JHR 76: 57–98 (2020)
doi: 10.3897/jhr.76.48004
http://jhr.pensoft.net

RESEARCH ARTICLE

JOURNAL OF Hymenoptera RESEARCH
A peer-reviewed open-access journal
The International Society of Hymenopterists

Two new species of *Ooencyrtus* (Hymenoptera, Encyrtidae), egg parasitoids of the bagrada bug *Bagrada hilaris* (Hemiptera, Pentatomidae), with taxonomic notes on *Ooencyrtus telenomicida*

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Academic editor: P. Jansta | Received 4 November 2019 | Accepted 28 February 2020 | Published 27 April 2020

http://zoobank.org/24B2A66D-D648-4854-B0B9-4C0489881233

Citation: Triapitsyn SV, Andreason SA, Power N, Ganjisaffar F, Fusu L, Dominguez C, Perring TM (2020) Two new species of *Ooencyrtus* (Hymenoptera, Encyrtidae), egg parasitoids of the bagrada bug *Bagrada hilaris* (Hemiptera, Pentatomidae), with taxonomic notes on *Ooencyrtus telenomicida*. Journal of Hymenoptera Research 76: 57–98. https://doi.org/10.3897/jhr.76.48004

Abstract

In support of a biological control program in California, USA, against the bagrada bug, *Bagrada hilaris* (Burmeister) (Hemiptera, Pentatomidae), an invasive pest of Asian origin, colonies of two species of *Ooencyrtus* Ashmead (Hymenoptera, Encyrtidae) are maintained using *B. hilaris* eggs as host. One of them, *Ooencyrtus mirus* Triapitsyn & Power, **sp. nov.**, is of Pakistani origin. It displays natural preference for bagrada bug eggs and is being evaluated in quarantine as a candidate for classical biological control. The other, *Ooencyrtus lucidus* Triapitsyn & Ganjisaffar, **sp. nov.**, appears to be native to California, and we believe it switched to *B. hilaris* from native pentatomid hosts. Both new species are described and illustrated, as is the Old World species *Ooencyrtus telenomicida* (Vassiliev), for which a neotype is designated. The presented morphometric evidence as well as mitochondrial and nuclear ribosomal DNA sequence data separate *Ooencyrtus mirus* from *O. telenomicida*. A lectotype is designated for *Ooencyrtus californicus* Girault from California, which is morphologically similar to *O. lucidus*.

Keywords

Chalcidoidea, natural enemy, invasive pest, California, Pakistan, biological control, genetic analysis, taxonomy

Introduction

The painted bug, also known as bagrada bug, *Bagrada hilaris* (Burmeister) (Hemiptera, Pentatomidae) (Fig. 1A), is native from western Asia to southern Africa (Howard 1907; Mahmood et al. 2015). It first was discovered in the United States in 2008 in Los Angeles County, California (Arakelian 2008). Since then, its range in the United States has expanded: it has been reported from other regions of California (Reed et al. 2013), Arizona (Palumbo and Natwick 2010; Palumbo et al. 2016), New Mexico (Bundy et al. 2012), Texas (Vitanza 2012), Nevada (Perring et al. 2013), Utah (Reed et al. 2013), and Hawaii (Matsunaga 2014). It also has been identified in Mexico (Sánchez-Peña 2014; Lomeli-Flores et al. 2019). *Bagrada hilaris* is a major pest of cole crops (Palumbo et al. 2016; Bundy et al. 2018), and there is no established biological control program for it. In 2014, in response to the rapid spread of *B. hilaris*, a search was conducted for egg parasitoids within its native range in Pakistan. Three species of parasitoid wasps, *Trissolcus hyalinipennis* Rajmohana & Narendran (Hymenoptera, Scelionidae, Telenominae) (Rajmohana 2006; Ganjisaffar et al. 2018), *Gryon gonikopalense* Sharma (Scelionidae, Scelioninae) (Sharma 1982; Martel et al. 2019), and *Ooencyrtus* sp. (Hymenoptera, Encyrtidae), were collected from *B. hilaris* eggs (Mahmood et al. 2015). Live specimens of the recovered parasitoids were shipped to the United States Department of Agriculture, Agricultural Research Service Quarantine Facility at the National Biological Control Laboratory in Stoneville, Mississippi (Mahmood et al. 2015) to be evaluated as classical biological control candidate agents for *B. hilaris*. In 2016, *Ooencyrtus* sp. was transported under permit to the University of California Riverside (UCR) Quarantine Facility, and we have been studying its biology, host specificity, and risk assessment. In concert with these biological studies, we addressed the taxonomic identification of this insect and describe it as a new species herein.

In addition to the classical biological control efforts with the exotic *Ooencyrtus* sp., surveys for resident egg parasitoids of *B. hilaris* have been conducted throughout California since 2017 using sentinel egg cards. In this project, we collected a variety of parasitoid species from the *B. hilaris* eggs. A species of *Ooencyrtus* Ashmead was recovered from sentinel cards in Riverside, California. Because the taxonomy of the Nearctic species of *Ooencyrtus* is in flux and there are no keys to the 11 described species, the first author physically compared specimens collected in our study to the types and specimens of all described species from North America. In addition, he tried to identify our specimens with keys from other regions (i.e. Neotropical, Oriental, and Palearctic). These efforts were unsuccessful, suggesting that our insect was an undescribed native species. Therefore, we also describe this parasitoid as a new taxon.

During our investigations, we attempted to compare our insects to *Ooencyrtus telenomicida* (Vassiliev) and to other egg parasitoids in the *O. telenomicida* species complex. However, we were unable to locate the type series of *Encyrtus telenomicida* Vassiliev (now *Ooencyrtus telenomicida*). Therefore, we collected *Ooencyrtus* sp. from eggs of *Eurygaster* Laporte, the host genus of *Encyrtus telenomicida*, in the same general habitat as indicated in the original description and matched the morphology of the new collections both with that description and with non-type specimens reared before

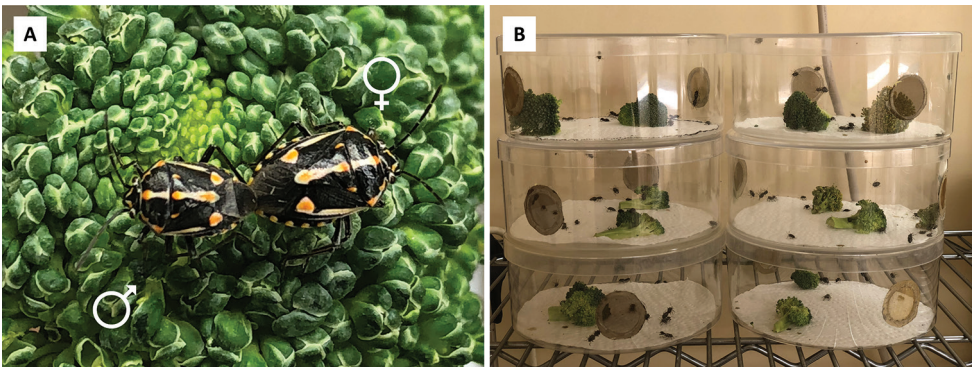

Figure 1. A *Bagrada hilaris* female and male **B** rearing cages for *Bagrada hilaris* mating pairs and egg production.

1950 from the original host in both Russia and Ukraine, not too far from its type locality. Based on the morphological congruence, we designated a neotype for *Encyrtus telenomicida* and supplement this designation with DNA sequence data necessary for the differentiation of *Ooencyrtus telenomicida* from morphologically similar species in the complex. Throughout the paper we are using the term '*O. telenomicida* species complex' for those species that are genetically and morphologically close to *O. telenomicida*. The group was defined morphologically by Hayat et al. (2014) and later expanded by Samra et al. (2018) using molecular and morphological data.

Materials and methods

Sources of specimens
Ooencyrtus species of Pakistan origin

Compared to other pentatomids, *B. hilaris* has an unusual ovipositional behavior of laying eggs singly or in small clusters on live plant material, in detritus and in soil (Taylor et al. 2014). Knowing this behavior, researchers working in the Toba Tek Singh District of the Punjab Plain in Pakistan noticed *B. hilaris* adults congregating on dry debris of *Brassica juncea* (L.) Czernajew and *Brassica napus* L. The plant debris was collected and shaken onto a plastic sheet, and the resulting leaves, stems and soil were transported to the laboratory and examined for *B. hilaris* eggs (Mahmood et al. 2015). Eggs were collected and placed in glass vials to wait for parasitoid emergence. Mahmood et al. (2015) recovered a uniparental strain of *Ooencyrtus* sp. from the *B. hilaris* eggs on *B. napus* but not from the eggs on *B. juncea*. The emerging parasitoids were reared and shipped to Dr. Walker Jones at the USDA-ARS Quarantine Facility, National Biological Control Laboratory in Stoneville, Mississippi, USA (Mahmood et al. 2015). From there a colony was sent to the UCR Quarantine Facility, where it has been reared continuously on *B. hilaris* eggs since January 2016. The colony is maintained at 26 °C, 14:10 L:D and ~50% RH.

Ooencyrtus species native to California

Sampling surveys for *B. hilaris* parasitoids were initiated in October 2017 and are still in progress. For the present study, *B. hilaris* adults were collected from a greenhouse colony where they were raised on seedlings of broccoli (*Brassica oleracea* L. var. *italica*), canola (*Brassica napus* L.), mustard greens (*Brassica juncea* L.), and sweet alyssum (*Lobularia maritima* (L.) Desvaux). Thirty adult mating pairs were placed in round plastic containers (15 cm diameter × 6 cm depth) (Durphy Packaging Co., Warminster, Pennsylvania, USA) with 2 screen openings on opposite sides for air circulation (Fig. 1B). Paper towels were placed in the bottom of the containers as an ovipositional substrate. The insects were provided organic broccoli florets and moved to new containers daily. Eggs were collected from the plastic containers and paper towels and glued to sentinel cards as described by Ganjisaffar et al. (2018). The cards were deployed in a squash field infested with shortpod mustard weeds, *Hirschfeldia incana* (L.) at the UCR Agricultural Operations on October 26, 2018. One of the cards with 15 glued *B. hilaris* eggs had 12 eggs parasitized, and from these 11 adult parasitoids emerged between November 13 and 15, 2018. These adults were placed in a vial, provided with honey, and given access for 24 hours to 50 *B. hilaris* eggs that had been glued (Elmer's) to a 1.5 × 4 cm white card. Following the 24 hour access period, the card with parasitized eggs was transferred to a new vial for rearing the parasitoids. The original egg parasitoids then were collected and placed in vials containing 95% ethanol and stored in a freezer at -20 °C until they were used for morphological studies or DNA extraction. Primary molecular voucher specimens were slide-mounted in Canada balsam.

Ooencyrtus telenomicida (Vassiliev) from Europe

Specimens of *O. telenomicida*, reared in Russia and Ukraine from eggs of *Eurygaster integriceps* Puton (Hemiptera, Scutelleridae) in 1948 and 1950, were borrowed from the Zoological Institute, Russian Academy of Sciences, Saint Petersburg, Russia. Unfortunately, PCRs failed on all the specimens that were extracted. Therefore, new specimens of *O. telenomicida* were reared in Ipatele, Iaşi County, Romania, from eggs of *Eurygaster* sp. found on wheat. We were able to extract DNA from these specimens, and the primary molecular vouchers were individually slide-mounted in Canada balsam or chemically dried and point mounted.

Taxonomic studies

For the taxonomic descriptions of the new species, the morphological terms of Gibson (1997) were used, with a few modifications. All wing measurements of length or length:width are given in micrometres (μm). Abbreviations used in the descriptions are: F = funicle segment of the female antenna or flagellomere of the male antenna; mps = multiporous plate sensillum or sensilla on the antennal flagellar segments (= longitudinal sensillum or sensilla, or sensory ridge(s)).

Specimens for morphometric studies were dried from ethanol using a critical point drier, then point-mounted and labeled. Selected specimens then were dissected and slide-mounted in Canada balsam. Slide mounts were examined under a Zeiss Axioskop 2 plus compound microscope (Carl Zeiss Microscopy, LLC, Thornwood, New York, USA) and photographed using the Auto-Montage system (Syncroscopy, Princeton, New Jersey, USA). Photographs were retouched where necessary using Adobe Photoshop (Adobe Systems, Inc., San Jose, California, USA). In addition, the body length of 24 male and 24 female *O. mirus* wasps were measured from the anterior end of the head to the posterior end of the gaster, not including the ovipositor or aedeagus, with a Leica Wild M10 stereoscope using a Bausch & Lomb 0.1 mm and 0.01 mm micrometer.

For the morphometric analysis, we measured characters in adult females and males to determine the following ratios: 1) ovipositor length to mesotibia length; 2) fore wing length to maximum width; 3) scape length to width (excluding the radicle); 4) clava length to width; 5) F1 length to pedicel length; and 6) F2 length to F1 length. For all parameters, the ranges, means, and standard deviations were determined.

For *O. mirus* and *O. telenomicida* we also used multivariate ratio analysis (MRA) (Baur and Leuenberger 2011) because these two species differ mostly in color and very little in body ratios. For this analysis, 15 measurements per specimen were used and 9 female specimens for each species were included in the analysis. Following the approach of Baur et al. (2014) the MRA also served to place the neotype of *O. telenomicida* in the morphospace of specimens from Russia and Ukraine. The complete set of measurements is available as Suppl. material 1 data from the publisher's website.

Specimens examined are deposited in the collections with the following acronyms:

AICF Alexandru Ioan Cuza University, Iaşi, Romania (Lucian Fusu collection);
BMNH The Natural History Museum, London, UK;
EMEC Essig Museum of Entomology, University of California, Berkeley, California, USA;
UCRC Entomology Research Museum, Department of Entomology, University of California, Riverside, California, USA;
USNM National Museum of Natural History, Washington, District of Columbia, USA;
ZIN Zoological Institute, Russian Academy of Sciences, Saint Petersburg, Russia.

*DNA extra***ction***, ampli***fication, and sequencing**

Paratype specimens of O. lucidus and O. mirus were selected for genetic analysis. Genomic DNA from three females *(UCRC ENT 311756, 311757, and 311769) and one male (UCRC ENT 311770) O. lucidus* and three female *O. mirus* (UCRC_ENT 00506189–00506191) was extracted using the non-destructive HotShot method (Truett et al. 2000) as described in Andreason et al. (2019a, 2019b) to preserve specimen integrity for subsequent slide mounting and morphological study. DNA from the neotype female specimen of *O. telenomicida* (UCRC ENT 311776) and a non-type female from the same collection event (UCRC ENT 311775) was extracted using

the non-destructive method described in Triapitsyn et al. (2019). Three more females and one male from the same collection event (AICF vouchers OoIs0101, OoIs0102, OoIs0201, OoIs0202) were extracted with another non-destructive method as detailed in Cruaud et al. (2019); several other specimens are kept at AICF in 96% ethanol at -20 °C to preserve DNA integrity for future investigations. DNA extracts were stored at -20 °C until PCR amplification was performed.

Paired with morphological descriptions, confirmation of novel species was based on analysis of a fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene and the nuclear internal transcribed spacer 2 (ITS2) region. PCRs were performed using the reagents at concentrations described in Andreason et al. (2019b) with the primers of Samra et al. (2018), synthesized by Integrated DNA Technologies, Inc. (Coralville, IA, USA), with modified thermal cycling conditions. For COI amplification, thermal cycling was performed at 95 °C for 3 m, 40 cycles of 95 °C for 30 s, 30 °C for 1 m, and 72 °C for 1 m 15 s, with a final extension at 72 °C for 5 m. For ITS2 reactions, thermal cycling was performed similarly but at a 54 °C annealing temperature. Confirmation of amplification by gel electrophoresis, product cleaning, and sequencing were all performed according to Andreason et al. (2019b).

For *O. telenomicida* sequencing of the ITS2 with the primers in Samra et al. (2018) failed, thus we used the primers described in Yara (2006) to obtain a sequence from specimen OoIs0102. For the same species we obtained standard DNA barcodes for specimens OoIs0101, OoIs0102 and OoIs0201 with the primers LCO1490 and HCO2198 (Folmer et al. 1994). Molecular protocols described in Fusu and Ribes (2017) were used, except for the annealing temperature for ITS2 that was set at 45 °C. All sequences were deposited in GenBank (accession numbers MN933499–MN933500; MN935769–MN935775; MN947512–MN947518; MN945949–MN945951 [CO1]; MN946502 [ITS2]).

