

***Aprostocetus neglectus* (Hymenoptera: Eulophidae)
is the only species of Tetrastichinae wasps parasitizing
the ladybird beetle *Adalia bipunctata*
(Coleoptera: Coccinellidae)**

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ABSTRACT. This paper presents the morphological characteristics of *Aprostocetus neglectus* wasps that emerged from the pupae of *Adalia bipunctata* and *A. decempunctata* ladybirds. According to literature, 3 species of Tetrastichinae wasps (*A. neglectus*, *Oomyzus scaposus*, and *Tetrastichus epilachnae*) can parasitize in *A. bipunctata* pupae; however, our findings indicate that *A. neglectus* parasitizes exclusively in *A. bipunctata*. It is a gregarious parasitoid that usually infects 2.3–6.9% of *A. bipunctata* ladybird's pupae. Between 2 and 26 (typically 11–16) imagoes of *A. neglectus* can emerge from one pupa of *A. bipunctata*. The diversity of the mitochondrial DNA (mtDNA) of *A. neglectus* has also been studied, finding that genetic distances for the *cox1* gene ranged from 0.2 to 4.9%, a range indicative of subspecies or close related species. Given the high mtDNA polymorphism observed across all studied populations of *A. neglectus*, there is no justification for distinguishing subspecies. We identified 26 mitochondrial haplotypes, yet found no haplotypes common across different populations suggesting reproductive isolation among *A. neglectus* populations. Imagoes of *A. neglectus* across the surveyed regions are infected with the symbiotic bacterium *Wolbachia*.

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KEY WORDS: parasitism, mitochondrial DNA polymorphism, gene flow, *Wolbachia*.

***Aprostocetus neglectus* (Hymenoptera: Eulophidae)
— единственный вид наездников-тетрастихин,
паразитирующий на коровке *Adalia bipunctata*
(Coleoptera: Coccinellidae)**

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РЕЗЮМЕ. Дана морфологическая характеристика наездников *Aprostocetus neglectus*, выведенных из куколок коровок *Adalia bipunctata* и *A. decempunctata*. Согласно литературным данным, в куколках *A. bipunctata* могут паразитировать три вида наездников-тетрастихин (*A. neglectus*, *Oomyzus scaposus* и *Tetrastichus epilachnae*), однако наши данные свидетельствуют, что в *A. bipunctata* паразитирует исключительно *A. neglectus*. Это гregarный паразитоид, инфицирующий обычно 2,3–6,9% куколок коровок *A. bipunctata*. Из одной куколки *A. bipunctata* может быть выведено от 2 до 26 (обычно 11–16) имаго *A. neglectus*. Изучено разнообразие митохондриальной ДНК (мтДНК) *A. neglectus*, значения генетических расстояний по гену *cox1* варьируют от 0,2 до 4,9%, достигая уровня, характерного для подвидов или близких видов. Поскольку высокие значения полиморфизма мтДНК наблюдаются во всех изученных популяциях *A. neglectus*, то нет оснований для выделения подвидов. Нами описано 26 митохондриальных гаплотипов. Однако гаплотипов, общих для разных популяций, не было найдено, что указывает на репродуктивную изоляцию популяций *A. neglectus*. Имаго *A. neglectus* по всему изученному нами ареалу инфицированы симбиотической бактерией *Wolbachia*.

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КЛЮЧЕВЫЕ СЛОВА: паразитизм, полиморфизм митохондриальной ДНК, поток генов, *Wolbachia*.

Introduction

Aprostocetus (Westwood, 1833) is among the largest genera of the Tetrastichinae subfamily (Hymenoptera: Eulophidae), with a worldwide distribution. *Aprostocetus* wasps parasitize a diverse range of hosts including mites and insects such as butterflies, beetles, hemipterans, dipterans, hymenopterans, cockroaches, dragonflies, orthopterans, and thrips. Notably, parasitization by *A. neglectus* (Domenichini, 1957) and *Aprostocetus* sp. from Brazil on ladybird beetles (Coleoptera: Coccinellidae), has been documented (Graham, 1987; Togni *et al.*, 2015).

