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Orijinal araştırma (Original article)

A new species of the *menes* species group of the genus *Ceranisus* (Hymenoptera: Eulophidae) from Turkey[1]

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Summary

A new species of the *menes* species group of *Ceranisus* Walker, 1841 (Hymenoptera: Eulophidae: Entedoninae) was described from Hatay, Turkey as a part of the work on thrips parasitoids of Turkey conducted in 2005-2009. The new species, *Ceranisus amanosus* Doğanlar, Gumovsky, Doğanlar was compared with the closely related species, *Ceranisus menes* (Walker, 1839), of the genus. The composition of the setal rows on the pregenital sclerites is proposed for the first time as a diagnostic charater separating two similar species in the genus. DNA sequences of 28S D2 (nuclear), Cytochrome Oxidase I (COI, mitochondrial) and Cytochrome B (CytB, mitochondrial) genes are provided for the new species as a barcoding data.

Key words: Hymenoptera, Eulophidae, menes group, amanosus n.sp., Turkey

Anahtar sözcükler: Hymenoptera, Eulophidae, menes grup, amanosus n.sp., Türkiye

Introduction

The species of the genus *Ceranisus* Walker, 1841 (Hymenoptera, Eulophidae: Entedoninae) are among of the most tiny chalcid wasps (about 1.0 mm long). The identity of these wasps is still based on limited morphological characters, intraspecific variation of which within different populations is still poorly known.

Loomans & van Lenteren (1995) and Triapitsyn (2005) provided a global list of the described species of *Ceranisus* and their associated host thrips, and

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Triapitsyn & Headrick (1995) and Triapitsyn & Morse (2005) provided revisions of *Ceranisus* species from various regions.

Graham (1963) was first, who keyed out the species of *Ceranisus* in Europe, and he also described a new species, *Ceranisus. lepidotus* Graham, from Great Britain. Erdös (1966) described then *Ceranisus planitianus* Erdös from Hungary. More recently, Doğanlar (2003) described *Ceranisus bozovaensis* (Doğanlar, 2003) from Turkey in the genus *Urfacus* Doğanlar [the generic synonymy was then provided by Doğanlar & Triapitsyn (2007)]. Triapitsyn described a new species, *Ceranisus antalyacus*, from Antalya, Turkey (Cameron et al., 2004). Doğanlar & Triapitsyn (2007) added an unusual new species, *Ceranisus hirsitus* Doğanlar & Triapitsyn, from Turkey, and provided keys to both sexes of the European and Turkish species.

Triapitsyn (2005) provided a list of 14 known species of *Ceranisus*, allocated in the *pacuvius*; *russeli*, *barsoomensis* and *menes* species groups. *Ceranisus menes* Walker belongs to the latter group and is probably the most widely distributed species (Noyes, 2007), actively involved in biocontrol species (Murai, 1994; Loomans & Pakozdi, 1996). This species is rather distinguishable within the European fauna by the possession of the yellowish metasoma in female, and this character is mostly mentioned in the identification keys for the species of the genus. However, more species possessing the same or similar character combination are found so far. For example, Triapitsyn (2005) described *Ceranisus udnamtak* Triapitsyn, which reminds *C. menes* in general color, though differs in length of antennal joints and longer postmarginal vein. It may be expected that many regional records for *C. menes* will happen rather to concern various sibling species as it was proven to be with some other widespread and practically important chalcids, *Diglyphus isaea* (Walker) to list some (Sha et al., 2006).

Below we describe a new species of the *menes* species group of *Ceranisus* from Turkey, which is also similar to and easily confusable with *C. menes*. The new species differs in the possession of number of fine characters, some of which have not been used in taxonomy of the genus. Molecular characteristics provided below are aimed to facilitate further recognition of the new species.

Material and Methods

Materials

Specimens were collected by sweeping and putting the whole contents of the swept materials directly in 96 % ethanol. After sorting the materials out, individuals were stored in absolute ethanol at deep freeze (-20˚C). Some specimens were used for both, morphological and molecular studies, and treated by the mixture of 20 µl Proteinase K and 180 25 µl Buffer ATL (DNeasy

Kit, Quiagen). The exosceleton of the insects appeared rather transparent after the treatment (Figures 1B,C) what facilitated light-microscopic studies, and the lysed tissues might be used further for DNA extraction (see below).