*Genetic a***na***lysis*

*In*terspecific variation among O. lucidus, O. mirus, *O. telenomicida*, and other populations and species of *Ooencyrtus* was estimated by calculating uncorrected pairwise distances (p-distances) of the COI fragment and ITS2 region and by phylogenetic analysis. MEGA X (Kumar et al. 2018) was used to trim and analyze sequence files obtained in this study, to perform multiple sequence alignments using ClustalW (Thompson et al. 2003), and to construct a phylogenetic tree. For comparisons of the COI and ITS2 regions, *Ooencyrtus* sequences deposited in GenBank by Samra et al. (2018) were aligned with *O. lucidus*, *O. mirus*, and *O. telenomicida* from Romania, and p-distances were calculated using the p-distance model with pairwise deletion of gaps. When comparing COI fragments, all available sequences were analyzed to account for intraspecific differences within species; for ITS2, one sequence was selected from GenBank because the ITS2 region generally has very little, if any, intraspecific variation in Hymenopterans (Campbell et al. 1994; Stouthamer et al. 1999). A phylogeny of the

studied species based on concatenated and unpartitioned COI and ITS2 sequences was inferred using the Maximum Likelihood method based on the Tamura-Nei model with 1000 bootstrap replications (Tamura and Nei 1993). The tree was drawn to scale with the number of substitutions per site estimating the branch lengths. *Ooencyrtus kuvanae* (Howard) was included as an outgroup because it is not part of the *O. telenomicida* complex and for which reliable sequences are available (Samra et al. 2018).

Results

Taxonomy

***Ooencyrtus lucidus* Triapitsyn & Ganjisaffar, sp. nov.**
http://zoobank.org/98066B3C-9BBB-4BAE-AC92-13A559D58817
Figs 2–4

Ooencyrtus californicus Girault: Noyes 2010: *402 (mis*identification of specimens from Texas).

Type material. Holotype female, deposited in UCRC, on slide (Fig. 2B) labeled: 1. "USA: California, Riverside Co. Riverside, T. M. Perring laboratory at UCR, F3 on bagrada bug eggs From colony, ii.2019, F. Ganjisaffar Originally from: UCR Ag. *Ops. 33.966002*N, 117.343198W Cards with fresh sentinel eggs of Bagrada hilaris (Burmeister) placed in squash field 26–29.x.2018 Parasitoids emerged 13–15.xi.2018, F. Ganji*saffar"; 2. "V. V. Berezovskiy 2019 in Canada balsam"; 3. [red] "Ooencyrtus lucidus* Triapitsyn & Ganjisaffar Holotype ♀"; 4. "Det. by S. V. Triapitsyn 2019"; 5. [barcode database label/unique identifier] "UCRC [bold] UCRC ENT 311771". The holotype (Figs 2C, 3) is in good condition, complete, dissected under 4 coverslips.

Paratypes. USA, California, Riverside County, Riverside, University of California at Riverside (UCR): Agricultural Operations, 33.966002N, 117.343198W, 304 m, cards with fresh sentinel eggs of *Bagrada hilaris* placed in squash field 26–29.x.2018, parasitoids emerged 13–15.xi.2018, F. Ganjisaffar [4 females on points, 4 females on slides (including 3 molecular vouchers of S. A. Andreason, UCRC ENT 311756, 311757, and 311769) and 1 male on slide (molecular voucher UCRC ENT 311770), UCRC]; T. M. Perring laboratory, from colony, third generation (F3) on bagrada bug eggs, ii.2019, F. Ganjisaffar, originated from the above collection [7 females (1 in BMNH, 1 in EMEC, 3 in UCRC, 1 in USNM, 1 in ZIN), 14 males (2 in BMNH, 2 in EMEC, 6 in UCRC, 2 in USNM, 2 in ZIN) on points and 5 females, 2 males on slides, UCRC].

Other (non-type) material examined. USA: California, Merced County, Merced, 24.viii.1938, R. Rose, "Ex eggs of *Acrosternum hilaris*" [1 female, 1 male, USNM] (misidentified as *O. californicus* Girault by A. B. Gahan). Texas, Presidio County, Presidio, 14.viii.1941, L. W. Noble (from eggs of *Chlorochroa sayi* Stål) (misidentified as *O. californicus* by A. B. Gahan) [2 females, 1 male, UCRC; 6 females, 4 males, USNM].

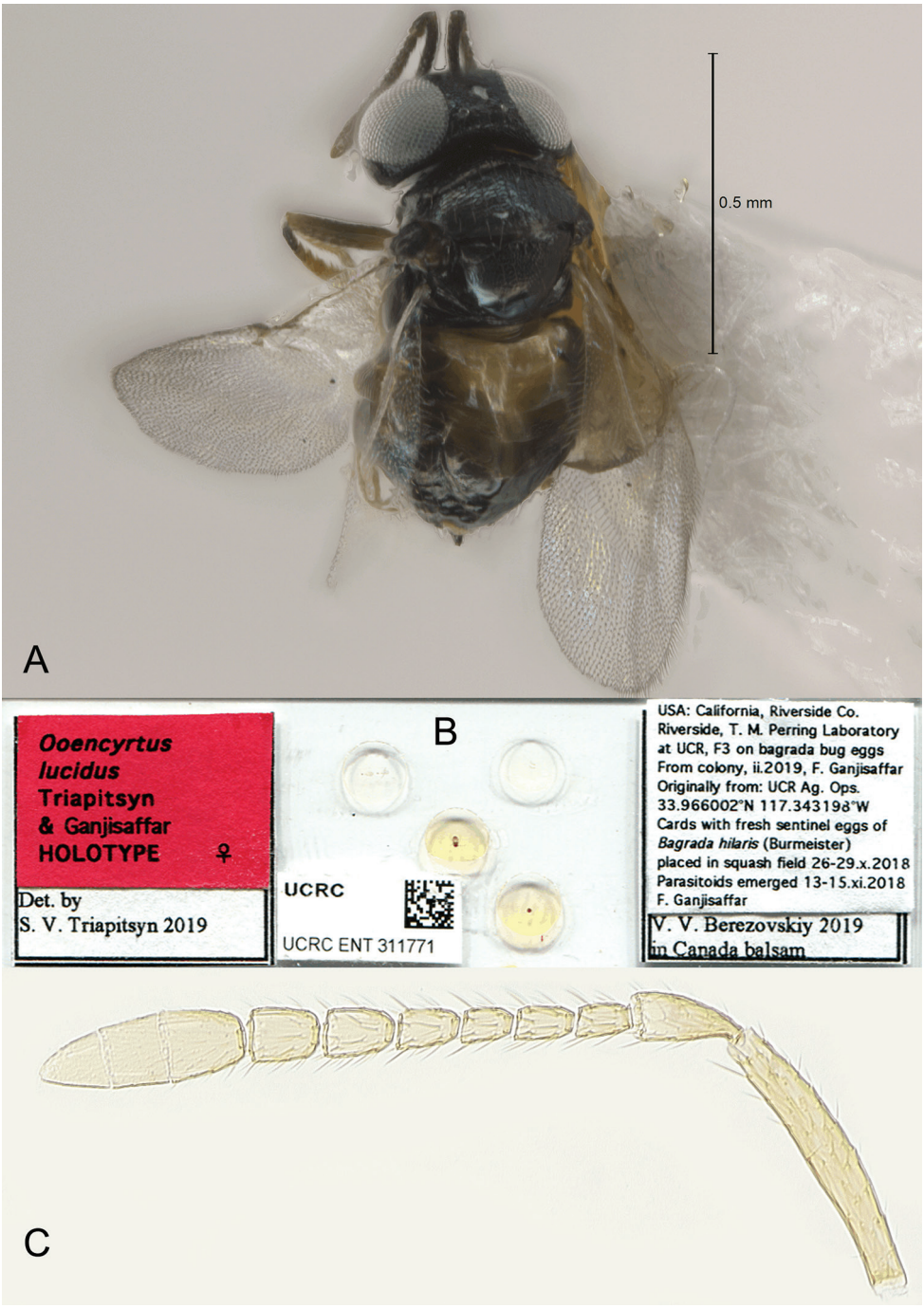

Figure 2. *Ooencyrtus lucidus* sp. nov. female **A** habitus in dorsal view (paratype) **B** holotype slide **C** antenna (holotype).

Diagnosis. There are no comprehensive keys for *Ooencyrtus* in North America and only 3 described species have been identified from California (Zuparko 2015, 2018). Therefore, to confirm that *O. lucidus* was not already collected in North America, the first author visited USNM in February 2019 and compared *O. lucidus* specimens with all the available types of *Ooencyrtus* species; no match was found. Morphologically, *O. lucidus* is most similar to the Nearctic species *O. californicus*, to which its female specimens key in both Noyes (2010) (to the Neotropical species) and Zuparko (2018) (to species in California). However, females of *O. lucidus* differ from *O. californicus* in having the scape at most 7.5× as long as wide (average of 6.6× as long as wide, Table 1) and the F1 is about 1.5× as long as wide (Fig. 2C). For *O. californicus* the scape (Fig. 5C) is about 8.8× as long as wide (as measured from the slide-mounted syntypes, with no significant difference between the four scapes measured; however, these measurements could very well be inaccurate because of the way the specimens were crushed, and the antennae were slide-mounted), and the F1 is a little more than 2.0× as long as wide. In addition, the "base of abdomen encircled by a narrow golden band" described by Girault (1917: 22) for *O. californicus* is not present in *O. lucidus*. Unfortunately, the metasoma of both extant types of *O. californicus* is missing (see below under comments). Instead the base of the gaster has a distinct yellow spot medially. Furthermore, although it is a minor difference, F1 of the female antenna is about 0.5× the length of the pedicel on average in *O. lucidus* (Table 1) whereas in the type specimens of *O. californicus* it is about 0.6× the length of the pedicel. Thus, we are unable to positively attribute our specimens of *O. lucidus* to *O. californicus* based on the available, very limited comparable morphological data.

In Noyes (1985), *O. lucidus* keys to the New World species *O. johnsoni* (Howard), whose entire gaster is shining black, perhaps with a slight greenish tinge. The entire type series of the latter taxon, 2 females and 1 male syntypes, were examined by the first author at USNM; the females are on points, with some parts of them mounted on a slide, and the male is on a slide. It also does not fit any of the described Old World species keyed in the publications mentioned below in the diagnosis of *O. mirus*, and is presumed to be native to the USA.

Description. Female (holotype and paratypes). ***Body length*** of dry-mounted, critical point-dried paratypes 825–1025 μm, and of slide-mounted paratypes 1045–1125 μm.

Color. Body (Fig. 2A) mostly shining black with some metallic reflections, particularly on mesosoma, except base of gaster always with a distinct yellow, dorsal spot medially (on gastral tergites 1–3) and often with either yellow or light brown areas laterally and ventrally (always separated from medial yellow spot by a brown area); antenna brown; legs mostly yellow to light brown except coxae brown to dark brown basally and protibia and tarsi brownish.

Sculpture. Head with faint, inconspicuous sculpturing; mesoscutum reticulate, with sculpture cells mostly wider than long; axilla and anterior 1/3 or so of scutellum with a rather weak cell-like sculpture, remainder of body smooth.

Table 1. Morphometric ratios and measurements (μm) of *Ooencyrtus lucidus* female morphological characters. All measurements are from slide-mounted specimens.

	Length ovipositor: length mesotibia	Length: width fore wing	Length: width hind wing	Length: width scape	Length: width clava	Length F1: length pedicel	Length F2: length F1
Range	1.0–1.2	2.2–2.5	4.7–5.3	5.7–7.5	2.9–3.6	0.45–0.55	0.9–1.1
Mean	1.1	2.4	4.9	6.6	3.2	0.5	1.0
n	10	9	8	10	10	10	10
	Length F1	Length F2	Length F3	Length F4	Length F5	Length F6	
Range	29–35	27–38	24–36	34.85–39.39	41–45	38–42	
Mean	33	33	32	37	43	41	
n	10	10	10	10	10	10	

Pubescence. Frontovertex, pronotum, mesoscutum, axilla, and scutellum with short, dark setae except scutellum with a few pairs of long, dark setae in posterior half.

Head (Fig. 3A) about 1.2× as wide as high. Minimum width of frontovertex about 0.3× head width. Toruli just below level of lower eye margin. Ocelli in an obtuse triangle. Maxillary palpus 4-segmented, labial palpus 3-segmented. Mandible with 2 teeth and a broad truncation.

Antenna (Fig. 2C) with radicle about 3.2× as long as wide, rest of scape slender, slightly wider in the middle, 5.7–7.5× (5.9× in the holotype) as long as wide; pedicel about 2.2× as long as wide, notably longer than any funicular segment (F1 0.45–0.55× length of pedicel, Table 1); funicle segments all longer than wide, F1–F3 usually subequal in length (F2 0.9–1.1× length of F1, Table 1) although often F3 the shortest, F5 the longest funicular segment (Table 1), F1–F3 without mps, F4 with 1 mps, F5–F6 each with 2 mps; clava 3-segmented, 2.9–3.6× (2.9× in the holotype) as long as wide and about as long as combined length of F4–F6, each claval segment with several mps.

Mesosoma (Fig. 3B, C). Mesoscutum about 2.5× as wide as long; scutellum a little shorter than wide and slightly longer than mesoscutum, placoid sensilla close to each other and about in the middle of scutellum.

Wings (Fig. 3D) not abbreviated, fore wing extending beyond apex of gaster. Fore wing 2.2–2.5× as long as wide (2.3× in the holotype), disc hyaline; costal cell about 12× as long as wide; marginal vein punctiform; inconspicuous postmarginal vein much shorter than stigmal vein; linea calva closed posteriorly by 2 rows of short, inconspicuous setae; filum spinosum usually with 3 setae, rarely with 4 or 5 setae; longest marginal seta about 0.09× maximum wing width. Hind wing 4.7–5.3× as long as wide (4.9× in the holotype), disc hyaline.

Legs. Mesotibial spur about as long as mesobasitarsus.

Gaster (Fig. 3C) longer than mesosoma. Ovipositor occupying 0.6–0.7 length of gaster, a little exserted beyond its apex, and 1.0–1.2× (about 1.1× in the holotype) as long as mesotibia.

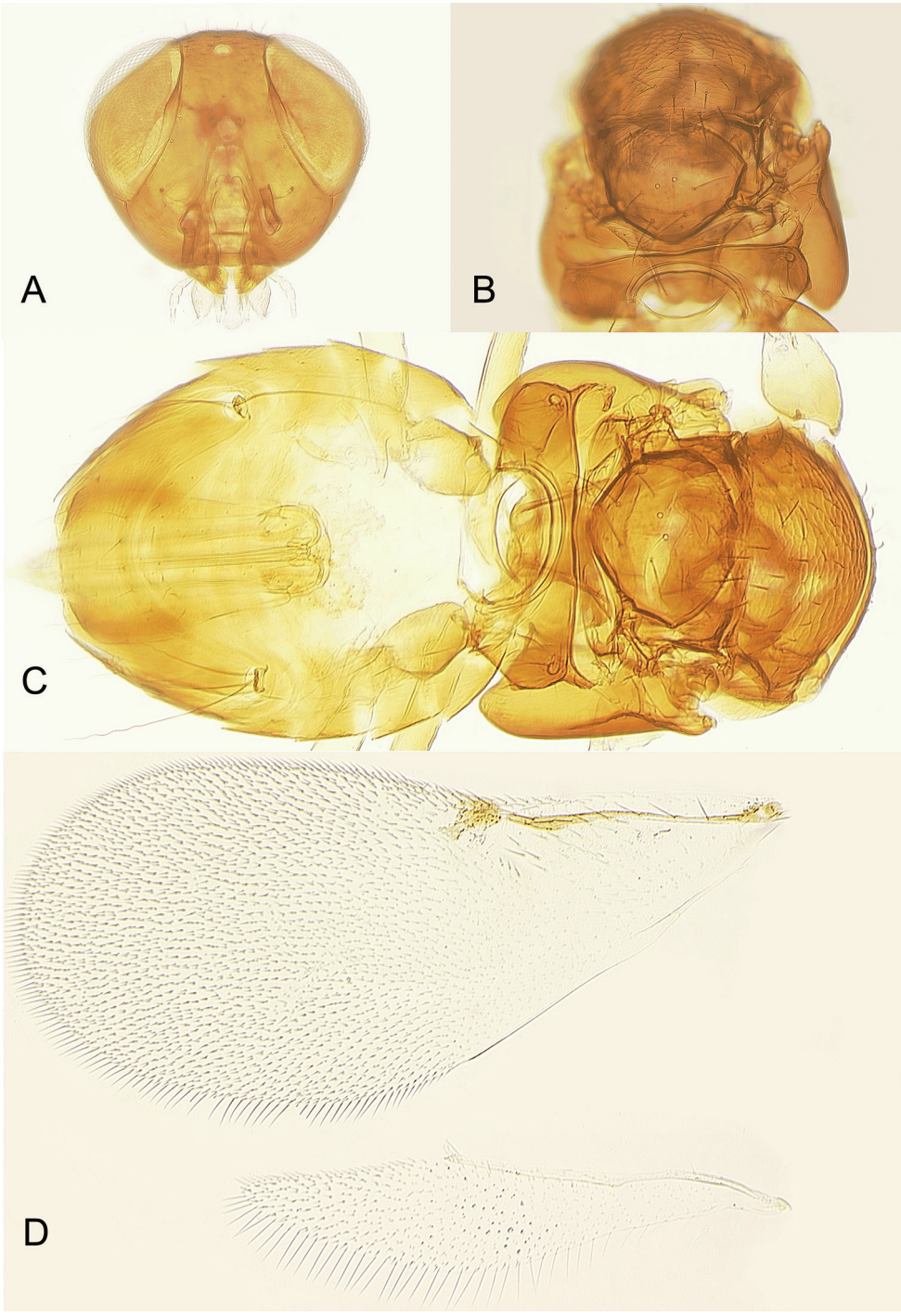

Figure 3. *Ooencyrtus lucidus* sp. nov. female (holotype) **A** head in frontal view **B** mesosoma **C** mesosoma and metasoma **D** fore and hind wings.

