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A new species of the genus *Tramaforda* Manheim, 2007

(Hemiptera, Aphididae, Eriosomatinae, Fordini)

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Tramaforda wooli Manheim belongs to a monotypic genus that induces galls on *Pistacia atlantica* Desf. in Israel. Based on distinct gall characteristics, differences in morphometrics and molecular markers (COI, COII and microsatellite analysis), we recognized a new species in this genus, *Tramaforda koachi* sp. nov. which is currently endemic to north-central Israel and the Golan Heights. Fall migrants of *Tramaforda koachi* sp. nov. are smaller (1.62–2.02 mm) than the same form of *T. wooli* (>2.14 mm). However, no distinct qualitative morphological differences were found between fall migrants of these two species.

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Introduction

The tribe Fordini (Hemiptera, Eriosomatinae) comprises aphids which produce species-specific gall types on the leaves and buds of the primary host plants, *Pistacia* L. and *Rhus* L. (Zhang & Qiao 2007). Nearly 21 aphid species, from eleven genera (tribe Fordini) induce galls on three wild *Pistacia* spp. (*P. atlantica* Desf., *P. lentiscus* L. and *P. palaestina* Bois.) in Israel (Koach & Wool 1977, Swirski & Amitai 2001, Barjadze et al. 2018). Each aphid species can be accurately recognized by their distinct gall morphology (Inbar et al. 2004), excluding the species of *Geoica* (Brown & Blackman 1994, Ben-Shlomo & Inbar, unpublished data).

Based on gall morphology, two forms designated as "Fordini sp. A" and "Fordini sp. B" had been recorded on *P. atlantica* in Israel (Koach & Wool 1977, Wool et al. 1994, Inbar & Wool 1995, Inbar et al. 2004). Differences between single populations of "Fordini sp. A" and "Fordini sp. B" in COI and COII sequences were shown in Inbar et al. (2004). Later, "Fordini sp. B" was described as *Tramaforda wooli* (Manheim 2007). The genus *Tramaforda* was erected based on the very long hind legs and rostrum of embryos inside the abdomen of alate emigrants, which is unique among eriosomatid genera (Manheim 2007).

DNA barcoding is a useful discriminatory method used to investigate differences between organisms and for species identification in Aphididae (Foottit et al. 2008, Piffaretti et al. 2012, Massimino Cocuzza & Cavalieri 2014, Lee et al. 2015, 2017, Zhu et al. 2017). Similarly, multivariate analysis makes it possible to distinguish morphologically similar species and, associated with DNA barcode, can improve the correct recognition and identification of aphid species (Kim et al. 2010, Rakauskas et al. 2014, Massimino Cocuzza et al. 2015). The aim of this study is to clarify the taxonomic status of the rare species "Fordini sp. A".

Fig. 1. Sampling sites and the distribution of *Tramaforda* spp. in Israel (few records are based on Koach & Wool 1977 and Manheim 2007). Symbols: *Tramaforda koachi* sp. nov. (■): 1. Ein Zivan; 2. Malkia; 3. Baram; 4. Katzrin; 5. Gamla; 6. Dalia; 7. Givat Ada and *Tramaforda wooli* (▲); 8. Mishmar Ayalon; 9. Givat Brenner; 10. Canada Park; 11. Jerusalem; 12. Agur; 13. Adullam; 14. Beit Guvrin. ●, location of sympatric populations of both species in the lower Galilee in Israel (e.g. Ein Ulam 32.668533, 35.499737).

Material and methods

Morphometrics. Fifteen specimens of alate emigrants of *T. wooli* from Jerusalem were collected in October 2012, and twenty one specimens of *T. koachi* from Gamla, sampled on *P. atlantica* in 12 October 2012 were measured. The measurements were done according to Ilharco & van Harten (1987) and Blackman & Eastop (2006) using a Leica DM LB2 microscope fitted with microscope ocular micrometer. In total, for each specimen we measured nine morphological characters that are often used in Fordini taxonomy (Brown & Blackman 1994, Remaudière et al. 2004, Manheim 2007).

The mean value and standard deviation (SD) for each morphological character and ratios between them were calculated. Patterns of morphometric variation were analysed using principal component analysis (PCA) (Tabachnick & Fidell 2006). PCA assesses components of the total of variation among all specimens by calculating a linear combination of the variables that explains the maximum amount of total variation, and then iteratively calculates new combinations to explain any residual variation. This procedure does not assume any a priori groupings. PCA was based on the correlation matrix of the coefficients (Tabachnick & Fidell 2006, Abdi & Williams 2010). The analyses were performed using the software packages Past ver. 2.16 (Hammer et al. 2001).

Molecular analysis. *Tramaforda* spp. specimens for barcoding were sampled in Givat Ada on 27.06.2015, in Gamla on 30.06.2015, in Katzrin on 29.06.2015, in Adullam on 28.06.2015 and in Jerusalem on 28.06.2015 by M. Inbar (Fig. 1).

DNA was extracted from intact whole aphid bodies using a modified CTAB procedure: specimen were ground in 350 µl of CTAB solution in the bottom of a 1.5 ml microcentrifuge tube using a polypropylene tissue grinder and then rinsed with an additional 350 µl of CTAB solution. 650 µl chloroform were added and centrifuged at 1300 rpm for 15 minutes; the aqueous supernatant was transferred and 650 µl of an equivolume phenol-chloroform-isoamyl alcohol mixture (Fisher Scientific BP1752I) were added, mixed and centrifuged. The supernatant was retreated with chloroform; and DNA was precipitate by adding 1 ml absolute ethanol and 40 µl 3M sodium acetate to the supernatant, and incubating at −20 °C for at least 8 h; centrifuged at 13 000 rpm for 30 min, and rinsed pellet with cold 80 % ethanol; the pellet was dried and resuspended in 15 to 30 µl TE buffer (10 mM tris, 1 mM EDTA).

