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Reproductive behavior of *Urostylis annulicornis* Scott (Heteroptera, Urostylididae) in the southern Far East of Russia

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Abstract

The reproductive behavior of the oak bug *Urostylis annulicornis* Scott, 1874 was studied for the first time. This species lives in the crown of tall *Quercus mongolica* Fisch. ex Ledeb. trees making it difficult to access for study and observation. Our study aimed to elucidate the timing of mating and oviposition of *U. annulicornis* and the effect of temperature variations on the lifespan of adults in natural conditions. Nymphs grown from eggs that had overwintered on rhytidome fragments were placed in stationary netting cages installed on oak saplings, where the bugs were kept until adulthood. The behavior of the control pairs was monitored for 15 hours a day (from 06:00 to 21:00). The duration of an uninterrupted tandem during copulation was confirmed by additional observations at night. To facilitate egg-laying, artificial shelters were created from pieces of oak bark stacked together, attached to the stem of the plant, and placed inside cages. The number of eggs laid by the female was counted and photographed. The process of oviposition by a female on the oak tree bark in natural conditions was recorded and documented for the first time. This study of the biology of *U. annulicornis,* from oviposition through the sequence of postembryonic development, documenting all stages of metamorphosis, was carried out on a single plant. A summer reproductive diapause was observed in the seasonal cycle of the species. The timing of mating in *U. annulicornis* was shifted to autumn, the laying of overwintering eggs occurred in October, as night temperatures decreased. Males were observed performing postcopulatory mate-guarding. The effect of night cold spells on the lifespan of bugs was recorded: natural mortality of adults occurred when the night temperatures dropped below zero.

Key words: true bugs, Heteroptera, Urostylididae, *Urostylis annulicornis*, overwintering, ootheca, nymphs, Primorsky Krai, *Quercus mongolica*

Introduction

Urostylis annulicornis Scott (Urostylididae) is distributed in the south of the Russian Far East, with its range covering the Jewish Autonomous Oblast, the south of Khabarovsk Krai, and Primorsky Krai. In the east of Russia, the species is known from an area extending up to Kunashir Island, and is also recorded from northern and northeastern China, the Korean Peninsula, and Japan (Rider 2006; Kanyukova 2010). The oak *Quercus mongolica* Fisch. ex Ledeb. and *Q. dentana* Thunb., has been identified as the main forage plant for this species, however they are occasionally observed on other deciduous plants (Kerzhner 1966; Kanyukova 1988).

The species of the family Urostylididae live and develop in tree crowns and are therefore rarely observed, but in the summer months they occasionally appear more conspicuous when they gather together to migrate or are attracted to artificial lights. During mass breeding they can cause damage (Kuznetsov 2000). Species of this family exhibit a level of care for their offsprings: when laying eggs, the female coats them with a jelly-like secretion, which forms an ootheca that protects the eggs from drying out. In addition to protection against mechanical damage and bacteria, the gelatinous membrane of the ootheca is a nutritive substance, which contains symbiotic intestinal bacteria that is transferred to

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nymphs (Kobayashi 1965; Kaiwa *et al*. 2014; Ranade & Ghate 2023). In autumn, eggs are laid into bark crevices on the outside or on the inside of the detached bark (rhytidome) of the host plant, where the egg clutch overwinters.

We studied the timing of development of *U. annulicornis* nymphs reared from overwintered egg clutches under conditions in the southern Far East of Russia (Primorsky Krai), describing the oviposition, the sequence of molts of nymphs at all ages, their feeding at different instar stages, and the timing of ontogenetic development in the species (Kanyukova *et al*., 2023a). Prior to this, we carried out studies on ontogeny and a comparative analysis of development from clutches from two other species, *Urostylis trullata* Kerzhner, 1966 and *U. lateralis* Walker, 1867 (Kanyukova *et al*. 2023a, b; 2024).

This study aims to clarify the reproductive behavior of *U. annulicornis*, the timing of mating and oviposition, and the effect of temperature variations on lifespan in natural conditions. The effect of daylight duration on these processes was beyond the scope of our study.