Measurements (μm) of the holotype. Mesosoma 394; gaster 480; ovipositor 379; mesotibia 358. Antenna: radicle 48; rest of scape 179; pedicel 70; F1 35; F2 38; F3 30; F4 38; F5 45; F6 42; clava 129. Fore wing 852:369; longest marginal seta 33. Hind wing 603:123; longest marginal seta 48.

Male (paratypes). ***Body length*** of dry-mounted, critical point-dried paratypes 595–795 μm, and of slide-mounted paratype 940 μm. ***Head*** and ***mesosoma*** shining black with metallic reflections (Fig. 4B), ***gaster*** dark brown; legs mostly yellow or light brown except coxae brown to dark brown and tarsi brownish. ***Head*** with toruli slightly above lower eye margin. ***Antenna*** (Fig. 4C) with scape minus short radicle 3.7–4.0× as long as wide (Table 2); funicle segments all longer than wide and more or less subequal in length (proximal segments a little shorter), F1–F3 apparently without mps, F4–F6 with at least 2 mps each; clava entire, 3.1–3.2× as long as wide, with several mps; flagellar segments all with numerous long setae. Fore ***wing*** (Fig. 4D) 2.25–3.1× as long as wide, with linea calva open posteriorly; hind wing 4.1–4.2× as long as wide. ***Genitalia*** (Fig. 4A) length 171–182 μm.

Etymology. *Bagrada hilaris* populations have declined in California. We believe that parasitoids like *O. lucidus* are responsible for this decline. "Lucidus" is an adjective derived from Latin, meaning "lucid, clear." It is chosen for this species name referring to the elucidation of why populations of *B. hilaris* have declined in California.

Distribution. Nearctic region: USA (California and Texas).

Hosts. Pentatomidae: *Bagrada hilaris* (Burmeister), *Chinavia hilaris* (Say), and *Chlorochroa sayi* Stål. In California, *O. lucidus* apparently switched from its native host(s), such as the green stink bug *Chinavia hilaris*, to parasitize eggs of the invasive bagrada bug.

Comments. The following specimens of *O. californicus* have been examined. Lectotype female [USNM], here designated to avoid the existing ambiguity regarding the status of the type specimens of this species, on slide (Fig. 5A) labeled: 1. [red] "Type no. 20859 U.S.N.M."; 2. "*Ooencyrtus californicus* Girault. ♀ type.". Of the two crushed type female specimens (Fig. 5B) on this slide (because 4 scapes are present), only parts of 4 antennae and a slightly damaged fore wing (Fig. 5D) remain; the lectotype is constituted by the remains of one of them, circled in India ink, with the most intact antenna (Fig. 5C); remains of the other specimen are those of the paralectotype, and the single fore wing (Fig. 5D) can belong to either of them. The species was poorly described (Girault 1917: 22 [as *Oenocyrtus californicus*, sic]) from the unspecified number of "Types" under this catalog number in USNM; the type series was reared in Sacramento, California, USA from bug eggs on *Pinus sabiniana* (Douglas) D. Don (Pinaceae). The whereabouts of the other specimens of the type

Table 2. Morphometric ratios and measurements (μm) of *Ooencyrtus lucidus* male morphological characters. All measurements are from slide-mounted specimens.

	Length genitalia	Length: width fore wing	Length: width scape
Range	171–182	2.25–2.3	3.7–4.0
Mean	176	2.3	3.9
n	3	3	2

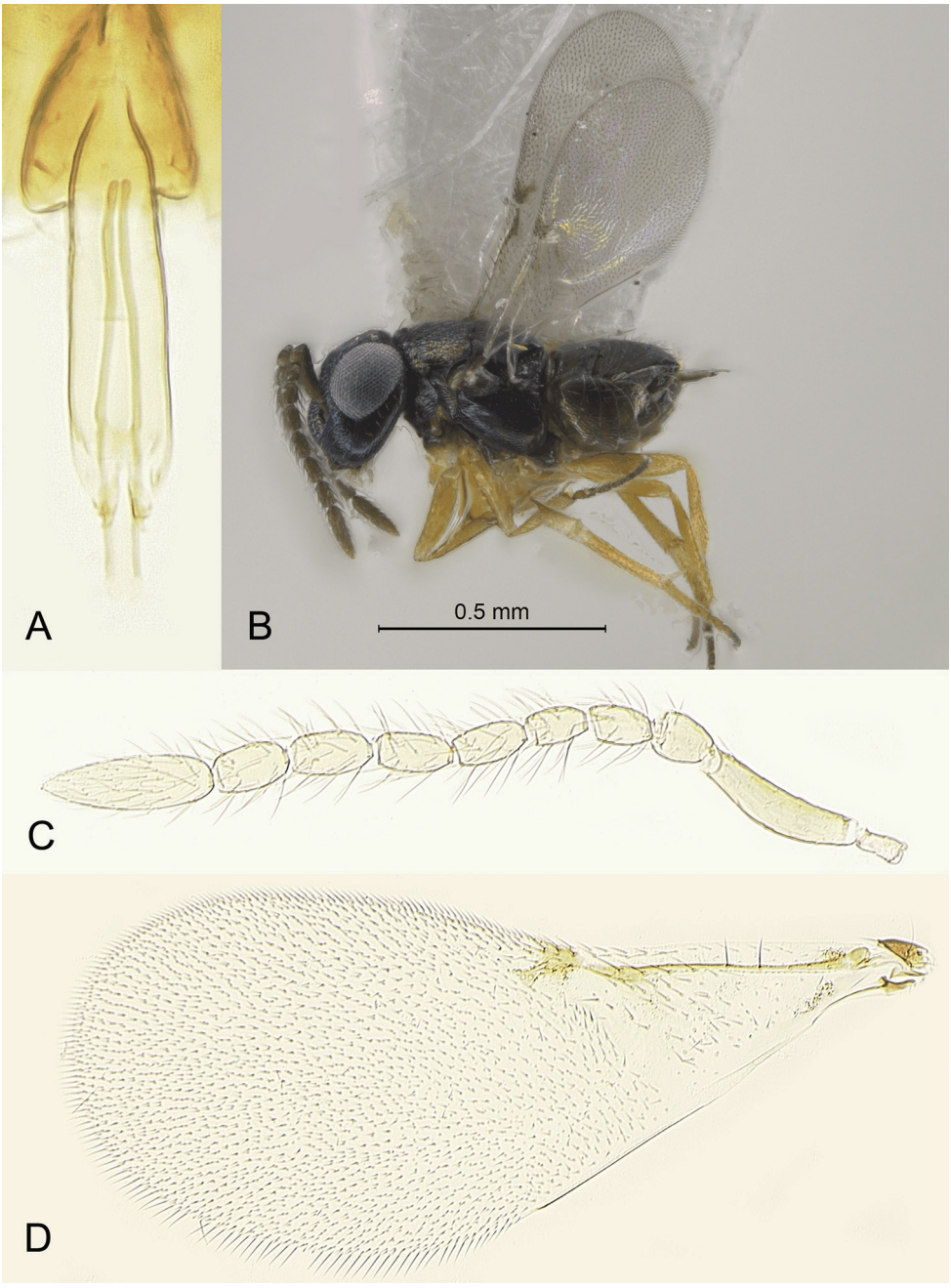

Figure 4. *Ooencyrtus lucidus* sp. nov. male (paratypes) **A** genitalia **B** habitus in lateral view **C** antenna **D** fore wing.

s*eries, if* they ever ex*isted, are unkno*wn; however, it is quite likely that these two females were the only original "types". Thus, all other identifications of this species co*uld be regarded* to be tentative at best: for instance, specimens belonging to

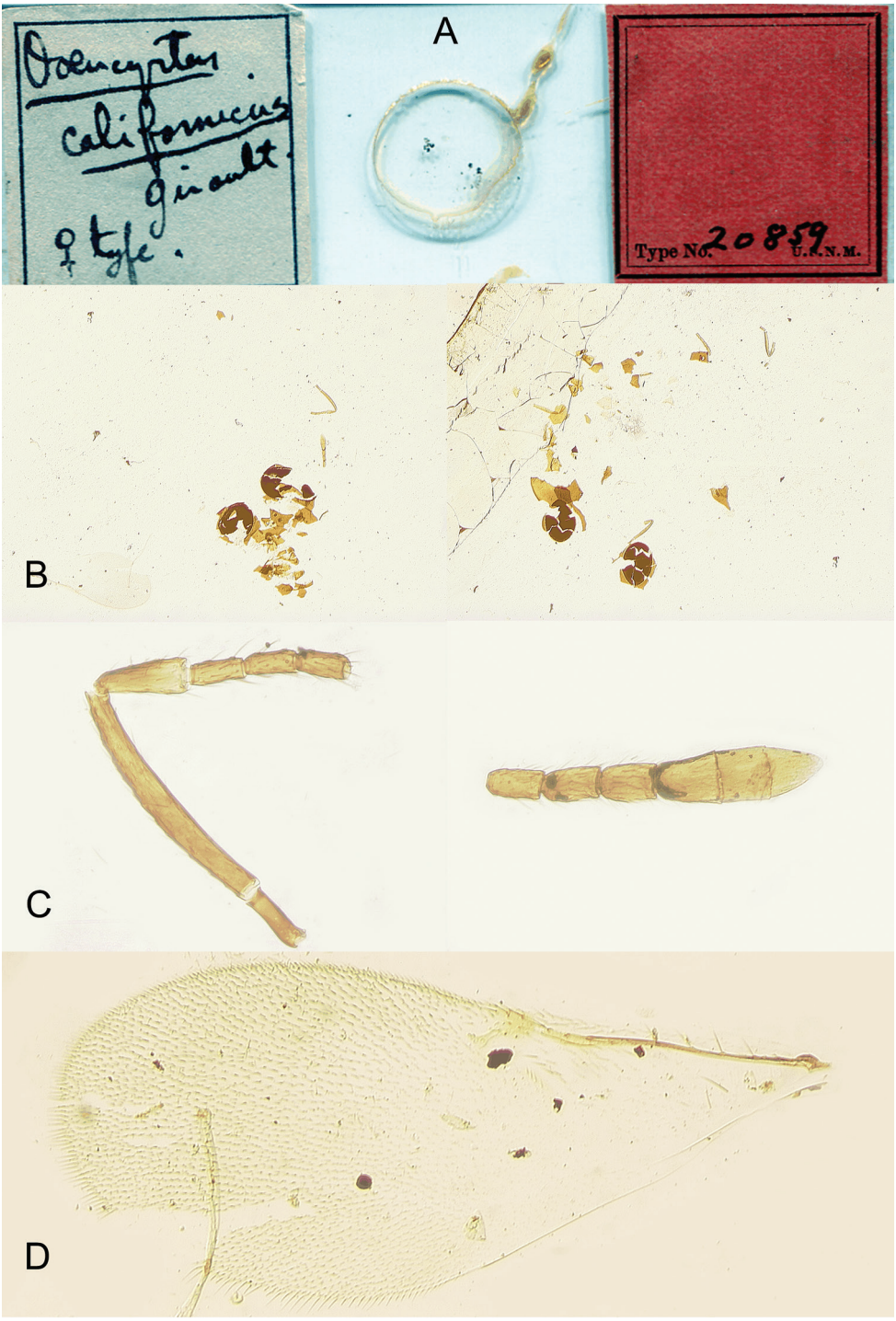

Figure 5. *Ooencyrtus californicus* female **A** lectotype/paralectotype slide **B** lectotype and paralectotype **C** antenna (lectotype) **D** fore wing (lectotype or paralectotype).

perhaps three different species of Ooencyrtus stand under O. californicus in UCRC. Zuparko (2015: 44) commented on the difficulties *of identifying this species and the poor condition of the "holotype" female of O. californicus in USNM, noting that a species similar to it was collected in several counties in California including Riverside County. To be fully recognizable (since the original syntypes are incomplete), O. californicus* will need to be re-described and thoroughly illustrated based on fresh specimens collected in the Sacramento area of California on the original host plant. DNA sequences will need to be compared with those of *O. lucidus* and other species of *Ooencyrtus*. Until that happens (keeping in mind that the true *O. californicus* may never be re-collected and thus would be impossible to be properly recognized), this species is treated as a *nomen dubium*, and making positive identifications of any specimens as *O. californicus* is not currently feasible. Therefore, we chose to describe *O. lucidus*, for which many good quality specimens and DNA sequences are available, as a new species that can be easily and positively recognized using a combination of morphological features and genetic data. The other option, i.e. trying to match our specimens with the incomplete original syntypes of *O. californicus* for which desired DNA sequences are not available, is impossible given the latter nominal species cannot be positively identified.

Noyes (2010) reported 2 females of *O. californicus* (determined as such by A. B. Gahan) from Presidio, Texas, USA, reared from eggs of *Chlorochroa sayi*, but closer examination of the specimens from the same series revealed that they are conspecific with *O. lucidus*.

Also present in UCRC is a series of 9 females misidentified (probably by H. Compere) as *O. californicus*, reared 1.ix.1937 in Riverside, Riverside County, California, USA by J. D. Maple from eggs of *Anasa tristis* (De Geer) (Hemiptera: Coreidae) and reported as *O. californicus* by Maple (1947: 105); these are neither *O. californicus* nor *O. lucidus* because their entire gaster is dark, without any yellow spot or band, and in this regard are more similar to *O. johnsoni*.

***Ooencyrtus mirus* Triapitsyn & Power, sp. nov.**
http://zoobank.org/C22A1533-33B2-43F5-84E4-CAEC9A986247
Figs 6–9

Type material. Holotype female, deposited in UCRC, on slide (Fig. 7A) labeled: 1. "USA: California, Riverside Co. Riverside, UCR Q*uarantine Lab. 27.ii.2019, N. Power, from colony on bagrada bug, Bagrada hilaris* (Burmeister). Of Pakistan origin via USDA-ARS Lab., Stoneville, Mississippi, USA. Received 3.xii.2015, S&R # N-15–30 *Ooencyrtus* sp., females, ca. F44"; 2. "Mounted by V. V. Berezovskiy 2018 in Canada balsam"; 3. [red] "*Ooencyrtus mirus* Triapitsyn & Power Holotype ♀"; 4. "Det. by S. V. Triapitsyn 2018"; 5. [barcode database label/unique identifier] "UCRC [bold] UCRC ENT 311772". The holotype (Figs 7B, D, E, 8A) is in good condition, complete, dissected under 4 coverslips.