Aprostocetus neglectus is found across Europe (France, Germany, Czech Republic, Austria, Hungary, Italy, Moldova, Ukraine, European part of Russia, Georgia), North Africa (Morocco) and Asia (Turkey, Israel, Iran, Tajikistan, Pakistan, Korea, Russia (Primorye Territory)) (Trjapitzin, Kostjukov, 1978; Graham, 1987; Storozheva *et al.*, 1995; Muštu, 2010). *A. neglectus* was also introduced to North America during the introduction of the ladybird *Chilocorus kuwanae* (Silvestri, 1909) from Korea and Japan to the USA, first discovered in Delaware and Maryland in 1988–1989, and subsequently in New Jersey and North Carolina (Hendrickson *et al.*, 1991; Nalepa *et al.*, 1993; LaSalle, 1994; Matadha *et*

al., 2003). As a gregarious endoparasitoid with a koinobiont larva (Kosheleva, Kostjukov, 2014) *A. neglectus* targets several ladybird species including *Chilocorus bipustulatus* (Linnaeus, 1758), *C. bijugus* (Mulsant, 1853), *C. kuwanae*, *Exochomus quadripustulatus* (Linnaeus, 1758), *Scymnus subvillosus* (Goeze, 1777), and *Adalia bipunctata* (Linnaeus, 1758) (Graham, 1987; Romanov, Matveikina, 2022). The parasitization of *A. neglectus* in *Coccinella septempunctata* (Linnaeus, 1758) (Richerson, 1970; Graham, 1987) refuted by V.P. Semyanov (Semyanov, 1981; Shaefer, Semyanov, 1992). Females of *A. neglectus* lay eggs in the larvae of ladybird beetles during their 3rd and 4th instars. Infected larvae of the ladybird pupate and subsequently die, with *A. neglectus* larvae development to the imago stage ranging from 15 to 32 days, temperature dependent (the higher the temperature, the faster the development). Imagoes of the parasitoid wasps exit the host pupa through a dorsal opening (Ceryngier *et al.*, 2012).

Adalia bipunctata is a holarctic species found in Western Europe, North Africa, Russia (European part, Caucasus, Siberia, Far East), West Asia, west part of South Asia, Central Asia, north part of East Asia, and North America. *A. decempunctata* (Linnaeus, 1758) is distributed in Europe to the Ural Mountains, North Africa,

Table 1. Sampling of *Adalia* ladybird pupae and their infestation by *Aprostocetus neglectus* parasitic wasps.

Таблица 1. Сборы куколок божьих коровок рода *Adalia* и их заражённость наездниками *Aprostocetus neglectus*.

Location	Sampling's time	Host's species	Ladybird pupae		Infestation, %
			collected	infected	
Vologda	June 2022	<i>A. bipunctata</i>	517	16	3.1±0.76
Yaroslavl	July 2023	<i>A. bipunctata</i>	232	16	6.9±1.66
Moscow	June – August 2020	<i>A. bipunctata</i>	701	28	4.0±0.74
		<i>A. decempunctata</i>	118	3	2.5±1.44
	June 2021	<i>A. bipunctata</i>	192	9	4.7±1.53
	June – July 2022	<i>A. bipunctata</i>	180	7	3.9±1.44
	June – July 2023	<i>A. bipunctata</i>	385	9	2.3±0.76
Yalta	June 2018	<i>A. bipunctata</i>	24	5	20.8±8.28

West Asia, and west part of South Asia. Both species are aphidophagous, and inhabit trees, shrubs, and sometimes grasses (Kuznetsov, 1991; Zakharov, 2023).

The aim of this study was to identify parasitic wasps emerging from *A. bipunctata* and *A. decempunctata* pupae collected across several European Russian cities, and to clarify the list of host species for Tetrastichinae wasps.

Materials and methods

The imagoes of *Aprostocetus neglectus* were obtained from the pupae of the ladybird beetles *Adalia bipunctata* and *A. decempunctata*. The sampling of ladybird pupae was conducted in urban plantations across several locations: in Vologda (Oktyabrskaya St., Torgovaya Square, Prechistenskaya Embankment, Veterans of Labour Park, Tchaikovsky Square), during 2022, Yaroslavl (Sovetskaya St., Pervomayskaya St., Bolshaya Oktyabrskaya St., Krasnoperekopskaya St.) (2023), Moscow (Saranskaya St., Privolnaya St., Zhulebinsky Boulevard, General Kuznetsov St., Gubkin St., Fersman St.) (2020–2023), and Yalta (Lenin Embankment) in 2018 (Table 1).