A 658 base-pair (after primer trimming) fragment at 5'end of the mitochondrial cytochrome c oxidase subunit 1 (COI-5P, 'barcode' region) was amplified with GoTaq mastermix (Promega) using metazoan invertebrates primers: LCO1490 (GGTCAACAAATCATAAAGATATTGG) and HCO2198 (TAAACTTCAGGGTGACCAAAAAATCA) (Folmer et al. 1994) on an Eppendorf Mastercycler using 5 cycles of 40 s at 95 °C + 40 s at 45 °C + 60 s at 72 °C, followed by 30 cycles with the annealing temperature raised to 51 °C.

PCR products were sequenced bidirectionally with the same primers using BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems) and processed on an ABI PRISM 3130xl Genetic Analyser operated by the Microbiology Molecular Technologies Laboratory, Ottawa Research and Development Centre, Agriculture and Agri-Food Canada.

Sequences were assembled and trimmed using Geneious v. 8 software (Biomatters Ltd, Auckland, New Zealand) and translated to amino acids to check for the presence of stop codons and phase shifting insertion/deletions.

Pairwise sequence divergences were calculated using a Kimura 2-parameter (K2P) distance model (Kimura 1980). Neighbour-joining (NJ) analyses were conducted in MEGA 7.0 (Kumar et al. 2016).

Sequences are deposited at the GenBank with accession numbers: KY322813.1, KY322814.1, KY322815.1, KY322816.1 and KY322817.1 (Table 1).

Sequences of COII of Fordini sp. A (= *Tramaforda koachi* sp. nov.) and Fordini sp. B (= *Tramaforda wooli*) were used from the GenBank with accession numbers: AY227099.1 and AY227100.1 respectively.

Microsatellite analysis. *Tramaforda* samples collected in the autumn (September 2017) from 10 sites in Israel and the Golan Heights (Table 2): 26 showing *T. wooli* galls morphology (B) and additional 45 showing *T. koachi* galls morphology (A) were analysed using eight known nuclear microsatellite loci (Gu-1, 2, 3, 4, 6, 11 and Fm-4, 6; Ivens et al. 2011). The F-primer of each microsatellite was labeled with florescent dye (6-Fam, Vic, Ned or Pet) and amplification products were read by ABI 3130xl Florescence-Reader (Applied Biosystem).

Population clustering was performed using a Bayesian clustering method that divided the samples into possible homogenous groups (sub populations) according to their degree of similarity (STRUCTURE 2.3.4; Pritchard et al. 2000, 2010). The analysis followed an admixture model (burn-in of 500 000 steps and 500 000 iterations; K values from 1 to 11; ten replicates for each K). The inference of the probable number of clusters was extracted by the log likelihood for each putative number of populations (K), Ln P(D) = L(K), and by the delta K method (Evanno et al. 2005), using the program Structure Harvester (Earl & von Holdt 2012).

The following abbreviations are used in the paper: ANT, length of antenna; ANTVB, length of basal part of antennal V segment; ANTIII, length of antennal III segment; BL, length of body; HFEM, length of hind femora; HTIB, length of hind tibia; HTII, length of second segment of hind tarsus; PT, processus terminalis; URS, length of ultimate rostral segments.

Table 1. GenBank accession numbers of COI sequences of *Forda* spp. and *Tramaforda* spp.

Species	Locality	COI sequences obtained and GenBank accession number
Tramaforda koachi sp. nov. as Fordini sp. A 'M Wink 18451'	Unknown, Israel	AY227087.1
Tramaforda wooli as Fordini sp. B 'M Wink 18456'	Unknown, Israel	AY227088.1
Tramaforda koachi sp. nov. as *Tramaforda* sp. SB2015-A voucher SB2015-17091	Katzrin, the Golan Heights	KY322813.1
Tramaforda koachi sp. nov. as *Tramaforda* sp. SB2015-A voucher SB2015-17088	Givat Ada, Israel	KY322814.1
Tramaforda koachi sp. nov. as *Tramaforda* sp. SB2015-A voucher SB2015-17089	Gamla, the Golan Heights	KY322815.1
Tramaforda wooli as *Tramaforda* sp. SB2015-B voucher SB2015-17092	Adullam, Israel	KY322816.1
Tramaforda wooli as *Tramaforda* sp. SB2015-B voucher SB2015-17090	Jerusalem, Israel	KY322817.1
Forda marginata	Europe: France?	KF639396.1
Forda marginata	Canada	EU701668.1
Forda formicaria	Israel	AY227086.1
Forda ricobboni	Israel	AY227076.1

Table 2. Sampling sites in Israel and the Golan Heights, sampling sizes of *Tramaforda* for microsatellite investigation. Numbers of population correspond to the numbers at the bottom of the bar-plot in Figure 6.

Species	Number of population	Population	Number of investigated individuals
Tramaforda koachi sp. nov.	1	Baram	6
Tramaforda koachi sp. nov.	2	Malcia	5
Tramaforda koachi sp. nov.	3	Ein Zivan	5
Tramaforda koachi sp. nov.	4	Kazerin	7
Tramaforda koachi sp. nov.	5	Gamla	6
Tramaforda koachi sp. nov.	6	Dalia	11
Tramaforda koachi sp. nov.	7	Givat Ada	5
Tramaforda wooli	8	Mishmar Ayalon	6
Tramaforda wooli	9	Jerusalem	4
Tramaforda wooli	10	Adolam	16

Results

Tramaforda koachi sp. nov.
Fig. 2, Tables 3, 4, 6

Type material. Holotype: alate viviparous female (fall migrant), coll. no. ISGAM 20121012-1, the Golan Heights, Gamla, 32°54'10"N 35°44'26"E, 268 m a.s.l., 12.10.2012, in the galls of *Pistacia atlantica*, leg. M. Inbar. – Paratypes: 20 alate viviparous females on 20 slides, coll. no. ISGAM 20121012-2 to ISGAM 20121012-21, the same data as for holotype; 2 first instar larvae of exule on separate slides, ISGAM 20121012-22–23, the same data as for holotype.

