apical seta on anal lobe 240 µm long. Multilocular disc pores, each 6.2–7.5 µm in diameter, present around vulva, in single rows across posterior edges of abdominal segments V to IX and across anterior edges of segments VI to IX; and occasionally 0–4 pores associated with fore coxae, and 0–6 on the whole ventral thorax; number on each abdominal segment as follows: segments V with 18–42, VI 36–66, VII 52–72, VIII + IX 90–124. Trilocular pores scattered, each about 3.75 µm in diameter. Minute discoidal pores scattered. Oral collar tubular ducts of 2 sizes: (i) larger ducts, each about 10.00 µm long and 3.75 µm wide, present as segmental clusters on margin of abdominal segments II–VIII, in rows across median areas of abdominal segments, as follows: segment I with 12–38, II 10–23, III 22–34, IV 30–46, V 30–58, VI 49–80, VII 60–110, VIII + IX 52–122, scattered over median area of thorax, a single pore sometimes present on head, and up to 3 pores sometimes present on each margin of the thoracic segments; and (ii) smaller ducts, each 7 µm long and about 3 µm wide, present on marginal and submarginal areas of the abdominal segments.

Discussion

The molecular analyses reported herein represent the largest taxonomic work to date on *Planococcus* populations from the eastern Mediterranean, which constitute a separate genetic clade. Previous studies have reported five haplotypes belonging to this clade: two from USA (one of them shared with Mexico), two from Israel, and one from Egypt (Daane et al. 2018b). We substantially improve upon this existing knowledge of the Group I (*P. vitis*) by sequencing 233 specimens and describing 19 new haplotypes from Egypt. The high genetic diversity found suggests that Egypt may be part of the species native area, but a larger sampling effort should be carried out in other geographical areas. The observed genetic structure of the Egyptian population appears to be mainly driven by the Nile River and human-made barriers such as highways (Fig. 2). Previous studies on mealybug population genetics also found geographic barriers as the main structuring factors (Correa et al. 2015). These results suggest that mealybug dispersion between regions, countries and continents is mainly caused by human management of vineyards rather than by natural dispersion (Miller et al. 2005), even though control measures are in place to reduce the movement of mealybugs (Haviland et al. 2005; Daane et al. 2018a).

The genetic analyses carried out here, including worldwide samples from previous studies, provide strong support for the presence of cryptic taxa within *P. ficus*. A first clade (Group I in Fig. 3A) corresponds mainly to samples from Egypt obtained in this study and samples from North America (California) obtained by Park et al. (2011). It has been suggested that populations of the so-called *P. ficus* arrived in North America from vines imported from the Middle East (Daane et al. 2018b), in agreement with our results. The second clade (Group II) includes samples from France and South Africa and corresponds to the "Mediterranean group" of Daane et al. (2018b). Malausa et al. (2011) already reported *P. ficus* specimens collected from vineyards in Limoux (Aude, France) and their genetic clustering with material from South Africa. This is also supported by historical evidence since South African vineyards originated from vines imported from Western Europe, particularly France (Dickenson 1992).

The COI genetic distance between the two main *P. ficus* clades agrees with the distance separating *P. minor* and *P. citri* (Rung et al. 2009) and with the average interspecific

distance described in other mealybugs using the same DNA barcoding region (Park et al. 2011). Interestingly, the nuclear marker 28S also presents the same genetic distance separating the two *P. ficus* clades (1 bp) and the pair *P. minor* and *P. citri* (Fig. S1; see also Abd-Rabou et al. 2012). The further characterization of the genetic diversity of the Middle Eastern group and the thorough morphological re-evaluation of specimens from both groups of putative *P. ficus* suggest that these two clades represent cryptic species. A revision of the available literature also revealed that Group II must in fact correspond to *P. ficus s.str.*, whereas the eastern Mediterranean clade (Group I) corresponds to *P. vitis*.

These results have direct implications for the management of *P. ficus* and could explain the lack of success in previous control programs to fight this pest (i.e., use of specific sexual pheromones and/or parasitoids for biological control). For example, it has been shown that populations of so-called *P. ficus* from California (Hinkens et al. 2001) and Israel (Zada et al. 2003) produce different sexual pheromones. The presence of more than one sexual pheromone as evidence for the presence of different strains or races has been already reported in other insect species (Malausa et al. 2007). The variable response to these compounds of male mealybugs from Israel and Portugal provides further evidence of the necessity of recognizing the new eastern Mediterranean taxon (Kol-Maimon et al. 2010).

The integrative taxonomy approach used here provides further support to the validity of a previously described *Planococcus* species from eastern Mediterranean vineyards and has direct implications on the biological control of mealybugs reported as *P. ficus*, which has been commonly based on the encyrtid *Anagyrus pseudococci* (Girault). Parasitoids constitute the main biological agents used against scale insects because they tend to be highly specific. However, a recent study showed that this parasitoid consists of two cryptic species: *A. pseudococci* and *A. vladimiri* Triapitsyn (Triapitsyn et al. 2007; Andreason et al. 2019). Further studies on the specificity of *Anagyrus* spp. against *P. ficus s.str.* and *P. vitis* will improve current biological control practices. The search for candidate biological control agents is best conducted within the mealybug's native area (Malausa et al. 2016) and our results suggest that candidate biological control agents against this pest may be found in Egypt. Further efforts to characterize genetic diversity using larger samples from other geographical areas should help to narrow down the native area and identify suitable parasitoids.

Author's contributions

EP and TM designed the study. TM and KMD provided the first insights on Group I and Group II separation. ShAR carried out the fieldwork in Egypt. AC, KMD, MBK, and VCPS collected samples in vineyards. MCGC completed the molecular biology work and prepared figures. JFG, VCPS, and MBK conducted the morphological analyses. MCGC and FP analyzed the data and wrote the first draft of the manuscript. MCGC, FP, ShAR, KMD, JFG, VCPS, MBK, AC, EP, and TM contributed to the writing, review, and editing of the manuscript.

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Declarations

Conflict of interest The authors declare no competing interests.

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