In central Russia, a sampling of *Adalia* spp. pupae occurred in June on various plant species including bird cherry *Prunus padus*, *Philadelphus* sp., *Cotoneaster* sp., apple *Malus* sp., cherry *Cerasus* sp., during July–August — on lime trees *Tilia* sp.; in Yalta — on *Tamarix* sp. Ladybirds pupae attached to plant leaves were placed in plastic Petri dishes with a diameter of 2 cm until the imagoes of the parasitic wasps emerged. These imagoes of *A. neglectus* were preserved in 98% ethanol.

The study also incorporated samples of another *Aprostocetus* species — *A. xanthopus* (Nees, 1834) collected from the Kalacheyevsky district of the Voronezh Region in April 2022. Data on *A. xantho-*

pus were not available in the GenBank and BOLD databases.

Total DNA isolation from the imagoes of *A. neglectus* and *A. xanthopus* was performed using a phenol-chloroform extraction method following a standard protocol (Sambrook *et al.*, 1989).

The amplification reaction with each DNA preparation was conducted in a volume of 25 µl using the universal Encyclo Plus PCR kit (Evrogen, Russia) in accordance with the manufacturer's protocol. All PCR was performed on a MiniAmp Plus amplifier (Applied Biosystems, USA). Primers LCO1490 and HCO2198 were used to amplify a fragment of the mitochondrial *cox1* gene (Folmer *et al.*, 1994). Amplification conditions consisted of an initial denaturation at 94 °C for 4 minutes, followed by 5 cycles of denaturation at 94 °C for 20 s, annealing at 45 °C for 20 s, and polymerization at 72 °C for 40 s, then 30 cycles of denaturation at 94 °C for 20 s, annealing at 55 °C for 20 s, and polymerization at 72 °C for 40 s. PCR was completed by final polymerization for 5 min at 72 °C. The ITS2 region was amplified using primers complementary to the 5.8S and 28S rRNA (Porter, Collins, 1991). PCR conditions for these two primers sets were: initial denaturation at 94 °C for 4 min; then 35 cycles: denaturation at 94 °C for 20 s, annealing at 55°C for 20 s, and polymerization at 72 °C for 40 s; final polymerization at 72 °C for 5 min. The search for symbiotic bacteria *Wolbachia* was carried out by the *ftsZ* gene with *ftsZ*-F1ms primers (5'-ATTATGGAGCATATAAAAAGATAG-3') and *ftsZ*-R1ms (5'-TCAAGCAATGGATTAGATAT-3') amplifying a fragment with a length of 524 bp, and by the *fbpA* gene with *fbpA*-F1ms primers (5'-GCT-GCTCCACTTGGTATGAT-3') and *fbpA*-R1ms (5'-CCACCAGAAAAACTACTATTC-3'), amplifying a fragment with a length of 509 bp. Amplification conditions as described for ITS2. Each pair of primers was applied separately.

The PCR results were analyzed by electrophoresis in 1.0% agarose gel. The elution of selected

DNA fragments from the gel was performed using a DNA extraction kit from agarose gels Cleanup Mini (Evrogen, Russia) in accordance with the manufacturer's instructions. The purified DNA fragments were transferred to the Syntol (Russia) for sequencing. The nucleotide sequences obtained as a result of sequencing were registered in the GenBank database under the numbers OR977965–OR977984 (for mtDNA) and PP516378–PP516379 (for ITS2). The mitochondrial haplotypes of *A. neglectus* H1–H6 obtained earlier were registered in GenBank under the numbers OL889903–OL889908. Haplotypes of *A. xanthopus* are registered in GenBank under the numbers OR977985–OR977986.

Chromatograms of nucleotide sequences were analyzed using the DNASTAR Lasergene 6 software package (Clewley, 1995; Burland, 2000).

The calculation of genetic distances (according to the Kimura model) and the creation of phylogenetic trees (using the Neighbor-Joining method, the Kimura evolutionary model) were carried out in the MEGA11 program (Tamura *et al.*, 2021).

To construct a common dendrogram, sequences of the mitochondrial gene *cox1* of all representatives of the genus *Aprostocetus*, identified to species level, were used in the GenBank and BOLD databases.

Photos of imagoes parasitoids were performed using a Canon EOS 70D digital camera mounted on an Olympus SZX10 microscope (Zoological Institute of the Russian Academy of Sciences, St. Petersburg, Russia). Some parts of the samples were placed in Canadian balsam and their photographs were taken using a modular stereomicroscope ZEISS SteREO Discovery.V12 and AxioCam MRc3 cameras (All-Russian Institute of Plant Protection, St. Petersburg, Pushkin, Russia).