Holotype and paratypes coll. no. ISGAM 20121012-2 to 6 and ISGAM 20121012-22–23 are deposited at Ilia State University, Tbilisi, Georgia; paratypes coll. no. ISGAM 20121012-7 to12 are deposited at the Steinhardt Museum of Natural History, Tel Aviv University, Israel; paratypes coll. no. ISGAM 20121012-13 to 18 are deposited at Canadian National Collection of Insects, Ottawa, Canada; paratypes coll. no. ISGAM 20121012-19 to 21 are deposited at the Natural History Museum, London, UK.

Diagnosis. Specimens of fall migrants of *Tramaforda koachi* sp. nov. are significantly smaller (1.62–2.02 mm) than those of the same form of *T. wooli* (2.14–2.45 mm in this study, 2.780 ± 0.101 mm in Manheim 2007). The best discrimination between fall migrants of *T. koachi* and *T. wooli* is achieved by HTIB less than 0.475 mm in *T. koachi* vs. greater

Table 3. Measurements of morphological characters of emigrants of *Tramaforda wooli* and *Tramaforda koachi* sp. nov. Measurements are given in mm. The following abbreviations are used in the table: ANT, length of antenna; ANTVB, length of basal part of antennal V segment; ANTIII, length of antennal III segment; BL, length of body; HFEM, length of hind femora; HTIB, length of hind tibia; HTII, length of second segment of hind tarsus; PT, processus terminalis; URS, length of ultimate rostral segments.

Variable	*Tramaforda wooli* (n = 15)		*Tramaforda wooli* from Manheim 2007 (n = 10)	*Tramaforda koachi* sp. nov. (n = 21)	
	range	mean value ± sd	mean value ± sd	range	mean value ± sd
BL	2.138–2.450	2.302 ± 0.115	2.780 ± 0.101	1.625–2.025	1.867 ± 0.119
ANT	0.466–0.533	0.500 ± 0.024	–	0.409–0.452	0.430 ± 0.013
PT	0.013–0.022	0.018 ± 0.002	0.020 ± 0.004	0.010–0.018	0.014 ± 0.002
ANTVB	0.098–0.110	0.104 ± 0.005	0.140 ± 0.009	0.074–0.093	0.086 ± 0.005
ANTIII	0.190–0.223	0.205 ± 0.013	0.220 ± 0.008	0.163–0.195	0.181 ± 0.011
URS	0.075–0.083	0.079 ± 0.002	0.080 ± 0.003	0.063–0.075	0.069 ± 0.004
HFEM	0.280–0.310	0.300 ± 0.012	0.400 ± 0.016	0.238–0.263	0.249 ± 0.009
HTIB	0.495–0.552	0.513 ± 0.020	0.590 ± 0.028	0.414–0.447	0.431 ± 0.011
HTII	0.138–0.173	0.157 ± 0.010	0.180 ± 0.010	0.122–0.138	0.129 ± 0.006

Table 4. Ratios of morphological characters of emigrants of *Tramaforda wooli* and *Tramaforda koachi* sp. nov. Variable names are defined in Table 3.

Variable	*Tramaforda wooli* (n = 15)		*Tramaforda koachi* sp. nov. (n = 21)	
	range	mean value ± sd	range	mean value ± sd
ANT/BL	0.20–0.24	0.22 ± 0.01	0.20–0.25	0.23 ± 0.01
ANT/HFEM	1.51–1.79	1.69 ± 0.09	1.67–1.90	1.74 ± 0.06
ANT/HTIB	0.90–1.08	0.98 ± 0.05	0.94–1.09	1.01 ± 0.04
PT/ANTVB	0.12–0.21	0.17 ± 0.02	0.12–0.21	0.17 ± 0.03
ANTVB/HTII	0.57–0.78	0.66 ± 0.05	0.57–0.74	0.67 ± 0.06
ANTIII/BL	0.08–0.10	0.09 ± 0.01	0.08–0.11	0.10 ± 0.01
ANTIII/HFEM	0.61–0.75	0.69 ± 0.04	0.67–0.80	0.73 ± 0.04
ANTIII/HTIB	0.37–0.44	0.40 ± 0.02	0.38–0.46	0.42 ± 0.03
URS/BL	0.03–0.04	0.03 ± 0.01	0.03–0.04	0.04 ± 0.01
URS/ANTVB	0.69–0.85	0.76 ± 0.05	0.71–0.95	0.81 ± 0.06
URS/HTII	0.46–0.57	0.51 ± 0.04	0.47–0.60	0.54 ± 0.03
HFEM/BL	0.12–0.14	0.13 ± 0.01	0.12–0.15	0.13 ± 0.01
HTIB/BL	0.20–0.25	0.22 ± 0.01	0.21–0.26	0.23 ± 0.01

