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Studies on the identity of the parasitoids *Aphidius colemani* and *Aphidius transcaspicus* (Hymenoptera: Braconidae)

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Abstract. The taxonomic status of the aphid parasitoid *Aphidius colemani* Viereck has been questioned, especially in regard to *Aphidius transcaspicus* Telenga (Hymenoptera: Braconidae). The genetic association between *A. colemani* and *A. transcaspicus* was studied by cross mating individuals of *A. colemani* and *A. transcaspicus* (A.c.♀ × A.t.♂ and A.c.♂ × A.t.♀) and applying appropriate molecular methods. The cross mating resulted in offspring (female and males) that were fertile. Therefore, the cross mating assays performed in an artificial environment showed that these two populations are potentially compatible. The mean number of mummies that developed and the sex ratio of the offspring of each cross were similar. Most of the male and female offspring from each cross were assigned to *A. transcaspicus.* Furthermore, the genetic divergence between the ribosomal internal transcribed spacers (ITS2) of the *A. colemani* and *A. transcaspicus* studied was 16%. These results indicate that *A. colemani* might be a complex of species with different morphological and biological characters attacking different host aphids.

INTRODUCTION

In the Aphidiinae, a subfamily of Braconidae, 400 species are described in 60 genera and subgenera (Starý, 1988). Members of this subfamily are endoparasitoids of aphids and include genera in which many species are important biological control agents. The genus *Aphidius* Nees is the largest genus of aphid parasitoids with about 70 species recorded throughout the world (Pungerl, 1986).

Aphidius colemani Viereck (Hymenoptera: Aphidiinae) is presumed to be a species of Indian origin. Its hosts belong to the family Aphididae (Starý, 1975). It is a polyphagous parasitoid, which is widely used in the biological control of *Aphis gossypii* (Glover) (Homoptera: Aphididae) and *Myzus persicae* (Sulzer) infesting protected crops (van Schelt et al., 1990; Grasswitz & Reese, 1998; Elenberg et al., 2000; Bolckmans & Tetteroo, 2002; van Lenteren, 2003; Yano, 2006).

Aphidius transcaspicus Telenga (Hymenoptera: Aphidiinae) is recorded as a parasitoid of the following aphid genera *Hyalopterus* Koch, *Melanaphis* (Van der Goot) and *Rhopalosiphum* (Koch) (Starý, 1964, 1966). It is widely distributed throughout the Mediterranean, Asia Minor, Transcaucasia, and central Asia (Starý, 1964). It is an effective natural enemy of *Hyalopterus persikonus* (= *Hyalopterus pruni*) (Geoffroy) (Homoptera: Aphididae) on peach, according to Starý (1965) and Lozier et al. (2008a). Recently, it has attracted interest as a potential candidate for use in the biological control of aphids on prunes in California (Mills, 2002).

A. colemani and *A. transcaspicus* are very similar morphologically and difficult to separate. Starý (1975) considered *A. colemani* as synonymous with *A. transcaspicus*. Rabasse et al. (1985) compared populations of *A. colemani* from southern France and Brazil reared in *Melanaphis donacis* (Passerini) and *Aphis nerii* (Boyer), respectively. Their detailed investigation indicates that these populations differed in the number of antennal segments; the females of the population from southern France had 16–17 segments (rarely 15) and those from Brazil 15–16 segments (rarely 14). These populations also differed in their esterase patterns detected by electrophoresis. The R^F of the population from southern France was 0.80 and that of the Brazil population 0.63 and 0.70.

Pennacchio (1990) describes *A. colemani* reared from *A. nerii* and *Hyalopterus* spp. The *A. colemani* females have 15–16 antennal segments and their labial palps are 2 segmented (rarely 3 segmented). Takada (1998) states that *Aphidius magdae* Meschloff and Rosen (possibly = *A. transcaspicus*) is a species of Palearctic origin and occurs in Japan and Israel. The antennae of females of *A. magdae* have 16 (rarely 15 or 17) segments and their labial palps are 3 segmented. This species mainly specializes in parasitizing *Hyalopterus* spp., but has also been reared from *Schizaphis rotundiventris* (Signoret) and *Toxoptera aurantii* (Boyer de Fonscolombe). Kavallieratos & Lykouressis (1999) examined specimens of *A. transcaspicus* that emerged from *Hyalopterus* spp. and compared them with specimens of *A. colemani* reared from several aphid species in Greece. They report that *A. transcaspicus* females can easily be distinguished from those of *A. colemani* because the antennae of the former