Material and methods

Studies on the biology of bugs of the family Urostilididae were conducted in the south of Primorsky Krai (Russian Far East) from April 2022 to April 2024. In forest biotopes, we examined *Quercus mongolica* trees in order to estimate their occupancy by bugs, described and collected their egg clutches, and took photographs. For stationary observations, we collected pieces of rhytidome with clutches from oak trunks and placed them in portable netting cages in natural conditions until instar II–III nymphs emerged. We used water-soaked moss to maintain the humidity level (Kanyukova *et al*. 2023 a, b; 2024). Nymphs that reached instars II and III were transferred on fragments of rhytidome with egg mass residues to stationary cages (Markova *et al*. 2018) installed on one-meter tall oak saplings, where the bugs were kept until adulthood. Observations of the reproductive behavior of the control pairs were conducted during daylight hours for 15 hours per day (from 06:00 to 21:00). The duration of the uninterrupted tandem during copulation was confirmed by additional observations at night.

To facilitate egg-laying, we made artificial shelters of oak bark pieces, stacked together and attached to the trunk of this plant, and placed them into the cages (Fig. 1). The number of eggs laid by the female was counted and the objects were photographed.

In addition to our own collections, we also took into account data from the collection materials of the following institutions: Zoological Institute, Russian Academy of Sciences (St. Petersburg, ZIN); Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch, Russian Academy of Sciences (Vladivostok, FSC); and Zoological Museum, Far Eastern Federal University (Vladivostok, FEFU). In the text below, *m* represents the mean value.

Material. Russia. Primorsky Krai, Ussuriysky Urban Okrug, protected area of the Land of the Leopard National Park, Ussuriysky Nature Reserve (43°40′00″ N; 132°30′00″ E), Korean pine/broad-leaved forest.

A clutch was found in the forest under rhytidome of *Q. mongolica* on November 11, 2022 and left to overwinter in natural conditions (we attached the fragment to the trunk of this tree). In the spring of 2023, the ootheca was taken back from the forest for further observations of nymphal development. Since April 21, the study was carried out in a netting cage on a Mongolian oak in natural conditions (village of Kaymanovka).

Winged adults of *U. annulicornis* emerged from June 14 to 22, 2023 (10 males, 2 females) with a range of temperatures from +11.3 to +27.7°C (m = +19.5°C).

Results

We took control adults from the cage-reared *U. annulicornis* nymphs whose adult molt occurred on June 14, 2023. On June 20, 2023, we transferred a few bug specimens (3 males and 2 females) to a separate netting cage to observe their breeding behavior, where they were in an active state and fed on oak leaves. In the seasonal cycle of *U. annulicornis*, we found a summer breeding diapause, which lasted for about 2.5 months when the bugs did not exhibit mating behavior. Nevertheless, the bugs were active and fed intensively during this period.

Figure 1. Artificial shelter made from bark of *Quercus mongolica* Fisch. ex Ledeb., attached to the trunk in a netting cage under natural conditions (October 13, 2023).

Copulation and oviposition

We observed manifestations of breeding activity beginning from early September: on September 1 and 3, 2023, with a temperature range from +15.5 to +27.5°C (*m* = +20.8°C), the first cases of copulation were recorded. Copulation of *U. annulicornis* was in the opposed position (Honek & Martinkova 2019) or tail to tail (Socha & Zemek 2004) (Fig. 2A). During September, there was an increase in the mating duration. From the beginning to the middle of the month, the duration of uninterrupted tandem in pairs ranged from 2.5 to 7 h during the day at a temperature range from +8.6 to +27.5°C (*m* = +19.8°C). Since September 21, 2023, we transferred the copulating pairs to separate cages for detailed observation (1 male and 1 female in one cage and 2 males and 1 female in the other). From the middle of September, the duration of copulation reached 24–36 h at a temperature range from +2.3 to +24.4°C (*m* = +16.3°C). By the end of the third ten days of the month, the duration of uninterrupted tandem reached 96 h. A forced replacement of the male in the interval between copulations did not affect the subsequent process, which suggests that a change of partner in natural conditions may occur.

From October 12, 2023, single females began to move into the artificial shelters. On October 13, 2023, we recorded the beginning of oviposition at a temperature range in mid-October of +3.0 to +20.7°C (m = +11.7°C). With a decrease in night temperatures to –2°C at the end of the second ten days of October, mating couples also moved to shelters (Table 1; Fig. 3). We observed 2–3 egg clutches from each female with the number of eggs ranging from 15 to 43 in each. The time of laying of 15–26 eggs was stretched to 24 h, and a greater portion of eggs were laid within 1.5 days (see Table 1). During this time, the female stayed in the shelter, not leaving the clutch and not feeding (Fig. 4).