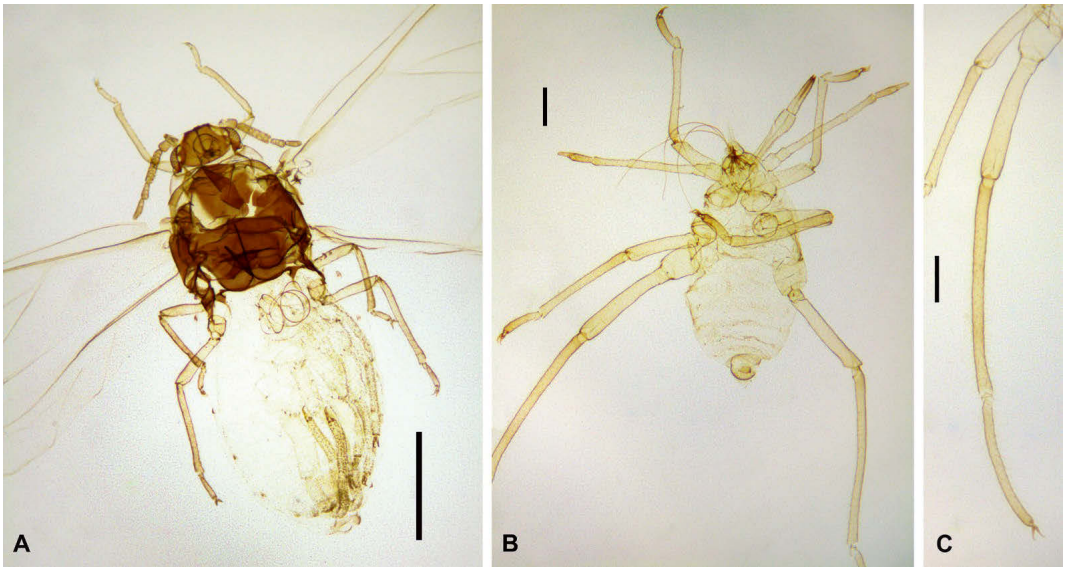

Fig. 2. *Tramaforda koachi* sp. nov. **A.** Habitus of fall migrant (scale bar: 0.5 mm); **B.** habitus of first instar larva of exule (scale bar: 0.2 mm); **C.** hind leg of first instar larva of exule (scale bar: 0.2 mm).

than 0.475 mm in *T. wooli*. Diagnostic differences in mitochondrial COI and COII sequences are given in Table 8.

Etymology. The specific name is given in honor of the late Dr. Jacob Koach, who recognized this species as Fordini sp. A with Prof. David Wool (see Koach & Wool 1977).

Morphology. Fall migrants of *T. koachi* (Fig. 2) contain embryos with the characters diagnostic for the genus *Tramaforda*. Specimens of *T. koachi* are significantly smaller (1.62–2.02 mm) than those of

Table 5. Proportion of contribution and variable coefficients of the first two eigenvectors (principal components) for PCA in emigrants of *Tramaforda wooli* and *Tramaforda koachi* sp. nov. (n = 36). Variable names are defined in Table 3.

Variable	PC 1	PC 2
ANT	0.3489	−0.1039
PT	0.2694	0.9008
ANTVB	0.3254	−0.0407
ANTIII	0.3124	−0.3629
BL	0.3515	−0.0071
URS	0.3185	0.0821
HFEM	0.3607	−0.0237
HTIB	0.3555	−0.1525
HTII	0.3474	−0.1172
Percentage of total variations	79 %	6 %

Table 6. Measurements of morphological characters and their ratios in first instar larvae of exules born by fall migrants of *Tramaforda wooli* and *Tramaforda koachi* sp. nov. Measurements are given in mm. Variable names are defined in Table 3.

	Variable	*T. wooli* (n = 8)	*T. koachi* sp. nov. (n = 2)
		range	range
length	ANT	0.971–1.095	0.866–0.885
	BL	1.171–1.409	1.114–1.209
	ANTIII	0.409–0.495	0.333–0.352
	ANTVB	0.120–0.138	0.115–0.116
	PT	0.068–0.075	0.065–0.069
	URSL	0.243–0.270	0.213–0.235
	HFEM	0.533–0.628	0.447–0.466
	HTIB	1.085–1.247	0.895–0.947
	HTII L	0.609–0.704	0.543–0.566
ratio	ANT/BL	0.75–0.87	0.73–0.78
	ANT/HFEM	1.65–1.88	1.90–1.94
	ANT/HTIB	0.85–0.92	0.93–0.97
	ANTVB/HTII	0.17–0.20	0.20–0.21
	HFEM/BL	0.43–0.53	0.39–0.40
	HTIB/BL	0.82–0.98	0.78–0.80
	URSL/HTII	0.38–0.40	0.39–0.42
	URSL/ANTVB	1.92–2.15	1.85–2.03
	ANTIII/BL	0.33–0.39	0.29–0.30
	ANTIII/HTIB	0.38–0.41	0.37
	ANTIII/HFEM	0.71–0.84	0.74–0.76
	PT/ANTVB	0.54–0.58	0.57–0.59

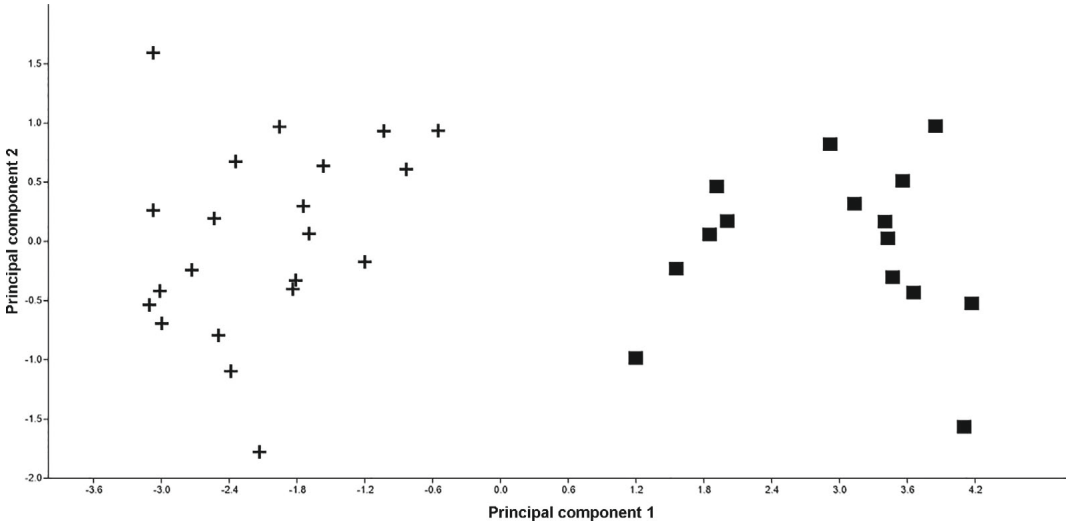

Fig. 3. Principal component ordination of 36 fall migrant individuals of *Tramaforda* spp. based on the analysis of nine morphological characters onto the first and second principal axes. Symbols: individuals of *Tramaforda wooli* (■) and *Tramaforda koachi* sp. nov. (+).