have 16–17 segments (rarely 15) and the latter 15 segments. The labial palps of *A. transcaspicus* are 3 segmented (rarely 2 segmented) rather than 2 segmented as in *A. colemani*. In addition, those authors state that *A. transcaspicus* differs morphologically from *A. colemani* in having a lighter F1, lighter coloured posterior terga, more slender pterostigma, and a less triangular and shorter metacarpus.

The above shows that despite the intense effort to separate *A. colemani* from *A. transcaspicus* uncertainties remain because their morphological characteristics are very variable and overlap. Thus, *A. colemani* and *A. transcaspicus* are not readily distinguished from each other. Even more, recent data indicates that *A. transcaspicus* can exploit a wider host range than previously thought. In the laboratory, *A. transcaspicus* has been successfully reared on *Aphis craccivora* (Koch), *Aphis fabae* (Scopoli), *A. gossypii*, and *M. persicae* (Mackauer & Starý, 1967; Starý, 1970; Takada, 2002; Li & Mills, 2004; Starý, 2006; Wang & Messing, 2006). *Aphidius trancaspicus* successfully attacks and parasitizes *M. persicae* infesting cabbage plants in field cages (Wang & Messing, 2006).

In fact, in terms of systematics *Aphidius* is a difficult genus (Pungerl, 1983, 1986; Mescheloff & Rosen, 1990). Some species within this genus are separated on the basis of their host aphids, despite being very difficult to distinguish morphologically, whilst laboratory experiments have shown that some of the morphological characters used for adult parasitoid identification can display considerable environmentally-induced variation (Pungerl, 1986). This approach was followed in the case of *A. colemani* and *A. transcaspicus* but has caused difficulties and given inconsistent results.

Molecular studies are becoming increasingly important in resolving taxonomic relationships in insects. Different genes are used to evaluate the genetic divergence of closely related species or populations (Caterino et al., 2000). The nuclear ribosomal repeat cistrons are widely used for phylogenetic studies of protistan, plant, and animal species. Nuclear RNA gene sequences have already proven valuable for studying higher taxonomic levels of insects (Pelendakis & Solignac, 1993; Malafronte et al., 2007; Nelson et al., 2008). A region of these repeats more suitable for genus and species comparisons is the second Internal Trancribed Spacer region (ITS2), i.e., the one between the 5.8S and 28S RNA genes. The ITS2, a noncoding rapidly evolving region, has highly repetitive sequences that can differ among closely related populations, which have proven useful for comparing closely related insect species, subspecies, or populations (Collins & Paskewitz, 1996; Fenton et al., 1998; Silva et al., 1999; Rokas et al., 2002; Rozenberg et al., 2006).

Studying genetic associations between closely related organisms is a topic that has recently attracted a large amount of attention. This is associated with the evolutionary concept concerning the genetic structure of populations and the process of speciation. This is especially important when using insect parasitoids in the biological control of aphid pests. This was recognized as a main element hindering the control of *Hyalopterus* spp. by *A. transcaspicus* in California (Lozier et al., 2008b).

The aim of the current study is to investigate the genetic association between *A. colemani* and *A. transcaspicus*. In particular two questions were addressed. Firstly, whether cross mating between individuals of *A. colemani* and *A. transcaspicus* is possible since cross mating indicates compatibility and thus, a close genetic relationship between two populations. Secondly, the genetic divergence between *A. colemani* and *A. transcaspicus* was determined using molecular methods. The combined use of these procedures provides a further contribution to the investigation of this taxonomic problem.

