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Molecular and morphological identification of pistachio armored scale insects (Hemiptera: Diaspididae), with description of a new species

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Abstract

Members of the family Diaspididae (Hemiptera: Coccomorpha) can be devastating pests that suck parenchyma cell contents from crops and cause severe damage to pistachio trees (*Pistacia vera* L.). The current research collected and characterized diaspidid species from pistachio orchards in Kerman province, Iran, according to their morphological and molecular features. *Lepidosaphes pistaciae* Archangelskaya, *Suturaspis davatchi* (Balachowsky & Kaussari) and *Melanaspis inopinata* (Leonardi) are redescribed and a new species, *Melanaspis pistaciae* Hosseininaveh & Kaydan **sp. n.**, is described. Phylogenetic trees based on molecular analysis of COI and 28S rDNA fragments placed all the species in separated clades and confirmed *M. pistaciae* as a new taxon which is concluded by morphological differences. Molecular analysis suggests non-monophyly of the populations of each species. *Melanaspis pistaciae* **sp. n.** has spread to most cultivated pistachio areas in Iran and has probably been misidentified as *M. inopinata* in the past. Further investigation of the biology of this species may lead to development of more effective approaches for controlling this pest.

Key words: COI, 28S, *Lepidosaphes, Melanaspis, Suturaspis, Pistacia vera*, pest

Introduction

Scale insects (Hemiptera: Coccomorpha) are some of the important hemipteran pests on agricultural crops and ornamentals. These parenchyma cell-feeders are closely related to aphids, whiteflies and jumping plant lice in the suborder Sternorrhyncha (Kondo *et al.* 2008). Most scale insects are less than 5 mm in length and cryptic, resembling a part of their host plants. Some secrete waxy compounds that cover their bodies as a separate scale or adhere to their host cuticles (Gullan & Cook 2007). Female scale insects are paedomorphic, with simple morphology; several posterior segments of the abdomen are fused to form a pygidium and no traces of wings are visible (Andersen *et al.* 2010). After the pupal stage, adult males (if present) become alate with non-functional mouthparts (Kondo *et al.* 2008).

The scale insects include 50 families (34 extant and 16 extinct families) and about 8,196 known species belonging to 1190 genera (García *et al.* 2016). In Iran, the first coccoid species was reported in 1902 by Rübsaamen. Since then, additional species have been reported by Lindinger (1905; 1911; 1931), Green (1919), Archangelskaya (1937), Afchar (1937), Bodenheimer (1944), Borchsenius (1952), Balachowsky (1951; 1953; 1954), Kaussari (1957; 1970), Kaussari & Farahbakhsh (1968), Asadeh & Mosaddegh (1991), Farahbakhsh (1961), Vahedi (2002; 2007), Torabi *et al.* (2010) and Moghaddam (2004; 2009; 2013a). Afchar (1937), Kozár *et al.* (1996), Seghatoleslami (1977) and Moghaddam (2004, 2009) have published checklists for the scale insects of Iran. Moghaddam (2013b) recently reported 275 scale species belonging to 113 genera and 13 families in Iran.

The family Diaspididae are armored scale insects, characterized by atrophied eyes and antennae and a lack of

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legs (Andersen *et al.* 2010). This family contains the largest number of described species of the coccoid families (more than 2590 belonging to 418 genera) (García *et al.* 2016). Some armored scales are major pests; some polyphagous species feed on 100 families of plants. Their small size make them difficult to detect and identify, creating a serious problem for plant quarantine specialists (Kondo *et al.* 2008). The Diaspididae is the most well-represented scale family in Iran, with 151 species in 45 genera. Among these species, 16 belong to the genus *Lepidosaphes* Shimer, two belong to *Melanaspis* Cockerell, and four belong to *Suturaspis* Lindinger (Moghaddam 2013b).

Pistachio nuts (*Pistacia vera* L., Anacardiaceae) are an important agricultural product of Iran. The trees are attacked by many pests including scale insects, some of which cause major economic damage annually. Eight diaspidid species were reported on *Pistacia* species by Taghizadeh & Safavi (1960) and Esmaeili (1991). Three pest species have been identified, the first being *Lepidosaphes pistaciae* Archangelskaya, commonly called yellow pistachio scale but known locally in Iran as pistachio twig and fruit scale, which lives on the leaves, fruit, twigs and branches of *P. vera* (Mehrnejad 2014; García *et al.* 2016). The microscopic cuticular features of the adult females differ according to their location on the tree, which led Borchsenius (1949) to describe it as two species (*L. pistaciae* and *L. pistacicola*). Subsequently, these morphs were named as biological forms of the same species, *L. pistaciae* forma *typica* and *L. pistaciae* forma *pistacicola,* by Balachowsky (1954). The second species is *Melanaspis inopinata* (Leonardi), locally known as pistachio trunk and branch scale; this is another pistachio pest that lives on the branches and trunks of trees in most pistachio-cultivation areas in Iran (Esmaeili 1991). *Suturaspis davatchi* (Balachowsky & Kaussari) is the third species, locally called Nooghi scale, and was first reported on *P. khinjuk* Stocks and *P. atlantica* Desf. in Iran (Esmaeili 1991). It now forms relatively large populations on the trunks and branches of *P. vera* in some of the orchards of Kerman province (Mehrnejad 2014).

Classification of scale insects at the species level is mainly based on adult female cuticular features; however, distinguishing species with only slight inter-specific differences is difficult (Park *et al.* 2011). Only scale insect specialists are able to identify taxa accurately because it requires specific skills that are achieved only through extensive experience (Jinbo *et al.* 2011). Due to increased interest in biodiversity, species identification has become increasingly important; therefore, alternative and accurate identification methods are required for use by non-expert researchers. One the most promising methods is the use of molecular data instead of morphological identification (Jinbo *et al.* 2011).

Recent molecular phylogenetic research has been used successfully for molecular identification of members of the family Diaspididae. In such studies, the phylogenetic relationship between species are usually analyzed based on sequences of the following gene regions: 28S rDNA; EF1a; mitochondrial COI and COII,; and 16S rDNA of bacterial endosymbionts (Morse & Normark 2006; Andersen *et al.* 2010). Edwards *et al.* (2008) identified three diaspidid species using the mitochondrial genes COI and COII, , resulting in quick, easy identification. As far as the authors of the present study are aware, there is no published molecular data on Iranian diaspidid species.

The area under pistachio cultivation has steadily increased in different parts of Iran, the main pistachio-producing country worldwide; however, updates on the fauna on this crop have not kept pace with this increase. Revisions of pistachio diaspidids are outdated and there appear to be differences between populations within each species. The present study updates, revises and redefines pistachio scale insects found in Iran, using morphological characteristics and molecular data (28S rDNA and mitochondrial COI genes) to resolve ambiguities in some of the diaspidid species found in Kerman province.

Materials and methods

Taxa examined. Scale-infested stems and branches of pistachio trees were collected from orchards in 10 pistachio-producing locations in Kerman province in Iran (Anar, Baghin, Davaran, Koshkoiye, Shahrebabak, Kabotarkhan, Noogh, Rafsanjan, Sirjan and Zarand) between October 2012 and October 2013. In total, 10 populations of *Lepidosaphes pistaciae,* 6 populations of *Melanaspis pistaciae*, 2 populations of *Melanaspis inopinata* and 1 population of *Suturaspis davatchi* were collected (Table 1). A needle that had been put in glacial acetic acid to remove any DNA contamination was used to take adult female scale insects from under their protective scales; they were then preserved in 100% ethanol and stored at -21°C. Each adult female was then slide-mounted using the method recommended by Kosztarab & Kozár (1988), and identified using morphological characters, based on the

TABLE 1. Voucher information and GenBank accession numbers for 4 species and related taxa.