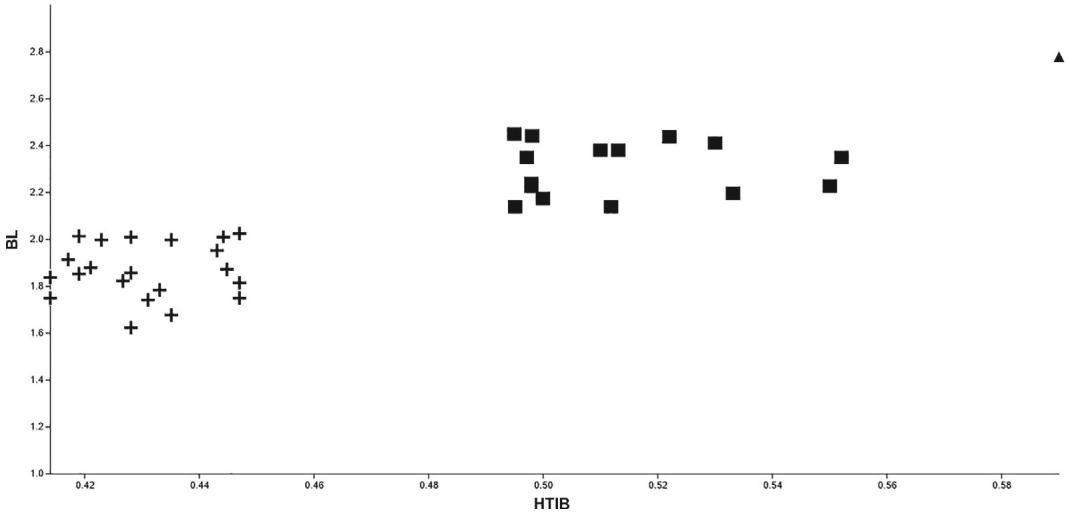

Fig. 4. Bivariate plot of the lengths of hind tibia vs. body length in mm in fall migrants between *Tramaforda wooli* and *Tramaforda koachi* (n = 36). Symbols: individuals of our investigated *Tramaforda wooli* (■) and mean values of the lengths of hind tibia and body in type material (▲) from Manheim (2007) and *Tramaforda koachi* sp. nov. (+).

the same form of *T. wooli* (2.14–2.45 mm). In original description of *T. wooli* length of body for fall migrants (n = 10) was 2.780 ± 0.101 mm (Manheim 2007). No differences in qualitative morphological characters were found between fall migrants of *T. wooli* and *T. koachi*. Measurements and ratios of morphological characters of fall migrants in *T. wooli* and *T. koachi* are provided in Tables 3 and 4.

Morphometrics. Examination of the raw data shows that the ranges of measurements of morphological variables mostly discriminate these two taxa (Table 3), while their ratios are mostly overlapping (Table 4).

Contributions of the variables to the first two principal components (PCs), accounting for 85 % of total variation, are given in Table 5. PC 1 (79 % of total

variation) reflects generalized body size, because contribution by all variables are of the same sign and with approximately the same magnitude. The main contributions to PC 2 (6 % of total variation) are PT, contrasted (opposite sign) with ANTIII (Table 5). A plot of PC 1 against PC 2 shows a clear separation between individuals of *T. koachi* and *T. wooli* (Fig. 3) on the first axis (size), but no difference in distribution of individuals on axis 2, or on any subsequent axes (not shown).

The best discrimination between fall migrants of *T. koachi* and *T. wooli* is achieved by HTIB vs. BL (Fig. 4). Note, however, that HTIB within each groups tends to be more or less constant independent of body size (i. e. the hind tibia is relatively shorter in large versus small individuals) but that this constant differs between the two groups. This effect is apparent in the other measurements of distal appendage segments (not shown). However, the mean values reported by Manheim (2007; see Fig. 4) do not seem to support this pattern. It remains to be determined if this trend holds if more extensive sampling across a greater range of body sizes.

Measurements of first instar larvae of exules deposited in the gall by fall migrants are given in Table 6. Some *T. wooli* larvae are of similar body size to the available *T. koachi* larvae. As in the adult measurements, appendage length seems to be constant in *T. wooli*, and the *T. koachi* larvae have shorter appendages than similarly sized *T. wooli* specimens. Again, additional samples are required to determine if this effect is real or an artefact of the limited sample.

Molecular analysis. Our COI data set covers the 658 base pair standard DNA barcode region. There are also single sequences of COI for *Tramaforda koachi* (AY227087.1) and *T. wooli* (AY227088.1) with length of 1278 and 1279 bp in the GenBank respectively, which have a 430 base pair overlap with the standard barcode region. Interspecific pairwise distances in COI sequences between three populations of *T. koachi* (Gamla, Givat Ada, Katzrin) and two populations of *T. wooli* (Jerusalem, Adullam) based on the standard barcode region is 3.8 % (4.4 % in the 430 bp region of overlap), while intraspecific differences is 0 % (Table 7). In COI sequences of *T. wooli* and *T. koachi* there are 25 nucleotide substitutions in the 658 base pairs of the standard barcode region (see Table 8): T→C in 11 sites; C→T in 9 sites; G→A in 2 sites; A→G in 1 site; C→A in 1 site; T→A in 1 site. The genus *Tramaforda* is closely related to the genus *Forda*. Genetic distances based on COI sequences (430 bp) for *Forda* and *Tramaforda* species are given in Table 7 and a neighbour-joining tree illustrating these distances is shown on Figure 5.

Interspecific pairwise distances in COII sequences between the two *Tramaforda* species based on 673 bp aligned positions is 3.4 % and there are 23 nucleotide substitutions (see Table 8): T→C in 12 sites, C→T in 8 sites, G→A in 2 sites and C→A in 1 site.