DNA extraction and sequencing

DNA extraction, amplification and data analysis were performed by the third author. Genomic DNA was extracted from ethanol-preserved individuals using a protocol largely based on those described in the DNeasy Tissue Handbook provided by Qiagen. The specimens were not grinded, but kept overnight in a deep freeze (- 20ºC), then the sample was kept for some minutes at room temperature until it is melted, then 20 µl of Proteinase K was added and incubated at 50-56ºC for about 2 hours. Then, the specimens were taken off from the mixture and further operations were done following the DNeasy protocol. Final elution elution of DNA was conducted in 100 µl of AE Buffer to increase the extracted DNA concentration.

Sequence fragments displaying an increasing degree of variability were analyzed in the conserved D2 expansion of the 28S nuclear gene. Standard 50 µl PCR reactions were performed using 0.2 U *Taq* DNA polymerase (Fermentas), 5 µl 10x *Taq* Buffer with KCl (Fermentas), 3 µl, 25mM $MgCl_2$ (Fermentas) , 1 µl 10x dNTPs (Fermentas), 1 µl of each primer, 2 µl template DNA and 36.8 µl dH_2O (Sigma). Primer sequences for the 28S rDNA D1F (ACC CGC TGA ATT TAA GCA TAT), D2R (TTG GTC CGT GTT TCA AGA CGG) were from Campbell et al. (1993; 2000) and from Simon et al.(1994). PCR conditions for *C. hirsutus* and *C. amanosus* were: 30 cycles of 94°C denaturation (30 s), 55°C annealing (30 s) and 72°C elongation (1:30 s) with an initial 94°C denaturation (3 min) and a final 72°C extension (30 min). For *C. menes* was: 30 cycles of 96°C denaturation (30 s), 53°C annealing (30 s) and 72°C elongation (60 s) with an initial 94°C denaturation (3 min) and a final 72°C extension (10 min). After DNA amplification, 5 µl product with 1 µl loading dye (Fermentas) was loaded on a 1% agarose gel to check DNA amplification. The remaining DNA was loaded on a 1.5 % agarose gel with ethidium bromide, separated by electrophoresis at 140 V for 1 h, and visualised under UV. The amplified product was purified using a Qiaquick Gel Extraction Kit (Qiagen GmbH, Leusden, The Netherlands). DNA fragments were run on ABI 3100 Avant Genetic Analyzer (Applied Biosystems, Foster City, CA).

Sequence alignment and gene analysis

Sequences were aligned with the program Clustal W (Thompson et al., 1996) in MEGA 3.1 (Kumar et al., 2001) using the default setting (Open gap penalty=15; extend gap penalty =6.66). The alignments were checked manually.

Equally weighted maximum parsimony (MP) and maximum likelihood (ML) analyses were performed using PAUP (4.0 beta version) (Swofford, 1998). For MP analysis, a heuristic search procedure was used with the following default settings: 10 replicates of random taxon addition, tree-bisection-reconnection branch swapping, multiple trees retained, no steepest descent and accelerated transformation. Gaps were treated as missing data. For ML analysis, the appropriate substitution model of DNA evolution that best fit (GTR+Gamma model) the data-set were determined by the Akaike Information Criterion with Model Test 3.06 (Posada & Crandall, 1998). 28S rDNA: base frequencies = (A=0.15609 C=0.29665 G=0.33296 T=0.21431). Bootstrap analysis with 1000 replicates was calculated as a measure of support for individual clades for MP and ML trees. As the MP and ML analyses usually gave multiple trees, we reduced the set of trees to one consensus tree. The sequences of *Chrysorcharis* sp. (Eulophidae; Eulophinae) (AJ274438, Gauthier et al., 2005) was used as out-group and *C.hirsutus* was used as control group (EU557274) for these analyses. The access number of the specis are as follows: *Ceranisus amanosus* GQ452244; *C. hirsutus*2 GQ452245 ; *C. hirsutus*3 GQ452246; *C. menes* (Turkey 1) GQ452247; *C. menes* (Turkey 2) GQ452248.