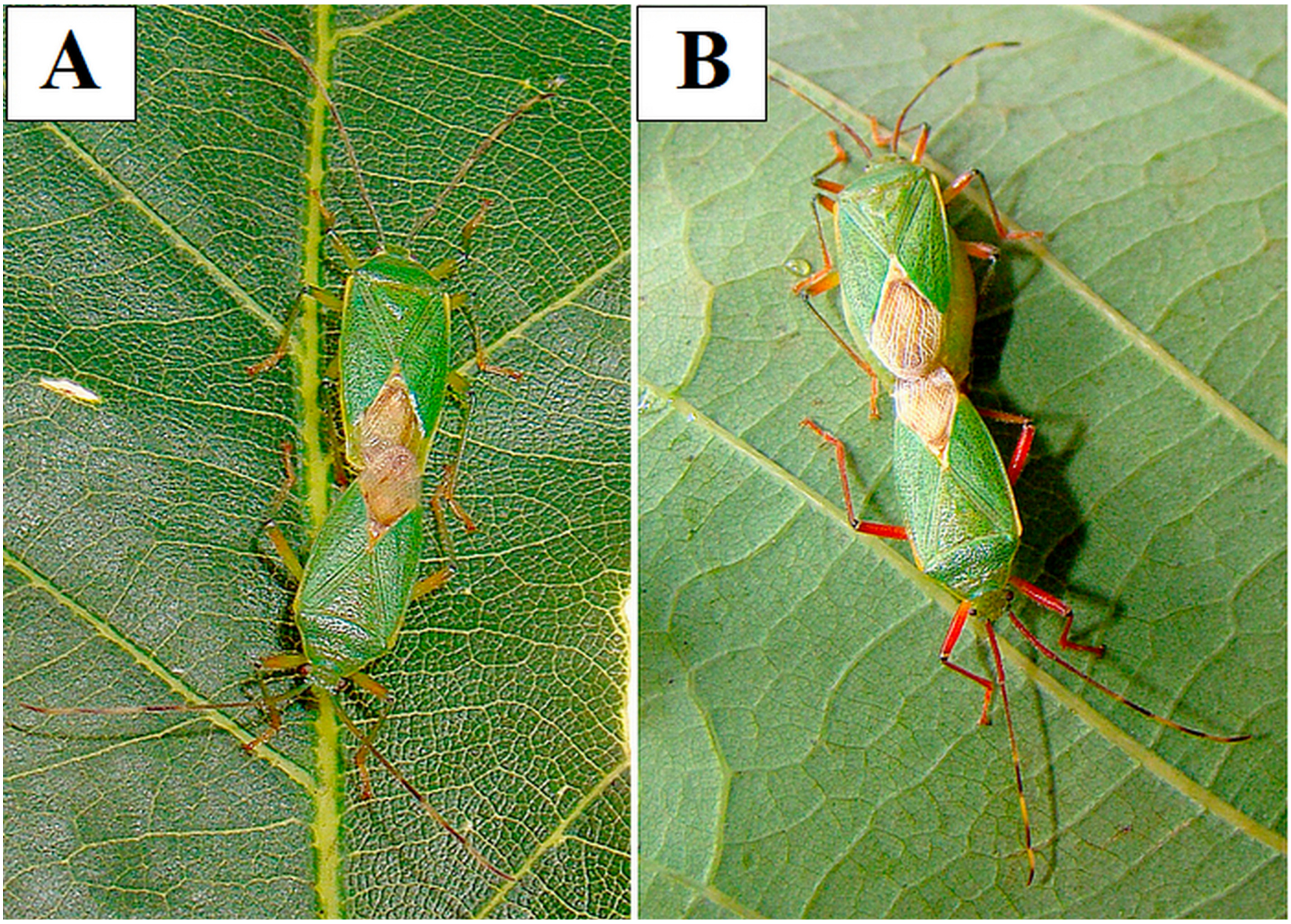

Figure 2. Changes in color pattern of *Urostylis annulicornis* Scott related to the breeding season: **A,** adult at the onset of mating season (September 3, 2023); **B,** marked change of color of appendages and first antennal segment and increase in size of female's abdomen (October 1, 2023).