MATERIAL AND METHODS

Experimental material

A. colemani and *A. transcaspicus* were reared on *M. persicae* infesting pepper plants (cv. Vidi). The *A. colemani* culture was initiated by adults reared from *A. gossypii* mummies collected on *Hibiscus syriacus* L. (Malvaceae) and that of *A. transcaspicus* from *Hyalopterus* mummies collected on *Prunus persica* L. (Rosaceae) in Athens. The aphid species was named following Lozier et al. (2008a), with aphids of the genus *Hyalopterus* infesting peach most likely belonging to *H. persikonus*. *M. persicae* colonies were initiated from aphids collected from pepper plants growing on the Campus of the Agricultural University of Athens. Aphid colonies were maintained in wooden framed cages 80 × 80 × 70 cm in a glasshouse at an average temperature of 22.5 ± 2.5°C (mean ± S.D.) and under natural lighting.

Potted pepper plants infested with *M. persicae* were each covered with a plastic cage. Each cage (11 cm in diameter and 30 cm high) was made of PVC (0.4 mm thick) with 2 openings of 9 × 9 cm each in the sides and the opening at the top covered with fine muslin for ventilation. Newly emerged parasitoids (15–20) (*A. colemani* or *A. transcaspicus*) were released into each cage and provided with food in the form of dilute drops of honey placed on the muslin covering the top of each cage. The cages were placed in a growth cabinet at 25 ± 0.5°C, 65 ± 5% R.H. and a 16L : 8D photoperiod. The parasitoid cultures were maintained by transferring, by means of an insect aspirator, two day old parasitoids to another cage.

Experimental procedure

Cross mating

Mummified aphids were removed from the cultures and transferred individually to small plastic vials (1 cm diameter × 4 cm height). The emerging parasitoids (<1 day old) were sorted by sex. One female (*A. colemani* or *A. transcaspicus*) together with one male (*A. transcaspicus* or *A. colemani*) were placed in a plastic Petri dish (9 cm in diameter and 1 cm in height, with a 3 cm in diameter opening in its lid covered with fine muslin, on which a droplet of honey was placed). After 24 h, the female parasitoid was transferred to another Petri dish containing about 200 individuals of *M. persicae* (nymphs of all instars and adults) on an egg plant leaf. The leaf was on a layer of cotton wool, moistened with water, in the base of the dish. The dish was then placed in a growth cabinet at 25 ± 0.5°C, 65 ± 5% R.H. and a 16L : 8D photoperiod. The parasitoid was removed from the dish after 24 h. The leaf in each dish was replaced with a new one every other day and the aphids carefully transferred to the new leaf and reared until mummification. In total, there were thirty replicates (15 with a *A. colemani* female and 15 with a *A.*

transcaspicus female). The number of parasitoids that emerged from the mummies was recorded for each female.

The number of antennal segments, the number of labial palps of the offspring with 15 antennal segments and the sex of the emerged offspring of each maternal female were recorded.

The fertility of one female offspring from each of the cross mated females was determined. The offspring of each cross mated female were kept in a dish for 24 h after emergence. Then one of the female offspring was transferred to a Petri dish containing approximately 200 nymphs and adults of *M. persicae* feeding on the leaf of an egg plant and its fertility recorded as described above. The sex of the offspring of each female was recorded.

The number of mummies and number of offspring resulting from the two crosses (*A. colemani*♀ × *A. transcaspicus*♂ and *A. colemani*♂ × *A. transcaspicus*♀) were compared using one way ANOVA of logarithmically transformed data. The emergence rate and sex ratio were compared using one way ANOVA. The percentage of offspring assigned to *A. colemani* or *A. transcaspicus* in the two crosses was compared using a two-way ANOVA, with the species of the maternal female and the morphology of the offspring as factors. Similarly, the sex ratio of the offspring of each maternal female was tested for significance using a two-way ANOVA. Data on percentages were arcsine transformed prior to analysis. Means were separated using a Tukey-Kramer HSD test. Analyzes were done using the statistical package JMP (SAS Institute Inc., 2007)

DNA extraction and amplification

Five *A. collemani* females were reared from *A. gossypii* mummies collected from five H. *syriacus* plants and five *A. transcaspicus* females from *H. persiconus* mummies collected from five *P. persica* trees in Athens.

