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Article

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A new species of scuttle flies (Diptera, Phoridae) from the genus *Menozziola* Schmitz that's a parasitoid of ants (Hymenoptera, Formicidae) *Camponotus vagus* Scopoli

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Abstract
A new species of scuttle flies, *Menozziola tanaitica* sp. n. that's a parasitoid of ants *Camponotus vagus* Scopoli was found in the lower reaches of the Don River in the Rostov region, Russia. The new species is similar to *Menozziola obscuripes* (Schmitz, 1927) but differs in large number of hairs on epandrium and anal tube. This is the first confirmed case of parasitization of scuttle flies on ants in Russia.

Key words Parazitisation, infected queens, parasitoid, *Camponotus*.

Introduction

Scuttle flies (Diptera, Phoridae) occupy a significant place among the parasitoids of ants. They parasitize 182 species of ants from 40 genera (Quevillon, 2018). Among European phorids, two species - *Menozziola obscuripes* (Schmitz, 1927) and *Microselia southwoodi* Disney, 1988 - parasiting larvae in ants *Camponotus vagus* (Scopoli, 1763) (Carles-Tolra, Rivera, 2008).

Information about the biological features of parasitoids of the Phoridae family and their distribution remains fragmentary. It is known that in most cases these flies parasitize worker ants. Information on queen infection during or after nuptial flight is limited to isolated observations (Smith, 1928; Gadau, Disney, 1996). There is no information about the symbiotic relationship between scuttle flies and ants of the fauna of Russia.

Phoridae is a large family from the order Diptera, including more than 3,800 species belonging to approximately 265 genera (Disney, 1994). Phorids are closely related by various symbiotic relationships with ants: many species of scuttle flies are parasitoids, parasitizing worker ants and sometimes reproductive individuals or brood, and occur together with ant families as scavengers eating detritus in nests, or as predators hunting brood or weakened or injured ants (Feener, Brown, 1997; Quevillon, 2018). According to L. Quevillon (2018), 182 species of ants from 40 genera are known to be parasitized by these flies.

About 45% of all species of the Phoridae family are members of the genus *Megaselia* (at least 1700 species), many of which lead a parasitoid lifestyle using ants as hosts (Disney, 2006). There is evidence that in most cases phorids infect worker ants. Information on queen infection during or immediately after the nuptial flight is extremely fragmentary (Gadau, Disney, 1996; Smith, 1928).

Parasitoid flies tend to specialize in infecting certain species of ants. For example, representatives of a small number of species of the genus *Menozziola*, parasitize ants of the genus *Camponotus* Mayr, widely distributed in the Palearctic. *Menozziola obscuripes* has been found to parasitize *C. herculeanus* (Linnaeus, 1758), *C. vagus*, and *C. ligniperda* (Latreille, 1802) (Gadau, Disney, 1996; Nielsen, 1934; Quirvin, Disney, Duvallet, 2009). Some *Menozziola* parasitize representatives of other ant genera. Menozzi (1921) reported on the parasitism of *M. schmitzi* on ants *Crematogaster scutellaris* (Olivier, 1792). In Russia, there have been no cases of parasitism of scuttle flies on ants. Flies of the genus *Menozziola* have not been recorded here before.

Materials and methods

The collection of *Camponotus vagus* queens was carried out in the period from 2018 to 2023 in the second to third decade of May in various local areas in the Lower Don Valley in the territory located between Bagaevskaya village (47°20’52.2”N 40°22’28.9”E) and Rostov-on-Don city (47.215787, 39.790240). The dealate queens of this species who landed after mating were selected. They were placed in test tube incubators for further maintenance in laboratory conditions. The females were fed with a 20% glucose syrup. In total, more than 100 queens were captured during the entire 5-year period (about 20-30 females annually).

The collected live ants were placed in individual test tube incubators. In the event of the death of queens and the release of phorid larvae, they continued to be stored in incubators at room temperature in a darkened place.