Taxon	Location Iran, Kerman,	Longitude	latitude	Altitude	Date	Voucher No.	Host plant	COI Gen Bank accession no.	28S Gen Bank accession no.
Lepidosaphes pistaciae	Anar	N30° 53' 41"	E55° 12' 18"	1434	16/1/2013	LA-1	*Pistacia vera*	LC175357	LC175407
Lepidosaphes pistaciae	Anar	N30° 53' 41"	E55° 12' 18"	1434	16/1/2013	LA-2	*Pistacia vera*	LC175358	LC175408
Lepidosaphes pistaciae	Anar	N30° 53' 41"	E55° 12' 18"	1434	16/1/2013	LA-3	*Pistacia vera*	LC175359	LC175409
Lepidosaphes pistaciae	Baghin	N30° 04' 56"	E56° 44' 54"	1858	12/1/2013	LB-1	*Pistacia vera*	-	LC175410
Lepidosaphes pistaciae	Baghin	N30° 04' 56"	E56° 44' 54"	1858	12/1/2013	LB-2	*Pistacia vera*	LC175360	LC175411
Lepidosaphes pistaciae	Baghin	N30° 04' 56"	E56° 44' 54"	1858	12/1/2013	LB-3	*Pistacia vera*	LC175361	LC175412
Lepidosaphes pistaciae	Davaran	N30° 34' 32"	E56° 10' 44"	1848	5/1/2013	LD-1	*Pistacia vera*	LC175362	LC175413
Lepidosaphes pistaciae	Davaran	N30° 34' 32"	E56° 10' 44"	1848	5/1/2013	LD-2	*Pistacia vera*	LC175363	LC175414
Lepidosaphes pistaciae	Davaran	N30° 34' 32"	E56° 10' 44"	1848	5/1/2013	LD-3	*Pistacia vera*	LC175364	LC175415
Lepidosaphes pistaciae	Koshkoiye	N30°33'52"	E 55°36'43"	1429	1/1/2013	LF-1	*Pistacia vera*	LC175365	-
Lepidosaphes pistaciae	Koshkoiye	N30°33'52"	E 55°36'43"	1429	1/1/2013	LF-2	*Pistacia vera*	LC175366	LC175416
Lepidosaphes pistaciae	Koshkoiye	N30°33'52"	E 55°36'43"	1429	1/1/2013	LF-3	*Pistacia vera*	LC175367	LC175417
Lepidosaphes pistaciae	Shahrebabak	N 29°59'56"	E 55°42'57"	2355	12/1/2013	LH-1	*Pistacia vera*	-	LC175418
Lepidosaphes pistaciae	Shahrebabak	N 29°59'56"	E 55°42'57"	2355	12/1/2013	LH-2	*Pistacia vera*	LC175368	LC175419
Lepidosaphes pistaciae	Shahrebabak	N 29°59'56"	E 55°42'57"	2355	12/1/2013	LH-3	*Pistacia vera*	LC175369	LC175420
Lepidosaphes pistaciae	Kabotarkhan	N30°18 26"	E56° 22' 51"	1650	6/1/2013	LK-1	*Pistacia vera*	LC175370	LC175421
Lepidosaphes pistaciae	Kabotarkhan	N30°18 26"	E56° 22' 51"	1650	6/1/2013	LK-2	*Pistacia vera*	LC175371	LC175422
Lepidosaphes pistaciae	Kabotarkhan	N30°18 26"	E56° 22' 51"	1650	6/1/2013	LK-3	*Pistacia vera*	LC175372	LC175423
Lepidosaphes pistaciae	Noogh	N30° 49' 00"	E55° 47' 43"	1348	6/1/2013	LN-1	*Pistacia vera*	LC175373	LC175424
Lepidosaphes pistaciae	Noogh	N30° 49' 00"	E55° 47' 43"	1348	6/1/2013	LN-2	*Pistacia vera*	LC175374	LC175425
Lepidosaphes pistaciae	Noogh	N30° 49' 00"	E55° 47' 43"	1348	6/1/2013	LN-3	*Pistacia vera*	LC175375	LC175426
Lepidosaphes pistaciae	Rafsanjan	N30° 22' 28"	E56' 00' 16"	1539	6/1/2013	LR-1	*Pistacia vera*	LC175376	LC175427
Lepidosaphes pistaciae	Rafsanjan	N30° 22' 28"	E56' 00' 16"	1539	6/1/2013	LR-2	*Pistacia vera*	LC175377	LC175428
Lepidosaphes pistaciae	Rafsanjan	N30° 22' 28"	E56' 00' 16"	1539	6/1/2013	LR-3	*Pistacia vera*	LC175378	-
Lepidosaphes pistaciae	Sirjan	N29° 22' 29"	E55° 44' 06"	1731	12/1/2013	LS-1	*Pistacia vera*	LC175379	LC175429
Lepidosaphes pistaciae	Sirjan	N29° 22' 29"	E55° 44' 06"	1731	12/1/2013	LS-2	*Pistacia vera*	LC175380	LC175430
Lepidosaphes pistaciae	Sirjan	N29° 22' 29"	E55° 44' 06"	1731	12/1/2013	LS-3	*Pistacia vera*	LC175381	LC175431
Lepidosaphes pistaciae	Zarnd	N30° 50' 00"	E56° 20' 58"	1600	5/1/2013	LZ-1	*Pistacia vera*	LC175382	LC175432

……continued on the next page

TABLE 1. (Continued)

Taxon	Location Iran, Kerman,	Longitude	latitude	Altitude	Date	Voucher No.	Host plant	COI Gen Bank accession no.	28S Gen Bank accession no.
Lepidosaphes pistaciae	Zarnd	N30° 50' 00"	E56° 20' 58"	1600	5/1/2013	LZ-2	*Pistacia vera*	LC175383	LC175433
Lepidosaphes pistaciae	Zarnd	N30° 50' 00"	E56° 20' 58"	1600	5/1/2013	LZ-3	*Pistacia vera*	-	-
Melanaspis inopinata	Baghin	N30° 10' 52"	E56° 48' 20"	1733	22/10/2013	MB-1	*Pistacia vera*	LC175384	LC175434
Melanaspis inopinata	Baghin	N30° 10' 52"	E56° 48' 20"	1733	22/10/2013	MB-2	*Pistacia vera*	LC175385	LC175435
Melanaspis inopinata	Baghin	N30° 10' 52"	E56° 48' 20"	1733	22/10/2013	MB-3	*Pistacia vera*	-	-
Melanaspis inopinata	Sirjan	N29° 22' 29"	E55° 44' 06"	1726	22/10/2013	MS-1	*Pistacia vera*	LC175386	LC175436
Melanaspis inopinata	Sirjan	N29° 22' 29"	E55° 44' 06"	1726	22/10/2013	MS-2	*Pistacia vera*	LC175387	LC175437
Melanaspis inopinata	Sirjan	N29° 22' 29"	E55° 44' 06"	1726	22/10/2013	MS-3	*Pistacia vera*	LC175388	LC175438
Melanaspis pistaciae	Davaran	N30° 34' 32"	E56° 10' 44"	1848	18/9/2012	MD-1	*Pistacia vera*	LC175389	LC175439
Melanaspis pistaciae	Davaran	N30° 34' 32"	E56° 10' 44"	1848	18/9/2012	MD-2	*Pistacia vera*	LC175390	LC175440
Melanaspis pistaciae	Davaran	N30° 34' 32"	E56° 10' 44"	1848	18/9/2012	MD-3	*Pistacia vera*	LC175391	LC175441
Melanaspis pistaciae	Koshkoiye	N30°33'52"	E 55°36'43"	1429	17/9/2012	MF-1	*Pistacia vera*	LC175392	LC175442
Melanaspis pistaciae	Koshkoiye	N30°33'52"	E 55°36'43"	1429	17/9/2012	MF-2	*Pistacia vera*	LC175393	LC175443
Melanaspis pistaciae	Koshkoiye	N30°33'52"	E 55°36'43"	1429	17/9/2012	MF-3	*Pistacia vera*	LC175394	LC175444
Melanaspis pistaciae	Kabotarkhan	N30°18 26"	E56° 22' 51"	1650	26/8/2012	MK-1	*Pistacia vera*	-	LC175445
Melanaspis pistaciae	Kabotarkhan	N30°18 26"	E56° 22' 51"	1650	26/8/2012	MK-2	*Pistacia vera*	LC175395	LC175446
Melanaspis pistaciae	Kabotarkhan	N30°18 26"	E56° 22' 51"	1650	26/8/2012	MK-3	*Pistacia vera*	-	LC175447
Melanaspis pistaciae	Rafsanjan	N30° 22' 30"	E56° 00' 15"	1530	26/8/2012	MR-1	*Pistacia vera*	LC175396	LC175448
Melanaspis pistaciae	Rafsanjan	N30° 22' 30"	E56° 00' 15"	1530	26/8/2012	MR-2	*Pistacia vera*	LC175397	LC175449
Melanaspis pistaciae	Rafsanjan	N30° 22' 30"	E56° 00' 15"	1530	26/8/2012	MR-3	*Pistacia vera*	LC175398	LC175450
Melanaspis pistaciae	Sarcheshme	N 29°59'56"	E 55°42'57"	2355	20/9/2012	MV-1	*Pistacia atlantica*	LC175399	LC175451
Melanaspis pistaciae	Sarcheshme	N 29°59'56"	E 55°42'57"	2355	20/9/2012	MV-2	*Pistacia atlantica*	-	LC175452
Melanaspis pistaciae	Sarcheshme	N 29°59'56"	E 55°42'57"	2355	20/9/2012	MV-3	*Pistacia atlantica*	LC175400	LC175453
Melanaspis pistaciae	Zarnd	N30° 50' 00"	E56° 20' 58"	1600	19/9/2012	MZ-1	*Pistacia vera*	LC175401	LC175454
Melanaspis pistaciae	Zarnd	N30° 50' 00"	E56° 20' 58"	1600	19/9/2012	MZ-2	*Pistacia vera*	LC175402	LC175455
Melanaspis pistaciae	Zarnd	N30° 50' 00"	E56° 20' 58"	1600	19/9/2012	MZ-3	*Pistacia vera*	LC175403	LC175456
Suturaspis davatchi	Noogh	N30° 46' 48"	E55° 50' 30"	1372	6/1/2013	SN-1	*Pistacia vera*	LC175404	LC175457
Suturaspis davatchi	Noogh	N30° 46' 48"	E55° 50' 30"	1372	6/1/2013	SN-2	*Pistacia vera*	LC175405	LC175458
Suturaspis davatchi	Noogh	N30° 46' 48"	E55° 50' 30"	1372	6/1/2013	SN-3	*Pistacia vera*	LC175406	LC175459

keys in Balachowsky (1951; 1953; 1954) and Danzig (1993). The morphological terminology used follows that of Miller & Davidson (2005). Measurements for each species were taken from five specimens in each population and the mean range for each species is given in brackets. The slides are stored in the Zoological Museum in the Plant Protection Department at the University of Tehran.

DNA sequences. Three specimens from each populations used for molecular studies were preserved in 100% ethanol (Table 1). Prior to DNA extraction, all specimens were examined under a microscope for the presence of parasitoids, and were discarded if any parasitoid was present. DNA was extracted from single, parasitoid-free, adult females using the DNEasy Blood & Tissue Kit (Qiagen; USA). For each specimen, the cuticle was recovered and stored in ethanol after DNA extraction so that vouchers could be slide-mounted and kept in the zoological museum. Among the sequences that have been used for scale insects molecular studies, here 28S rDNA and COI genes were chosen due to their improved appropriate efficiency as the molecular identification tools, also for their less difficulty sequencing and analyzing than the others. PCR products were generated from a mitochondrial gene, cytochrome oxidase I (COI), and one nuclear gene, using fragments of the D2 and D3 regions of the large subunit ribosomal DNA gene (28S). The primers for amplification and sequencing were 5'-CAA CAT TTA TTT TGA TTT TTT GG-3' (C1-J-2183 aka Jerry) and 5'-GCW ACW ACR TAA TAK GTA TCA TG-3' (C1-N-2568 aka BEN3R as designed by Schultz; Smithsonian Institute) for COI (which was used in several studies for species determination for different genus (Gullan *et al.* 2010)), and 5'-TCG GAR GGA ACC AGC TAC TA-3' (A335 REVERSE) and 5'-GAG AGT TMA ASA GTA CGT GAA AC-3' (s3660 FORWARD) for 28S.