Results

Systematics

Order Hymenoptera Linnaeus, 1758
 Suprafamily Chalcidoidea Latreille, 1817
 Family Eulophidae Westwood, 1829
 Subfamily Tetrastichinae Foerster, 1856

Aprostocetus neglectus (Domenichini, 1957)

Fig. 1–6.

DIAGNOSIS. The body is black, without yellow patches. The marginal vein of the forewing is 3.9–4.2 times longer than the radial vein. The pivoting segment of the antennae is equal to or slightly longer than the first segment of the antennal flagellum. The thorax is 1.5 times longer than its width. The shield of the mesonotum is without a longitudinal median furrow,

with a row of 3–4 bristles along the inner side of each parapsidal furrow. The scutellum of the mesonotum is convex, the median furrows are weak. The forewings are about 2 times longer than their width; submarginal vein of the forewing has 4–5 bristles on the dorsal side; the marginal vein is 3.9–4.2 times longer than the radial vein, with 10–12 marginal bristles; the length of the postmarginal vein ranges from 0.3–0.5 of the length of the radial vein. The abdomen is short-oval, 1.2–1.5 times longer than its width, rather flat, equal to or slightly shorter than the total length of the head and thorax; the last tergite of the abdomen is 2–3 times wider than its length, the sheath of the ovipositor almost protrudes. Body length 1.2–1.5 mm.

ECOLOGICAL ROLE.

Most eulophids perform a useful function, since their hosts are phytophages — agricultural and forestry pests. However, some species of this family have a negative effect, since they parasitize beneficial insects, including *A. neglectus*, which reduces the beneficial activity of predatory ladybirds.

The infection rate of *A. bipunctata* pupae by *A. neglectus* in the central part of Russia (Vologda, Yaroslavl, and Moscow) varies from 2.3 to 6.9% (Table 1). In the Crimea, this value is higher and can reach up to 20.8%; however, it is considered unreliable due to the small sample size (Table 1).

From 2 to 26 imagoes of *A. neglectus* can emerge from a single pupa of *Adalia* ladybirds (Table 2).

Both males and females are present among the imagoes of *A. neglectus* that emerged from a host pupa, but males are fewer in number. In two cases, the male and female imagoes were recorded: 1) 10 females and 1 male (pupa of *A. bipunctata*, Moscow, 2023), 2) 19 females and 5 males (pupa of *A. bipunctata*, Yaroslavl, 2023). Females and males are able to copulate with each other almost immediately after leaving the pupa of a ladybird.

Typically, 11 to 16 parasitoid imagoes emerge. The eclosion of more than 18 *A. neglectus* imagoes from one pupa of the host (since such events are infrequent) may result from the laying of different numbers of eggs in the larva of the host by one female, and from superparasitism (infection of a single host by multiple females of the same species). To investigate these hypotheses, 5 imagoes were selected from *A. neglectus* broods, comprising 16–18 individuals (sampling in 2020, Moscow) and 19–26 individuals (sampling in 2023, Yaroslavl). DNA was isolated from imagoes and PCR with primers for the *cox1* gene was carried out to determine their mitochondrial haplotypes. Since offspring inherit mitochondrial DNA from the mother, identifying identical haplotypes in all imagoes from the same host pupa supports the first hypothesis, while identifying different haplotypes supports the second. In only one case (a brood of 21 *A. neglectus* imagoes in Yaroslavl), carriers of two different haplotypes (H21 and H22) were found among the descendants.