The emerging imagos of scuttle flies (about 20 males and 30 females) are preserved in 70% ethanol. Some of them (4 males and 4 females) were used for microscopic examination of features of the external structure (Fig. 2, 3, 5). Microscopic examination was carried out on the equipment of the Research Park of St. Petersburg State University (“Resource Centre for Microscopy and Microanalysis” and “Centre for Molecular and Cell Technologies”, project No. 2404-045). Light (Leica M 205 C) and scanning electronic (Hitachi TM3000; FEI Quanta 200 3D) microscopes were used. A critical point dryer (Leica EM CPD300) was used to prepare the fly larvae for examination in a scanning electron microscope (Fig. 4). Morphometric analysis of the ratio of the lengths of the wing veins was carried out using photographs of the wings of males and females obtained on a light microscope (according to Disney, 1994) (Fig. 5).

Results

Infected queens of *C. vagus* were found on May 27, 2022 in the lower reaches of the Don River at a local site in the vicinity of the village of Bagaevskaya (47°20’52.2”N 40°22’28.9”E) (Fig. 1, A). They were localized under the bark of old fallen tree trunks, where they got immediately after the end of the nuptial flight. There were dead individuals near the live queens (Fig. 1, B). In total, 10 trunks of relatively dry fallen trees were examined and 20 live and about 10 dead *C. vagus* queens were found.
The site where the ants were collected is located on a low and gentle slope of the right bank of the Don River near the coastal sandy strip. In May-June, after the end of the spring flood, it turned out to be

raised above the surface of the river to a height of 1–1.5 meters and removed from the water's edge at 20-25 meters. Here, along the coastal slope (width from 10 to 30 m), fragments of woody and shrubby vegetation are preserved, represented mainly by white poplar (*Populus alba* L.), willow (*Salix alba* L.), elm (*Ulmus laevis* Pall.) and birch bark (*Euonymus verrucosus* Scop.). The mentioned coastal belt of tree plantations contained many fallen trees at various stages of decomposition of wood, and there were numerous open areas occupied by ruderal vegetation.

The first dead queens were found in incubators in 10-15 hours after their collection. On the second day, the number of dead ants exceeded 80%. As a result, out of 20 queens collected alive, only one was uninfected. She was successfully able to lay eggs and raise the first worker ants.

On May 30, 2023, a re-collection of *C. vagus* queens was carried out within this local area. In this case, the ants were caught in the first minutes after the end of the landing after the nuptial flight, before their entry into the trunks of dead trees. All queens of *C. vagus* (15 specimens) collected here immediately after landing turned out to be free of scuttle flies' larvae. They successfully laid eggs and raised worker ants in test tube incubators.

In the dead individuals, 5-8 hours after death, the abdomen was torn along the seam between the first and second tergite and larvae began to emerge from it (Fig. 1, C, D). There were from 50 to 60 fly larvae in the abdomen of each dead ant. Before pupation, which took place on the third day, the larvae actively moved around the test tube. The release of adult flies occurred on the 15-16 day after pupation. The incubators were all this time in a dark place at a temperature of 25-27 ° C.

Specimens of preserved flies are kept at the Zoological Institute of the Russian Academy of Sciences (ZISP) and in the personal collection of Dmitry Shevchenko (PCSH).