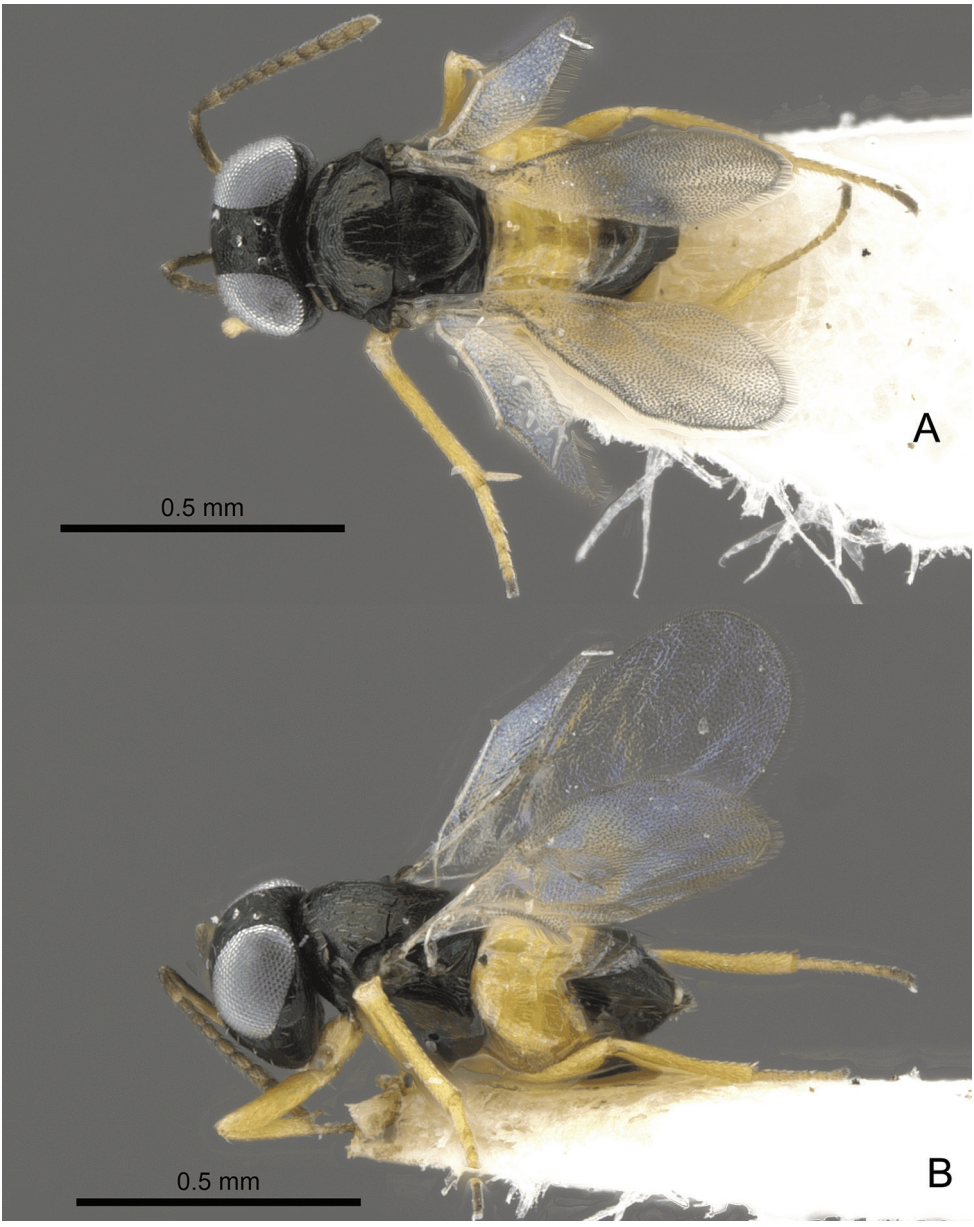

Figure 6. *Ooencyrtus mirus* sp. nov. female (paratype) **A** habitus in dorsal view **B** habitus in lateral view.

Paratypes. USA: California, Riverside Co., Riverside, UCR Quarantine laboratory, N. Power, from colony on *Bagrada hilaris* of Pakistan origin (via USDA ARS laboratory, Stoneville, Mississippi, USA), received 3.xii.2015, S&R # N-15–30: 3.ii.2017 [6 females on points and 2 females on slides, UCRC]; 13.ii.2017 [1 male on point and 2 males on slides, UCRC] (obtained by rearing females with a small dose of antibiotic at 30 °C); 8.iii.2017 [3 females, 7 males on points, UCRC]; 1–7.ii.2019, F. Ganjisaffar [3 females in 95% ethanol in the freezer (molecular vouchers UCRC_ENT 00506189–

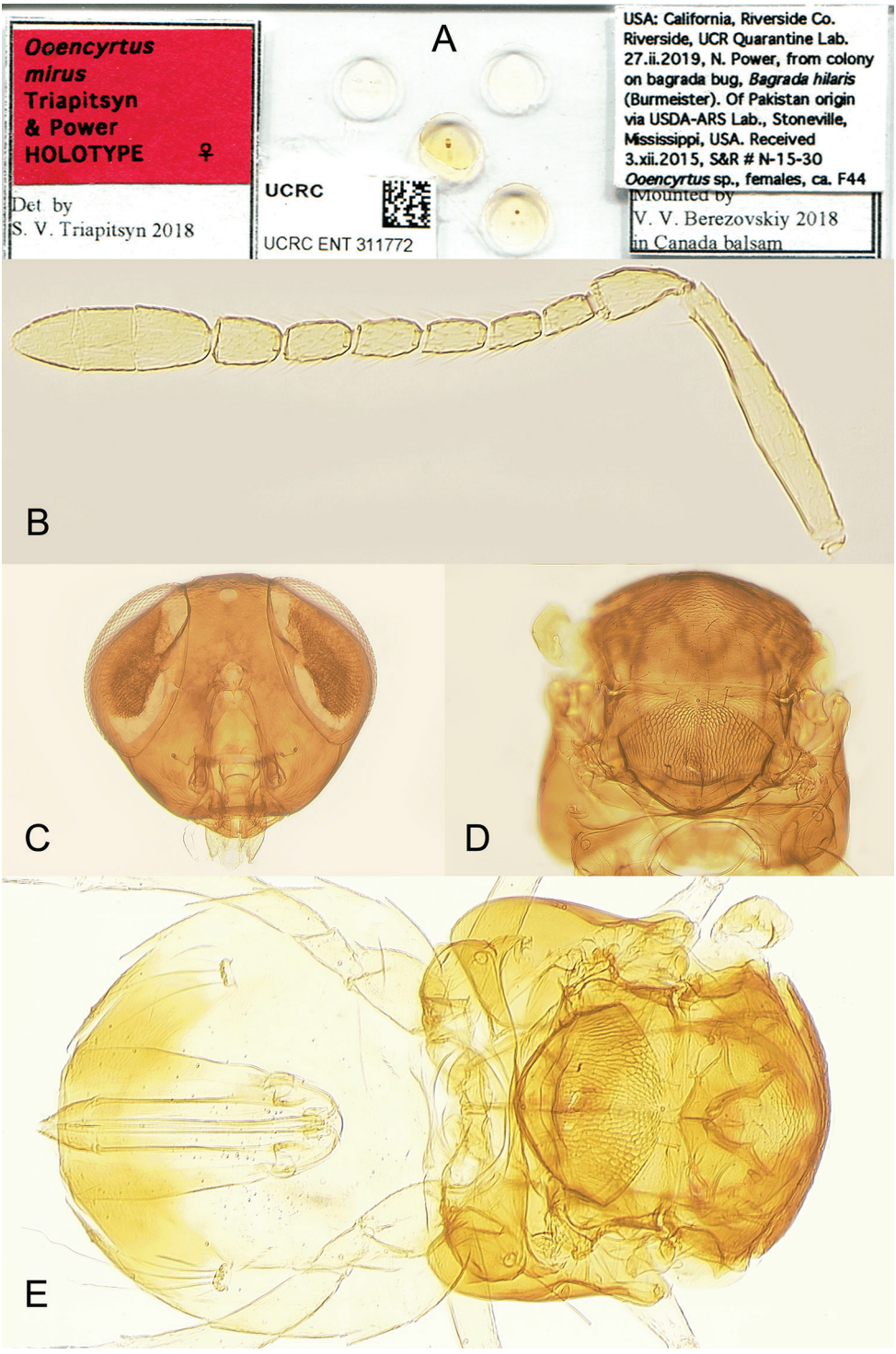

Figure 7. *Ooencyrtus mirus* sp. nov. female **A** holotype slide **B** antenna (holotype) **C** head in frontal view (paratype) **D** mesosoma (holotype) **E** mesosoma and metasoma (holotype).

00506191), UCRC]; 27.ii.2019, ca. F44 [30 females (2 in BMNH, 2 in EMEC, 22 in UCRC, 2 in USNM, 2 in ZIN), 18 males on points (2 in BMNH, 2 in EMEC, 10 in UCRC, 2 in USNM, 2 in ZIN) and 7 females, 2 males on slides, UCRC] (males obtained by rearing females at 36 °C).

Diagnosis. This new species is close to a small group of species of *Ooencyrtus* which are similar to *O. telenomicida* (Vassiliev), as defined by Hayat et al. (2014), although its female legs are entirely yellow. *Ooencyrtus mirus* keys to *O. telenomicida* in Ferrière and Voegelé (1961), Trjapitzin (1989), Huang and Noyes (1994), Zhang et al. (2005), Hayat and Mehrnejad (2016), and Samra et al. (2018). Morphologically, females of *O. mirus* differ from those of *O. telenomicida* mainly in having at least the proximal half of the gaster yellow, with only the apex (from the cercal plates) being brown to dark brown (Figs 6, 7E). In *O. telenomicida*, the yellow or light brown is present as a narrow, transverse basal band (Figs 10A, C, 12A, B, 13C), and this band is practically never extending to the cercal plates. Otherwise, females of these two species are quite similar although there are some differences in the lengths of their funicular segments (Table 3). In the multivariate ratio analysis *O. mirus* is well separated from *O. telenomicida* using the shape PCA (Fig. 16B). However, the scatterplot of isosize against the first shape PC (Fig. 16A) shows that *O. mirus* is also slightly smaller than *O. telenomicida*. This plot thus shows a certain amount of allometric variation and part of the separation is probably based on size rather than shape, and this might be a case of allometric scaling rather than true separation. The next two analyses indicated the same aspect. The PCA ratio spectrum for PC1 (Fig. 16C) identified as most relevant the ratio between propodeum length and scape width (variables lying at the opposite ends of the spectrum are the most relevant), while at the same time this is also the most allometric ratio as shown by the allometry ratio spectrum (Fig. 16D).

The LDA ratio extractor, which is a tool for identifying the best ratios for separating two groups, found that the best ratio to separate the two species is scape width / F5 length, the ratios being almost non-overlapping (Table 8).

Because the commonly used morphometric parameters and ratios of *O. telenomicida* and *O. mirus* are so similar, the importance of their clear separation based on the presented genetic data can not be overestimated.

Table 3. Morphometric ratios and measurements (μm) of *Ooencyrtus mirus* female morphological characters. All measurements are from slide-mounted specimens.

	Length ovipositor: length mesotibia	Length: width fore wing	Length: width hind wing	Length: width scape	Length: width clava	Length F1: length pedicel	Length F2: length F1
Range	0.92–1.02	2.28–2.48	4.52–4.97	5.64–6.89	3.00–3.71	0.48–0.60	0.91–1.08
Mean	0.95	2.36	4.75	6.33	3.30	0.54	1.00
n	10	10	10	10	10	10	10
	Length F1	Length F2	Length F3	Length F4	Length F5	Length F6	
Range	28–40	28–40	34–46	39–51	40–49	40–50	
Mean	35	35	41	45	45	45	
n	10	10	10	10	10	10	

I*n Hayat et al. (2017), the female of O. mirus* keys to *O. utuna* Hayat & Zeya from southern India (Karnataka and Tamil Nadu), but the latter has a linea calva closed posteriorly by 1–2 lines of setae (the linea calva is open posteriorly in *O. mirus*).

Description. Female (holotype and paratypes). ***Body length*** of dry-mounted, critical point-dried paratypes 595–1025 μm.

Color. Head and mesosoma (Fig. 6) mostly black with some metallic reflections, particularly on mesosoma, except mesopleuron with a strong violet luster; most of gaster yellow except brown to dark brown apically (from cercal plates); antenna brown; legs yellow.

Sculpture. Head with faint cell-like sculpture; mesoscutum reticulate, more so anteriorly; axilla reticulate; scutellum more strongly reticulate than mesoscutum or axilla (except sometimes almost smooth at apex), remainder of body more or less smooth.

Pubescence. Frontovertex, pronotum, mesoscutum, axilla, and scutellum with short, inconspicuous, not very dark setae except scutellum with a few pairs of long, dark setae.

Head (Fig. 7C) about 1.1× as wide as high. Minimum width of frontovertex 0.26–0.28× head width. Toruli just below level of lower eye margin. Ocelli in an obtuse triangle. Maxillary palpus 4-segmented, labial palpus 3-segmented. Mandible with 1 larger tooth, 1 very small, inconspicuous tooth and a broad truncation.

Antenna (Fig. 7B) with radicle about 2.8× as long as wide, rest of scape slender, a little wider in the middle and narrowing towards apex, 5.6–6.9× (6.3× in the holotype) as long as wide; pedicel about 2.0× as long as wide, longer than any funicular segment (F1 0.5–0.6× length of pedicel, Table 3); funicle segments all longer than wide, F1 usually about as long as F2 and slightly shorter than following funicular segments (F2 0.9–1.1× length of F1, Table 3), F3–F6 subequal in length although F3 usually slightly shorter than following funicular segments (Table 3), F1–F2 without mps, F3–F4 each with 1 mps, F5–F6 each with 2 mps; clava 3-segmented, 3.0–3.7× (3.1× in the holotype) as long as wide and almost as long as combined length of F4–F6, each claval segment with several mps.

Mesosoma (Fig. 7D, E). Mesoscutum about 2.8× as wide as long; scutellum wider than long and a little shorter than mesoscutum, placoid sensilla close to each other and closer to posterior margin of scutellum. Propodeum smooth and very narrow medially, less than 0.1× as long as scutellum.

Wings (Fig. 8A) not abbreviated, fore wing extending well beyond apex of gaster. Fore wing 2.3–2.5× as long as wide (2.3× in the holotype), disc hyaline; costal cell about 12× as long as wide; marginal vein punctiform; postmarginal vein shorter than stigmal vein; linea calva open posteriorly; filum spinosum usually with 3 setae, sometimes with 4 or, rarely, with 2 setae; longest marginal seta about 0.1× maximum wing width. Hind wing 4.5–6.7× as long as wide (4.65× in the holotype), disc hyaline.

Legs. Mesotibial spur about as long as mesobasitarsus.

Gaster (Fig. 7E) a little longer than mesosoma. Ovipositor occupying 0.6–0.7 length of gaster, at most barely exserted beyond its apex, and 0.9–1.0× (0.9× in the holotype) as long as mesotibia.