Terminology and abbreviations

Morphological terminology follows Gibson (1997). This study is based upon examination and identification of about 100 specimens collected from the southern and southeastern Anatolia (Turkey). Some of the studied species were slide-mounted in Canada balsam. The examined specimens were deposited in the collections indicated by the following acronyms: ICMKU, Insect Museum of Plant Protection Department, Agriculture Faculty, Mustafa Kemal University, Antakya, Hatay, Turkey, UCRC, Entomology Research Museum, Department of Entomology, University of California, Riverside, California, USA, BMNH, The Natural History Museum (London, Great Britain). MD, Mikdat Doğanlar (as a collector). Abbreviations used in the key and descriptions are: C = claval segment, and F = funicular segment, l_1= space between tip of forewing and the level of cross-point of stigmal vein and marginal vein, $l_{2=}$ space between tip of submarginal vein and the level of cross-point of stigmal vein and marginal vein.

Results

***Ceranisus amanosus* n. sp.** (Figures 1, 2)

Types.-Holotype ♀ (on slide, ICMKU), labeled: “TURKEY, Hatay (Antakya- Amanos Mnt.), 853 m, 22.IV.2007, 36°25'26" N, 36°12'05"E, (M. D). Mounted in Canada balsam”. Paratypes (same collection data as the holotype): 4 ♀♀ in entellelan, ICMKU); 2 ♀♀, (on points, ICMKU); 23 ♀♀ (in alcohol, ICMKU); some ♀♀ (in alcohol, NASU); Hatay (Belen- Kömür Çukuru), 815 m, 2. V. 2008; 36 24 49 N, 36 08 86 E, swept from pasture, 17 ♀♀; Kayseri (Akmescit), 835 m,

08.VI. 22008, 38 38 N, 35 52 E, swept from weath field with *Sinapis*, 3♀♀, 1♂ (in alcohol ICMKU) ; Niğde (Ulukışla- Gümüş), 1257 m, 07. VI. 2008, 1♀, (in alcohol ICMKU), swept from pasture.

Female (Figures 1A, B, C). Body bicolor: head and mesosoma brown; metasoma pale brown to yellow; antennae and legs pale yellow, except coxae basally pale brown, venation pale yellow.

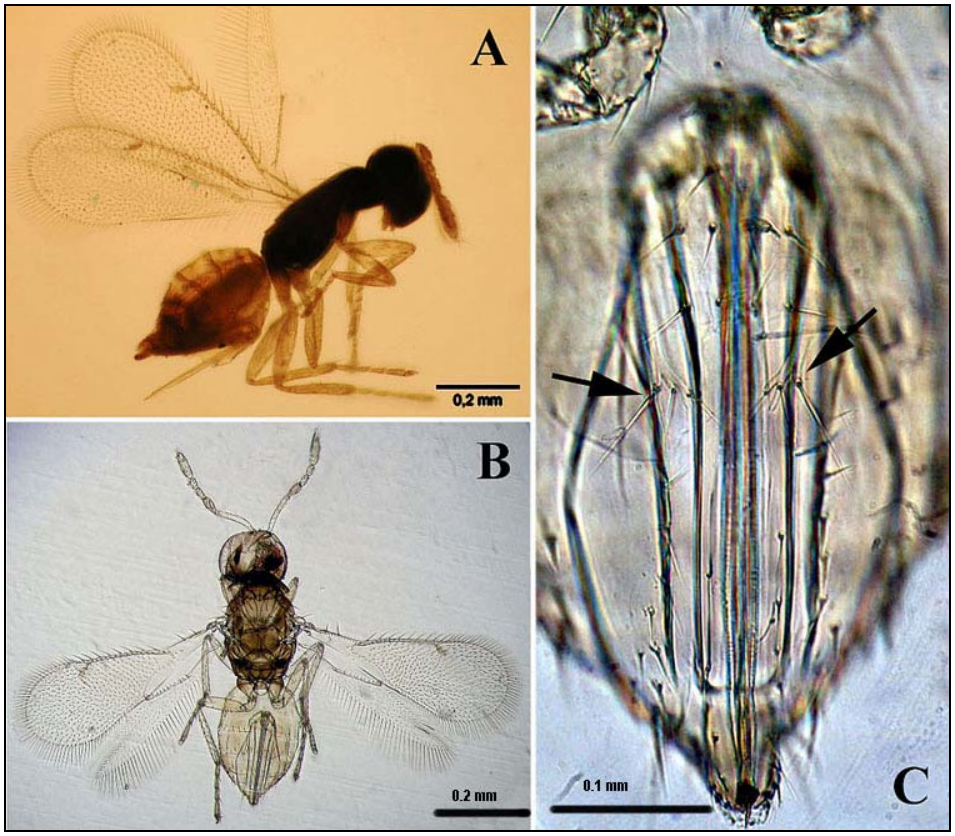

Figure 1. *Ceranisus amanosus* n.sp., Female, A-B. body, A. in lateral view; B. in dorsal view; C. pregenital area.