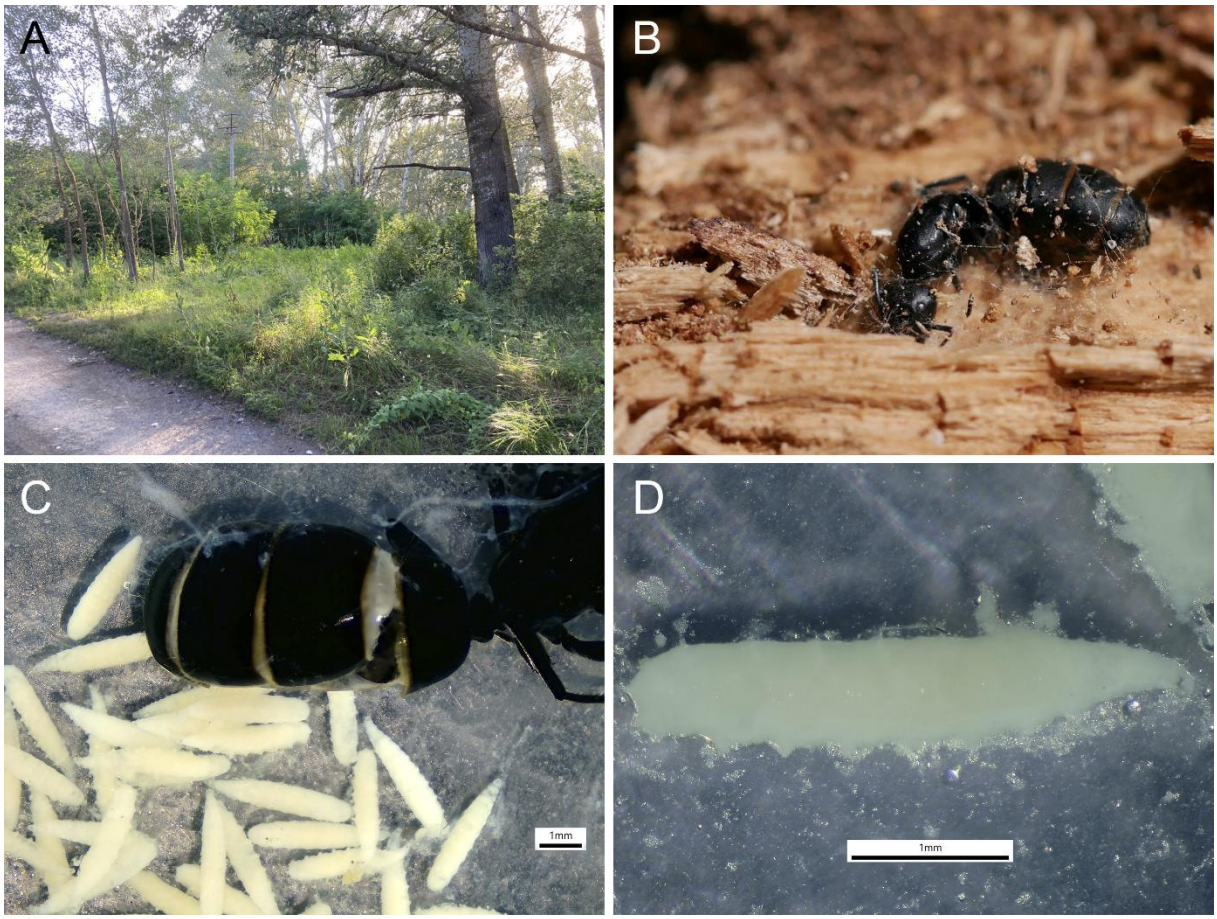

Figure 1. A - Type locality of *Menozziola tanaitica* sp. n.; B - dead *Camponotus vagus* queen inside the trunk; C - fly larvae emerging from the ant abdomen; D - larva of the scuttle fly.

Systematics

Insecta: Diptera: Brachycera: Muscomorpha: Phoridae: Metopininae: Metopinini: *Menozziola*

Menozziola tanaitica sp. n. (Fig. 1C,D, 2, 3, 4, 5)
https://zoobank.org/urn:lsid:zoobank.org:act:09D0D700-D46F-4F60-BB4D-FD150137359C

Type material: Holotype 1♀ (ZISP) and 20 paratypes 11♀, 9 ♂ (ZISP, PCSH): "Россия, Ростовская обл., ст. Багаевская, пойма р. Дон, выведены из самок Camponotus vagus, собранных 27.05.2022. Мухи выведены 15.06.2022 (Д. М. Шевченко)" [Russia, Rostov region, Bagaevskaya village, floodplain of the Don river, bred from *Camponotus vagus* queens collected on 05/27/2022. The flies were bred on 06/15/2022 (D. M. Shevchenko)].