Total genomic DNA was extracted from each individual by mechanical trituration of the tissue (in buffer solution), followed by 1h of chemical digestion with proteinase K and then a salt and ethanol precipitation. PCR products were amplified using the following ITS2 ribosomal primers (Porte & Collins, 1991):

5.8SF: 5'-TGTGAACTGCAGGACACATGAAC and
28SR: 5'-ATGCTTAAATTTAGGGGGTA.

These primers anneal to highly conserved sequences in the 5.8S and 28S rDNA genes flanking the ITS2 region. The ITS2 PCR protocol was 40 cycles of 94°C for 45 s, 54°C for 45 s and 72°C for 60 s. The PCR products were cloned in a Blue Script KS+ plasmid vector (Agilent Technologies-Stratagene Products Division, La Jolla, CA, USA). Samples were sent for direct sequencing in both directions.

Sequence alignment and phylogenetic analysis

The boundaries of the ITS2 region were defined by comparison with previously determined Braconidae 5.8S and 28S rDNA sequences submitted to the GenBank database at the US National Centre for Biotechnical Information (NCBI) (http://www.ncbi.nlm.nih.gov). Nucleotide sequences were aligned using the ClustalW program (http://www.ebi.ac.uk/clustalw/) under default parameters. Mean sequence divergence of ITS2 variants within and between each *Aphidius* population was estimated using PAUP* by uncorrected P, which is the proportion of nucleotide sites differing between two compared sequences. The multiple alignment resulting from the ClustalW analysis was the input for BOXSHADE program (http://www.ch.embnet.org/software/BOX form.html), which was used to indicate residue similarity based on ITS2 sequences from various insects. All sequences were retrieved from GenBank. The retrieved sequences were aligned using ClustalX program and the multiple alignment method (Thompson et al., 1997). Gaps and missing data were excluded from the data analysis. The program package PHYLIP 3.6a2 (Felsenstein, 2001) was used for calculating trees. The tree was constructed using the Neighbour-Joining method.

RESULTS

Females of *A. colemani* used in the experiments had 15 (rarely 14) antennal segments and 2-segmented labial palps and males had 15 (rarely 16) antennal segments and 2-segmented labial palps. Females of *A. transcaspicus* had 16 (rarely 15) antennal segments and 3-segmented labial palps and males 17–19 antennal segments and 3-segmented labial palps.

Cross mating occurred between *A. colemani* and *A. transcaspicus* as offspring (both females and males) were produced by all pairs. The results of the crossings between A.c.♀ × A.t.♂ and A.c.♂ × A.t.♀ are shown in Table 1. The mean number of mummies, mean number of offspring, emergence rate, and sex ratio were similar in the two crossings ($F_{1,28} = 4.08$, $p < 0.052$; $F_{1,28} = 2.67$, $p > 0.11$; $F_{1,28} = 0.13$, $p > 0.72$ and $F_{1,28} = 0.07$, $p > 0.79$, respectively).

The offspring were separated into three groups based on the morphological characters used to separate *A. colemani* and *A. transcaspicus*. The individuals with 15 antennal segments and 2-segmented labial palps were assigned to *A. colemani*. Those with more than 15 antennal segments were assigned to *A. transcaspicus*. Finally, individuals with 15-segmented antennae but 3 segmented labial palps were also grouped under *A. transcaspicus*, but in a different category following Kavallieratos & Lykouressis (1999). The 3 categories are referred to as A.c., A.t.1, and A.t.2, respectively.

Crossing did not significantly affect the percentage of offspring assigned to A.c. or A.t. ($F_{1,56} = 0.16$, $p > 0.89$). However, a significantly higher percentage of the offspring were assigned to A.t. than A.c. ($F_{1,56} = 370.78$, $p < 0.001$). When A.c. mothers were used the percentage of the offspring assigned to A.c was 31 ± 3% and to A.t. 69 ± 4%. In the case of A.t. mothers the percentages were 25 ± 1% and 75 ± 2%, respectively.

The percentage of the offspring assigned to each morphological type (A.c., A.t.1 and A.t.2), was not affected by the crossing ($F_{1,84} = 0.82$, $p > 0.36$). However, there were significant differences among the percentages of

TABLE 1. Mean (± SE) number of mummies, mean number of offspring, emergence rate, and sex ratio of offspring resulting from cross mating *A. colemani* and *A. transcaspicus*.