Head. Vertexal suture straight behind hind ocelli and turn upward on both sides towards eyes; frontal suture broadly V-shaped; malar sulcus split (but seen on more heavily sclerotized specimens); Antenna (Figure 1B) with scape slender, about 6.5 times as long as wide; pedicel about 2.7 times longer than wide; F1 1.2 times longer than F2, 2.1-2.2 times as long as broad, with one long sensillum; F2 nearly twice as long as broad, club including spicula 2.8-3.0 times as long as broad, C1 distinctly shorter than C2 (7:10), C1 with two sensilla; C2 with two basal + two apical sensilla; stelex sensilla tip I of Hansson (1990) spicula 1/5 C2.

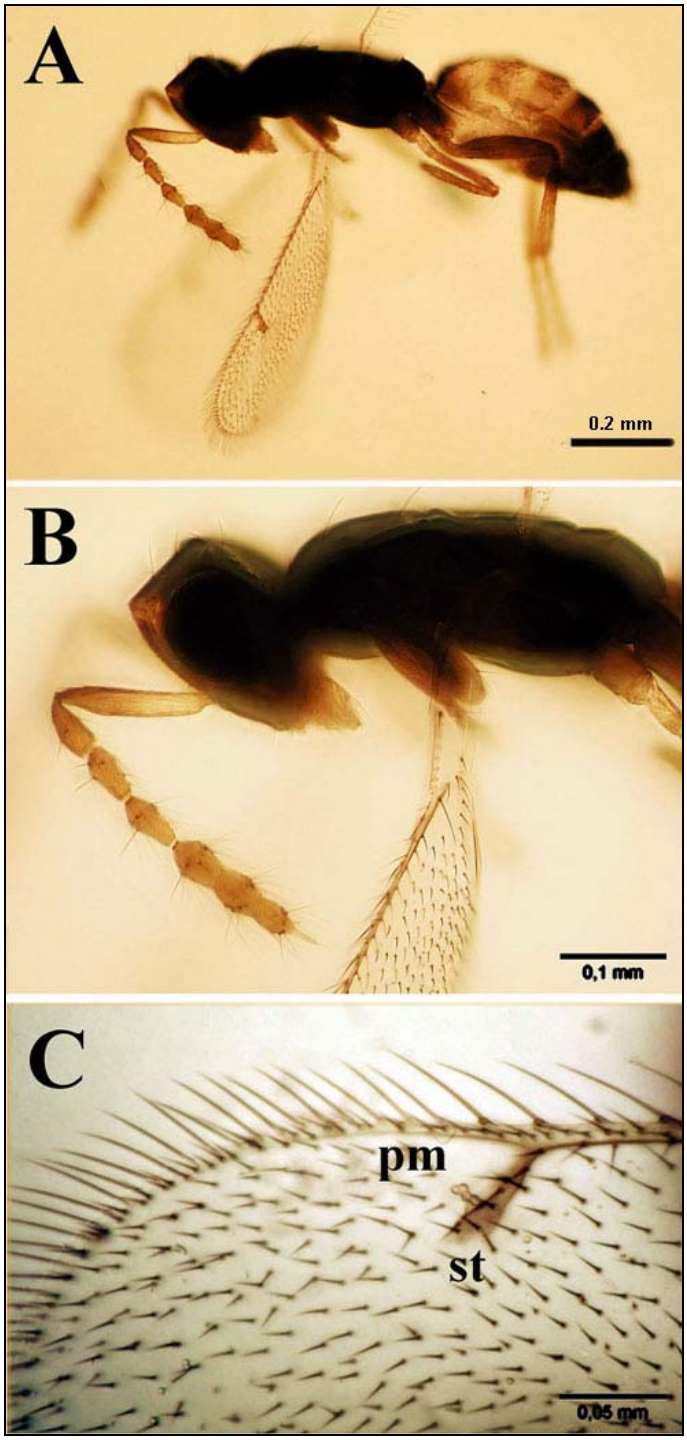

Figure 2. *Ceranisus amanosus* n.sp., Male, A. body; B. head with antenna and mesosoma; C. forewing's stigmal vein and postmarginal vein.