Measurements (μm) of the holotype. Mesosoma 400; gaster 431; ovipositor 321; mesotibia 351. Antenna: radicle 43; rest of scape 194; pedicel 68; F1 37; F2 40; F3 46;

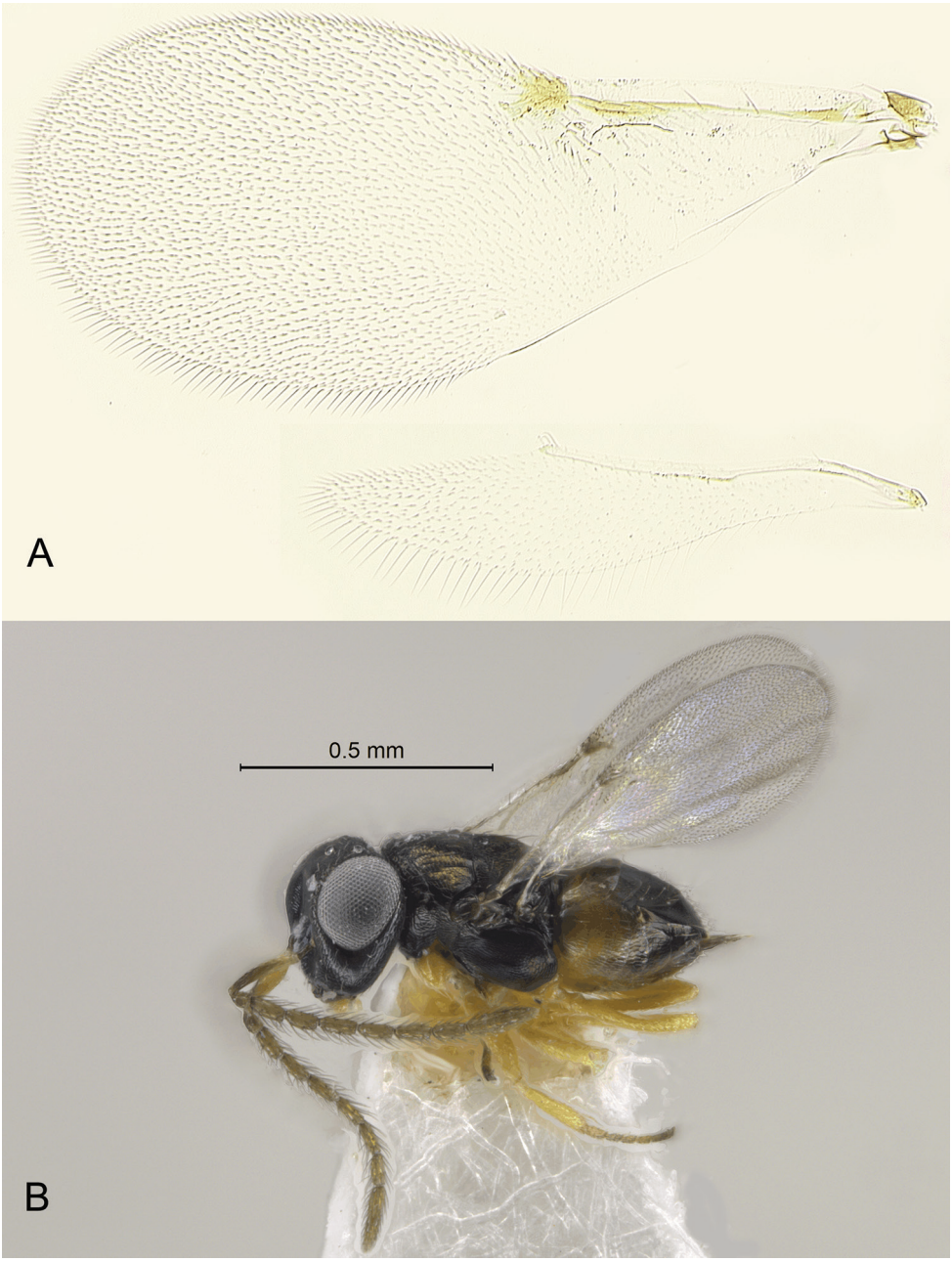

Figure 8. *Ooencyrtus mirus* sp. nov. **A** female fore and hind wings (holotype) **B** male habitus in lateral view (paratype).

F4 49; F5 48; F6 46; clava 135. Fore wing 839:369; longest marginal seta 36. Hind wing 601:129; longest marginal seta 51.

Male (paratypes). ***Body length*** of dry-mounted, critical point-dried paratypes 660–890 µm, and of slide-mounted paratypes 950–960 µm. ***Head*** and ***mesosoma*** black with

metallic reflections (Fig. 8B), ***gaster*** mostly dark brown to black except yellow to light brown or brown basally; antenna brown except scape light brown ventrally and often dark brown dorsally; legs yellow. ***Antenna*** (Fig. 9A) with scape minus short radicle 3.4–3.8× as long as wide (Table 4); funicle segments all longer than wide, more or less subequal in length and each with several mps; clava entire, 3.6–3.8× as long as wide, with several mps; flagellar segments all with numerous long setae. Fore ***wing*** (Fig. 9B) 2.2–2.4× as long as wide; hind wing 4.6–4.8× as long as wide. ***Genitalia*** (Fig. 9C) length 171–191 μm.

Variation (female and male body length, non-type specimens from the colony in UCR quarantine laboratory). The female body lengths, male body lengths, and paired differences, analyzed by the Shapiro-Wilks normality test in R (R Core Team 2018), all had normal distributions. The mean lengths were 849 μm for the females and 795 μm for the males, with a mean difference of 54 μm. A paired t-test in R showed that the males were significantly shorter in length than the females ($P<0.001$).

Etymology. The name is an adjective meaning "remarkable" or "amazing." The name is given to this species because the authors find its biology to be quite remarkable.

Distribution. Oriental region: Pakistan. The population in the quarantine laboratory in UC Riverside that served for the description of this species originated from the Toba Tek Singh District, Punjab, Pakistan.

Hosts. Pentatomidae: *Bagrada hilaris* (Burmeister). We conducted host studies on *O. mirus* and found it to reproduce on the eggs of eight other species in Pentatomidae, one species in Rhopalidae, and one species in Coreidae (Hemiptera), as well as on one species in Noctuidae (Lepidoptera). Of all the potential host species we evaluated, only one, in Pyralidae (Lepidoptera), was not utilized as a host, likely because its eggs were too small. These findings show *O. mirus* to be a generalist parasitoid, although it prefers and reproduces more successfully on *B. hilaris* than on the other hosts evaluated.

Biology. *Ooencyrtus mirus*, a uniparental species, typically produces about 99% females. However, the percentage of males can be increased by providing new eggs to the same female wasps daily for more than two weeks. This depletes the supply of *Wolbachia* bacteria in the ovaries (Lindsey and Stouthamer 2017), and the eggs, all unfertilized, then produce males instead of females.

Comments. This species was initially identified from digital images of both dry- and slide-mounted specimens as *Ooencyrtus telenomicida sensu lato* (J. S. Noyes and E. Guerrieri, personal communications). This determination was ambiguous, however, since *O. telenomicida* was not clearly defined prior to this communication, despite the availability of its numerous diagnoses and redescriptions (e.g., Ferrière and Voegelé 1961; Huang and Noyes 1994; Hayat and Mehrnejad 2016). Thus, until a neotype of *O. telenomicida* was properly designated, and respective DNA sequences were ob-

Table 4. Morphometric ratios and measurements (μm) of *Ooencyrtus mirus* male morphological characters. All measurements are from slide-mounted specimens.

	Length body	Length genitalia	Length: width fore wing	Length: width scape
Range	947–959	172–191	2.2–2.4	3.4–3.8
Mean	953	181	2.3	3.6
n	2	4	4	3

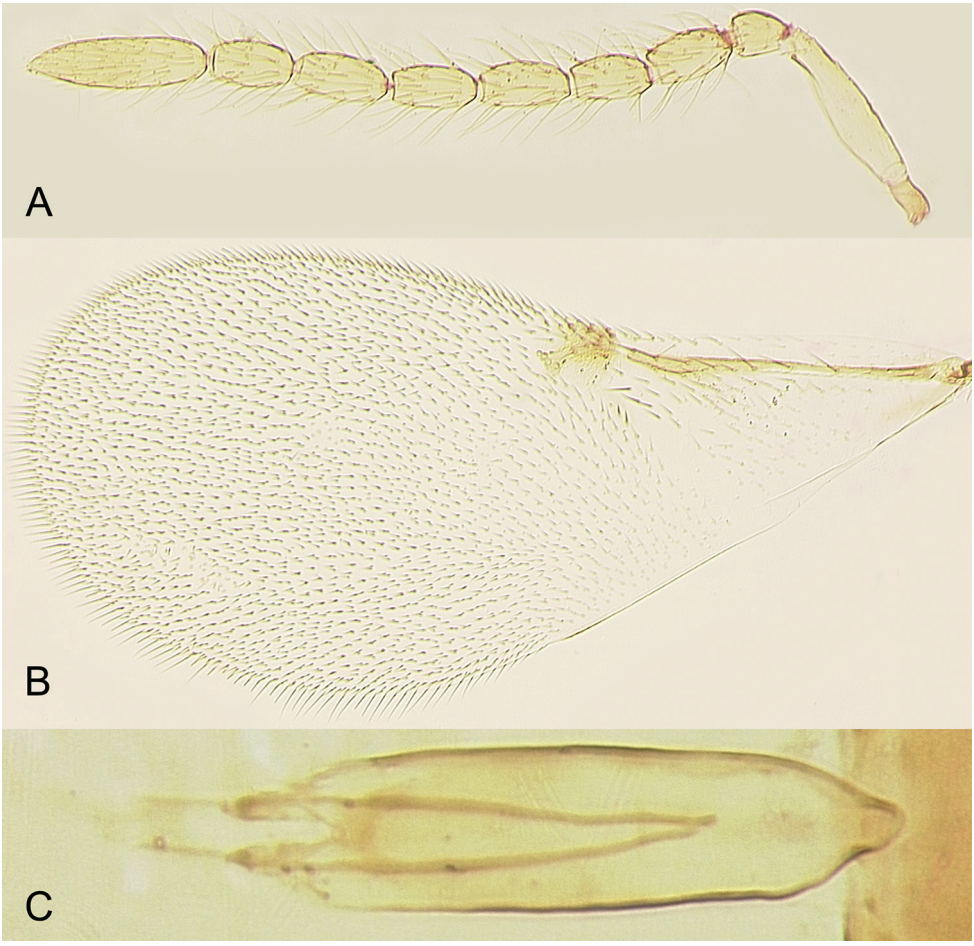

Figure 9. *Ooencyrtus mirus* sp. nov. male (paratypes) **A** antenna **B** fore wing **C** genitalia.

tained, *O. telenomicida* was not defined. We emphasize the importance of obtaining DNA sequences from the neotype since the only specimen defining this species is morphologically very similar to other species in the complex. Samra et al. (2018) provided a diagnosis and DNA sequences for "*O. telenomicida*" reared from Lepidoptera, rather than Pentatomidae, eggs collected in Israel and Turkey, countries with a different climate from that in the type locality. Thus, their conspecificity with *O. telenomicida* from Eastern Europe, reared from eggs of *Eurygaster integriceps*, needed confirmation.

Ooencyrtus telenomicida **(Vassiliev, 1904)**

Figs 10–15

Encyrtus telenomicida Vassiliev, 1904: 117–108. Original type locality: Kupiansk, Kharkov oblast', Ukraine (as "Kupjansk", "Gouvern. Charkov" [then Kharkov Governo-

r*ate of the Russian Emp*ire]). Unspecified number of syntype females and males [type depository not indicated in the original description], lost (not examined).

Schedius flavofasciatus García Mercet, 192*1: 315–318. Type locality* (of the lectotype designated by Noyes 1981: 182, not examined): Cercedilla, Madrid, Spain. Synonymy by Ferrière and Voegelé 1961: 32.

Ooencyrtus telenomicida (Vassiliev): Romanova 1953: 239–247 (host associations and biology)*; Ferrière an*d Voegelé 1961: 28 (key), 30 (illustrations), 32–35 (illustrations, redescription, distribution); Noyes 1978: 11–12 (illustration, comparison with O. brunneipes Noyes); Trjapitzin 1989: 202–203 (key, distribution, hosts); Huang and Noyes 1994: 78–79 (diagnosis, hosts, distribution), 130 (illustrations); Hayat a**nd Mehrnejad 2016: 200 (key), 207–209 (redescription, illustrations, hosts); Samra et al. 2018: 8 (key), 12–14 (illustrations, diagnosis, hosts, distribution).**

Type material. Neotype female [BMNH], here designated (see "Comments" below for the justification) to stabilize the usage of the name, on slide (Fig. 11A) labeled: 1. "Romania: Iaşi County, Ipatele 46.918781N, 27.442949E 317 m, 10.vi.2017, L. Fusu, O. A. Popovici, V. Chinan From eggs of *Eurygaster* sp. On wheat, egg mass # 32"; 2. [salmon] "DNA Voucher D # 6875 UCR, J. M. Heraty [Laboratory]"; 3. "Mounted by V. V. Berezovskiy 2019 in Canada balsam"; 4. [red] "*Encyrtus telenomicida* Vassiliev, 1904 Neotype ♀ = *Ooencyrtus telenomicida* (Vassiliev)"; 5. "Det. by S. V. Triapitsyn 2019"; 6. [barcode database label] "UCRC ENT 311776". The neotype (Figs 10A, C, 11B–F) is in good condition although lacking apex of one hind wing, dissected under 2 coverslips.

Material examined. Romania, Iaşi County, Ipatele, 46.918781N, 27.442949E, 317 m, 10.vi.2017, L. Fusu, O. A. Popovici, V. Chinan (from eggs of *Eurygaster* sp. on wheat) [3 females, two from egg mass # 22, one from # 32, BMNH, UCRC, including one from egg mass # 22 as DNA voucher D # 6874 (UCRC ENT 311775); 2 females from egg mass # 22 as DNA vouchers OoIs0101 and OoIs0102, AICF; 1 female and 1 male from egg mass # 32 as DNA vouchers OoIs0201 and OoIs0202, AICF]. Russia: Krasnodarskiy kray, Slavyansk-na-Kubani (as [stanitsa] "Slavyanskaya" on the original label), Karpova, 1950 (from eggs of *Eurygaster integriceps*; air dried specimens remounted in UCRC on points and slides from a small vial) [9 females, 5 males, UCRC, ZIN] Orenburgskaya oblast', Orsk, 5.vii.1935, G. Ya. Bey-Bienko (on *Elytrigia* sp.) [1 female, ZIN]. Stavropol'skiy kray: Karpova, Kamenkova 1950 (from eggs of *Eurygaster integriceps*; air dried specimens remounted in UCRC on points and slides from a small vial) [numerous females and males, AICF, UCRC, ZIN]. Spain, Madrid: Casa de Campo [park], 15–23.x.1978, J. S. Noyes [1 female, 2 males, UCRC] (determined by J. S. Noyes in 1979); Fuencarral-El Pardo, El Pardo, R. García Mercet [1 female, UCRC] (identified by R. García Mercet as *Schedius flavofasciatus* García Mercet). Ukraine, Nikolaevskaya oblast', 2.vi.1948 (from eggs of *E. integriceps*) [5 females, ZIN]. Taxonomic identifications of *O. telenomicida* from Russia and Ukraine were made by M. N. Nikol'skaya and/or V. A. Trjapitzin.

Description of the neotype female. ***Color. Body*** (Fig. 10A) mostly very dark brown with some metallic reflections (mainly dark bluish and some greenish) on fron-

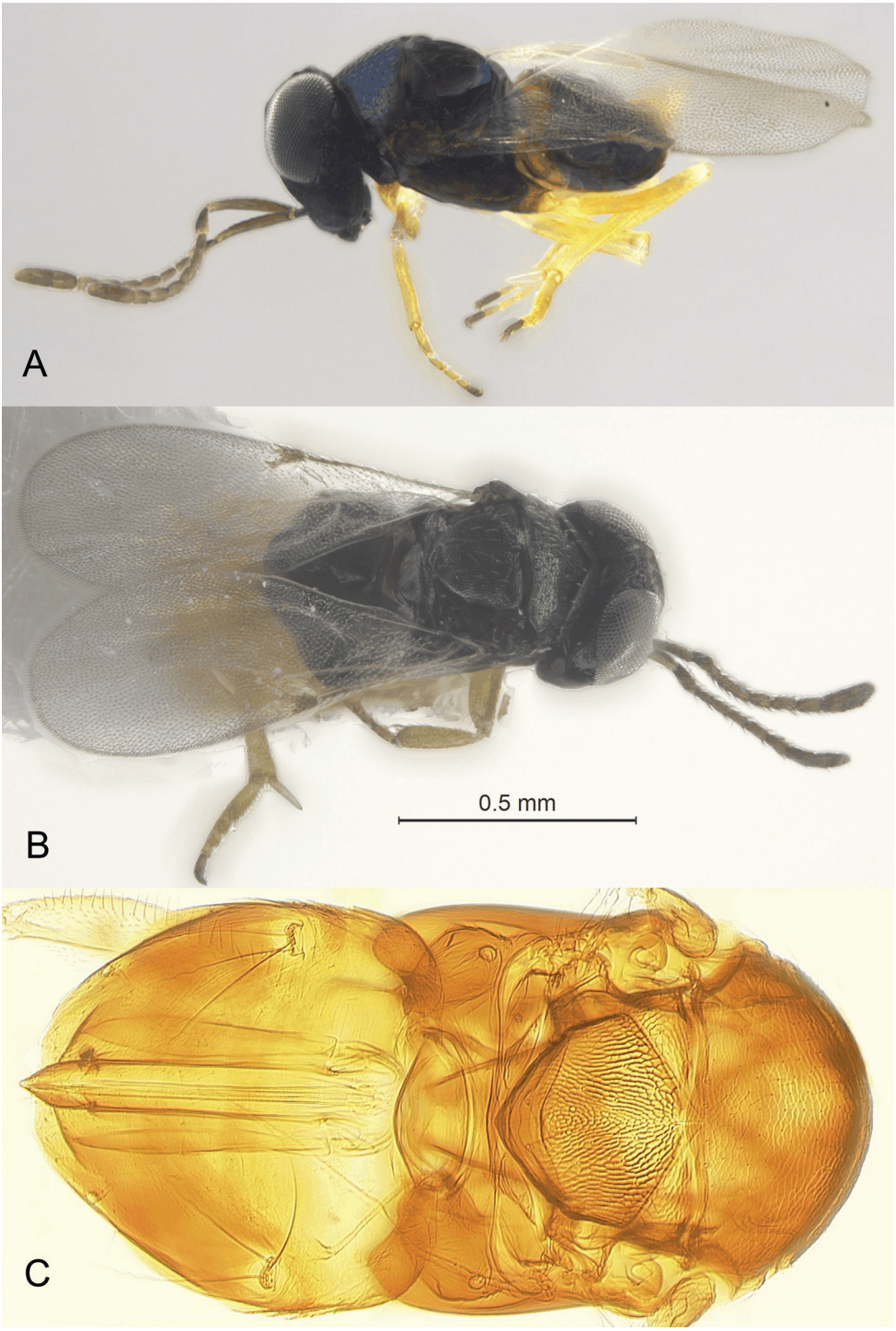

Figure 10. *Ooencyrtus telenomicida* female (from Romania) **A** habitus in lateral view (neotype, prior to DNA extraction) **B** habitus in dorsal view (non-type) **C** mesosoma and metasoma (neotype).

t***overtex,*** mesoscutum, and scutellum except tegula brown and base of gaster with a narrow, light brown band on the first gastral tergite; antenna brown except radicl***e dark brow***n; legs mostly yellow except meso- and metacoxa brown basally and tarsi partially light brown.