Crossing	Number of mummies /♀	Number of offspring /♀	Emergence rate	Sex ratio
A.c.♀ × A.t.♂	61.6 ± 2.8a	49.4 ± 2.5a	0.69 ± 0.02a	0.54 ± 0.01a
A.c.♂ × A.t.♀	69.7 ± 2.8a	55.7 ± 2.6a	0.82 ± 0.02a	0.54 ± 0.01a

Values within columns followed by the same lower case letter are not significantly different.

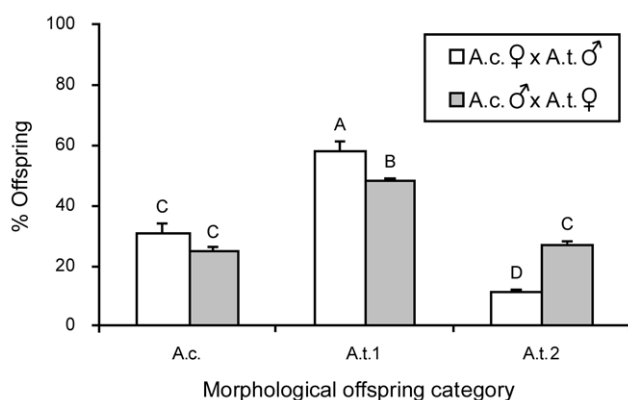

Fig. 1. Percentage (%) (mean ± SE) of the offspring of each cross that were assigned to the different morphological categories. The individuals with 15 antennal segments and 2-segmented labial palps were assigned to *A. colemani* (A.c.) and those with more than 15 antennal segments were assigned to *A. transcaspicus* (A.t.1). Finally, individuals that had 15-segmented antennae and 3 segmented labial palps were assigned *A. transcaspicus* (A.t.2). Columns topped with different letters indicate significantly different values.

offspring assigned to each of the three morphological types ($F_{2,84}$ = 194.14, p < 0.0001). The interaction between these two factors was significant ($F_{2,84}$ = 35.63, p < 0.0001). Significantly more of the offspring of A.c.♀ × A.t.♂ were assigned to A.t.1 than of the A.c.♂ × A.t.♀ (Fig. 1). The percentages of the other categories (A.c. and A.t.2) were similar when the maternal female was *A. transcaspicus*. However, in the case of *A. colemani* females the percentage of the offspring assigned to A.t.2 was much reduced compared to A.c. (Fig. 1).

The sex ratio of the offspring within each morphological category is shown in Table 2. Statistical analyses revealed that the effect of the crossing, the effect of the morphological category of the offspring and their interaction were insignificant ($F_{1,84}$ = 0.52 p > 0.47; $F_{2,84}$ = 1.72, p > 0.18 and $F_{2,84}$ = 0.63, p > 0.53, respectively). The sex ratios ranged between 0.53 ± 0.02 and 0.59 ± 0.05.

All the female offspring used in fertility tests produced offspring. The average sex ratio of the offspring was 0.54.

Molecular analysis

Analysis of the 5.8S, ITS2, and 28S regions

The primer pair (5.8SF and 28SR) amplified the ribosomal ITS2 of *A. colemani* and *A. transcaspicus*. Sequences were confirmed as ribosomal ITS2 by searching the GenBank database of the NCBI using BLAST protocol. Each sequence is available from GenBank (Accession number for *A. colemani* = DQ504298 and for *A. transcaspicus* = DQ504299).

Variability in the length of 5.8S-ITS2-28S was recorded both within and between populations of the two *Aphidius* examined. Lengths ranged from 739 to 745 bp for the five individuals of *A. colemani* and from 639 to 645 bp for the five of *A. transcaspicus* (Table 3).