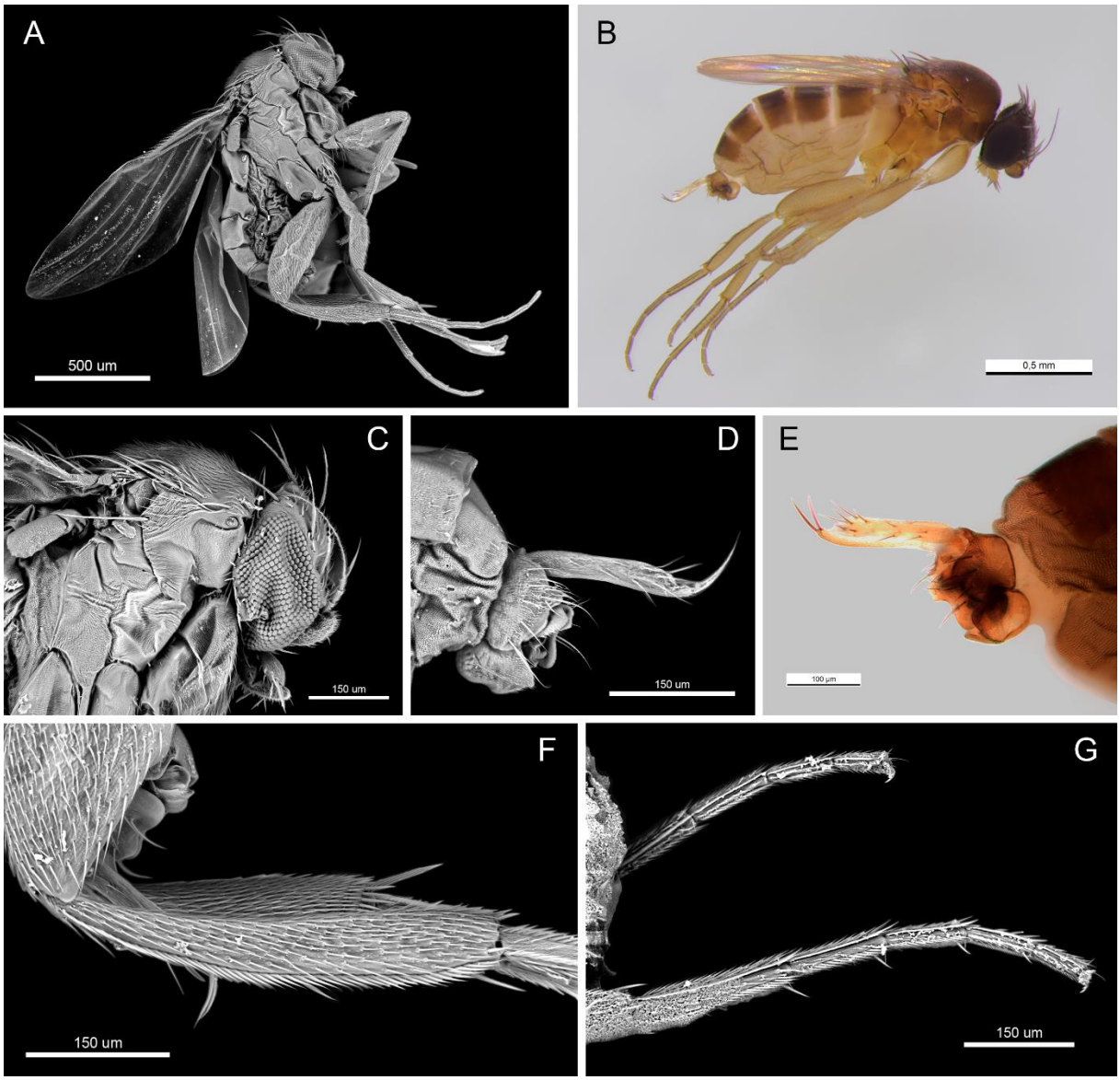

Figure 2. *Menozziola tanaitica* sp. n. male: A, B - lateral view; C - head and thorax lateral view; D, E - hypopigium; F - tibia; G - tarsus.

Description. Male (Fig. 2, 5). Thorax brown. Notopleuron with 2 bristles. Mesopleuron with about 14 hairs and one long bristle. Scutum with a pair of bristles in front of the scutellum, which has a posterior pair of bristles. Abdominal tergites 1-6 brown with small hairs mainly at rear margins. Hypopygium as Figs 2 D, E. Legs brownish yellow. Front tarsus as Fig. 2 G. Femur and tibia as Fig. 2 F. The average

length (n=4) of the wing (Fig. 5) is 1,12205 mm. Costal Index 0,3581. Costal ratios 3,929:1,154:1. Costal cilia 0,0273 mm long. Axillary bristle 0,0429 mm long. Haltere knob brown.

Female (Fig. 3). Thorax brown. Notopleuron, mesopleuron, scutum and scutellum: same as a male. Abdominal tergites 1-6 brown (slightly darker than the male's). Ovipositor as Figs 3 D. Legs brownish yellow. Femur, tibia and tarsus: same as a male. The average length (n=4) of the wing is 1,238 mm. Costal Index 0,356. Costal ratios 3,942:1,661:1. Costal cilia 0,0269 mm long. Axillary bristle 0,0431 mm long.