The PCR reaction components and final concentrations were 1.5 to 2.5 mM MgCl2, 0.2 mM dNTPs, and 1 unit Taq polymerase in a proprietary buffer (PCR Master Mix; Promega Biotechnology), 0.2 µM of each primer, and 3 µl DNA template in a final volume of 25 µl. The PCR cycling protocol for COI was 95°C for 7 m, followed by 40 cycles of 95°C for 1 m, 45°C for 1 m, and 72°C for 1 m 30 s with a final extension at 72°C for 5 m. The protocol for 28S was 94°C for 4 m followed by 35–45 cycles of 94°C for 1 m, 49–52 °C for 1 m, and 72°C for 1 m 30 s with a final extension at 72°C for 4 m. Phylogenetic analysis was carried out on PCR products purified and sequenced by Iontek (Turkey). DNA was extracted from 57 specimens and 50 sequences of COI fragment and 53 sequences of 28S fragment were aligned and analyzed. All sequences were deposited in GenBank (accession numbers: LC175357- 459).

The sequences were edited using BioEdit ver. 7.0.9.0 (Hall 1999) and aligned with MUSCLE using the default parameters (Edgar 2004) followed by manual adjustment. The positions of the indels were treated as missing data for all datasets. Pairwise genetic distances between sequences were calculated using the maximum composite likelihood model with pairwise deletions and gamma-distributed among-site rate variation as implemented in MEGA ver. 5.2 (Tamura *et al.* 2011).

Phylogenetic analysis. Maximum parsimony (MP) analysis for COI and 28S datasets individually was conducted using PAUP* ver. 4.0b10 (Swofford 2002) for phylogenetic analysis. The heuristic search option was employed for the dataset using tree bisection-reconnection branch swapping with 1000 replications of a random addition sequence and an automatic increase in the maximum number of trees. Uninformative characteristics were excluded from analysis yielding a dataset of 276 parsimony-informative characters for COI and 664 for 28S. Branch supports were assessed by 1000 bootstrap replicates (yielding bootstrap percentages, BP; Felsenstein 1985) with the same settings as for heuristic searches. The model of sequence evolution for the dataset was selected using the program MrModeltest ver. 2.3 (Nylander 2004) based on the Akaike information criterion (Posada & Buckley 2004). The COI dataset was analyzed using the GTR + G (six substitution types with rate variation across sites modeled using a gamma distribution), whereas GTR+ I (six substitution types with the proportion of sites being invariant) was identified as the best model for the 28S.

MrBayes ver. 3.2 (Ronquist *et al.* 2012) was used for Bayesian phylogenetic analysis. Posteriors of the model parameters were estimated from the data using the default priors. The analysis was done with ten million generations using the Markov chain Monte Carlo search. MrBayes performed two simultaneous analyses starting from different random trees (Nruns = 2), each with four Markov chains, and the trees were sampled every 100 generations. TRACER v.1.5 (Rambaut & Drummond 2007) was used to evaluate mixing of chains and to determine burn-in. The first 25% of trees were discarded as burn-in. The remaining trees were then used to build a 50% majority rule consensus tree accompanied with posterior probability (PP) values. Tree visualisation was carried out using TreeView ver. 1.6.6 (Page 2001). Maximum likelihood (ML) analysis was performed for the datasets in raxmlGUI ver. 1.3. (Silvestro & Michalak 2012). The model of evolution employed for the dataset was

the same as that of MP. Bootstrap values for ML (BP^{ML}) were calculated in raxmlGUI based on 1000 replicates with one search replicate per bootstrap replicate.

Results

Melanaspis Cockerell

Synonyms. *Pelomphala* MacGillivray, 1921; (García *et al.*, 2016).
Type species. *Melanaspis obscurus* (Comstock).

Field characters. Female scale usually thick, rough, dark, matte black, brown or dark grey, and sub-circular convex shape. Male puparium flattened oval, clearer.

Slide-mounted characteristics. Adult female pyriform, cephalothoracic cuticle without sclerotization. Thoracic tubercles absent or very low, but thick meta-pleural protrusion always present. Pygidium wide, always forming an apical angle greater than 90°, with 4 well-developed pairs of lobes (L_1, L_2, L_3, L_4), the fourth pair often reduced. Margin of segment V sclerotized, often to the level of segment IV. Medial and lateral pectinae small, short, degraded, rarely illustrated, present in the interlobular spaces. Paraphyses robust, thickened, fusiform, equal or unequal, developed on both sides of pygidium on either side of inter-lobular space 1 (between L_1 and $L_{2)}$; 2 present between L_2 and $L_{3;}$ and 2 between L_3 and L_4. The latter pair is less clear and is often confused with the marginal zone of sclerotization on segment V. Anal opening diameter equal to or less than that of L_1, located anterior to 1/3 the apical or in the central area of the pygidium. Dorsal macroducts relatively few, long, with oval pores and different diameter. Ventral microducts present, very small; dorsal area of the pygidium with large cuticular oblique grooves separating segments VI–VII and V–VI up to level of latero-basal apophysis.

Key to adult female *Melanaspis* species in the Palearctic Region

(adapted from Danzig 1993 and Balachowsky 1951).

1. Paraphyses on pygidial margin of unequal size; external paraphyses on segment 8 more developed than others 3
- Paraphyses of pygidial margin thick and fusiform, more or less all the same size. 2
2. Pygidial lobes each without medial notch; number of submarginal macroducts on segment V fewer than 20 . . . . . *M. inopinata* (Leonardi)
- Pygidial lobes each with one distinct medial notch; number of submarginal macroducts on segment V more than 20 . . . . . *M. pistaciae* Hosseininaveh & Kaydan **sp. n.**
3. Perivulvar pores absent; lobe size gradually increasing from L_1 to L_4; dorsal macroducts on segments VI–VII arranged in irregular rows or sometimes in two rows . . . . . *M. smilacis* Comstock
- Perivulvar pores present; L_1 larger than other lobes; dorsal macroducts of segments VI–VII arranged in oblique regular single row. . . . . *M. louristanus* Balachowsky & Kaussari

Melanaspis inopinata (Leonardi, 1913).

Figure 1.

Synonyms. *Aonidiella inopinata* Leonardi, 1913; *Aonidiella robusta* Grassi & Berlese in Berlese 1915; *Melanaspis inopinata* Bodenheimer, 1924; *Chrysomphalus robusta* Koroneos, 1934; *Aspidiotus* (*Aonidiella*) *inopinata* McKenzie, 1938; *Melanaspis robustus* McKenzie, 1939; *Chrysomphalus inopinatus* Ferris, 1941; *Pelomphala inopinata* Lupo, 1954; *Melanaspis inopinata* Borchsenius, 1966 (García *et al.*, 2016).

Material examined. 6 adult females on 1 slide, Iran, Kerman, Baghin, N: 30° 10' 52", E: 56° 48' 20", 1,733 m, on branches and trunk of *Pistacia vera*, coll: F. Hosseininaveh, 22.x.2013, coll. no: MB-127, one individual used for drawing; 6 adult females on 1 slide, Iran, Kerman, Sirjan, N: 29°22' 29", E: 55°44' 06", 1,726 m, on branches and trunk of *P. vera*, coll: F. Hosseininaveh, 22.x.2013, coll. no: MS-126.

Field characters. Female scale cover about 2.5 mm long, robust, subcircular, convex, usually dark brown in central area, greyish brown or dark grey toward edges; larval exuviae eccentric but not marginal, colour dark;

ventral scale well developed, but the greater part remains attached to the plant. Male puparium scale cover oval, about 1.8 mm long, convex anteriorly, flattened at apex, paler than female scale; larval exuviae eccentric, dark brown.

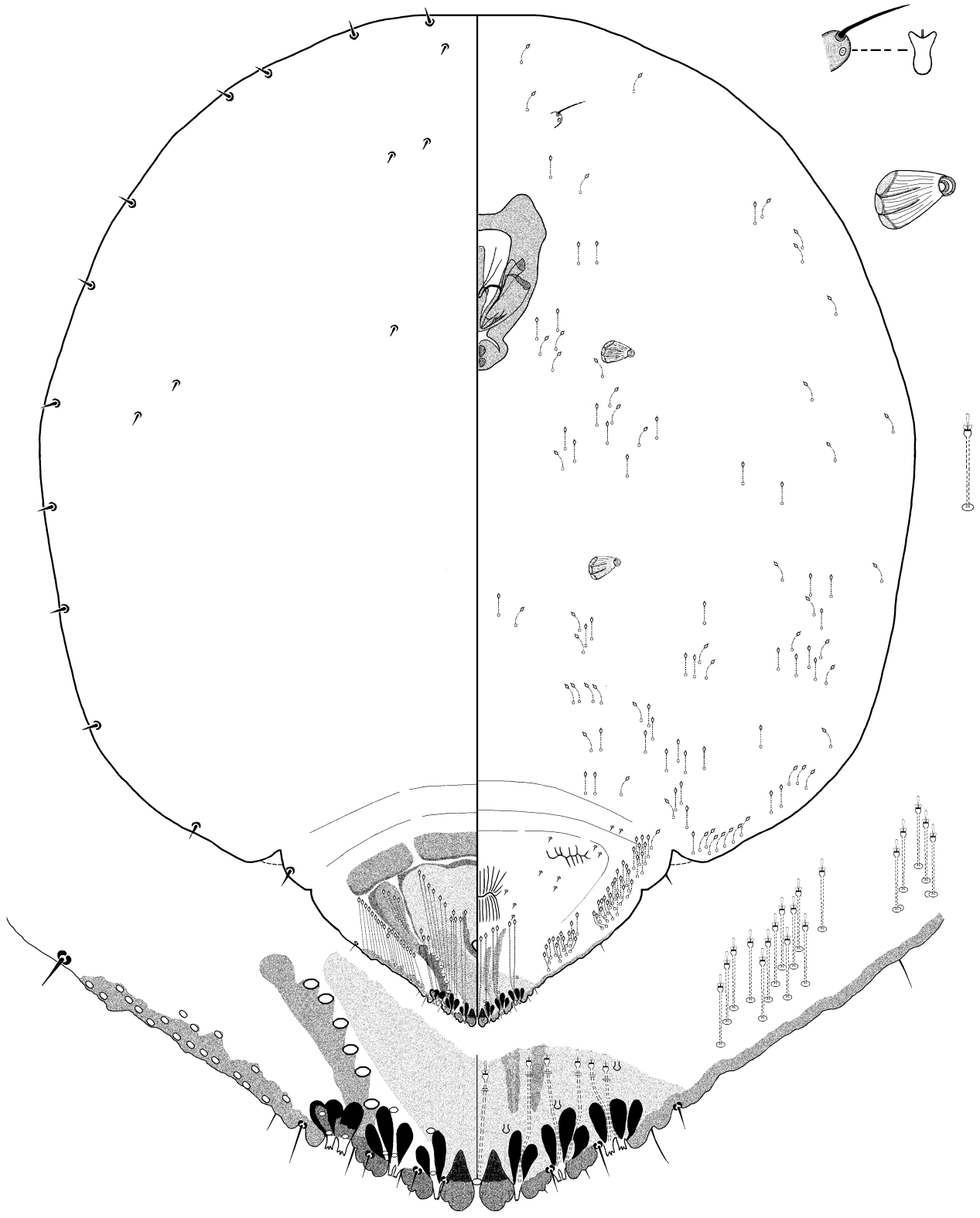


FIGURE 1. Adult female of *Melanaspis inopinata* (Leonardi), illustration by Hosseininaveh.