Microsatellites analysis. STRUCTURE analysis (500 000 burn-in period; 500 000 reps; 10 iterations; K = 1–11) of 71 *Tramaforda* samples collected from

Table 7. Uncorrected pairwise genetic distances for COI for *Tramaforda* species from different localities in Israel and the Golan Heights, and *Forda* species from Israel, France and Canada based on 430 aligned positions.

	*F. marg*_Fr.	*F. marg*_Can.	*F. ricc*_Israel	*F. form*_Israel	*Tr. wooli* –SB2015-17090	*Tr. wooli* –SB2015-17092	*Tr.* sp. A –SB2015-17089	*Tr.* sp. A –SB2015-17088	*Tr.* sp. A –SB2015-17091	Fordini sp. B 'M Wink 18456'	Fordini sp. A 'M Wink 18451'
*F. marg*_Fr.	*	*	*	*	*	*	*	*	*	*	*
*F. marg*_Can.	0.007	*	*	*	*	*	*	*	*	*	*
*F. ricc*_Israel	0.079	0.082	*	*	*	*	*	*	*	*	*
*F. form*_Israel	0.044	0.041	0.074	*	*	*	*	*	*	*	*
Tr. wooli – SB2015-17090	0.092	0.092	0.097	0.094	*	*	*	*	*	*	*
Tr. wooli – SB2015-17092	0.092	0.092	0.097	0.094	0.000	*	*	*	*	*	*
Tr. sp. A – SB2015-17089	0.087	0.092	0.105	0.095	0.044	0.044	*	*	*	*	*
Tr. sp. A – SB2015-17088	0.087	0.092	0.105	0.095	0.044	0.044	0.000	*	*	*	*
Tr. sp. A – SB2015-17091	0.087	0.092	0.105	0.095	0.044	0.044	0.000	0.000	*	*	*
Fordini sp. B 'M Wink 18456'	0.092	0.092	0.097	0.094	0.000	0.000	0.044	0.044	0.044	*	*
Fordini sp. A 'M Wink 18451'	0.087	0.092	0.105	0.095	0.044	0.044	0.000	0.000	0.000	0.044	*

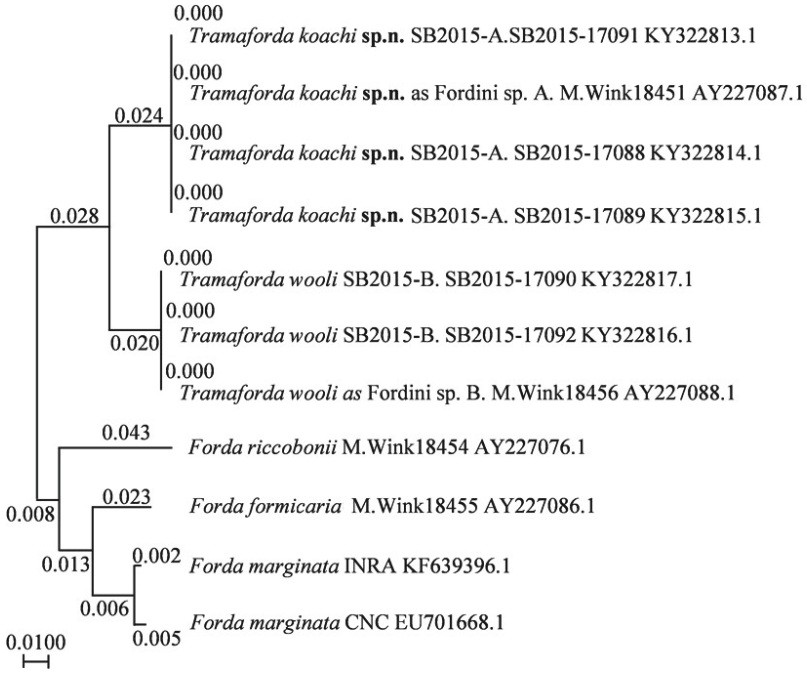

Fig. 5. A neighbour-joining tree using COI sequences (430 bp) of *Tramaforda* spp. and *Forda* spp. The evolutionary history was inferred using the Neighbour-Joining method. The optimal tree is shown (the sum of branch length = 0.18205290). The tree is drawn to scale, with branch lengths (next to the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. The analysis involved 11 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 430 positions in the final dataset. Evolutionary analyses were conducted with MEGA7.

Table 8. Variant sites in mitochondrial COI and COII distinguishing *Tramaforda koachi* sp. nov. and *T. wooli* Manheim. COI position is offset relative to the base preceding the standard barcoding region (position 38 in *Acyrthosiphon pisum* mitochondrial genome NC011594). Offsets 215 to 658 include 403 base overlap between barcodes presented here (*T. koachi*, n = 3; *T. wooli*, n = 2 sites) and Fordini sp. A and B sequences (GenBank accessions AY227087 and AY227088); offsets 659 to 1493 are represented only by single sequences AY227087 and AY227088. COII positions are offsets from position 1599 in *A. pisum* NC011594. COII sequences are represented by only a single sequence for each species (GenBank accessions AY227099 and AY227100).

COI position	+67	103	139	163	184	202	214	271	274	340	343	364	367	373	433	451	502	518	523	532
T. koachi	T	C	C	C	A	T	A	C	T	C	T	A	C	C	A	T	C	T	C	T
T. wooli	C	T	T	T	T	C	G	T	C	T	C	C	T	T	G	C	T	C	T	C
	542	548	589	616	625	688	689	694	700	728	747	757	760	763	809	847	898	906	955	961
T. koachi	C	T	T	C	G	C	C	C	A	A	A	T	G	C	A	C	T	T	C	C
T. wooli	T	C	C	T	A	T	T	T	C	C	C	C	A	T	C	T	C	C	T	T
	994	1006	1018	1057	1083	1105	1110	1135	1148	1178	1228	1240	1261	1273	1282	1291	1330	1450	1465	
T. koachi	A	A	G	T	T	A	A	C	G	A	C	T	A	C	T	T	A	C	T	
T. wooli	T	G	A	C	C	G	G	T	A	G	T	C	G	T	C	C	T	T	A	