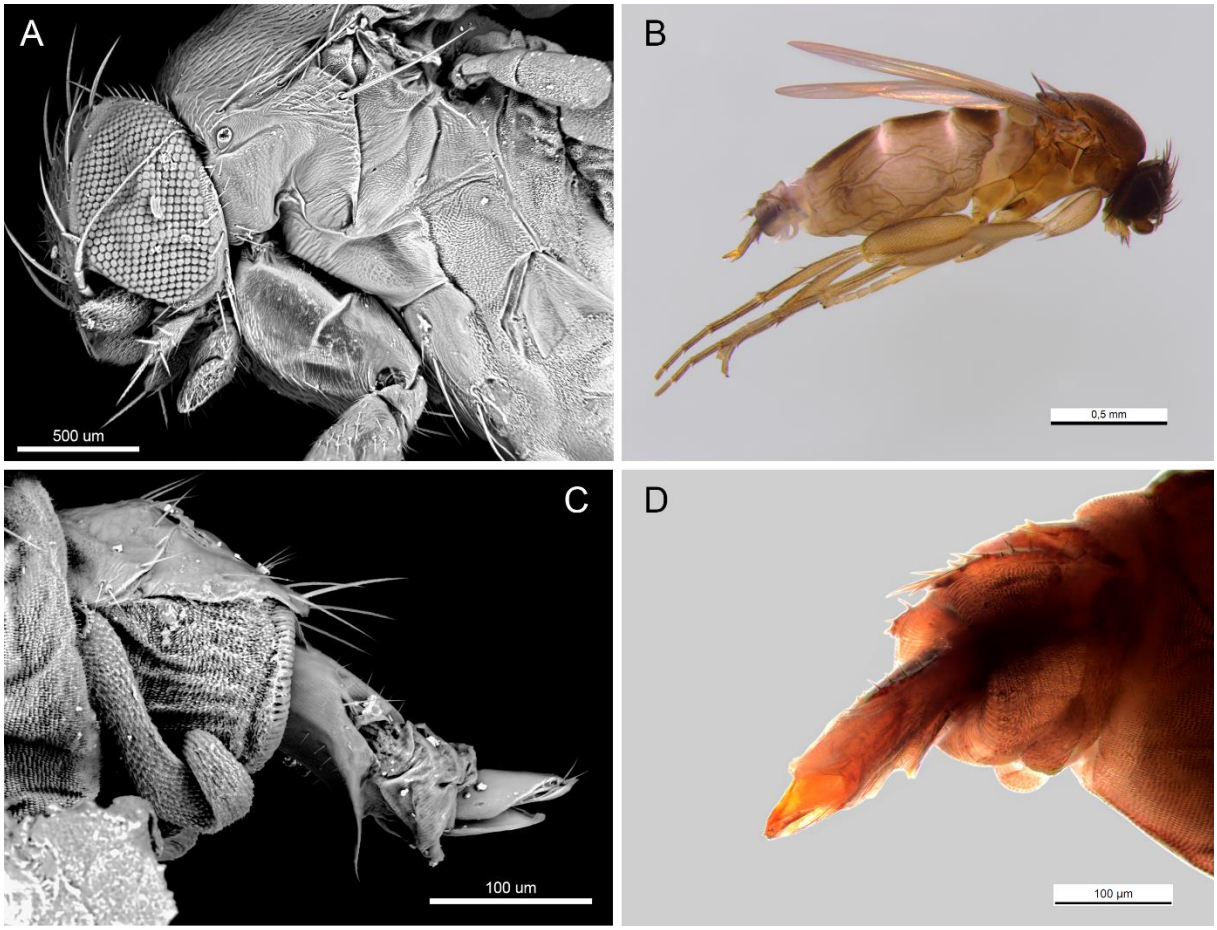

Figure 3. *Menozziola tanaitica* sp. n. female: A - head and thorax lateral view; B - lateral view; C, D - ovipositor.

Differential diagnosis. In the key of Gadau & Disney (1996) it runs to *M. schmitzi* (Menozzi), but the epandrium has more hairs and likewise the anal tube. It differs from the not described species of *Menozziola* from Turkey by a much smaller wing size, a larger costal index, smaller costal cilia and a different costal ratio.

Etymology. The species is named after the Greek name of the Don River (Τάναϊς), in the floodplain of which the type material was obtained.