Slide-mounted characteristics. Adult female pyriform, 1.70–1.85 (1.79) mm long and 1.49–1.70 (1.56) mm wide, cephalothorax and the anterior abdominal segments without sclerotized area. Thoracic tubercle absent. Antennae normally each with 1 long and 1 very small seta. Perispiracular pores absent. Distance from apex of the median lobe to anteriormost edge of pygidial sclerotized area 0.2 times body length. Pygidium wide and obtuse, with 4 pairs of lobes (L_1, L_2, L_3, L_4). Median lobes (L_1) each 15–16 (15) µm wide, lobes parallel, symmetrical, rounded and without notches or each with low lateral notch; separated by space 0.2–0.3 (0.2) times width of median lobe; medial margins with axes parallel or diverging apically, lateral margins converging apically. Second lobes (L_2) each 16–18 (17) µm wide, asymmetrical, barely wider than median lobe, with 2 lateral notches, medial notches absent. Third lobes (L_3) each 16–18 (17) µm wide, asymmetrical, about same size as second lobe, with 3 lateral notches, medial notches absent. Fourth lobes (L_4) simple, each 8–10 (9) µm wide, generally reduced, pressed into the margin of segment V and slightly protruding. Pectinae very short, reduced to small protruding plates, the lateralmost generally most developed, formula commonly 1-2-2. Marginal paraphyses robust, fusiform and approximately all same size; paraphysis formula always 2-3-3. Margin of segment V heavily sclerotized. Anal opening oval, diameter 0.7 times less than width of L_1, located relatively high, almost in central area of pygidium. Dorsal macroducts very long, with oval orifices; ducts from some groups extend over entire height of pygidium; with 2 macroducts between L_1 and L_2 each 173–178 (177) µm long ; an oblique row of 6 or 7 large macroducts each 178–181 (180) µm long in cuticular groove between L_2 and L_3, each orifice surrounded externally by sclerotized area; 4 or 5 smaller macroducts present in sclerotized part of segment IV; marginal area of segment V with 13–19 (16) macroducts along its entire length, each 89–92 (90) µm long . Dorsal macroducts absent between prepygidial segments and head. Oblique and transverse dorsal cuticular grooves of pygidium well marked. Microducts absent from dorsum but numerous on venter of abdominal segments II and III, in groups 7–16, each 27–35 (31) µm long; 3 of these groups present in submarginal areas of segments IV and V. Apico-ventral area of pygidium with slightly oblique longitudinal sclerotization, extending almost to vulva. Three papillate appendages (each 2.5 µm in length and 1.5 µm in width) present in submarginal area of pygidial segments VI–VIII, situated anteriorly to L_1, L_2 and third group of paraphyses.

Host plants. *Acer* sp., *A. cinerascens* Boiss. (Aceraceae); *Pistacia atlantica* Desf., *P. khinjuk* Stocks, *P. lentiscus* L., *P. palaestina* Boiss., *P. terebinthus* L., *P. vera* (Anacardiaceae); *Arbutus unedo* L. (Ericaceae); *Astragalus* sp., *Cercis siliquastrum* L., *Sophora japonica* L. (Fabaceae); *Juglans regia* L. (Juglandaceae); *Fraxinus* sp., *Jasminum fruticans* L. (Oleaceae); *Rhamnus rhodopea* Velen. (Rhamnaceae); *Amygdalus orientalis* Mill., *Cotoneaster* sp., *Crataegus* sp., *C. monogyna* Jacq., *Malus* sp., *M. domestica* Borkh., *Prunus* sp., *P. armeniaca* L., *P. avium* L., *P. silvatica* Roxb., *Pyrus* sp., *P. communis* L., *P. elaeagnifolia* Pall., *P. malus* L., *P. nigra* (Willd.) Sarg. (Rosaceae); *Populus nigra* L. (Salicaceae); *Celtis tournefortii* Lam. (Ulmaceae) (García *et al.*, 2016).

Distribution. Armenia, Cyprus, Egypt, Greece, Iran, Iraq, Israel, Italy, Lebanon, Pakistan, Sardinia, Sicily, Turkey (García *et al.*, 2016).

Melanaspis pistaciae Hosseininaveh & Kaydan sp. n.

Figure 2.

Material examined. Holotype. Adult female, Iran, Kerman, Kabotarkhan, N: 30°18 26", E: 56° 22' 51", 1,650 m, on branches and trunk of *Pistacia vera*, coll: F. Hosseininaveh, 26.viii.2012, coll. No: MK-123. **Paratypes.** 4 females on same slide as holotype; 5 adult females on 1 slide, Iran, Kerman, Rafsanjan, N: 30°22'30", E: 56°00'15", 1,530 m, on branches and trunk of *P. vera*, coll: F. Hosseininaveh, 26.viii.2012, coll. no: MR-121; 5 adult females on 1 slide, Iran, Kerman, Koshkoiye, N: 30°33'52", E: 55°36'43", 1,429 m, on branches and trunk of *P. vera*, coll: F. Hosseininaveh, 17.ix.2012, coll. no: MF-122; 5 adult females on 1 slide, Iran, Kerman, Davaran, N: 30°34'32", E: 56°10'44", 1,848 m, on branches and trunk of *P. vera*, coll: F. Hosseininaveh, 18.ix.2012, coll. no: MD-124; 5 adult females on 1 slide, Iran, Kerman, Zarnd, N: 30°50'00", E: 56°20'58", 1,600 m, on branches and trunk of *Pistacia vera*, coll: F. Hosseininaveh, 19.ix.2012, coll. no: MZ-125; 5 adult females on 1 slide, Iran, Kerman, Sarcheshme, N: 29°59'56", E: 55°42'57", 2,355 m, on branches and trunk of *P. atlantica,* coll: F. Hosseininaveh, 20.ix.2012, coll. no: MV-128.

Field Characters. Female scale cover large, about 2.2 mm long, robust, subcircular, convex, usually dark brown in central area, greyish brown or dark grey toward edges; larval exuviae eccentric but not marginal, colour

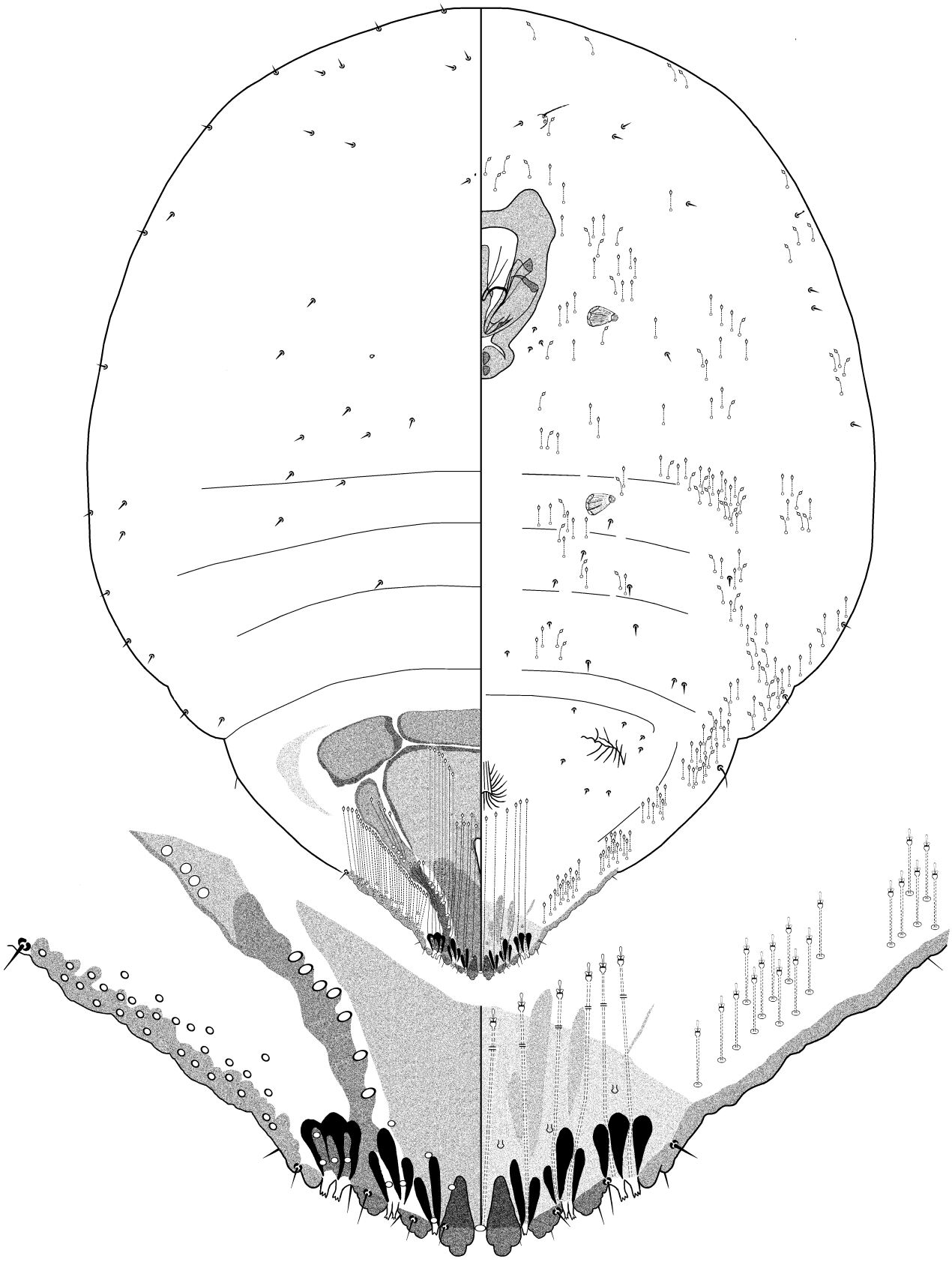


FIGURE 2. Adult female of *Melanaspis pistaciae* Hosseininaveh & Kaydan, **sp. n.** holotype.

dark; ventral scale well developed, but the greater part remains attached to the plant. Male puparium scale cover oval, about 1.8 mm long, convex anteriorly, flattened at apex, paler than female scale; larval exuviae eccentric, dark brown.