Sculpture. Head with stronger sculpture on frontovertex; meso***scutum and axilla reticulate; scutellum (Fig. 11D) more strongly reticulate but almost smooth at apex.***

Pubescence. Frontovertex, pronotum, mesoscutum, axilla, and scutellum with short, inconspicuous, fine, light setae except scutellum with a pair of longer, dark setae.

Head (Fig. 11C) about 1.1× as wide as high. Minimum width of frontovertex 0.25× head width. Toruli just below level of lower eye margin. Ocelli in slightly obtuse triangle, distance from posterior ocellus to eye margin about equal to ocellus diameter. Maxillary palpus 4-segmented, labial palpus 3-segmented. Mandible with 1 larger tooth, 1 smaller tooth and broad truncation.

Antenna (Fig. 11B) with radicle 2.5× as long as wide, rest of scape slender, slightly wider in the middle and narrowing towards apex, 6.3× as long as wide; pedicel 2.0× as long as wide, longer than any funicular segment (F1 0.6× length of pedicel); funicle segments all longer than wide, F1 as long as F2 and slightly shorter than following funicular segments, F3, F4 and F6 about equal in length, and F5 the longest funicular segment, F1–F2 without mps, F3–F4 each with 2 mps, F5–F6 each with 3 mps; clava 3-segmented, 3.0× as long as wide and almost as long as combined length of F4–F6, each claval segment with several mps.

Mesosoma (Fig. 10C). Mesoscutum about 2.3× as wide as long; scutellum (Fig. 11D) slightly wider than long and a little longer than mesoscutum, placoid sensilla close to each other and closer to posterior margin of scutellum. Propodeum (Fig. 11D) smooth and very narrow medially, less than 0.1× as long as scutellum.

Wings not abbreviated, fore wing extending well beyond apex of gaster. Fore wing (Fig. 11E) 2.4× as long as wide, its disc hyaline; costal cell about 11× as long as wide; marginal vein punctiform; postmarginal vein a little shorter than stigmal vein; linea calva almost closed posteriorly by a row of short, inconspicuous setae; filum spinosum with 3 setae on one wing and 5 on the other; longest marginal seta 0.09× maximum wing width. Hind wing 5.4× as long as wide, disc hyaline.

Legs. Mesotibial spur almost as long as mesobasitarsus (Fig. 11F).

Gaster (Fig. 10C) almost as long as mesosoma. Ovipositor occupying more than 0.9 length of gaster, not exserted beyond its apex, and almost 1.0× as long as mesotibia.

Measurements (μm) of the neotype. Mesosoma 418; gaster 400; ovipositor 370; mesotibia 375. Antenna: radicle 45; rest of scape 200; pedicel 70; F1 40; F2 40; F3 50; F4 50; F5 60; F6 50; clava 140. Fore wing 900:370; longest marginal seta 33. Hind wing 725:135; longest marginal seta 48.

Taxonomic notes. Female. Variation (non-type specimens from Romania, Russia, and Ukraine). Body length of dry-mounted, air-dried specimens 860–925 μm. Body (Figs 10B, 12A, B, 14B) mostly very dark brown with some bluish and greenish metallic reflections on mesoscutum, except tegula and mesopleuron brown and base of gaster usually with a complete, narrow, yellowish or light brown band (dorsally almost

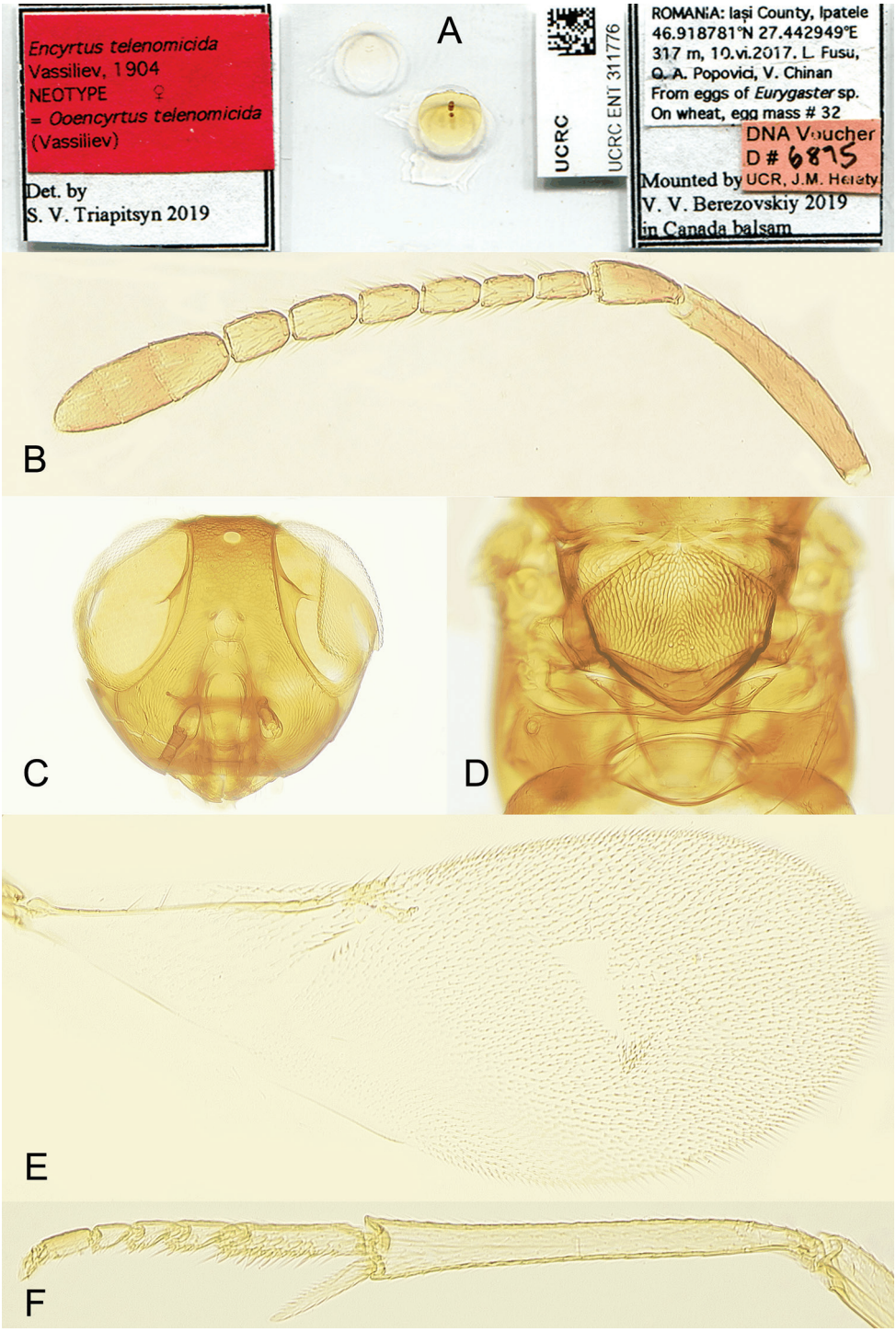

Figure 11. *Ooencyrtus telenomicida* female (neotype) **A** slide **B** antenna **C** head in frontal view **D** axillae, scutellum and propodeum **E** fore wing **F** mesotibia and mesotarsus.

always at most on the first and second gastral tergites, usually only on the first) and often brown (but never yellow) between the yellow basal band and cercal plates dorsally, but occasionally base of gaster entirely dark (Fig. 10B) or, very rarely (observed only in one specimen from Ukraine) the yellow band extends almost to cercal plates (in the absence of molecular data for this historical specimen, it cannot be excluded that it might belong to another species); antenna brown except apex of pedicel a little lighter (light brown); legs mostly yellow except meso- and metacoxa often brown basally, tarsi partially light brown. Minimum width of frontovertex 0.25–0.28× head width (Figs 13A, 14A). Antenna (Fig. 12C) with scape minus radicle 6.0–8.75× as long as wide; F1 the shortest funicular segment, 0.5–0.65× length of pedicel; F2 1.0–1.1× length of F1 (Tables 5, 7), F1–F2 without mps, F3–F6 each with at least 2 mps; clava 2.6–4.1× as long as wide. Fore wing (Fig. 13D) 2.2–2.7× as long as wide; filum spinosum with 3–5 setae. Hind wing 4.2–4.4× as long as wide, its disc hyaline. Ovipositor occupying 0.7–0.9 length of gaster (Fig. 13C), at most barely exerted beyond its apex, and 0.9–1.0× as long as mesotibia.

Male (non-type specimens from Russia). Body length of dry-mounted, air-dried specimens 600–900 µm. Body (Fig. 15A) black with metallic reflections, particularly on mesosoma; antenna brown except scape light brown ventrally and dark brown dorsally; legs yellow except most of coxae and metafemur brown. Antenna (Fig. 15B) with scape minus short radicle 3.6–3.7× as long as wide; funicle segments all longer than

Table 5. Morphometric ratios and measurements (µm) of morphological characters of female *Ooencyrtus telenomicida* from Russia and the Ukraine. All measurements are from slide-mounted specimens.

	Length ovipositor: length mesotibia	**Length: width fore wing**	**Length: width hind wing**	**Length: width scape**	**Length: width clava**	**Length F1: length pedicel**	**Length F2: Length F1**
Range	0.89–1.02	2.22–2.71	4.13–5.20	6.19–8.75	2.56–4.09	0.47–0.65	1.00–1.17
Mean	0.94	2.43	4.6	7.2	3.4	0.55	1.09
n	12	9	10	10	10	10	10
	Length F1	Length F2	Length F3	Length F4	Length F5	Length F6	
Range	34–49	37–51	40–55	43–58	49–65	46–62	
Mean	39	43	50	52	55	52	
n	10	10	10	10	10	10	

Table 6. Morphometric ratios and measurements (µm) of morphological characters of male *Ooencyrtus telenomicida* from Russia. All measurements are from slide-mounted specimens.

	Length body	**Length genitalia**	**Length: width scape**			
Range/Value	836	175	2.9–6.3			
Mean	–	–	4.6			
n	1	1	2			
	Length F1	Length F2	Length F3	Length F4	Length F5	Length F6
Range/Value	74	74–80	74	74	74–77	65
Mean	1	77	74	74	75	1
n	–	2	2	2	2	–

Table 7. Morphometric ratios and measurements of morphological characters of two female *Ooencyrtus telenomicida*, including the neotype, from Romania. All measurements are from slide-mounted specimens.

	Body length	Length ovipositor: length mesotibia	Length: width fore wing	Length: width scape	Length: width clava	Length F1: length pedicel	Length F2: length F1
Range	978	0.92–0.96	2.45–2.49	6.0–8.0	3.0–3.4	0.60–0.64	1.0
Mean	–	0.94	2.47	7.0	3.2	0.62	1.0
n	1	2	2	2	2	2	2
	Length F1	Length F2	Length F3	Length F4	Length F5	Length F6	
Range	43	43	49	49	52–55	49–54	
Mean	43	43	49	49	54	51	
n	2	2	2	2	2	2	

wide, more or less subequal in length (Table 6) and each with several mps; clava entire, 2.9–3.2× as long as wide, with several mps; flagellar segments all with numerous long setae. Fore wing (Fig. 15C) about 2.3× as long as wide; hind wing about 5.0× as long as wide. Genitalia (Fig. 13B) length 175–200 μm.

Distribution. Confirmed records of *O. telenomicida* are from Romania, Russia, Spain and Ukraine; those from other countries in the Palearctic and Oriental regions were summarized by Samra et al. (2018), but many of them will need to be verified using molecular methods.

Hosts. Scutelleridae (Hemiptera): *Eurygaster integriceps* Puton (Vassiliev 1904; Romanova 1953; Trjapitzin 1989), *Eurygaster* sp., as well as some Telenominae (Scelionidae) primary egg parasitoids of *E. integriceps*, such as *Telenomus* spp. and *Trissolcus* spp. (Vassiliev 1904; Romanova 1953), keeping in mind that their species identifications were likely incorrect. Samra et al. (2018) listed some other Heteroptera (Hemiptera) as hosts of *O. telenomicida*; however, identification of the parasitoids will need to be verified using molecular methods.

Biology. *Ooencyrtus telenomicida* is a facultative hyperparasitoid of *Eurygaster integriceps*, being either a primary egg parasitoid (more so earlier in the season when unparasitized eggs of the host are readily available and prevalent) or a secondary parasitoid via the telenomine primary egg parasitoids, particularly later in the season when many of the host eggs are parasitized (Romanova 1953).