Discussion

As a result of the conducted research, for the first time in Russia in the lower reaches of the Don River, the fact of parazitisation of phorids on ants was established. The discovery of *C. vagus* queens infected with larvae of scuttle flies here expands modern ideas about the biotic relationships of ants in the south of the European part of Russia with representatives of the order Diptera. Probably, within the study area, the infection of ants with Phoridae larvae is local in nature. During the five-year period of mass

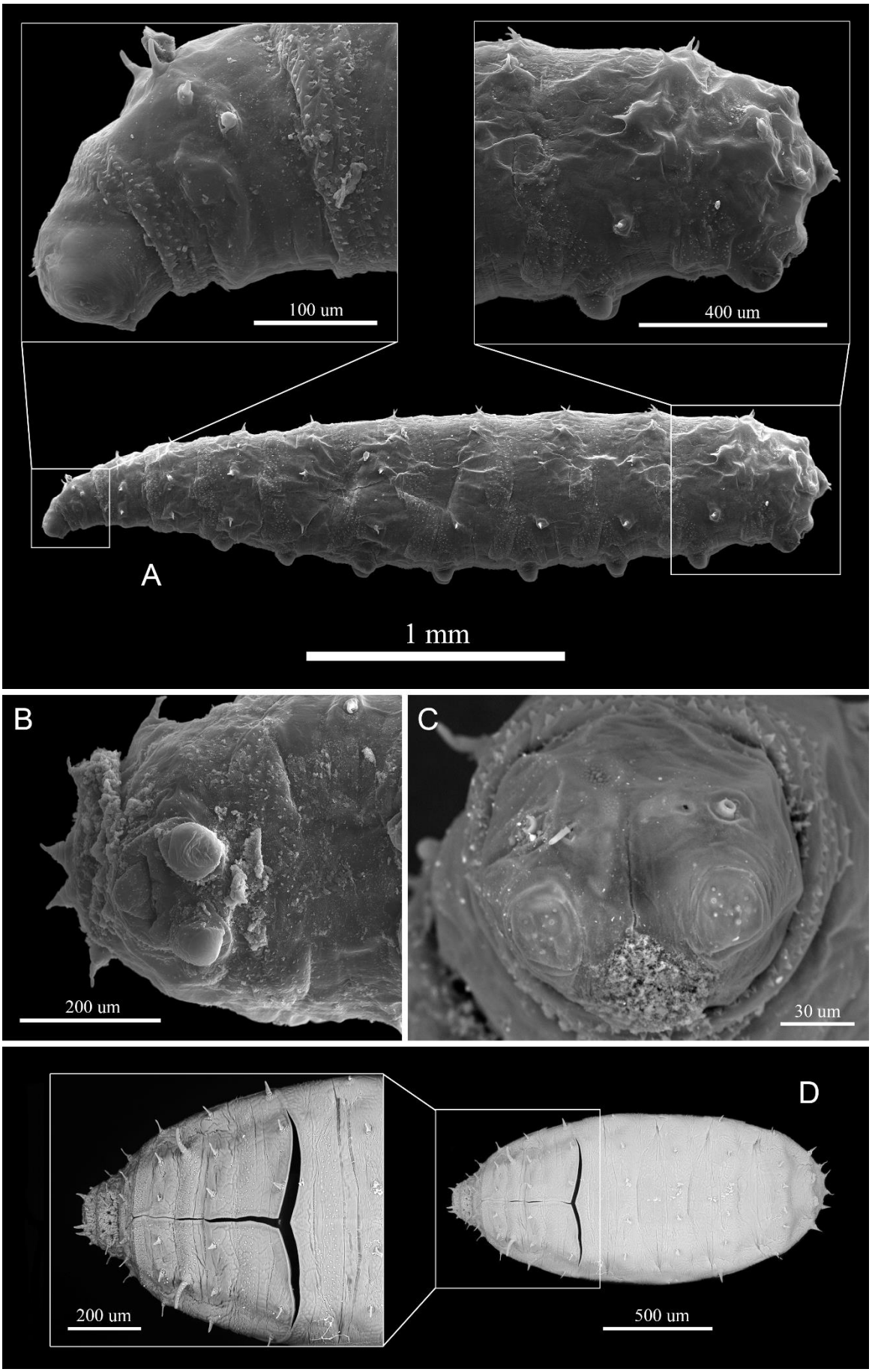

Figure 4. *Menozziola tanaitica* sp. n.: A-C - larva; D - puparium after emerging of the fly.