Aligned ITS2 sequences of *A. colemani* (DQ504298) and *A. transcaspicus* (DQ504299), contained several inferred insertions or deletions (indels), the largest being 60 bp (Fig. 2). Three different regions of the rRNA gene can be distinguished. Region I (~90 bp in length) corresponds to the 5.8S rRNA coding sequence. ITS2 covers Region II (~450 bp in *A. colemani* and ~385 bp in *A.*

Table 2. The sex ratio (mean ± SE) of offspring of crosses between *A. colemani* and *A. transcaspicus* assigned to each morphological category. The offspring with 15 antennal segments and 2-segmented labial palps were assigned to *A. colemani* (A.c.) and those with more than 15 antennal segments to *A. transcaspicus* (A.t.1). Finally, offspring that had 15-segmented antennae and 3 segmented labial palps were assigned to *A. transcaspicus* (A.t.2).

Crossing	Morphological category of the offspring		
	A.c.	A.t.1	A.t.2
A.c.♀ × A.t.♂	0.55 ± 0.02a	0.53 ± 0.01a	0.59 ± 0.05a
A.c.♂ × A.t.♀	0.53 ± 0.02a	0.54 ± 0.01a	0.56 ± 0.02a

Values followed by the same lower case letters are not significantly different.

Table 3. Sequence and length diversity of 5.8S-ITS2-28S variants from *A. colemani* and *A. transcaspicus* populations.

Species	GenBank Accession Nos	ITS2 length (bp)	GC content (%)	Sequence divergence	
				Between individuals	Between populations
A. colemani					
1	DQ504298	745	22.95		
2	FJ495547	744	22.85		
3	FJ495548	739	22.16	0.087	
4	FJ495549	740	22.22		
5	FJ495550	743	22.88		
A. transcaspicus					0.16
1	DQ504299	682	23.49		
2	FJ495551	683	23.72		
3	FJ495552	679	23.31	0.087	
4	FJ495553	677	23.63		
5	FJ495554	684	23.68		