Comments. According to V. A. Trjapitzin (personal communication), the entire type series of *O. telenomicida*, if such ever existed, has never been located and is certainly lost. The dire necessity of a proper recognition of this nominal species, which has been impossible with any confidence from some other members of the *O. telenomicida* species complex (e.g., according to Huang and Noyes (1994), from *O. gonoceri* Viggiani and *O. acastus* Trjapitzin), leaves no choice but to designate a neotype for *O. telenomicida*, complemented with the much needed DNA sequence data from it. That is done herein from the specimen reared from an egg of a species of *Eurygaster* Laporte,

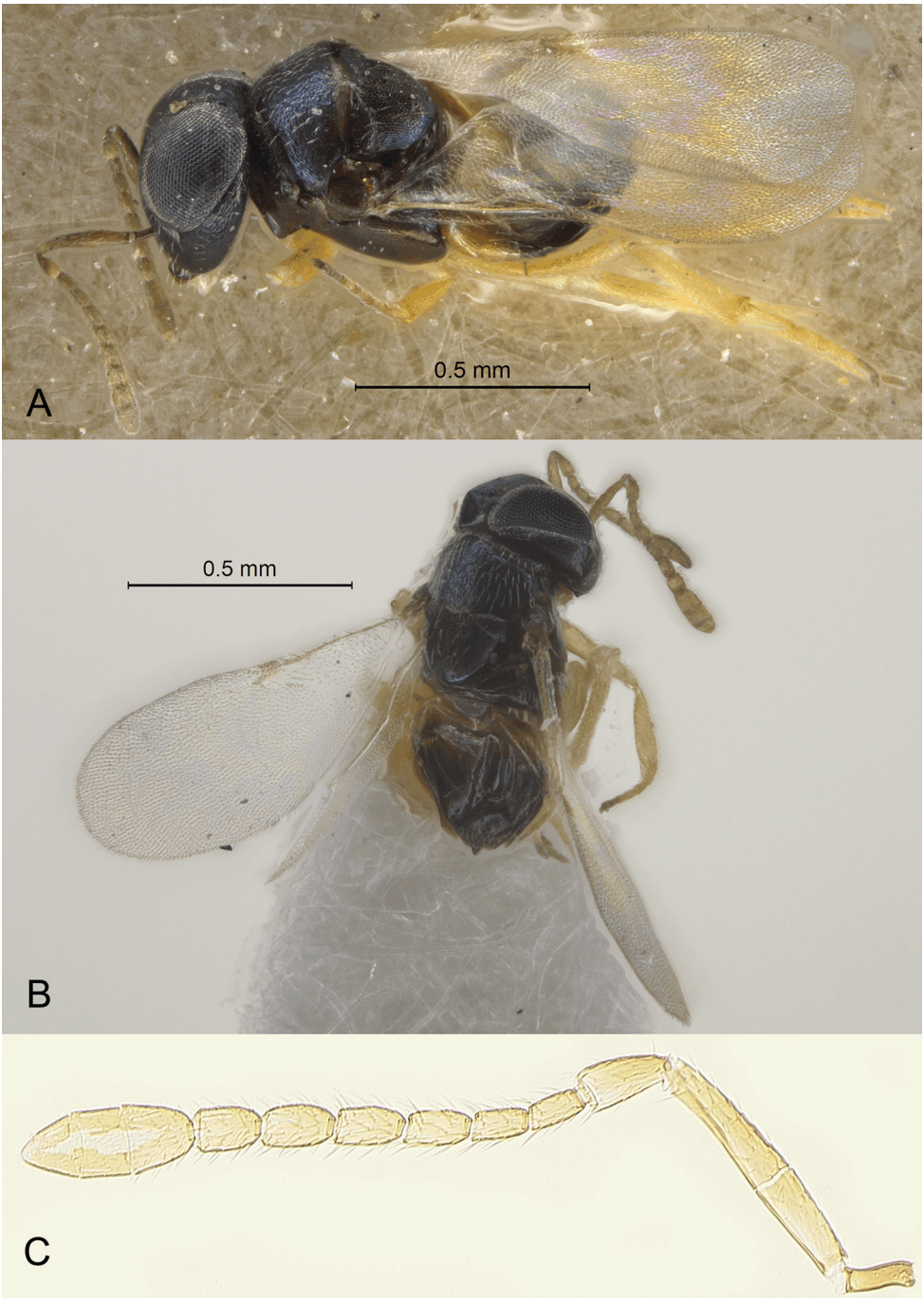

Figure 12. *Ooencyrtus telenomicida* female **A** habitus in lateral view (from Nikolaevskaya oblast', Ukraine) **B** habitus in dorsolateral view (from Krasnodarskiy kray, Russia) **C** antenna (from Stavropol'skiy kray, Russia).

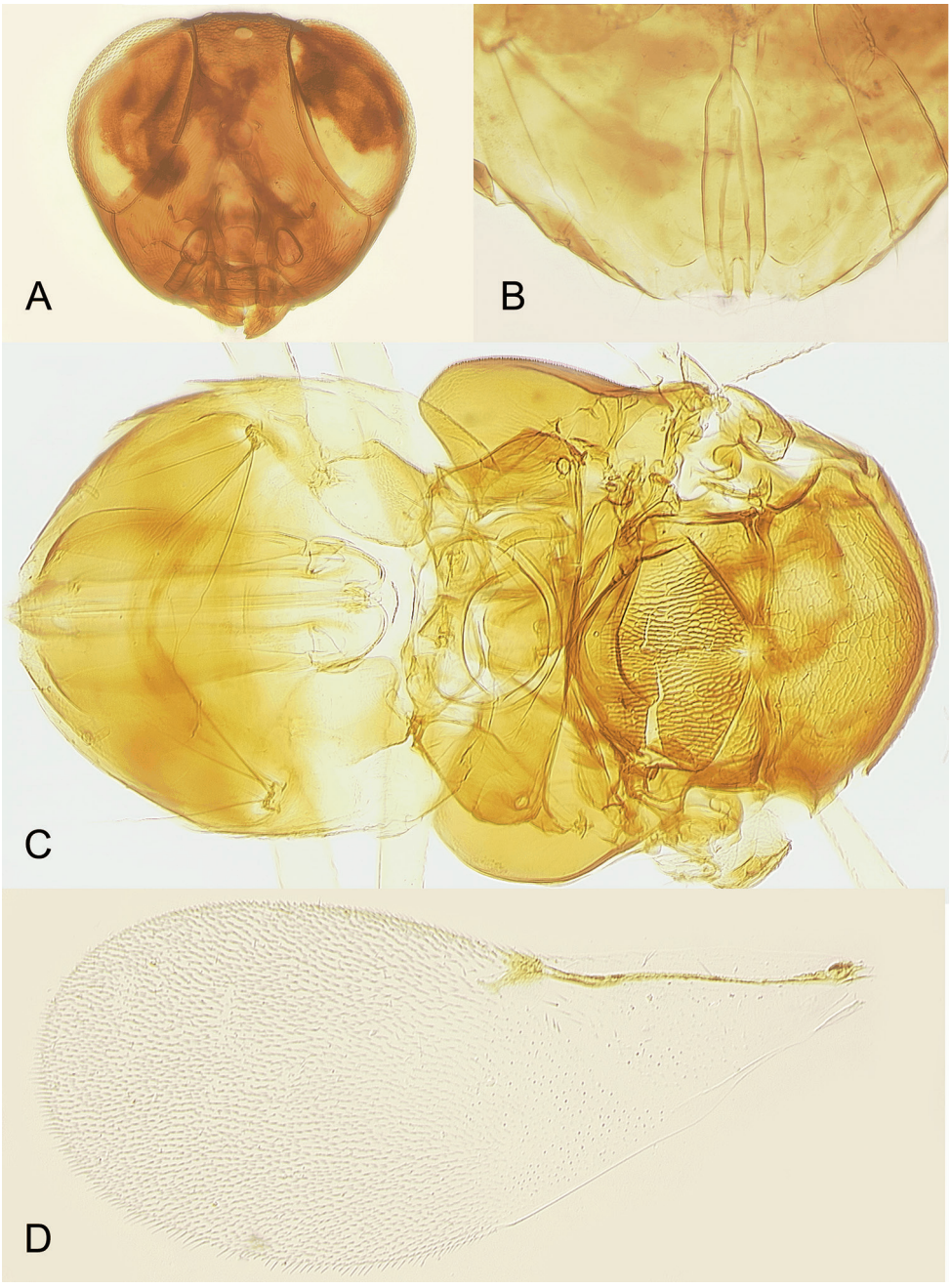

Figure 13. *Ooencyrtus telenomicida* (from Stavropol'skiy kray, Russia) **A** female head in frontal view **B** male genitalia **C** female mesosoma and metasoma **D** female fore wing.

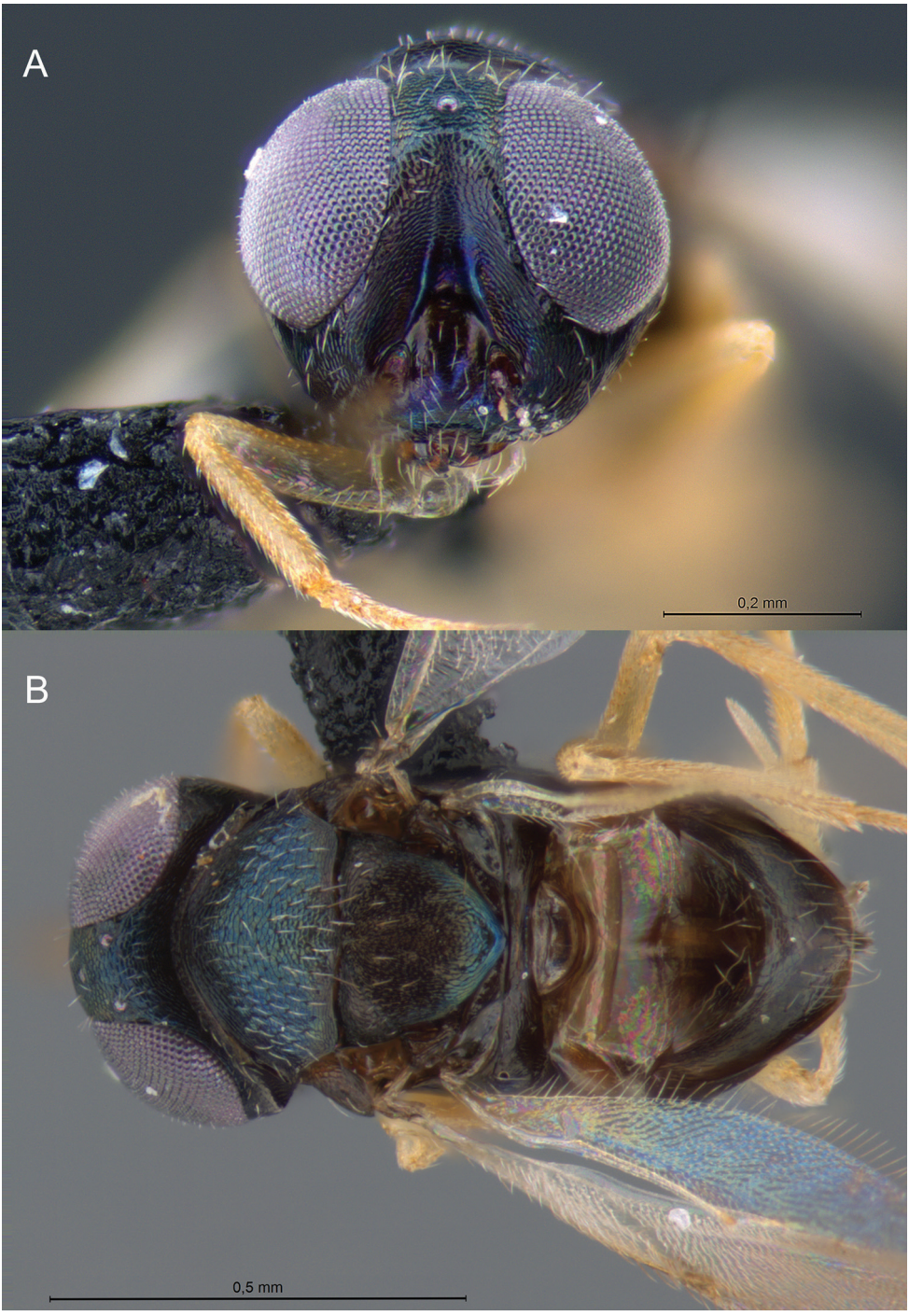

Figure 14. *Ooencyrtus telenomicida* female (from Romania, non-type) **A** head in frontal view **B** body in dorsal view.

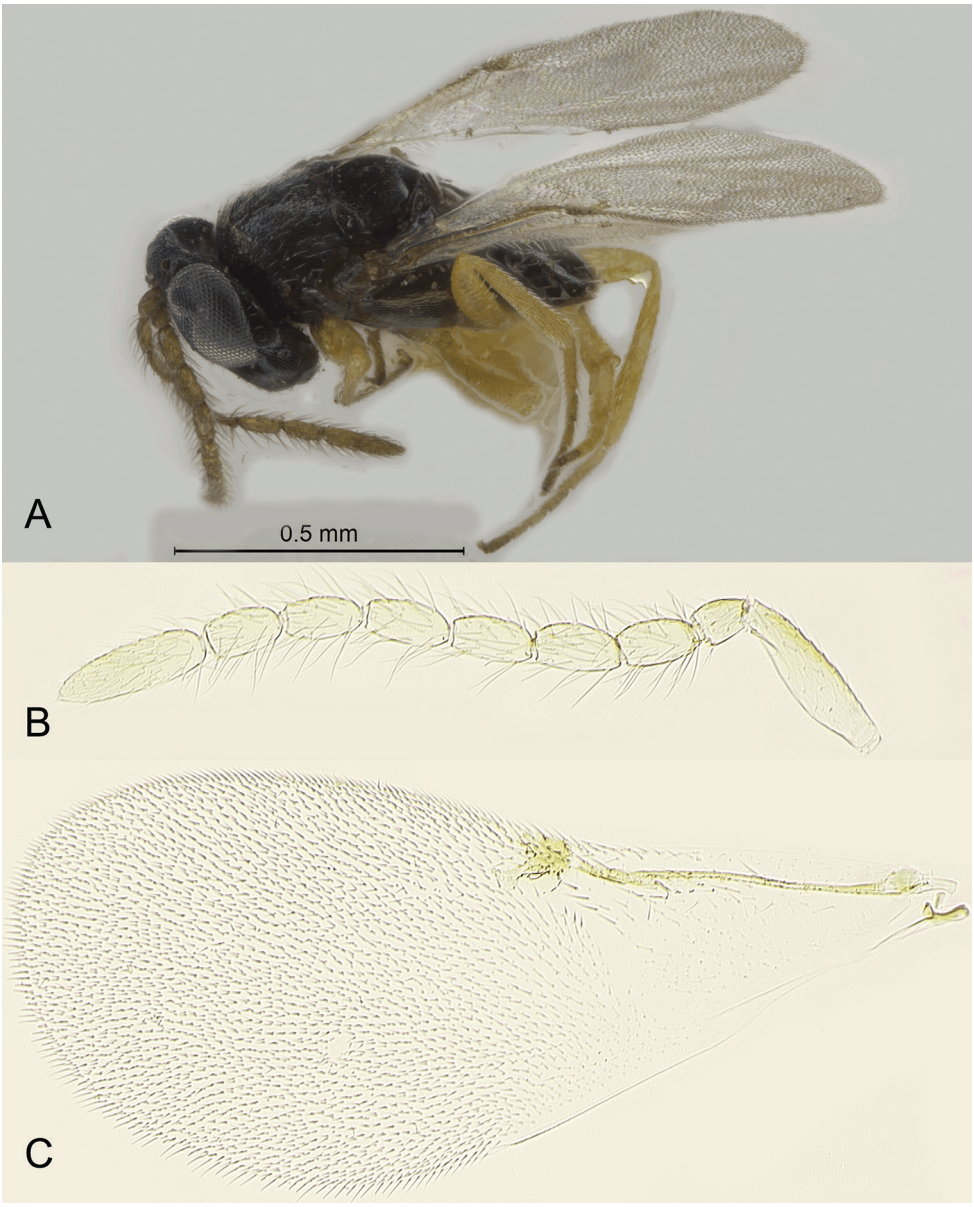

Figure 15. *Ooencyrtus telenomicida* male (from Stavropol'skiy kray, Russia) **A** habitus in lateral view **B** antenna **C** fore wing.

which is the genus from which the originally described *O. telenomicida* emerged. Furthermore, the insects were collected in northeastern Romania which is relatively close to the original collection site in Kharkov oblast' of Ukraine. Importantly, the collections were made in the same general habitat (sylvo-steppe biome) as the originally described species. Morphologically, female specimens from Romania (Figs 10, 11; Ta-

ble 7) are identical to those from Russia and Ukraine reared from eggs of *Eurygaster integriceps* in the late 1940s and early 1950s (Figs 12, 13A, C, D; Table 5). The neotype and especially a second specimen from the same collecting event ('topotype') grouped together with the specimens from Russia and Ukraine (Fig. 16A, B) in the shape PCA of the multivariate ratio analysis.

Based on this information, a genetic library of other members of the complex can be constructed, and their identity determined.

Molecular analyses

COI fragment sequences of *O. lucidus*, *O. mirus*, and *O. telenomicida* from Romania were trimmed for alignment and analysis with sequences of *O. telenomicida*, *O. pistaciae* Hayat & Mehmejad, *O. pityocampae* (García Mercet), *O. mevalbelus* Guerrieri & Samra, *O. zoeae* Guerrieri & Samra, and *O. kuvanae* (Howard) from Samra et al. (2018), to be able to provide continuity and to have comparable data. The four molecular vouchers of *O. lucidus* and those of *O. mirus* demonstrated no intraspecific sequence differences (Table 9). In contrast, the neotype and one non-type *O. telenomicida* specimens from Romania had 3.1% pairwise sequence differentiation (Table 9), indicating high intraspecific variation (these two specimens were obtained from two distinct egg masses found in close proximity). The standard barcode region, obtained from other three specimens but from the same two egg masses, confirms this genetic differentiation (4.4% p-distance). This genetic divergence is at a level that has been demonstrated for other *Ooencyrtus* species, e.g. *O. pistaciae* clades a and b at 4.4% (Samra et al. 2018). ITS2 sequences of *O. lucidus* and *O. mirus* also had no intraspecific differences among specimens. The ITS2 sequence of only one *O. telenomicida* specimen from Romania was obtained. Intraspecific variation could not be determined, although high variation in this region is not expected within the species.