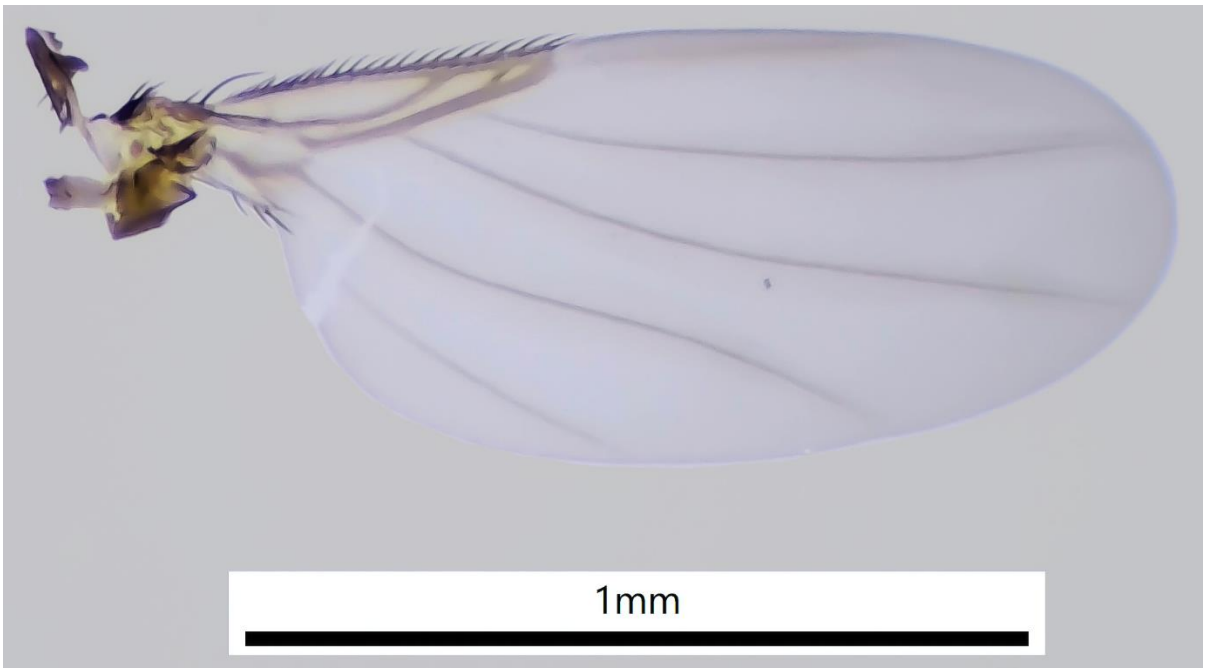

Figure 5. *Menozziola tanaitica* sp. n. wing, male.

collection of *C. vagus* queens in the Rostov urban agglomeration and in adjacent areas, only one case of ant infection has been found.

Obviously, ants are not available for parasitoids to enter them at all periods of their life. The observations suggest that mated *C. vagus* queens are available for infection after the end of the nuptial flight. Having descended to the ground, they spend 2-5 hours searching for shelter under the bark of a tree, in the decaying wood of which they make deep passages with the help of powerful mandibles. Queens moving in search of shelter in open spaces become convenient targets for attack by Phoridae parasitoids. Therefore, among the *C. vagus* caught immediately after the end of the mating at the location of the scuttle flies, there were no infected individuals. Ants who had made a "journey" in search of shelter turned out to be infected.

Experiments with the infection of ants with phorid parasitoids conducted by Duran (2012) shows that the attack of a fly on an ant takes a very short time. The fly sits on the queen's abdomen and inserts its ovipositor between the sternites. Obviously, in a limited time, she is able to inject only a small number of eggs into her victim's body.

However, in our case, several dozen fly larvae emerged from the abdomen of the dead *C. vagus* queens. To explain this phenomenon, two assumptions can be formulated: 1 - each ant is attacked by several dozen female flies; 2 - natural cloning of the phorid zygote occurs in the ant's body (the zygote splits into several cells, each of which turns into a separate egg). However, the presence of females and males who came from the same female makes us lean towards the second version.

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