**RESEARCH ARTICLE** 

# The first data on the nesting biology of the invasive blue nest-renting wasp, *Chalybion turanicum* (Gussakovskij, 1935) (Hymenoptera, Sphecidae, Sceliphrinae) in the Crimea

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#### Abstract

The nesting biology of *Chalybion turanicum* (Gussakovskij, 1935) has been studied, with a total of 31 nests being examined. All studied nests were located inside the old nest cells of *Sceliphron destillato-rium* (Illiger, 1807). Each nest of *Ch. turanicum* consisted of a single cell. Females hunted for spiders, with 18 species in five families being identified among their prey. Two most abundant victim groups were Theridiidae (eight species, 54% of specimens) and Araneidae (seven species, 33% of specimens) spiders. A spider number stored in a cell varied from five to 31 (mean = 17.6 ± 5.4). In the Crimea, *Ch. turanicum* has one generation per year with reproductive success of 67%. Two species of the nest parasites were reared from cells of *Ch. turanicum*: *Chrysis taczanovskii* Radoszkowski, 1876 and *Acroricnus seductor* (Scopoli, 1786). *Chalybion turanicum* is the seventh invasive species of Sphecidae naturalized in Europe.

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#### **Keywords**

Sphecid wasps, invasive species, bionomics, trophic relationships, prey, spiders, reproductive success, *Sceliphron destillatorium* 

#### Introduction

The apoid wasp genus *Chalybion* Dahlbom, 1843 is distributed in all zoogeographical realms and accounts for 49 extant species (Pulawski 2020), most of which are confined to tropical and subtropical regions. A few species of *Chalybion* have penetrated countries with the temperate climate, such as Russia, where just two native species occur: viz., *Chalybion japonicum* (Gribodo, 1882) in Primorskiy Territory and *Ch. walteri* (Kohl, 1889) in the North Caucasus (Danilov 2017). Similarly to other above-ground nesting Sphecidae (i.e., *Sceliphron* Klug, 1801 and *Isodontia* Patton, 1880), several species of *Chalybion* are known to have colonized new territories with the aid of man. For example, two species, i.e., the primarily Oriental *Chalybion bengalense* (Dahlbom, 1845) and the Nearctic *Ch. californicum* (de Saussure, 1867), were recently introduced to Europe (Mei et al. 2012, Mei and Boščík 2016).

Females of the majority of *Chalybion* species nest in various preexisting cavities: hollow stems, wood burrows, stone caverns, and abandoned or even operated nests of other hymenopterans, especially closely related genus Sceliphron. The mud is the principal material used for nest partitions and the plug, but instead of collecting already existing mud, the wasps carry water to a source of dry clay soil near their nests and then use it to dampen and plaster the soil (Bohart and Menke 1976, Iwata 1976, Kazenas 2001, Gess and Gess 2014, Pham 2018). In addition to mud, some species use a white material composed of either lime from whitewashed walls or uric acid obtained from bird or reptilian droppings (Jayakar and Spurway 1963, Barthélémy 2011, Pham 2019). Females of at least one species in the subgenus Hemichalybion Kohl, 1918 excavate nests in the non-friable soil of vertical banks with the aid of water (Gess et al. 1982). All species of the genus Chalybion hunt for spiders and store up to several tens of victims in each nest cell. The Chalybion species that are studied bionomically vary in nesting cavity preference, prey composition, number of generations per year, and other bionomical features, which could be important, for instance, the ability to colonize new territories. Thus, Chalybion spinolae (Lepeletier de Saint-Fargeau, 1845) nests in vertical banks and hunts exclusively for Black Widow spiders (the genus Latrodectus Walckenaer, 1805; Theridiidae) and therefore is not synanthropic (Gess et al. 1982, Nel et al. 2014), while some other species are known to nest exclusively in hollow stems of trap-nests and to hunt for Araneidae spiders (Ohl and Höhn 2011, Pham and Dang 2017). Other species hunt for various spiders with the preference to the families Araneidae or Theridiidae. Of them, Chalybion bengalense, Ch. japonicum, Ch. tibiale (Fabricius, 1781), and Ch. zimmermannii (Dahlbom, 1843) are versatile in the nesting substrate selection (Ward 1971, Barthélémy 2011, Gess and Gess 2014, Pham 2018, 2019), whereas Ch. californicum

nests almost exclusively in the *Sceliphron* nests (Rau 1928, Horner and Klein 1979, Landes et al. 1987). *Chalybion* is also one of the sphecid genera that commonly form "sleeping" aggregations, usually consisting of both sexes and sometimes even of two species (Landes and Hunt 1988, Pham 2020).