Mesosoma: Almost as long as metasoma; mesoscutum, scutellum, and axillae with light engraved sculpturing, without metallic tint; midlobe of mesoscutum with 2 pairs, scutellum with one pairs of setae. Forewing transparent, speculum closed, continuing along 1/3 basal part of marginal vein; disc uniformly covered with numerous short setae and with distinct semi-oval bare area at the posterior margin behind base of marginal vein, demarcated anteriorly by a sinuate line of setae; marginal setae at most 1.5 times longer than stigmal vein; the latter about 2.4 times as long as wide; forewing about as wide as l_2; l_1 equal to l_2 ; longest setae of marginal fringe about 3 times shorter than maximal forewing width; submarginal vein about as half as long as marginal vein plus parastigma, subcosta with 2 long setae and 3 shorter setae at underside, postmarginal vein twice as long as stigmal vein (Figure 1B), marginal vein plus parastigma 5 times as long as stigmal vein, the latter distincly petiolate. Hind wing about 5 times as long as wide, longest marginal cilia about as long as wing's maximal width. Coxae with light alutaceous sculpture consisted of elongate cells).

Metasoma: Petiole about twice as wide as long. Ovipositor occupying about 2/3 of gaster, its length and length of metatibia are in ratio 5:4. Pregenital sclerites of metasoma with four rows of setae on ventral surface of gaster. First row contains a pair of simple setae, second and third rows represented by rows of four simple setae and fourth row consting of two sockets of three digits bearing one setae each (Figure 1C, arrowed).

Measurements: Body length: 0.88-0.93 (Holotype:0.91 mm). Relative measure- ments, as length or length/width: Antenna: scape: 26/4; pedicel: 11/4; F1: 7.5/3.5; F2: 6.5/3.5; clava: 17+2/6, C1: 7, C2: 10, spicula: 2. Forewing: 59/25; longest seta of marginal fringe: 7. Hind wing: 45/9; longest marginal cilia: 8.5. Ovipositor: 22.

Male (Figure 2A, B, C): Similar to female except as follow: metasoma in apical 1.3 brownish black. Antenna (Figure 2B) with scape slender, about 5.3 times as long as wide; pedicel 2.1 times longer than wide; flagellum long, 1.58 times as long as width of head; funicular segments and club with distinct whorls of erect setae, F1 nearly as long as F2, about twice as long as broad; F2 slightly more than twice as long as broad; club including spicula 4.3 times as long as wide, C1 distinctly longer than C2 (3.7:3), C3 slightly shorter than C2 (2.6:3), spicula about 1/4 of C3.

Mesosoma, slightly shorter than metasoma, about 2.3 times as long as height. Forewing width of forewing slightly larger than l_2; l_1 nearly twice longer than l_2; submarginal vein slightly less than half of marginal vein plus parastigma; marginal vein plus parastigma about 5.5 times as long as stigmal vein, the latter distincly petiolate.

Metasoma, twice as long as broad.

Measurements (paratype): Body length, 0.84-0.90 mm. Relative measurements, as length or length/width: Antenna: scape: 9.5/1.8; pedicel: 4.0/1.9; F1: 3.5/1.8; F2: 3.9/1.8; club: 9.3+1/2.3, C1: 3.7, C2: 3.0, C3: 2.6, spicula: 1. Forewing: 101/53; longest seta of marginal fringe: 15.

Diagnosis: The females of this species are easily confusable with *C. menes* in coloration and relatively long marginal setae. However, it is easily distinguishable from the latter in, the possession of the marginal setae of fore wing at most 1.5 times longer than stigmal vein (more than twice longer than stigmal vein in *C. menes*), longer postmarginal vein (1.5 times as long as stigmal vein (Figure 2C), whereas about just as long as stigma in *C. menes*, (Figure 3A) and by the composition of the fourth row of setae on pregenital sclerites of metasoma (two pairs of three socketed digits bearing one setae each, (Figure 1C) whereas the fourth row is represented by two pairs of separate simple setae in *C. menes*, (Figure 3C). Also, the first two rows are different in these two species: first row is represented just by two and the second by four simple setae (Figure 1C), whereas these rows are subparallel and each contains four setae situated on individual digits of *C. menes*, (Figure 3C). Also, the malar sulcus is split in most specimens of the new species; where it is entire in *C. menes* (but this character is visible just under correct angle and light).