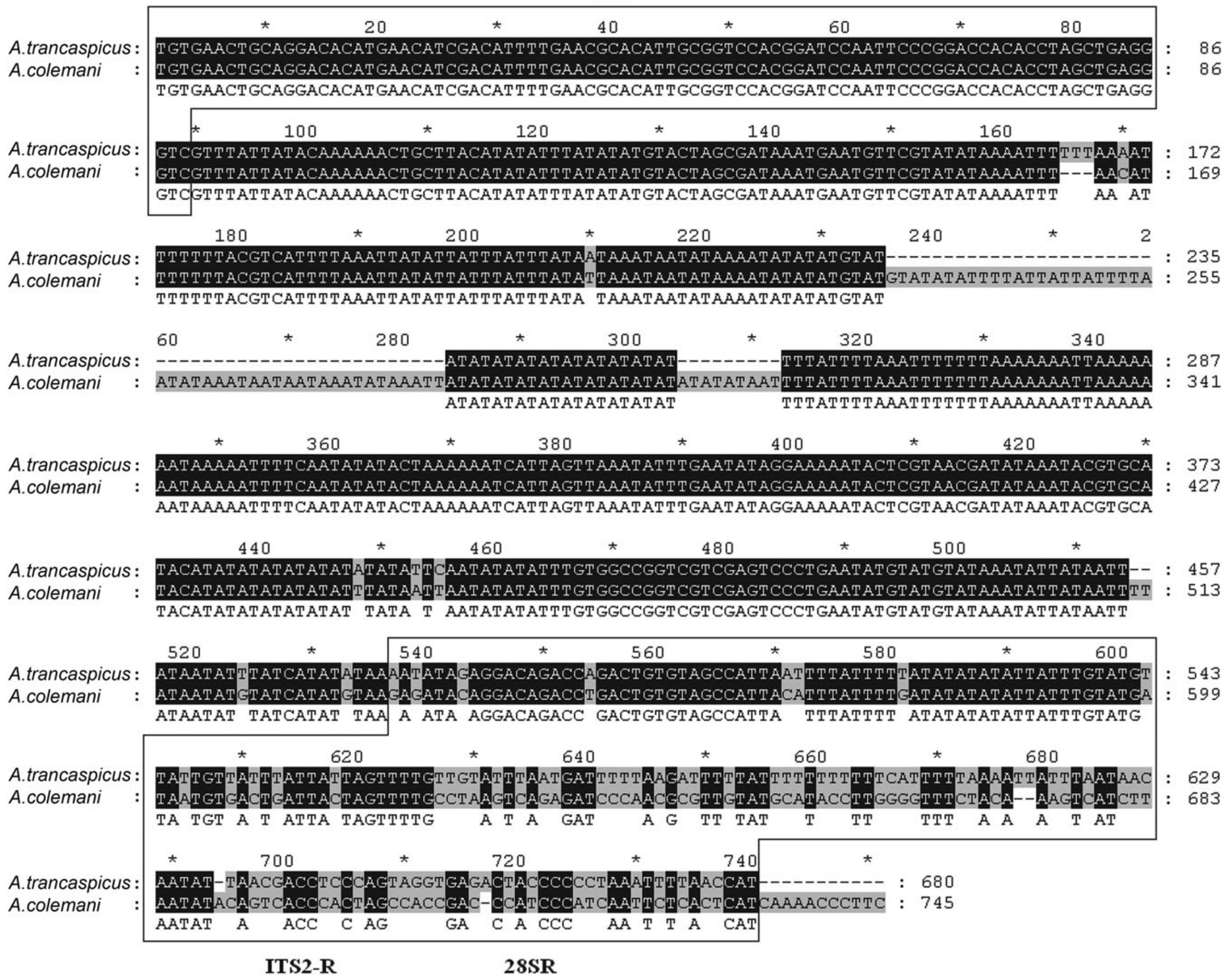

Fig. 2. Alignment of the sequences of the 5.8S, ITS2, and 28S regions of *A. colemani* (DQ504298) and *A. transcaspicus* (DQ504299). Identical nucleotides are shaded black. Dashes (–) indicate insertion/deletions.

transcaspicus). Region III covers a ~205 bp sequence at the 5' end of the 28S rRNA gene.

Mean sequence divergence of 5.8S-ITS2-28S within and between *A. colemani* and *A. transcaspicus* populations was estimated using PAUP* by uncorrected *P* (Table 3). The mean GC content percentage of *A. colemani* was 22.8 (SD = 0.19), whereas that of *A. transcaspicus* was 23.5 (SD = 0.21). Sequence divergence recorded between individuals of *A. colemani* was 0.087, the same as between *A. transcaspicus*. Sequence divergence (uncorrected *P*) between populations was 0.16.

ITS2 phylogenetic analysis

Complete ITS2 sequences of the ribosomal DNA of six specimes/species of Hymenoptera (Braconidae) were determined using a BLAST search of GenBank. Namely: *A. colemani* (Greece) (DQ504298), 2-*A. colemani* (USA) (EU561659), *A. transcaspicus* (DQ504299), *Lysiphlebus testaceipes* Cresson (Hymenoptera: Braconidae) (AY498555) and *Leptopilina boulardi* (Hymenoptera: Figitidae) (AY124568). ITS2 sequences were phylogenetically informative in separating Braconidae species (Fig. 3). Our results indicate that *Aphidius* populations (*A. colemani* and *A. transcaspicus*) do not cluster in the same clade as *Lysiphlebus*, within the Aphidiinae.

DISCUSSION

According to our results, *A. colemani* and *A. transcaspicus* can interbreed. The F_1 generation consists of both females and males and the F_1 females are fertile. Thus the *A. colemani* and *A. transcaspicus* populations used in this study are compatible and genetically very similar.

Cross-mating is the main criterion for species separation (cryptic or complex species) (Mackauer, 1969; Starý et al., 1980). However, more recent research has shown that interspecific crosses may occur in the laboratory as in this artificial mating environment the ecological or behavioural barriers active in nature are not effective (Smith et al., 1993). In fact, neither the sterility or viability of offspring of cross matings are useful criteria of species status (Paterson, 1988). Even when interbreeding occurs the hybrid population might be viable only for a few generations or hybridization might result in a significant post mating fitness cost such as a reduction in offspring fecundity. According to the Haldane's rule, in haplodiploid