Slide-mounted characteristics. Adult female pyriform, 1.28–1.67 (1.48) mm long and 1.10–1.45 (1.25) mm wide, cephalothorax and anterior abdominal segments without sclerotized area. Thoracic tubercle absent. Antennae normally each with 1 or 2 long setae and 1 very small seta. Perispiracular pores absent. Distance from apex of median lobe to anteriormost edge of pygidial sclerotized area 0.3 times body length. Pygidium wide and obtuse, with 4 pairs of lobes (L_1, L_2, L_3, L_4). Median lobes (L_1) each 14–18 (16) µm wide, lobes parallel, symmetrical, rounded, each with 1 lateral notch and 1 clear medial notch; separated by space 0.1–0.3 (0.3) times width of median lobe; medial margins with axes parallel or diverging apically, lateral margins converging apically. Second lobes (L_2) each 15–18 (17) µm wide, asymmetrical, barely wider than median lobe, with 3 lateral notches and 1 medial notch. Third lobes (L_3) each 16–19 (17) µm wide, asymmetrical, about same size as second lobe, with 4 lateral notches and 1 medial notch. Fourth lobes (L_4) simple, each 8–13 (10) µm wide, generally reduced, pressed into margin of segment V and slightly protruding. Pectinae very short, reduced to small and very protruding plates, the lateralmost ones generally most developed, formula commonly 1-2-2. Marginal paraphyses robust, fusiform and approximately all the same size; paraphysis formula always 2-3-3. Margin of segment V heavily sclerotized. Anal opening inverted pyriform, maximum width 0.7 times less than width of L_1, located relatively high, almost in the central area of the pygidium. Dorsal macroducts very long, with oval orifices, ducts from some groups extend over entire height of pygidium; with 2 macroducts between L_1 and L_2 each 173–208 (189) µm long; an oblique row of 6 or 7 large macroducts each 178–220 (201) µm long in cuticular groove between L_2 and L_3, each orifice surrounded by a clear membranous area; 4 or 5 smaller macroducts present in sclerotized part of segment IV; marginal area of segment V with 21–31 (26) macroducts along its entire length, each 84–99 (91) µm long. Dorsal macroducts absent between prepygidial segments and head. Oblique and transverse dorsal cuticular grooves of pygidium well marked. Microducts absent from dorsum but numerous on venter of abdominal segments II and III, in groups of 10–18, each 25–32 (29) µm long; 3 of these groups present in submarginal areas of segments IV and V. Apico-ventral area of pygidium with slightly oblique longitudinal sclerotization, extending almost to vulva. Three papillate appendages (each 2.5 µm long and 1.5 µm wide) present in submarginal area of pygidial segments VI–VIII, situated anteriorly to L_1, L_2 and third group of paraphyses.

Comment. *Melanaspis pistaciae* Hosseininaveh & Kaydan **sp. n.** closely resembles *M. inopinata* in having: (i) four pairs of pygidial lobes (L_1, L_2, L_3, L_4); (ii) reduced pectinae in the formula 1-2-2, with the lateralmost ones generally more developed; (iii) marginal paraphyses approximately all the same size in the formula 2-3-3, and (iv) margin of fifth abdominal segment heavily sclerotized with macroducts along its entire length. However, *M. pistaciae* differs from *M. inopinata* in having: (i) L_1, L_2 and L_3 each with a distinct notch in the medial margin (absent in *M. inopinata*); (ii) margin of fifth abdominal segment with 21–31 (26) macroducts (13–19 (16) in *M. inopinata*); and (iii) distance from the apex of the median lobe to the anteriormost edge of the sclerotized area 0.3 times body length (0.2 in *M. inopinata*).

Etymology. This species was named after its host plant, *Pistacia vera*, to reflect the high levels of infestation in pistachio orchards.

Hosts. *Pistacia vera* L., *P. atlantica* Desf. (Anacardiaceae).

Distribution. Iran.

Lepidosaphes pistaciae Archangelskaya, 1930.

Figure 3.

Common names. Yellow pistachio hard scale; yellow pistachio scale (García *et al.*, 2016)

Synonyms. *Mytilococcus pistaciae* Bodenheimer, 1943; *Lepidosaphes pistacicola* Borchsenius, 1949; *Pistaciaspis pistacicola*, Borchsenius, 1963; *Pistaciaspis pistaciae*, Borchsenius, 1963 (García *et al.* 2016).

Material examined. 5 adult females on 1 slide, Iran, Kerman, Zarnd, N: 30°50'00", E: 56°20'58", 1,600 m, on branches and twigs of *Pistacia vera*, coll: F. Hosseininaveh, 5.i.2013, coll. no: LZ-110, one individual used for drawing; 5 adult females on 1 slide, Iran, Kerman, Rafsanjan, N: 30° 22' 28", E: 56’ 00' 16", 1539 m, on branches

and twigs of *P. vera*, coll: F. Hosseininaveh, 6.i.2013, coll. no: LR-111; 5 adult females on 1 slide, Iran, Kerman, Shahrebabak, N: 29°59'56", E: 55°42'57", 2,355 m, on branches and twigs of *P. vera*, coll: F. Hosseininaveh, 12.i.2013, coll. no: LH-112; 5 adult females on 1 slide, Iran, Kerman, Sirjan, N: 29° 22' 29", E: 55° 44' 06", 1,731 m, on branches and twigs of *P. vera*, coll: F. Hosseininaveh, 12.i.2013, coll. no: LS-113; 5 adult females on 1 slide, Iran, Kerman, Noogh, N: 30° 49' 00", E: 55° 47' 43", 1,348 m, on branches and twigs of *P. vera*, coll: F. Hosseininaveh, 6.i.2013, coll. no: LN-114; 5 adult females on 1 slide, Iran, Kerman, Kabotarkhan, N: 30°18 26", E: 56° 22' 51", 1,650 m, on branches and twigs of *P. vera*, coll: F. Hosseininaveh, 6.i.2013, coll. no: LK-115; 5 adult females on 1 slide, Iran, Kerman, Koshkoiye, N: 30°33'52", E: 55°36'43", 1,429 m, on branches and twigs of *P. vera*, coll: F. Hosseininaveh, 1.i.2013, coll. no: LF-116; 5 adult females on 1 slide, Iran, Kerman, Anar, N: 30° 53' 41", E: 55° 12' 18", 1,434 m, on branches and twigs of *P. vera*, coll: F. Hosseininaveh, 16.i.2013, coll. no: LA-117; 5 adult females on 1 slide, Iran, Kerman, Baghin, N: 30° 04' 56", E: 56° 44' 54", 1,858 m, on branches and twigs of *P. vera*, coll: F. Hosseininaveh, 12.i.2013, coll. no: LB-118; 5 adult females on 1 slide, Iran, Kerman, Davaran, N: 30° 34' 32", E: 56° 10' 44", 1,848 m, on branches and twigs of *P. vera*, coll: F. Hosseininaveh, 5.i.2013, coll. no: LD-119.

Field characters. Female scale cover oyster like, 2.4–3.0 mm long, 1.0–1.8 mm wide, light or dark brown, flared posteriorly, flattened at edges, straight or wavy, central part moderately convex; larval exuviae on cephalic end, first shining yellow, second dark brown. Male scale cover about 1.6 mm long, narrow, linear, very light brown, almost white; larval exuviae on cephalic end, light yellow.

Slide-mounted characteristics. Adult female oval, 1.21–1.59 (1.39) mm long and 0.61–0.87 (0.75) mm wide, widest at abdominal segment II. Cuticle entirely membranous. Antenna with 2–4 (4) setae. Anterior spiracles each with 2–5 (3) pores, posterior spiracles each with 1–3 (1) pores, each pore with 3 loculi. Lateral abdominal lobules, formed as robust, pleural intersegmentary finger-like nipples, well developed between abdominal segments I–II, II–III and III–IV. Pygidium with 2 definite pairs of lobes; third and fourth pairs of lobes represented by low projections. Median lobes (L_1) each 20–27 (23) µm wide, strongly sclerotized, prominent, parallel and symmetrical, slightly rounded at apex with periapical indentation on either side of apex. Median lobes without basal yoke; space between the lobes wider than L_2, 0.4–0.6 (0.5) times width of median lobe, occupied by 2 short gland-spines, these sometimes broken. Second lobes (L_2) each 8–10 (9) µm wide, small, conical, narrow, protruding, lateral lobule (L_2b) absent. Third lobes (L_3) each 12–18(15) µm wide, small, slightly convex, associated with paired marginal macroducts 3 and 4. Gland spines each containing microduct 27–37 (33) µm long, without basal fringing. Pygidial gland-spine formula 2–3, 2–5 and 3–6, segments III and IV each with 3–6 (4) gland spines near each margin. Seven thick dorsal marginal macroducts each 22–25 (23) µm long, present on each side, present in pairs on the segments VI–VIII, single on segment V. Dorsal submarginal and submedial macroducts quite narrow, very numerous on pygidium, each duct short 20–25 (21) µm long, cylindrical, with orifice sub-circular with thickened rim, distributed in irregular rows in all pygidial areas, where they form tight groups on longitudinal segments VII, VI and V; arranged in more regular rows on segment V and prepygidial segments I–IV where they form distinct submarginal and submedial groups, extending along intersegmental lines. Ventral microducts very slender, barely visible, few in number, present in the submarginal area of pygidium and submedial areas of prepygidial segments. Ventral macroducts almost same size as dorsal macroducts each 15–22 (18) µm long, present in the pleuthoracic area and segments I and II of the abdomen, extending medially to posterior spiracles on meta-thorax. Perivulvar pores present, arranged into 5 groups, formula: median group 5–19 (9), submedial groups 7–20 (15) each, and lateral groups 8–20 (13) each; median group is situated anterior to level of anal opening. Anal Opening located 9–12 (10) times length of anal opening from base of medial lobes, anal Opening 12–15 (15) µm long.