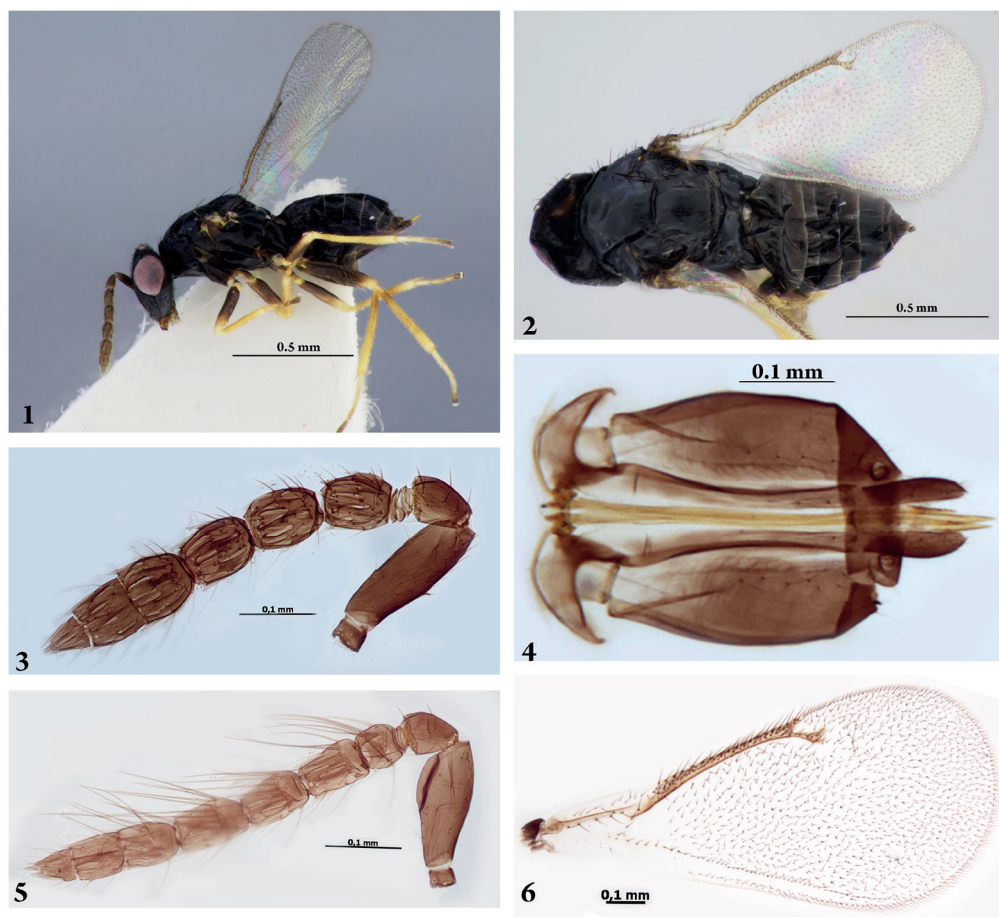


Fig. 1–6. *Aprostocetus neglectus* (Domenichini, 1957), habit view and details of the morphology. 1–2 — the female’s habit: 1 — lateral view; 2 — dorsal view; 3 — the female’s antenna, 4 — the female’s genitals; 5 — the male’s antenna; 6 — the female’s forewing.

Рис. 1–6. *Aprostocetus neglectus* (Domenichini, 1957), общий вид и детали строения. 1–2 — габитус самки: 1 — вид сбоку; 2 — вид сверху; 3 — антенна самки, 4 — гениталии самки; 5 — антенна самца; 6 — переднее крыло самки.

Nonetheless, the high diversity of mitochondrial haplotypes in *A. neglectus* implies that finding identical haplotypes in imagoes from other broods indicates their origination from a single female.

All *A. neglectus* imagoes from Yalta, Moscow, Yaroslavl, and Vologda are infected with the same line of the *Wolbachia* bacterium from supergroup A. The results of multilocus typing of this *Wolbachia* line by the *gatB*, *coxA*, *hcpA*, *ftsZ*, and *fbpA* genes were performed by us earlier (Shaikevich, Romanov, 2023) and are not presented in this work. The *Wolbachia* bacterium has not been detected in *A. xanthopus*.

MITOCHONDRIAL DNAPOLYMORPHISM

In total, 26 mitochondrial haplotypes were identified in *A. neglectus* imagoes that emerged from 28

different ladybird pupae with H1 found twice and H11 three times). Of these, H1 and H2 haplotypes were found in Yalta; H3–H8 and H24–H26 were found in Moscow; H11–H23 were found in Yaroslavl, and H9, H10 — in Vologda. No haplotypes common to samples from different cities were identified (Table 3).

The intrapopulation diversity in *A. neglectus* is very high; the number of identified haplotypes nearly matches the number of tested broods of the parasitoid, suggesting a uniform distribution of haplotype frequencies in populations.

The values of genetic distances in *A. neglectus* ranged from 0.2 to 4.9% (Table 3), with a value of 4.8% recorded in parasitoids collected from neighboring trees along the same street (haplotypes H13 and H16

Table 2. The number of *Aprostocetus neglectus* imagoes emerged from a single pupa of *Adalia* ladybirds.
Таблица 2. Количество имаго *Aprostocetus neglectus*, выведенных из одной куколки коровок
рода *Adalia*.