This species is similar to *Ceranisus udnamtak* Triapitsyn from Nepal in having forewing with very long postmarginal vein; with a distinct semi-oval bare area at the posterior margin behind base of marginal vein, demarcated anteriorly by a sinuate line of setae and a yellow metasoma, thus it would key together with *C. udnamtak* in the world key to females of *Ceranisus* by Triapitsyn (2005). It differs from *C. udnamtak* in having scapus 6.5-8.0 times as long as broad and club about 3.0 times as long as broad (in *C. udnamtak* scapus 5.25 times as long as broad and club twice as long as broad); the longest marginal seta about 0.3 times maximal width of forewing (in *C. udnamtak* the longest marginal seta 0.24 times maximal width of forewing). The longer clava is the best morphological character distinguishing the two species (according to the description of *C. udnamtak* Triapitsyn, 2005).

Etymology.-This species is named for its locality

Hosts.-Unknown.

***Ceranisus menes* (Walker, 1839)** (Figure 3)

Material examined (ICMKU).-Turkey: Adiyaman (Gölbaşı), 5.IX.2005, 5 ♀♀ (swept from lent field) (MD); 1 ♀ (on slide, ICMKU), labeled: "TURKEY, Adıyaman (Kahta), 567 m, 09.V.2005, 34 45 98 N, 38 39 21 E, swept from lent field (E. Çıkman), mounted in Canada balsam; Hatay (Antakya- Serinyol), 65 m, 17.III.2005, (MD), 25 ♀♀, (swept from leek field infested by *Thrips tabaci* Lindeman). 2 ♀♀, (on points, ICMKU); 23 ♀♀ (in alcohol, ICMKU); (Hassa-Saylak), 23. IV. 2008, 1 ♀, swept from pea field with *Sinapis*; (Reyhanlı-Atçana), 96 m, 03. v. 07, 36 14 30 N, 36 22 89 E, 1 ♀, (MD); Gaziantep **(**Islahiye), wheat field, 489 m, 15. v. 07, 37 01 65 N, 36 39 19 E, (MD); (Araban-Yukarımülk), lentil field, 921 m, 24. v. 07, 37 27 89 N, 37 26 91 E, 1 ♀, (MD); some ♀♀ (in alcohol, NASU); and also many uncounted specimens from various regions in BMNH and UCR.

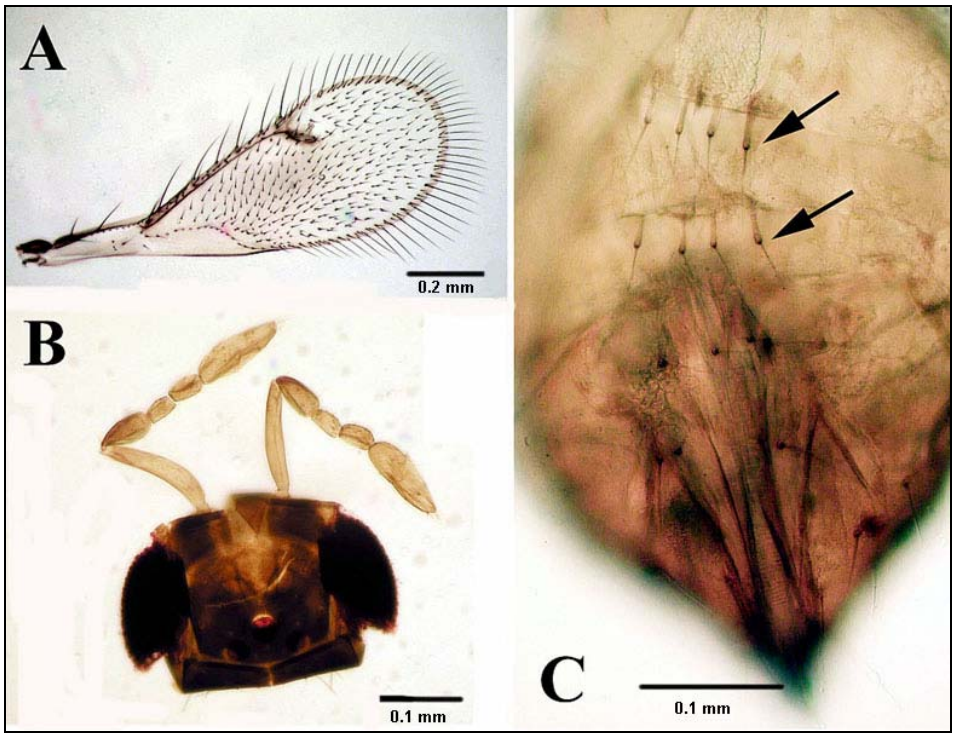

Figure 3. *Ceranisus menes* (Walker), Female, A. forewing; B. head with antennae; C. pregenital area.