Host plants. *Pistacia integerrima* J.L. Stewart ex Brandis, *P. khinjuk* Stocks, *P. atlantica* Desf., *P. terebinthus* L., *P. vera* L. (Anacardiaceae); *Rhododendron* sp. (Ericaceae); *Stillingia* sp. (Euphorbiaceae); *Ceratonia siliqua* L. (Fabaceae); *Sassafras* (Lauraceae); *Ceanothus* sp. (Rhamnaceae); *Malus pumila* Mill., *Pyrus* sp., *Prunus armeniaca* L., *Rosa* sp., *Sorbus* sp. (Rosaceae); *Populus* sp., *Salix* sp. (Salicaceae); *Alianthus* sp. (Simaroubaceae) (García *et al.*, 2016).

Distribution. Afghanistan, Armenia, Cyprus, Georgia, Iran, Iraq, Kazakhstan, Kyrgyzstan, Pakistan, Syria, Tajikistan, Turkey, Turkmenistan and Uzbekistan (García *et al.*, 2016).

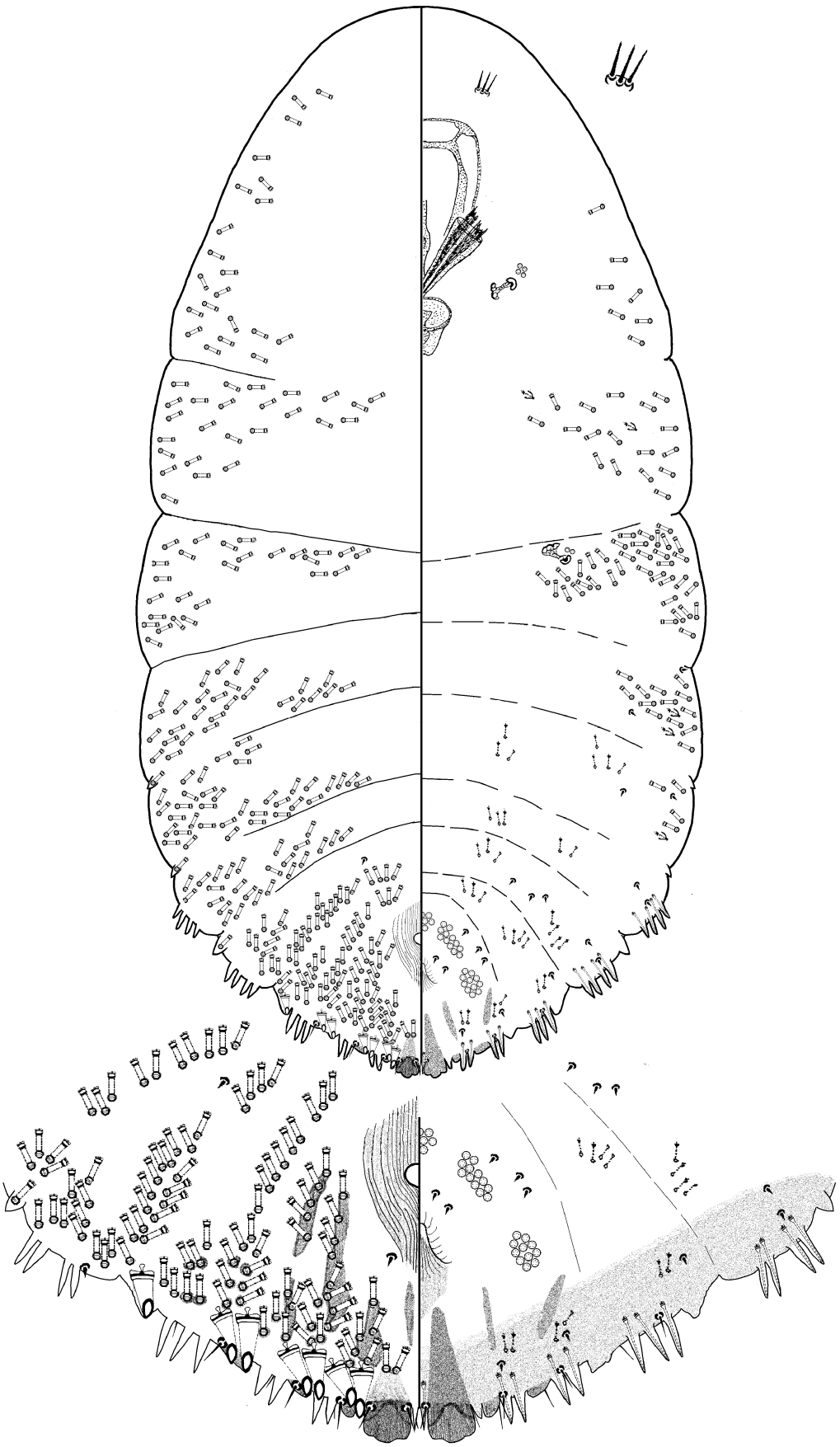


FIGURE 3. Adult female of *Lepidosaphes pistaciae* Archangelskaya, illustration by Hosseininaveh.

***Suturaspis davatchi* (Balachowsky & Kaussari, 1951)**
Figures 4 and 5.

Synonyms. *Salicicola davatchi*, Balachowsky & Kaussari 1951; *Salicicola davatschii* Balachowsky, 1953; *Suturaspis davatchi* Borchsenius, 1966; *Salicicola davatchii* Danzig & Pellizzari, 1998 (García *et al.* 2016).

Material examined. 5 adult females on 1 slide and 8 second-instar female on 1 slide, Iran, Kerman, Noogh, N: 30°46'48", E: 55°50'30", 1,372 m, on branches and trunk of *Pistacia vera*, coll: F. Hosseininaveh, 6.i.2013, coll. no: SN-130.

Field Characters. Female scale cover 1.2–1.4 mm long, 0.5–0.7 mm wide, straight or slightly curved, more or less convex, narrow or slightly wider towards posterior end; larval exuviae pale yellow, situated at narrow end, secreted part of cover pure white, matte, with slightly raised transverse ridges.

Slide-mounted characteristics. Adult female elongate, almost oval, slightly narrowed anterior to pygidium, 0.63–0.81 (0.74) mm long and 0.37–0.47 (0.42) mm wide (Fig. 4). Cuticle membranous, except on mid-line of ventral prepygidial segments as far anteriorly as mesothorax, and 2 ventral chitinous thickenings in submargins of abdominal segments VII and VIII. Antenna with 2 very long, soft setae. Anterior spiracles each with 2 pores. Posterior perispiracular pores absent. Pygidium short, narrow, semi-circular, membranous on both surfaces but finely spiculated; without any trace of pectinae; margin very finely wavy, with some short and separate submedial, submarginal and marginal setae. Anal opening thick-rimmed with large diameter, subcircular, 10–15 (12) µm long and 15–20 (17) µm wide. Dorsal macroducts very few, each duct short and narrow, with inconspicuous oval opening on margin; 3 single ducts 10 or 11 µm long on each side, on segments VI–VIII. Vulvar opening wide, surrounded by radial lines. Perivulvar pores and ventral tubular glands absent.

Second-instar female oval, 0.68–0.81 (0.74) mm long and 0.43–0.55 (0.49) mm wide, without prepygidial narrowing (Fig. 5). Antenna with 3 setae. Anterior spiracles each with 2 pores. Pygidium short, marginal ornamentation highly simplified, with a series of short, irregular and rounded prominences on segments VII and VIII. Pygidium without any distinct pectinae. Anal opening subcircular, 5–8 (7) µm long and 8–10 (10) µm wide, located at anterior end of pygidium. Submedial and submarginal macroducts numerous throughout dorsum and venter from prothorax to pygidium, all similar in size and shape each 8–12 (10) µm long. Ventral marginal area of abdominal segments V–VIII of pygidium containing slightly larger macroducts in groups of 2–3, total 10–11 on each side. A few patches of thickened cuticle present in submarginal area of pygidium on both surfaces.

Hosts. *Pistacia* sp., *P. khinjuk* Stocks; *P. vera* L. (Anacardiaceae); *Ficus carica* L. (Moraceae) (García *et al.* 2016).

Distribution. Afghanistan, Iran, Turkey (García *et al.* 2016).

Molecular results

Sequences characteristics, tree statistics and model choice of different data partitions are summarized in Table 2. The molecular trees constructed using the MP, ML and Bayesian inference showed nearly the same topology for each DNA fragment of COI and 28S rDNA. Bayesian trees (Figures 6 and 7) are presented along with PP, BP for ML (BP^{ML}) and MP (BP^{MP}) analysis.

In COI analysis, *L. pistaciae* formed a monophyletic group with high support (PP=0.94, BP^{ML}=95, BP^{MP}=100). *Melanaspis* genus formed a well-supported monophyletic group (PP=0.77, BP^{ML}=96, BP^{MP}=99) and *S. davatchi* allied with the *Melanaspis* clade as a sister group. *Suturaspis* formed a monophyletic clade with high support (PP=0.99, BP^{ML}=83, BP^{MP}=100). Highly supported monophyletic groups were formed for the species of *M. pistaciae* **sp. n.** (PP=0.76, BP^{ML}=81, BP^{MP}=82) and *M. inopinata* (PP=0.95, BP^{ML}=96, BP^{MP}=95). Among the populations of *M. pistaciae,* well supported monophyletic groups were formed only for MF (PP=0.98, BP^{ML}=90, BP^{MP}=70) and MV (PP=0.68, BP^{ML}=69, BP^{MP}=78) populations; however, monophyly in the populations of *M. inopinata* could not be determined. For *L. pistaciae*, highly supported monophyletic groups were depicted only for the populations of LK (PP=0.82, BP^{ML}=81, BP^{MP}=80) and LS (PP=0.82, BP^{ML}=95, BP^{MP}=70).