COII position	+63	96	120	132	165	204	207	279	291	300	327	336	382	411	451	459	496	519	540	582	594	613	636
T. koachi	C	T	C	C	T	C	C	T	T	C	A	C	A	C	C	C	C	T	C	T	T	A	T
T. wooli	T	C	T	T	C	T	T	C	C	T	G	T	C	T	T	T	T	C	T	C	C	G	C

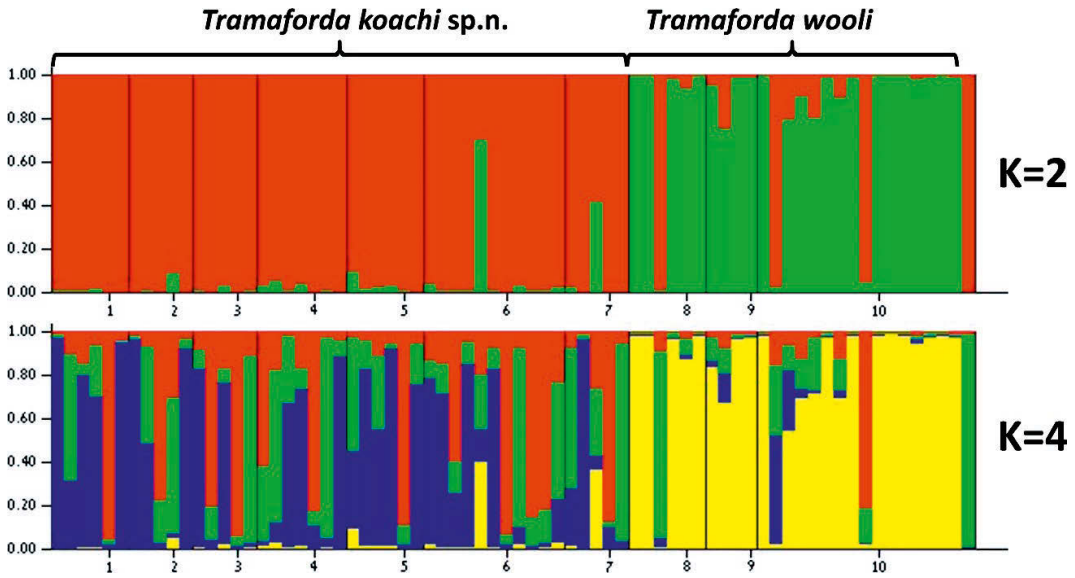

Fig. 6. Bayesian clustering bar-plot of *Tramaforda* microsatellite genotypes resulting from STRUCTURE analysis assuming 2 or 4 clusters (K value). The numbers at the bottom of the bar-plot match population numbers in Table 1.

P. atlantica in Israel and the Golan Heights indicated clustering to two groups, one of *T. koachi* sp. nov. and the second of *T. wooli* (Fig. 6).

Life cycle and general biology. The primary host of *T. koachi* is *P. atlantica*. The life cycle of *T. koachi* is similar to the life cycle of *T. wooli*. The galls are formed early in spring (late March – early April) on the margins of unfolding leaves. Then the galls can be usually seen on the first (basal) leaves on the shoot. This location is typical for Fordini species in which only the fundatrix induce galls (Wool & Burstein 1991a, Inbar & Wool 1995). At this stage, the gall may stop its development if the fundatrix is parasitized by *Monoctonia pistaciaecola* (Wool & Burstein 1991b). By mid-June, 3–4 apterae of the second generation were often found in the galls. The third generation begins to appear in the galls at the end of August. The total number of aphids per gall reaches at most 25. In early October, the alate fall migrants leave the galls. The secondary host of the species is not known yet. We observed (in Katzrin) sexuparae returning to the primary host (*P. atlantica*) on April–May; a clear indication for a two-year holocycle as in most Fordini species. The sexuales mate on the trunk of the primary host. As is in other Fordini a single fertilized, overwintering egg is formed in each female, from which a new fundatrix emerges next spring.

Distribution and gall morphology. The galls are rather rare. Even within a given site, only few trees routinely support galls every year (MI personal observations). Our extensive sampling and surveys confirm the earlier reports by Koach & Wool (1977) on the distribution of *Tramaforda* spp. in Israel. *Tramaforda wooli* has a very narrow distribution on *P. atlantica* trees in central Israel from the coastal plain, and the Judean Mountains. The distribution of *T. koachi* is in most cases allopatric with respect to *T. wooli*. Nevertheless, we recently found that both species may be found sympatrically, even on the same trees on the eastern part of the lower Galilee in Israel (see black circle in Fig. 1). It is found in Northern Israel and the Golan Heights (Fig. 1). This species was also recorded in southern Jordan (Moshe Inbar, personal observations). Although the galls of the two species are formed on the margin of the leaflet, the galls of *T. koachi* do not have the serrated outer margins typically found in *T. wooli* (Fig. 7). The length of the galls is ca. 1 cm. There are usually one or two (rarely three or four) galls on a single leaflet.

Discussion

Currently, the tribe Fordini (Hemiptera, Eriosomatinae) comprises 83 valid species, while 119 additional names are synonymized under suitable valid species (Favret 2020). One of the reasons of the high number of species level synonyms is the distinct differences in morphology between *Pistacia* gall producing and grass root-feeding forms of the same species (Brown & Blackman 1994). In some cases, these