COI alignment and p-distance calculations among the *Ooencyrtus* species revealed at least 5.9% and 7.3% genetic divergence in *O. mirus* and *O. lucidus*, respectively,

Table 8. First five best ratios found by the LDA ratio extractor for separating *O. mirus* sp. nov. and *O. telenomicida*. Standard distance indicates how well one ratio discriminates compared to another; δ indicates how well shape discriminates compared to size (values close to 0 indicate no influence of size and those close to 1 indicate separation based mainly on size). Ranges were calculated on all available measurements, not only on those from the complete dataset used in the analysis. The ratio marked with * has very little overlap.

Ratio	Ranges		Standard distance	δ
	O. telenomicida	*O. mirus*		
L.mesotibia/L.clava	7.56–12.36	7.29–9.66	11.9	0.18
W.scape/L.F5*	0.44–0.63	0.61–0.74	11.73	0.18
L.F4/L.scutel	0.22–0.31	0.25–0.28	11.5	0.18
W.clava/L.pedicel	0.46–0.70	0.53–0.67	11.11	0.19
L.F2/L.F3	0.75–0.92	0.78–1.00	10.7	0.19

Table 9. Uncorrected pairwise-distances between *Ooencyrtus lucidus* sp. nov., *O. mirus* sp. nov., *O. telenomicida*, and other congeneric species. Proportions were determined for a fragment of the mitochondrial cytochrome c oxidase I (COI) gene and the nuclear internal transcribed spacer 2 region (ITS2). Values to the left are the p-distances observed based on the COI gene region, while values to the right are p-distances observed based on the ITS2 region. Values on the diagonal element within the parentheses represent the intraspecific variation observed.

	1	2	3
1. *O. lucidus*	(0.000) / (0.000)	–	–
2. *O. mirus*	0.087 / 0.227	(0.000) / (0.000)	–
3. *O. telenomicida* Romanian	0.096 – 0.104 / 0.216	0.067 – 0.074 / 0.072	(0.031) / -
4. *O. telenomicida* East Mediterranean	0.080 – 0.086 / 0.224	0.060 – 0.070 / 0.077	0.056 – 0.076 / 0.062
5. *O. pistaciae*	0.073 – 0.078 / 0.223	0.059 – 0.065 / 0.069	0.063 – 0.079 / 0.064
6. *O. pityocampae*	0.082 – 0.085 / 0.226	0.062 – 0.066 / 0.144	0.073 – 0.079 / 0.134
7. *O. mevalbelus*	0.091 – 0.094 / 0.205	0.070 – 0.074 / 0.091	0.075 – 0.093 / 0.075
8. *O. zoeae*	0.078 – 0.084 / 0.202	0.062 – 0.066 / 0.096	0.074 – 0.087 / 0.083
9. *O. kuvanae*	0.097 / 0.442	0.091 / 0.428	0.098 – 0.109 / 0.425

f*rom O. telenom*icida and other anal*yzed Ooency*rtus species (Table 9), indicating species level differentiation in both cases. Sequence*s of O.* mirus were most similar to thos*e of O. pistaciae,* but the species is well differentiated with at least 5.9% difference. COI sequences of *O. lucidus* also were closest to *O. pistaciae*, but were divergent with a minimum of 7.3%. The Pakistani *O. mirus* was unequivocally supported as a distinct species from *O. telenomicida*, both from the Romanian neotype designated herein and the East Mediterranean populations studied by Samra et al. (2018). This is supported by 6.7–7.4% and 6.0–7.0% genetic divergence, respectively. *Ooencyrtus lucidus* had high genetic separation from all compared species.

Analysis of the ITS2 region further demonstrated genetic separation of these species. Intraspecific variation in this region is absent to extremely low, while interspecific variation is expected to be high. Our analysis was based on a partial fragment of the ITS2 region because the full sequence was not obtained for every specimen; however, the region analyzed was flanked by regions of congruence (16 bases at the 5' end and 22 bases at the 3' end; average 385 bp region analyzed for each species). The lowest p-distance between *O. mirus* and all compared species in this region was 0.069 (6.9% pairwise distance), which was demonstrated with *O. pistaciae* (Table 9). Sequence divergence for *O. lucidus* was extremely high with the lowest p-distance at 0.202.

Phylogenetic analysis of *Ooencyrtus* species, inferred using concatenated COI and ITS2 genetic regions, supported *O. mirus* as a sister taxon to *O. telenomicida* from Romania (Fig. 17). These two species formed a larger clade with the sister taxa *O. pistaciae* and East Mediterranean *O. telenomicida*, separated from *O. zoeae* and *O. mevalbelus*. Basal to this clade were *O. pityocampae* and *O. lucidus*; *O. kuvanae* was used to root the tree. Using concatenated COI and ITS2 sequences resulted in a phylogenetic tree with a topology that combined the two separate COI and ITS2 trees of Samra et al. (2018). East Mediterranean *Ooencyrtus telenomicida* and *O. pistaciae* branched in a clade separate from *O. zoeae* and *O. mevalbelus* as seen in both the COI and ITS2 phylogenetic

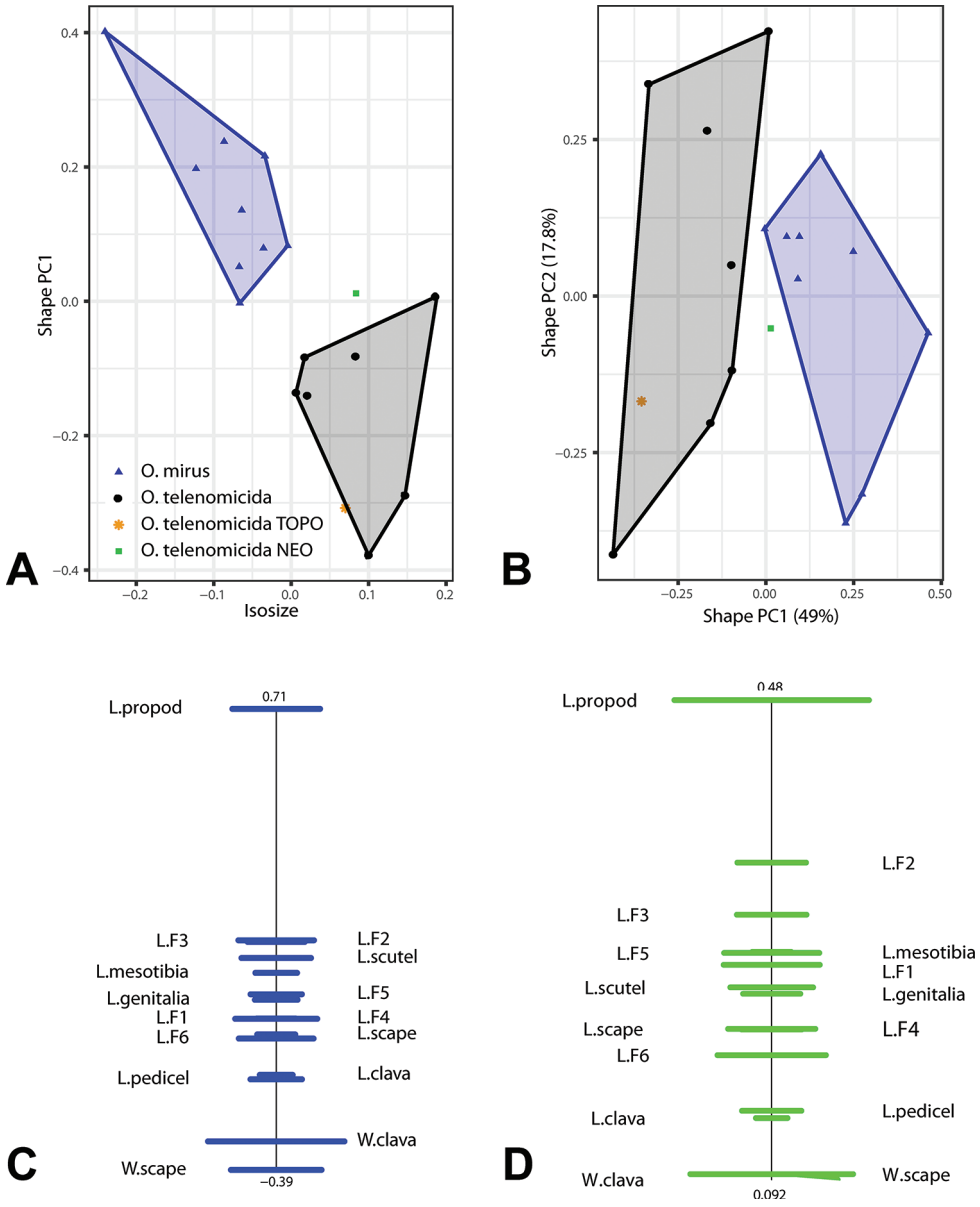

Figure 16. Multivariate ratio analysis for *Ooencyrtus mirus* sp. nov. and *O. telenomicida* **A** scatterplot of isosize against first shape PC **B** shape PCA, scatterplot of first against second shape PC **C** PCA ratio spectrum for PC1, bars represent 68% confidence intervals **D** allometry ratio spectrum; bars represent 68% confidence intervals.

t*rees, and O. pityocampae* branched basally as in the ITS2 tree. As suspected, inferring the phylogenetic placement of *O. mirus* and the Romanian *O. telenomicida* (including the neotype) with these species resulted in a clade for the *O. telenomicida* species

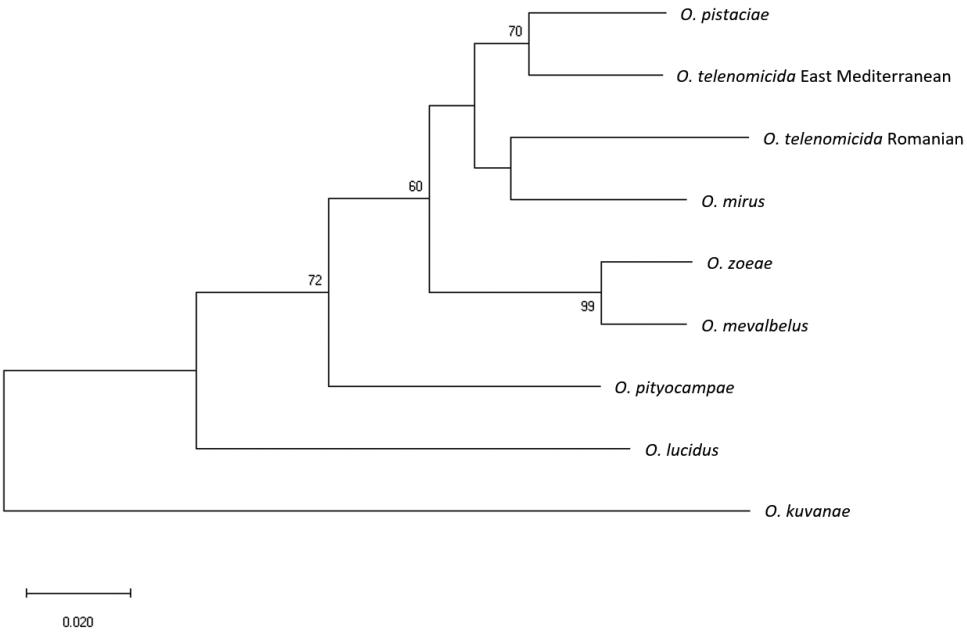

Figure 17. Relationship of *Ooencyrtus lucidus* sp. nov., *O. mirus* sp. nov., and *O. telenomicida* with other congeneric species based on concatenated partial regions of the mitochondrial cytochrome c oxidase I (COI) gene and the nuclear internal transcribed spacer 2 region (ITS2). Optimal maximum likelihood phylogenetic tree based on the Tamura-Nei model. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (values over 50 are shown), and the tree is drawn to scale with branch lengths indicating uncorrected p-distance.

complex. Almost certain of New World origin and completely different from the *O. telenomicida* species complex morphologically, *O. lucidus*, with its high genetic divergence in both COI and ITS2 regions, branched separately. It is basal to all other species in the concatenated phylogenetic tree (as well as in our earlier analyses on single-gene COI and ITS2 trees; data not shown).

Conclusion

Members of the speciose genus *Ooencyrtus*, in which more than 300 currently valid species are known, are notoriously difficult to identify morphologically. That is particularly true for the taxa within the *O. telenomicida* species complex in the Old World. In the New World, identification keys exist only for some Neotropical species (Noyes 1985, 2010) but not for those in the Nearctic region. Moreover, many undescribed species have been recognized (e.g., Zuparko 2015, 2018), and misidentifications of *Ooencyrtus* species are quite common. Whereas molecular methods with both mitochondrial and nuclear gene regions often are necessary for providing reliable identifica-

tion or separation of morphologically similar species, using these methods for positive identifications is useful only if those taxa were correctly identified based on morphological studies of the type specimens and reared material from known hosts. Here we provide both morphological and genetic evidence that has helped to untangle the true identity of the common Old World parasitoid, *O. telenomicida*, and also of two primary egg parasitoids of the bagrada bug, one from California and the other from Pakistan.

Traditionally, the standard DNA barcode region of the COI gene described in Folmer et al. (1994) is analyzed to estimate intraspecific and interspecific difference when comparing metazoan invertebrate species. However, in order to compare our new species with recently described *Ooencyrtus* species, we sequenced the COI region analyzed by Samra et al. (2018). This region is 946-bp in length, whereas the Folmer region is 648-bp long, and these regions share approximately 400-bp overlap allowing comparison of a portion of the two regions. However, we also obtained the standard barcode sequence for three specimens of *O. telenomicida* in order to maximize compatibility with standard DNA barcodes libraries. We found well-supported genetic separation of the two new species described herein from all compared *Ooencyrtus* species in both the full Samra et al. (2018) proposed COI region and the overlapping Folmer region. ITS2 sequences reinforced *O. lucidus* and *O. mirus* as distinct species with high levels of genetic differentiation. Our COI and ITS2 sequence analyses supported and confirmed the morphological differences and morphometric separation observed for these new species. Interestingly, our analysis also demonstrated significant genetic divergence of the neotype of *O. telenomicida* from the likely misidentified *O. telenomicida* specimens previously sequenced. This observation emphasizes the fact that additional work remains to sort out and properly describe and re-describe the species of the *O. telenomicida* species complex, including proposing possible synonymies. That difficult and laborious task, however, is well beyond the scope of this study.

Acknowledgments

We are very grateful to John S. Noyes (BMNH) and Emilio Guerrieri (Istituto per la Protezione Sostenibile delle Piante, Consiglio Nazionale delle Ricerche, Portici, Naples, Italy) for help with the initial identification of *O. mirus* as a species very similar to *O. telenomicida* and valuable discussions. Hannes Baur (Natural History Museum of Bern, Switzerland) is thanked for his advice on using the multivariate ratio analysis. We also thank Ekaterina V. Tselikh (ZIN) for the loans of specimens of *Ooencyrtus telenomicida*, Vladimir A. Trjapitzin (Moscow, Russia) and Robert L. Zuparko (California Academy of Sciences, San Francisco, California, USA) for important information and advice, Vladimir V. Berezovskiy (UCRC) for skillful mounting of specimens, Michael W. Gates (USNM) for providing access to the collection under his care and the loan of material, as well as Natalie Dale-Skey Pappillod (BMNH), Roger A. Burks and Shayla Hampel (UCR) for kind assistance. Thoughtful comments and suggestions by the reviewers helped to improve the manuscript significantly. This research was supported, in

part, by California Department of Food and Agriculture Specialty Crops Block Grant SBC16053. We thank Charlie Pickett (California Department of Food and Agriculture, Sacramento) for his collaboration on this grant. The search for *O. telenomicida* in Romania was supported by the Romanian Executive Agency for Higher Education, Research, Development and Innovation Funding (UEFISCDI) through the project PN-III-P4-ID-PCE-2016-0233.

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Supplementary material 1

Measurements used in the multivariate ratio analysis for *Ooencyrtus mirus* Triapitsyn & Power and *O. telenomicida* (Vassiliev) (Hymenoptera: Encyrtidae)

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Data type: measurement

Explanation note: The complete set of measurements used in the multivariate ratio analysis for *Ooencyrtus mirus* Triapitsyn & Power and *O. telenomicida* (Vassiliev).

Link: https://doi.org/10.3897/jhr.76.48004.suppl1