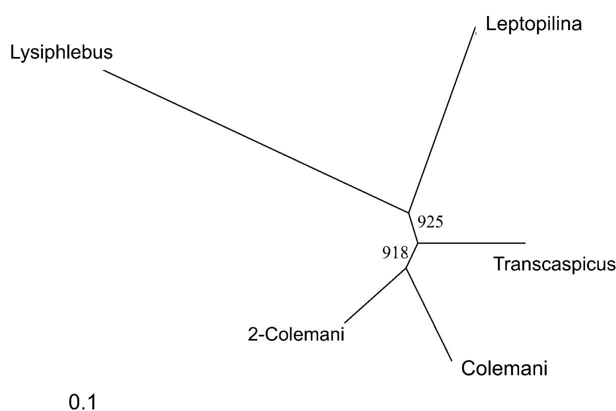

Fig. 3. Phylogenetic bootstrap neighbour-joining tree (unrooted) built using CLUSTALX vl.8 default alignment of ITS2 genes of *A. colemani* and *A. transcaspicus*, and relevant ITS2 genes of other species. Bootstrap values for branches are shown (1000 replicates). The following abbreviations are used: Colemani: (*A. colemani*, DQ504298), 2-Colemani (*A. colemani*, EU561659), Transcaspicus (*A. transcaspicus*, DQ504299), Lysiphlebus (*L. testaceipes*, AY498555), and Leptopilina (*L. boulardi*, AY124568) (as out group).

species, the fertility of F_2 males must be assessed since F_1 males are not hybrids (Koevoets & Beukeboom, 2009). These shortcomings weaken the reliability of mating assays for identifying species, when done in the laboratory.

However, if the offspring of the crosses are classified according to their morphology, then most of them are *A. trascaspicus* (Fig. 1). In addition, 26% of the offspring of A.t.♀ × A.c.♂ crosses and 9% of those of the A.c.♀ × A.t.♂ crosses had 15 antennal segments and 3-segmented labial palps and were assigned to *A. transcaspicus* (A.t.2). According to Kavallieratos & Lykouressis (1999) individuals of *A. transcaspicus* reared from *Hyalopterus* spp. collected from several areas in Greece, only very rarely have 15 antennal segments (1 female specimen in a total of 49 specimens collected). Therefore, a relatively high percentage of offspring of the crosses belong to a morphological type rarely occurring in nature. This indicates the potential for producing different morphological types, which could be used to evaluate the results of field surveys of variability in *A. colemani* and *A. transcaspicus*.

Based on morphological characters and cross mating it is difficult to determine conclusively the exact taxonomic association between *A. colemani* and *A. transcaspicus*. Therefore, a genetic approach was adopted based on quantifiable data obtained by characterization of rDNA sequences, including the second internal transcribed spacers, the inverting 5.8S and adjacent portions of the 28S coding regions. An advantage of this genomic target is that it includes highly conserved segments in the coding regions as well as hypervariable spacer sequences. The latter have repeatedly proved useful in distinguishing between taxonomic entities at and below the species level.

ITS2 sequences of *A. trancaspicus* were shorter than those of *A. colemani* and the difference in length was due to a deletion of ~60 bp at position 235 in the ITS2 fragment of *A. trancaspicus* (Fig. 2). The placement of indels was, in general, unambiguous and easily identifiable because they were sufficiently infrequent that they generally did not overlap (Lopez-Vaamonde et al., 2001). These consistent differences in length could be used to separate the two populations without the necessity of sequencing.

The ITS2 in the *A. colemani* and *A. transcaspicus* populations studied differed by 16%. This level of divergence is quite high for the same species or sub-species. In similar studies it was difficult to confirm the species level when the variability in ITS2 sequence between populations is of this order (Onyabe & Con, 1999; Romi et al., 2000; Lehr et al., 2005). Thus, we suggest that the DNA sequence divergence between *A. colemani* and *A. transcaspicus* populations might indicate a species-complex with different morphological and biological characters attacking different host aphids. Our results indicate that the *A. colemani*, *A. transcaspicus*-species complex is very complicated and requires further study. Future studies, including more nuclear and mtDNA genes, might further verify the species status of these two closely related aphid parasitoids.

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