Type locality.-Near London, England, UK.

See Triapitsyn & Headrick (2005) for the diagnosis and illustrations of this species, Triapitsyn (2005) for the list of its synonyms, distribution, etc., and

Loomans & van Lenteren (1995) for known hosts. This common cosmopolitan species was also recently recorded from Turkey (Kemer) by Triapitsyn (2005).

Some additional characters for the description of Triapitsyn & Headrick (2005) taken from the Turkish specimens are: Head (Figure 3B) with broad V-shaped occipital suture, and antennae having longer funicular segments; forewing (Figure 3A) about 3 x as long as wide; width of forewing 0.8-0.9 times l_2; l_1 distictly shorter than l_2; longest marginal cilia 0.55 times maximal forewing width; submarginal vein about 0.6 times marginal vein plus parastigma, subcosta with 2 long setae and 1 shorter setae at underside, postmarginal vein 0.65 x as long as stigmal vein, marginal vein plus parastigma 5.5-6.4 x as long as stigmal vein, the latter basally broad. Hind wing about 8.5 x as long as wide; blade uniformly setose, hyaline; longest marginal cilia about 1.5 x as long as wing's maximal width.

Metasoma (Figure 3C): Petiole about 1.2 x as wide as long. Ovipositor occupying about 4/5 x length of gaster, slightly excreted; ovipositor length/metatibia length ratio 4: 3. Pregenital sclerites with two distinct rows of four separate digits with one seta each, both rows situated above the base of ovipositor.

Measurements: Body length, 0.8-0.83 mm. Relative measurements, as length or length/width: Antenna: scape: 20/4; pedicel: 10/4; F1: 5.5/3; F2: 5.5/3; clava: 17+3/6, C1: 8, C2: 8, spicula: 4. Forewing: 56/18; longest marginal cilia: 10. Hind wing: 51/6; longest marginal cilia: 9. Ovipositor: 24.

Discussion

The new species is a distinct species which differs from closely related *C. menes* in posession of the number of characters (Table 1).

Table 1. The diagnostic characters of *Ceranisus menes* and *Ceranisus amanosus*

Characters	*Ceranisus menes*	*Ceranisus amanosus*
first row of setae on pregenital sclerites of metasoma	four setae situated on individual digits	two simple setae on individual digits
second row of setae on pregenital sclerites of metasoma	four setae situated on individual digits	four simple setae on individual digits
fourth row of setae on pregenital sclerites of metasoma	four setae situated on individual digits	two pairs of three socketed digits bearing one setae each
postmarginal vein	as long as stigma	more than twice stigmal vein
marginal setae	more than twice longer than stigmal vein	setae at most 1.5x longer than stigmal vein

Additionally, the both species occur in the same areas in Turkey, although *C. amanosus* is more common in highlands (850 m). The composition of the setal rows of pregenital sclerites (presence of digits and number of setae) proposed for the separation seems to be a good stable and thus reliable morphological marker and is expected to be used in taxonomy of *Ceranisus* and other groups. The obtained DNA sequences may be used as molecular "barcodes" in further studies and to clarify the specific affiliation of problematic specimens of the *menes* species group of *Ceranisus*.

The size of the amplified 28S D2 expansion region of the rDNA gene fragment ranged from 415 to 576 bp. The base composition of the sequence had a strong bias toward cytosine and guanine, which constituted approximately 62.3% of the total. The alignment of the sequenced fragment resulted in 464 characters, including gaps. Of these, 345 characters were constant, 59 characters were variable and parsimony-uninformative and 60 characters were parsimony-informative. The alignment was relatively straightforward and did not require insertion gaps. The base composition of the 28S D2 region was as follows: A, 0.156; C, 0.296; G, 0.332; T, 0.214. The slight G bias evident in the 28S sequences was noted in Chalcidoidea by Gillespie et al. (2005), and can be attributed to guanine's ability to base pair with both cytosine and uracil in RNA molecules (Gutell et al., 1994).