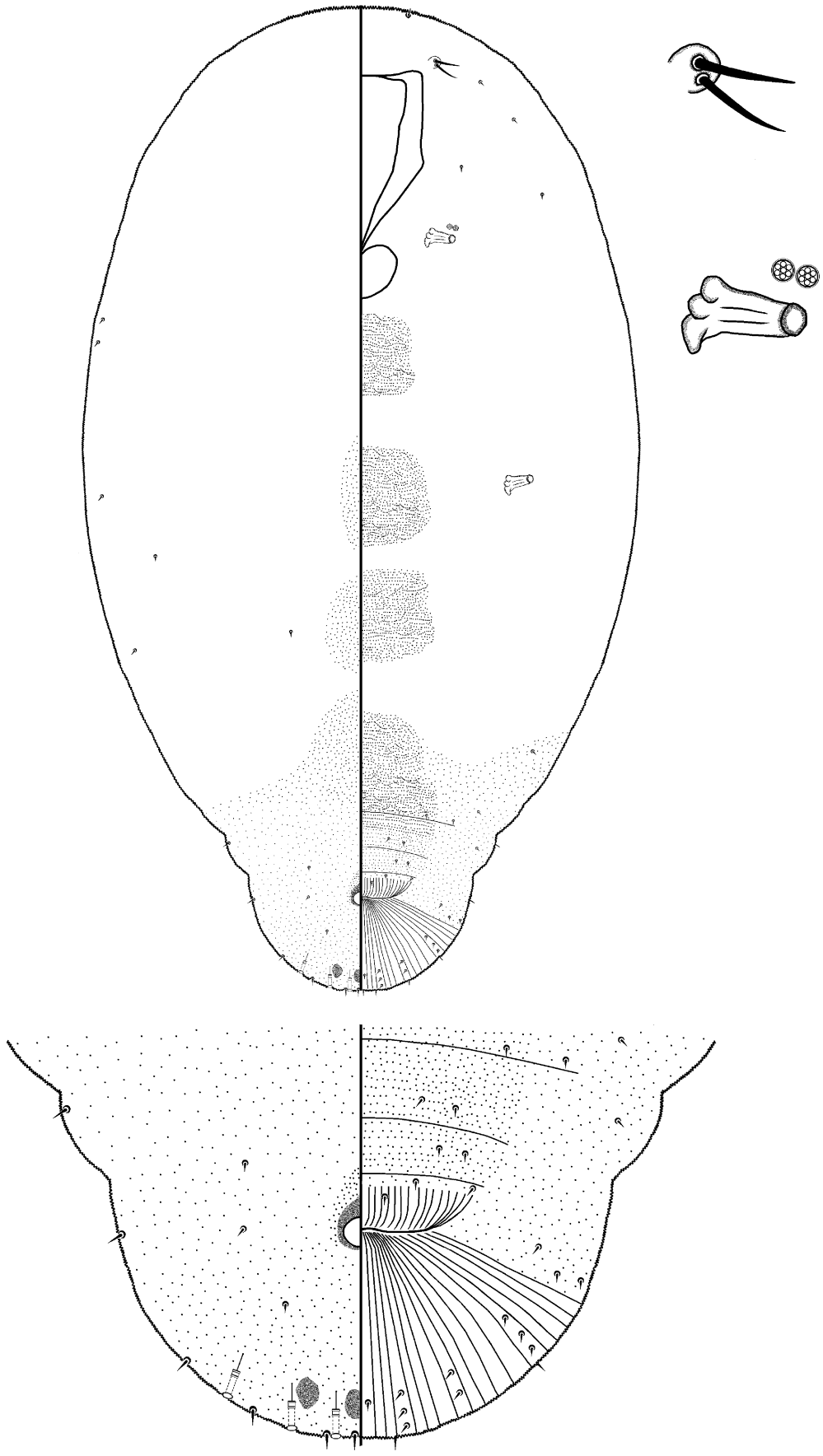


FIGURE 4. Adult female of *Suturaspis davatchi* Balachowsky & Kaussari, illustration by Hosseininaveh.

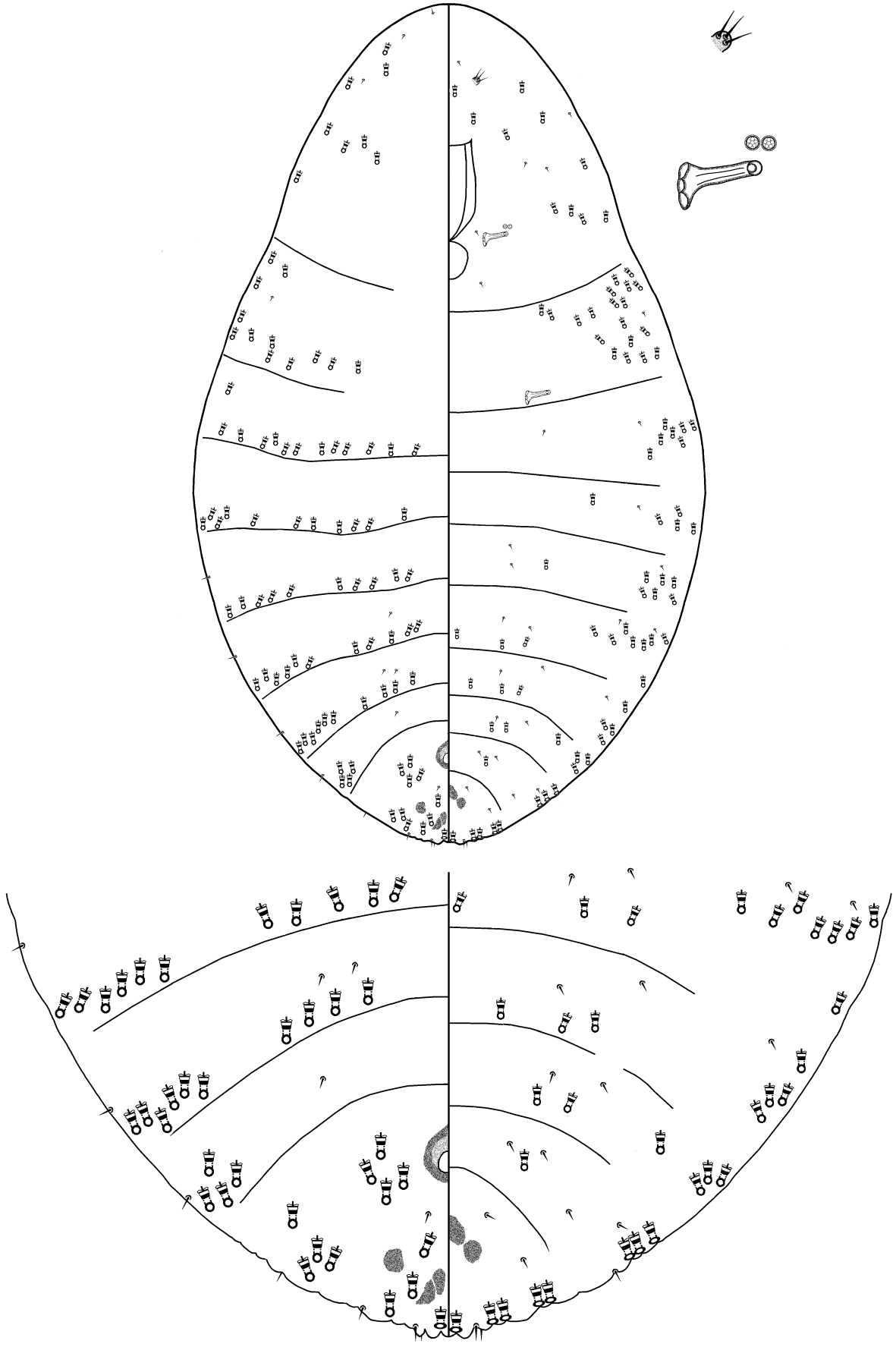


FIGURE 5. Second-instar female of *Suturaspis davatchi* Balachowsky & Kaussari, illustration by Hosseininaveh.

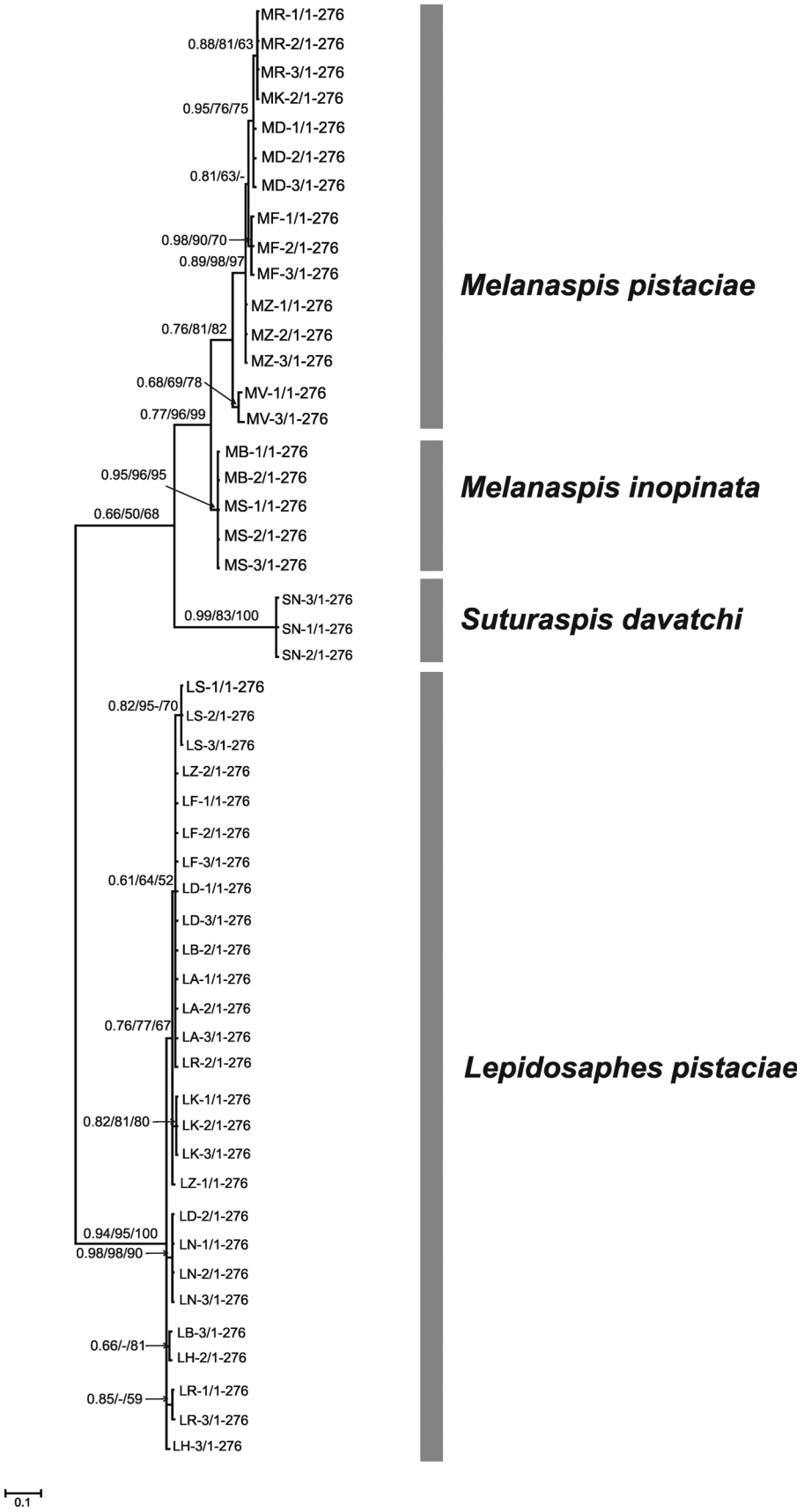


FIGURE 6. Fifty percent majority rule consensus tree resulting from Bayesian analysis of the COI dataset. Numbers above branches are posterior probability and likelihood as well as parsimony bootstrap values, respectively. Values <50 % are not shown.