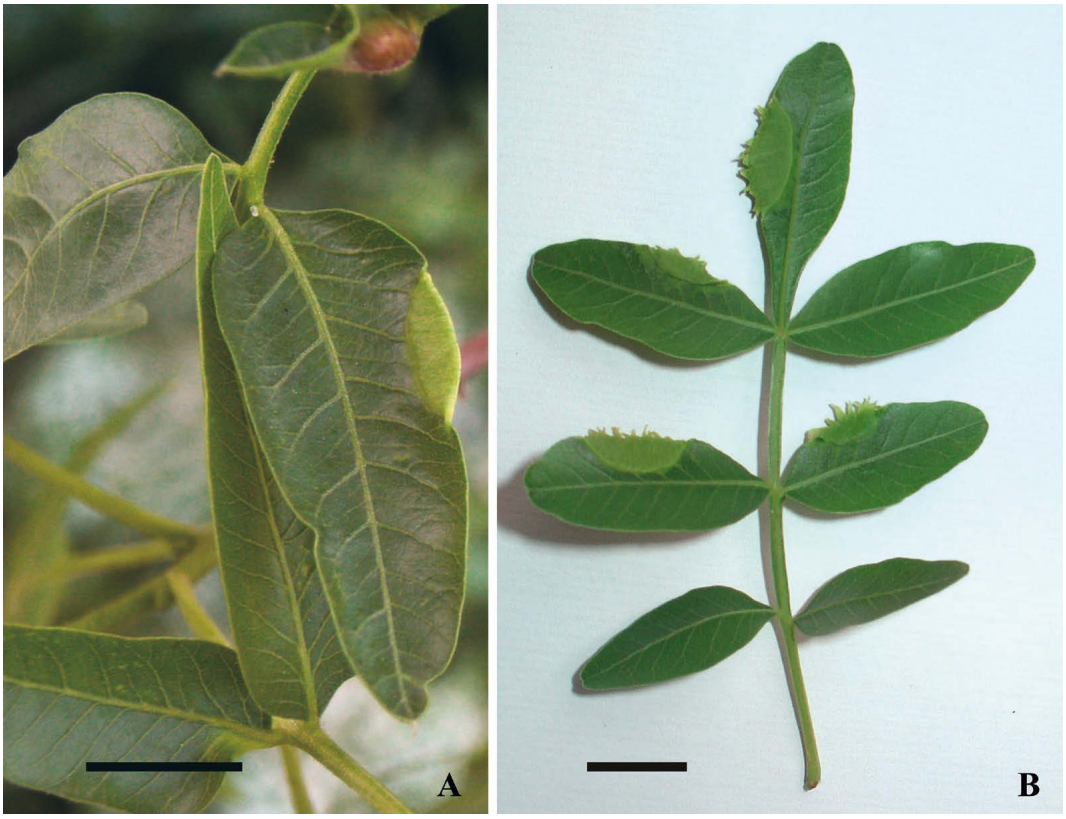

Fig. 7. Galls of *Tramaforda* on the leaflets' margin of *Pistacia atlantica*. **A.** Gall of *Tramaforda koachi* sp. nov.; **B.** gall of *Tramaforda wooli* (scale bar: 1 cm).

morphologically different forms were described in different genera (Brown & Blackman 1994). We did not find any distinct difference in morphology between fall migrants of two closely related *Tramaforda* spp., except **difference in the body size**: emigrants of *T. koachi* are smaller. It is difficult to decide if difference in body length is a good discriminating character between *Tramaforda* spp. or not, because we investigated only a single population. On the other hand, in Aphididae there are several species that develop morphological form of reduced size as consequence of environmental factors, such as, the summer form of *Brachycaudus persicae* erroneously described as *B. mimeuri* (Burger 1975, Coeur d'acier et al. 2008). Although, the fall migrants develop within the controlled environment of the gall, which minimizes to some extent the effect of environmental factors on aphid body size. However, given the rather large difference in size between the *T. wooli* samples presented here and the mean measurements provided by Manheim (2007), it is possible that the postulated size difference between these two species is a result of microenvironmental differences affecting host suitability. Nevertheless, barcoding and other molecular data clearly indicated a distinct **genetic difference** between these two forms large enough (3.8 % in COI and 3.4 % in COII) to suggest that they are distinct species.

Microsatellite analysis is widely used in population genetics analyses and is found to be informative in species identification were species diagnostic alleles between closely related species are found (Allendorf et al. 2013). Microsatellites as nuclear markers are also particularly useful in identifying interspecific hybridization (Allendorf et al. 2013). The microsatellite analyses of the *Tramaforda* spp. similarly indicated a clear differentiation between the two species.

The gall structure ("the extended phenotype of the insect") among Fordini aphids is usually species-specific (Koach & Wool 1977, Inbar et al. 2004, Zhang & Qiao 2007). The two *Tramaforda* taxa produce **species-specific galls** on the leaflet of *P. atlantica*: the outer margin of gall of *T. wooli* is

serrated, while it is smooth in *T. koachi* (Fig. 7). In addition, these closely related species are spatially separated (Fig. 1). Identification of species without reliable morphological differences could rely on molecular markers and gall characteristics as was found in other groups of gall-forming insect (e.g. Dorchin et al. 2015).

The Atlantic or the Atlas pistachio, *P. atlantica* has wide and fragmented distribution across different phytogeographical regions and climatic zones. It is found in Central Asia, the Levant, North Africa all the way to the Canary Islands in the Atlantic Ocean. Such distribution creates isolated populations, sometimes relicts with different genetic and phenotypic variation (Danin 1999, Inbar & Kark 2007, Avrani et al. 2012, Talebi et al. 2012, El Zerey-Belaskri et al. 2018). The gall-forming aphids that are entirely dependent on *P. atlantica* are also exposed to different selection pressures in variable environmental conditions and isolation in this region that may promote local adaption and speciation. Such isolation and speciation have been found in the related gall-forming aphid *Slavum wertheimae* Hille Ris Lambers (Fordini) in Israel and Jordan (Avrani et al. 2012). Similarly, a recently described gall-forming aphid, *Inbaria swirskii* (Remaudière & Inbar) (= *Geoica swirskii*) and two apparently undescribed lineages of *Inbaria*, all forming galls on *P. atlantica*, are restricted to a few locations in Israel and Jordan (Remaudière et al. 2004, Barjadze et al. 2018). The speciation and restricted distribution that we report in *Tramaforda* spp. are probably the results of their obligate interactions with their host trees along wide but fragmented distribution across heterogeneous habitats.

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