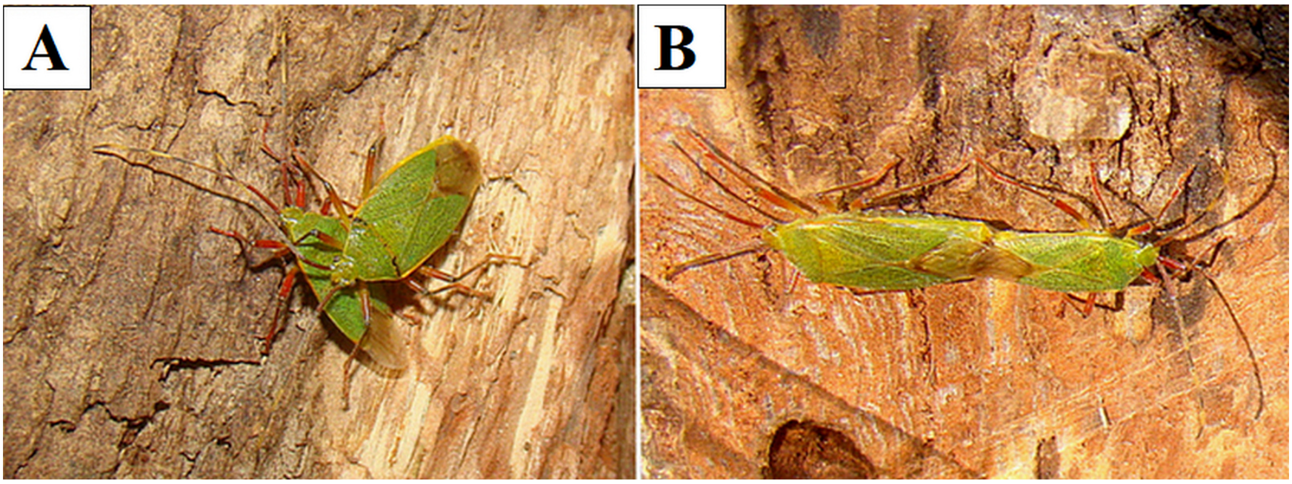

Figure 3. Reproductive behavior of *Urostylis annulicornis* Scott in shelters on *Quercus mongolica* Fisch. ex Ledeb. under natural conditions: **A**, males and females getting prepared to mate (October 17, 2023); **B,** a copulating pair (tandem) (October 21, 2023).

Table 1. Duration of mating in *Urostylis annulicornis* Scott with subsequent oviposition in stationary netting cages in natural conditions

Date	Pair 1		Pair 2		T (°C)
	Activity	Localization	Activity	Localization	Tn…Tx
Oct. 13, 2023	O (15)	Shelter (1♀), Netting cage (1♂)	–	Shelter (1♀), Netting cage (2♂)	+2.0…+23.4
Oct. 14, 2023	C	Netting cage (1♂, 1♀)	–	Netting cage (2♂, ♀)	+3.0…+20.7
Oct. 15, 2023	C_{cont} (36 h) O	Netting cage (1♂, 1♀), post-copulation Shelter (1♀)	C (24 h)	Netting cage (2♂, ♀)	+6.3…+19.0
Oct. 16, 2023	O_{cont} (43)	Post-oviposition Netting cage, – (1♂, 1♀)	O (17)	Post-oviposition Netting cage, – (2♂, 1♀)	–1.2…+13.5
Oct. 17, 2023	C	Shelter (1♂, 1♀)	C	Shelter (1♂, 1♀), Oak leaf (1♂)	–1.8…+15.7
Oct. 18, 2023	C_{cont}	Netting cage (1♂, 1♀)	C_{cont}	Netting cage (1♂, 1♀), Oak leaf (1♂)	+0.7…+16.9
Oct. 19, 2023	C_{cont} (64 h)	Netting cage (1♂,1♀)	C_{cont} (68 h)	Oak leaf (2♂,1♀)	+1.4…+15.5
Oct. 20, 2023	–	Netting cage (1♂, 1♀)	O	Shelter (1♀), Netting cage (1♂). death 1♂	–2.8…+10.1
Oct. 21, 2023	–	Shelter (1♀), Netting cage (1♂)	O_{cont} (32)	Shelter (1♀), Netting cage (1♂)	–3.0…+6.2
Oct. 22, 2023	–	Shelter (1♂,1♀)	–	Shelter (1♂, 1♀)	–0.4…+14.4
Oct. 23, 2023	C (16 h)	Oak leaf (1♂,1♀)	C	Oak leaf (1♂, 1♀)	–4.2…+15.9
Oct. 24, 2023	–	Oak leaf (1♂,1♀)	C_{cont} (36 h)	Netting cage (1♂,1♀)	+6.6…+16.6
Oct. 25, 2023	–	Oak leaf (1♂,1♀)	–	Oak leaf (1♂, 1♀)	+9.8…+18.7
Oct. 26, 2023	–	Oak leaf (1♂,1♀)	K	Netting cage (1♂, 1♀)	+3.4…+16.0
Oct. 27, 2023	O (26)	Shelter (1♀), Oak leaf (1♂)	C_{cont} (24 h)	Netting cage (1♂,1♀), death in copula	–4.5…+11.7
Oct. 29, 2023	–	Oak leaf, death (1♂, 1♀)	–	–	–6.0…+14.0
N	**84**		**49**		