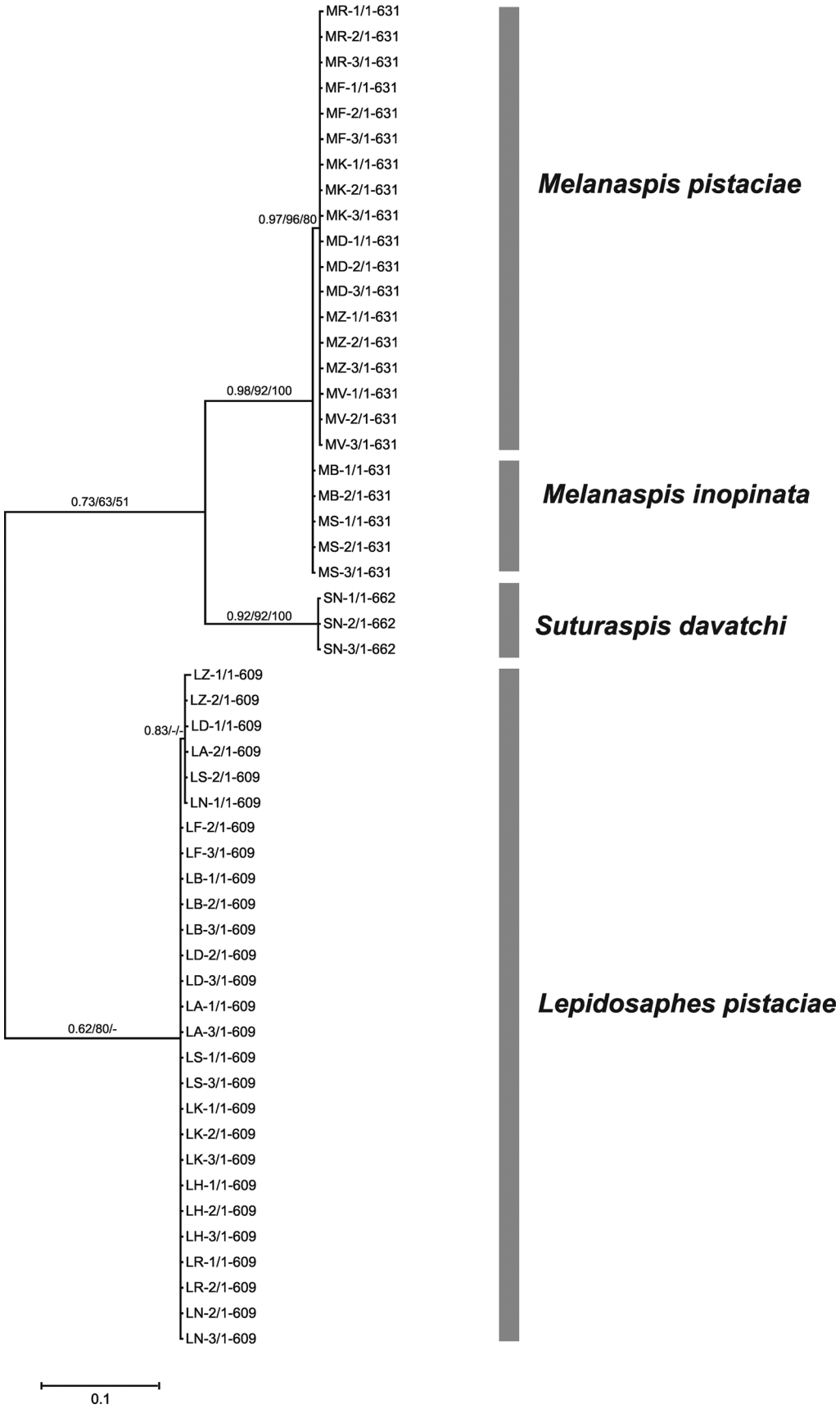


FIGURE 7. Fifty percent majority rule consensus tree resulting from Bayesian analysis of the 28S rDNA dataset. Numbers above branches are posterior probability and likelihood as well as parsimony bootstrap values, respectively. Values <50 % are not shown.

In 28S rDNA analysis, *L. pistaciae* formed a monophyletic group with good support (PP=0.62, BP^{ML}=80, BP^{MP}<50). *Melanaspis* formed a well-supported monophyletic group (PP=0.98, BP^{ML}=92, BP^{MP}=100) and *S. davatchi* allied with the *Melanaspis* clade as a sister group. *S. davatchi* formed a monophyletic clade with high support (PP=0.92, BP^{ML}=92, BP^{MP}=100) and *M. pistaciae* **sp. n.** formed a monophyletic group with high support (PP=0.97, BP^{ML}=96, BP^{MP}=80) nested within *M. inopinata*. None of the populations formed a monophyletic group.

TABLE 2. DNA sequence characteristics and phylogenetic statistics of data partition.

DNA fragment	CO1	28S
Number of sequences	53	56
Number of characters	276	664
GC contents (%)	23.5	60.1
Number of variable characters in the ingroup	81	133
Number of PI characters in the ingroup	81	132
Average sequence divergence in the ingroup	0.212	0.113
Number of MPTs	36	4
Length of MPTs	275	604
C.I. of MPT	0.87	0.97
R.I. of MPT	0.97	0.99
Evolutionary model selected (under AIC)	GTR + G	GTR + I

Discussion

The current research reports on diaspidids on *P. vera* in Kerman province: three previously reported diaspidid species, *M. inopinata*, *L. pistaciae* and *S. davatchi*, are redescribed and a new diaspidid, *M. pistaciae* **sp. n.**, is described for the first time. Regarding the monophyly of all four species, we can conclude that the fragments of COI and 28S rDNA are appropriate for molecular identification of diaspidid species. According to observed monophyly in some of the populations, it appears that the fragment of COI may be more convenient for studying the genetic structure of diaspidid populations.

Melanaspis inopinata has been reported as a polyphagous pest on different plants, including *P. vera* (Kaussari & Farahbakhsh, 1968) from Iran; however, in the most recent checklist of scale insects of Iran (Moghaddam 2013b), *P. vera* was not reported as a host of this species. The present study collected *M. inopinata* in about 30% of the *P. vera* cultivation area. Our description of *M. inopinata* (based on 10 individuals from 2 populations) differs slightly from that given by Balachowsky (1951) in the number of small antennal setae, marginal macroducts on the fifth abdominal segment, macroducts in the cuticular groove between the second and third lobes, and submarginal microducts on the venter of the fourth and fifth abdominal segments.

Based on the shape of the pygidial lobes, the number of marginal macroducts on the fifth abdominal segment and the ratio of the distance from the apex of the median lobe to the anterior-most edge of the sclerotized area to the body length, *M. pistaciae* **sp. n.** has been described as a new species (based on 30 individuals from 6 populations). The characteristic pygidial papilla has been observed for the first time in *M. pistaciae* and *M. inopinata*. So far as the authors are aware, this characteristic has not been used previously to describe other closely related species. In the molecular tree constructed by using the COI fragment, *M. pistaciae* placed in the separated clade (8% distance from closest one), confirms that *M. pistaciae* is a different species from *M. inopinata* (Figs 6 and 7) as morphological data was concluded. However, in the tree constructed using the 28SrDNA fragment, *M. pistaciae* formed the monophyletic group nested within *M. inopinata* clade, in this case, 28S tree was not resolved enough, that reason can be the less mutation rate fragment 28SrDNA, compared with COI and basically fewer numbers of informative characters and less genetic distance. About 70% of Iranian pistachio orchards are infested with *M. pistaciae*. No intraspecific variation has been observed in either of these *Melanaspis* species.

Morphometric measurements of *L. pistaciae* (50 specimens from 10 different populations) revealed

discrepancies in the number of anterior perispiracular pores, posterior perispiracular pores, antennal setae and perivulvar pores with the descriptions previously published by Balachowsky (1954) and Danzig (1993). The lateral lobule of the second lobe (L_2b) observed in *L. pistaciae* and used as a discriminating characteristic between *L. pistaciae* forms (Balachowsky 1954; Borchsenius 1949) was not observed in our specimens. According to these morphological assessments, there is only one form of *L. pistaciae* present in Kerman province. Furthermore, molecular analysis represents the non-monophylies in all populations of the species and clearly refute two form of *L. pistaciae*.

Suturaspis davatchi was found in only one region (Noogh) across the entire sampling area. The morphological traits of five adult females and five second instars (puparium) of this single *S. davatchi* population were assessed. The number of anterior perispiracular pores in the adult female, antennal setae and ventral marginal macroducts of segments fifth to eighth in second-instar female, differ from those described by Balachowsky (1953).

In this study, diaspidids were collected only on *P. vera* in Kerman province despite the fact that these species, especially *M. inopinata,* are polyphagous and abundant over a much larger range; sampling from across their entire geographic and host range would likely reveal more genetic variation that suggests a need for further molecular studies. *Melanaspis pistaciae* **sp. n.** has spread to more cultivated pistachio areas and probably has misidentified as *M. inopinata* in earlier studies. Further investigation of the biology of this species may lead to the development of more effective approaches for controlling this important pest.

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