Number of <i>A. neglectus</i> imagoes	Number of such events	Ladybird species	Location
2	2	<i>A. bipunctata</i>	Moscow, 2020
4	1	<i>A. bipunctata</i>	Moscow, 2020
5	2	<i>A. bipunctata</i>	Moscow, 2020
	1	<i>A. decempunctata</i>	Moscow, 2020
6	1	<i>A. bipunctata</i>	Moscow, 2020
7	1	<i>A. bipunctata</i>	Moscow, 2020
8	3	<i>A. bipunctata</i>	Moscow, 2020
	1	<i>A. decempunctata</i>	Moscow, 2020
9	1	<i>A. bipunctata</i>	Moscow, 2020
10	2	<i>A. bipunctata</i>	Moscow, 2020
	1	<i>A. bipunctata</i>	Moscow, 2023
11	4	<i>A. bipunctata</i>	Moscow, 2020
	1	<i>A. decempunctata</i>	Moscow, 2020
	1	<i>A. bipunctata</i>	Moscow, 2023
	1	<i>A. bipunctata</i>	Yaroslavl, 2023
12	1	<i>A. bipunctata</i>	Moscow, 2020
13	2	<i>A. bipunctata</i>	Moscow, 2020
	1	<i>A. bipunctata</i>	Moscow, 2023
	6	<i>A. bipunctata</i>	Yaroslavl, 2023
14	1	<i>A. bipunctata</i>	Yaroslavl, 2023
15	4	<i>A. bipunctata</i>	Moscow, 2020
16	2	<i>A. bipunctata</i>	Moscow, 2020
	2	<i>A. bipunctata</i>	Yaroslavl, 2023
17	1	<i>A. bipunctata</i>	Yaroslavl, 2023
18	2	<i>A. bipunctata</i>	Moscow, 2020
	1	<i>A. bipunctata</i>	Yaroslavl, 2023
19	1	<i>A. bipunctata</i>	Yaroslavl, 2023
21	1	<i>A. bipunctata</i>	Yaroslavl, 2023
24	1	<i>A. bipunctata</i>	Yaroslavl, 2023
26	1	<i>A. bipunctata</i>	Yaroslavl, 2023

from Yaroslavl, Sovetskaya St.). At the same time, the sequences of the second internal transcribed spacer ITS2 in all individuals of *A. neglectus* were identical, affirming their classification as a single species.

A comparison of *A. neglectus* with other representatives of the genus *Aprostocetus* revealed that *A. venustus* and *A. gala* were the closest species among those analyzed (Fig. 7).

Discussion

According to literary sources, *Adalia bipunctata* ladybirds are considered hosts for three species of Tetrastichinae wasps: *Aprostocetus neglectus*, *Oomyzus scaposus* (Thomson, 1878), and *Tetrastichus epilachnae* (Giard, 1896) (Richerson, 1970; Romanov, Matveikina, 2022). However, we analyzed the parasitic wasps that emerged from 93 *Adalia* pupae collected in different locations, and all of them belonged to the single species *A. neglectus*.

Parasitization of *A. neglectus* on *Adalia bipunctata* was previously documented only once in Dresden in July 1967. Out of 1,111 collected pupae, 103 pupae were infected by a parasitoid (9.3±0.87%). The minimum number of *A. neglectus* imagoes that emerged from a single pupa of the host was 3, while the maximum was 14 (Klausnitzer, 1969). Our observations align with Klausnitzer's data, serving to complement them.

Infection of *A. bipunctata* pupae by *O. scaposus* parasitoids has been noted in several studies (Iperti, 1964; Lipa, Semyanov, 1967). However, when sampling pupae of different coccinellid species (*A. bipunctata* and *Harmonia axyridis* (Pallas, 1773) in Feodosia, *A. bipunctata* and *C. septempunctata* in Moscow) on the same or neighbouring plants, we never observed parasitization of *O. scaposus* on *A. bipunctata*, unlike with the other two species of ladybirds (Romanov, 2018; Romanov, Matveikina, 2022).