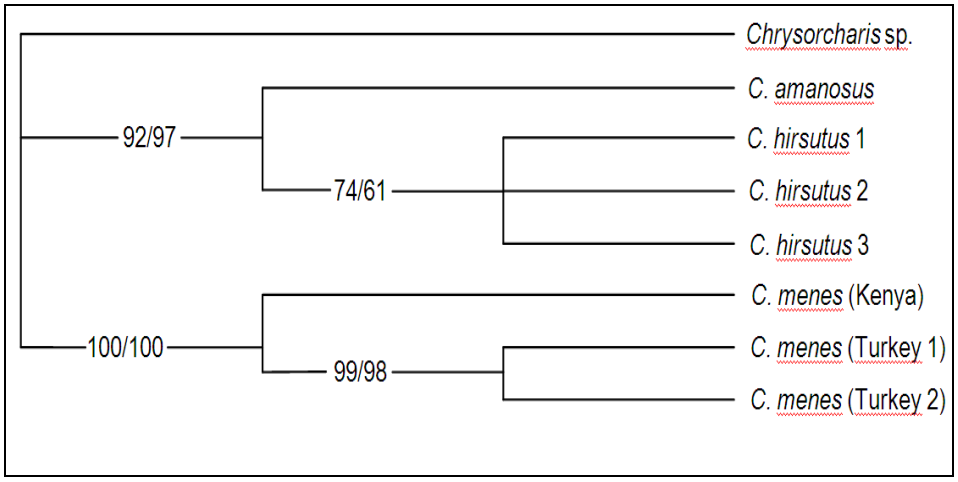

Figure 4. Phylogenetic MP and ML tree, inferred by 28S D2 rDNA sequences. Numbers represent bootstrap values from 1000 replicates on all parsimony-informative characters, only bootstrap values > 50% shown. Tree length 81, CI is0.9383, HI is 0.0617,RI is 0.9242, – Ln likelihood is 1079.28214.

Maximum parsimony analysis of the amplified 28S D2 expansion region of the rDNA gene fragment produced 136 equally parsimonious trees

[consistency index (CI) = 0.9383, retention index (RI) = 0.9242], and bootstrap (1000 replicates). The 50% majority-rule strict consensus tree is given in Figure 4.

There are some differences between the consensus tree generated under maximum parsimony and maximum likelihood, but in general, the topologies of these reconstructions are almost similar.

Two monophyletic group as *menes* group and *hirsutus-amanosus* group were clustered by maximum parsimony and maximum likelihood analysis. Molecular evidence supports the separation of *C. amanosus* from control group, *C. hirsutus* and *C. menes* and also the generic similarity of the Turkish *menes* species with the specimens previously identified as *C. menes* from Kenya (Gauthier et al., 2000).

Menes group clearly separated from other group with high bootstrap value (MP/ML;92/97) and 18 bp differences. There was no bootstap score among *hirsutus* species. *C. amanosus* was located different branch by the MP and ML analysis with 74/61 boot strap value and 8 bp differences.

The obtained DNA sequences may be used as molecular "barcodes" in further studies and to clarify the specific affiliation of problematic specimens of the *menes* species group of *Ceranisus*.

Özet

Türkiye'den *Ceranisus* cinsine (Hymenoptera: Eulophidae) ait *menes* tür grubundan yeni bir tür

Hatay (Türkiye) ilinden *Ceranisus* cinsine (Hymenoptera: Eulophidae) ait *menes* tür grubundan yeni bir tür, 2005-2009 yılları arasında yürütülen Türkiye'nin thrips parazitoitleri üzerinde yapılan çalışmanın bir parçası olarak tanımlanmıştır. Yeni tür, *Ceranisus amanosus* Doğanlar, Gumowsky, Doğanlar cins içinde kendisine çok benzeyen *Ceranisus menes* (Walker, 1839) ile mukayese edilmiştir. Pregenital skleritler üzerinde bulunan seta sıralarının kompozisyonları cins içindeki bu iki benzer türü birbirinden ayırmada ilk kez kullanılmıştır. 28S D2 (nuclear), Cytochrome Oxidase I (COI, mitochondrial) ve Cytochrome B (CytB, mitochondrial) genlerinin DNA sekansları yeni tür için onu belirleyen veriler olarak saptanmıştır. Maksimum likelihood ve parsimony inference metotlarla türlerin filogenetik ilgileri ortaya konmuştur.

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