Note. C—copulation; C_{cont}—continuous copulation (values in parentheses are duration in hours); O—oviposition (values in parentheses are number of eggs laid); O_{cont}—continuous oviposition; en-dash (–) means lack of breeding activity; N—number of eggs laid; T—air temperature at 2 m above ground; Tn—minimum air temperature for the past period; Tx—maximum air temperature for the past period (air temperature data were provided by the Timiryazevsky Meteorological Station, Russia).

The duration of uninterrupted tandem in the observed pairs decreased in October compared to September. From the beginning of the first ten days to the end of the second ten days of the month, it lasted from 24–68 h; in the third ten days of October, it decreased to 16–36 h (see Table 1). Mating pairs remained together until the natural death of individuals.

The period from the onset of breeding activity to egg ripening and the onset of oviposition lasted 1.5 months. In forest biotopes, we found adults in shelters under bark of oak trunks during the same period, on October 15 and November 11, 2022 their presence given away by a strong odor.

Color changes and feeding patterns of adults during the breeding season

By early October, we observed a pronounced change in the color of the appendages and the first antennal segment of *U. annulicornis*, this was more pronounced in males. In addition, the females showed a marked increase in the size of their abdomens, which was associated with the breeding season (see Fig. 2B). The bright red femurs of all the legs looked especially conspicuous and the green color of the upper body also became more intense and brighter compared to that at the beginning of the mating season.

Both males and females fed throughout the breeding season. The most common feeding areas were the bugs made punctures with their proboscises for sucking sap, were the primary veins, which perform a conductive function, on the abaxial side of leaf blades of the Mongolian oak. Congealing sap drops were clearly visible at the sites of damage. From the beginning of the second ten days of October, the breaks in feeding, during the period of oviposition and staying in shelters, lasted more than a day.

Figure 4. An egg clutch of *Urostylis annulicornis* Scott in a shelter in a netting cage on *Quercus mongolica* Fisch. ex Ledeb. under natural conditions (October 21, 2023).

The total lifespan and death of adults

The total lifespan of adult *U. annulicornis* in the south of the Russian Far East, according to the data from the collection materials, is approximately five months (160 days), from mid-June to mid-November. We did record an effect of nighttime cold spells on the activity and lifespan of bugs; nevertheless, the insects' activity was observed to increase during the daytime under sunlight and temperature values of +11.7 to +14.0°C. Death of the bugs was recorded in the mating state on October 27 in one pair and after oviposition on October 29 in another. According to our data, the natural mortality of adults occurred when night temperatures dropped to –4.5 to –6.0°C.

Conclusion

For *U. annulicornis*, we recorded a monovoltine seasonal cycle with two periods of rest: winter obligate diapause at the embryonic stage and summer breeding diapause (estivation). These adaptations, previously reported for certain species of the family Pentatomidae, shift the timing of oviposition to a more favorable season for overwintering eggs (Saulich & Musolin 2014). The adult diapause of the precopulation period of the control *U. annulicornis* individual lasts about 2.5 months. The timing of mating and oviposition in bugs are shifted to the end of the summer months. The breeding season begins in autumn, with the onset of nighttime cold spells in early September, and lasts until late October. In the field, we found active adults under bark of an oak trunk in forest biotopes until mid-November, where their presence could be detected by a specific strong odor.

Males perform postcopulatory mate-guarding, maintaining a tandem (pair) for a long period of time, thereby preventing the females from mating with other males. At the onset of the breeding season, the duration of time that male's guarded females increased. We also found that the mating activity (propensity to mate and the duration of the pair-bond) decreases with the age of bugs.

Laying of overwintering eggs begins after the first ten days of October, at a temperature range of +2.0 to +23.4°C, and occurs repeatedly. Females lays several clutches nearby to each other, without returning to the previous clutch. The oviposition process is stretched to 1–1.5 days depending on the number of eggs.

We have documented an effect of nighttime cold spells on the lifespan of bugs: natural mortality of adults occurred when night temperatures dropped to below-zero values, for example, –4.5°C.

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