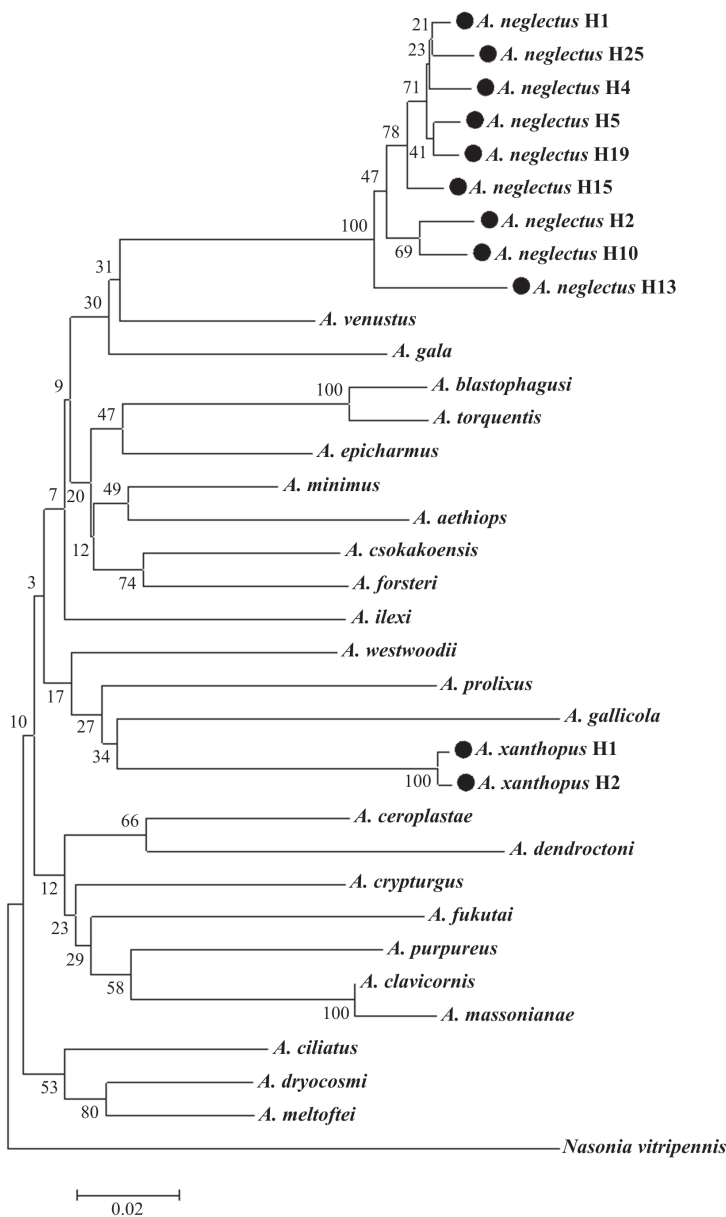


Fig. 7. Comparison of *Aprostocetus neglectus* with other species of the genus *Aprostocetus*. To construct the dendrogram, nucleotide sequences from the BOLD database (Barcode of Life Database) of all *Aprostocetus* wasps, identified to species level, were used. The nucleotide sequences marked with black circles were obtained by the authors. *Nasonia vitripennis* sequence was used as an external group. A fragment of the *cox1* gene with a length of 590 bp was used to build the tree.

Рис. 7. Сравнение *Aprostocetus neglectus* с другими видами рода *Aprostocetus*. Для построения дендрограммы были использованы нуклеотидные последовательности из базы данных BOLD (Barcode of Life Database) всех ос рода *Aprostocetus*, определенных до вида. Нуклеотидные последовательности видов, отмеченных чёрными кружками, были получены авторами. В качестве внешней группы использована последовательность *Nasonia vitripennis*. Для построения дерева использовался фрагмент гена *cox1* размером 590 п.н.

Since the morphological definition of Tetrastichinae parasitoids is difficult, earlier information (Iperti, 1964; Lipa, Semyanov, 1967) may have been incorrect. It is obvious that the selectivity of host selection observed for *A. neglectus* and *O. scaposus* parasitic wasps is not random and indicates a much narrower specialization of these parasitoids than previously assumed.

The parasitization of *T. epilachnae* on *A. bipunctata* was mentioned in the works of European researchers published in 1924 and 1943 (Richerson, 1970), when *A. neglectus* had not yet been described, so this information cannot be entirely reliable. *T. epilachnae* has been identified as a parasitoid of herbivorous ladybird beetles from the Epilachnini tribe (*Henosepilachna argus* (Geoffroy in Fourcroy, 1762), *Subcoccinella vigintiquatuorpunctata* (Linnaeus, 1758)), with members of this tribe remaining its primary hosts (Hansson, Schmidt, 2020).

We noted 8 events out of 50 when the number of *A. neglectus* imagoes emerging from the ladybird's pupa exceeded 16 individuals (Table 2). However, in only one case it was the result of eggs being laid by different females. It has been noted that *O. scaposus* females can lay eggs up to three times in the same host (Semyanov, 1986). Consequently, the females of *A. neglectus* exhibit similar behavior. Even in the case of superparasitism, where a simultaneous emergence of parasitoids occurs, it can be assumed that the "instinct error" of females has very narrow boundaries: they cannot distinguish between a larva of a ladybird that was infected only a few hours earlier and an uninfected one.

Mitochondrial DNA sequence analysis is widely used in phylogenetic studies and often allows the identification of cryptic species (Gokhman, 2018). In various families of parasitic Hymenoptera, there have been descriptions of individuals with differing chromosome numbers within populations that appear identical (Gokhman, 2005), indicating that genetic and morphological divergence levels can be unequal. The mtDNA polymorphism we discovered in *A. neglectus* (based on the *cox1* gene sequences) reaches values that are typically indicative of subspecies and twin species (Kartavtsev, 2013). However, given the absence of differences in the nucleotide sequences of the ITS2 spacer, and considering that significantly different mitochondrial haplotypes (e.g., H1 and H2 in

Yalta, H9 and H10 in Vologda, H13 and H16 in Yaroslavl) occur in imagoes sharing the same habitats and parasitizing the same host species, we see no basis for distinguishing subspecies.

It should also be noted that we have not identified haplotypes common to different populations. This observation suggests that populations of *A. neglectus* occupy small areas with no gene flows between them, essentially making them isolates — populations with complete reproductive isolation from other populations of the same species. The absence of gene flows leads to an increase in population polymorphism (Logue *et al.*, 2015; Oh, Oh, 2022). However, instances have been recorded where populations exhibit high levels of mtDNA polymorphism without reproductive isolation (Dai *et al.*, 2017). Thus, our hypothesis remains preliminary and requires further validation, potentially through the analysis of several nuclear genes.

The symbiotic bacterium *Wolbachia* can serve as an additional source of data used to identify species. *Wolbachia* in *A. neglectus* belongs to supergroup A, in *O. scaposus* belongs to supergroup B (Shaikevich, Romanov, 2023), and it is absent in *A. xanthopus*. The *Wolbachia* strain from *A. neglectus* proved to be very similar to that from one of two *Homalotylus* sp. This *Homalotylus* sp. is parasitized in *Chilocorus renipustulatus* (Scriba, 1791) and *Calvia quatuordecimguttata* (Linnaeus, 1758) ladybirds. In another *Homalotylus* sp., parasitizing in *C. septempunctata*, *Wolbachia* from supergroup B was found, which differs from *Wolbachia* of *O. scaposus* (Shaikevich, Romanov, 2023). Since *Wolbachia* often proved to be species-specific, the detection of identical bacterial strains in individuals that differ greatly in mtDNA will indicate that they belong to the same species (as in *A. neglectus*), and the identification of different strains of *Wolbachia* will indicate the presence of diverse species. For instance, two species of *Homalotylus* genus, with a genetic distance of 6%, calculated by the *cox1* gene, are infected with different strains of *Wolbachia*.

Conclusion

In the pupae of *A. bipunctata* and, possibly, *A. decempunctata* ladybirds, only *A. neglectus* among Tetrastichinae wasps have been observed to parasitize. This parasitoid does not pose a sig-

nificant threat to the populations of *A. bipunctata* and *A. decempunctata*, as it infects only a small fraction of individuals (2.3–6.9%). This limited infection rate may be attributed to the fact that *A. bipunctata* and *A. decempunctata*, belonging to the Coccinellini tribe, are not typical hosts for the development of *A. neglectus* larvae, which more commonly parasitize species from the Chilocorini tribe. The reproductive isolation of *A. neglectus* populations is underscored by the absence of shared mitochondrial haplotypes, likely contributing to the notable increase in intraspecific polymorphism, with the maximum genetic distance for the *cox1* gene reaching up to 4.9%. Both male and female *A. neglectus* are infected with the *Wolbachia* bacterium, though the significance of this infection for the species remains to be fully understood.

Conflicts of interest: The authors declare that they have no conflicts of interest.

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