Chalybion turanicum (Gussakovskij, 1935) is a Palaearctic species native to Iran, Turkmenistan, Uzbekistan, Kyrgyzstan, Tajikistan, and southern and south-eastern Kazakhstan (Kazenas 2001, Danilov 2017). It was accidentally introduced to Russia, where two female individuals were first collected in 2010 from Altai Territory (Baghirov 2011). The next record was made in 2019 from the Crimea, again based on two females (Mokrousov et al. 2019). Little is known about the bionomics of *Ch. turanicum*. Kazenas (2001) reported that the nest he found in Kazakhstan was made inside 8 mm wood burrow and females hunted for spiders in tree canopies. On the other hand, Marikovsky (1990) reported on a female of *Chalybion* sp. (as "синий сцелифрон" [Blue *Sceliphron*]) hunting for *Latrodectus* sp. that occurred on the ground. According to Kazenas (2001), "Blue *Sceliphron*" was most likely to be *Ch. turanicum*. No further details were published on the nesting habits of this species. The report on the bionomics of *Ch. turanicum* by Baghirov (2011), cited also by Pham (2018), can be considered as an extrapolation of the data known for other *Chalybion* species.

The purpose of our contribution is to provide the first data on the nesting biology of *Ch. turanicum* in the Crimea, where the species was recently introduced.

#### Material and methods

The nests of *Chalybion turanicum* were initially found in the old nest cells of *Sceliphron destillatorium* (Illiger, 1807) in Koktebel (44°57.9′N, 35°15.1′E) in March 2020. The nests of *S. destillatorium* were located under a shed roof and were abandoned by the host species many years ago. Several nests were removed and dissected in the laboratory with a knife, by cutting mud surface layer by layer. A total of 13 nest cells were occupied by *Ch. turanicum*. Cell contents (cocoons of *Ch. turanicum* and other insects) from the dissected nests were placed into glass tubes sealed with cotton plugs and kept under outdoor conditions until the emergence of imagoes. Then, the reared wasps were identified. Specimens of *Ch. turanicum* were deposited in the research collection of M.V. Mokrousov, Nizhny Novgorod.

Nesting females and males of *Ch. turanicum* were also observed near the dolphinarium of the T.I. Vyazemsky Karadag Scientific Station (44°54.7′N, 35°12.1′E) in June 2020. The observations were made during one full day and during an hour on two other days within a week. There were several old nests of *S. destillatorium* on the south-facing wall of the dolphinarium, but the wasps did not use them. Instead, they entered a room with freezers through an inoperative exhaust fan (Fig. 1). Five clusters of the old nests of *S. destillatorium* were found on the ceiling of that room, with each cluster consisting of three to five nests made one above another (Figs 3,

4). Three of these clusters were removed and studied in the laboratory on 29 June (two clusters) and 12 July (the remaining one) that resulted in obtaining additional 18 nest cells of *Ch. turanicum*, of which 13 were sealed by females in 2020 and the remaining other five represented last-year nests. A total of 12 cells (11 "fresh" and one last-year cell with a dead host's egg) contained the prey items – 211 spider specimens, which were stored in 70% ethanol and deposited in the arachnological collection of the V.I. Vernadsky Crimean Federal University, Simferopol.

## Results

The studied nests of *Chalybion turanicum* were found exclusively in the old nest cells of *Sceliphron destillatorium*. Yet, one nest was found inside an abandoned nest cell of *Megachile (Pseudomegachile) ericetorum* Lepeletier de Saint-Fargeau, 1841 (Hymenoptera, Megachilidae), which in turn was built up inside a cell of *S. destillatorium*.



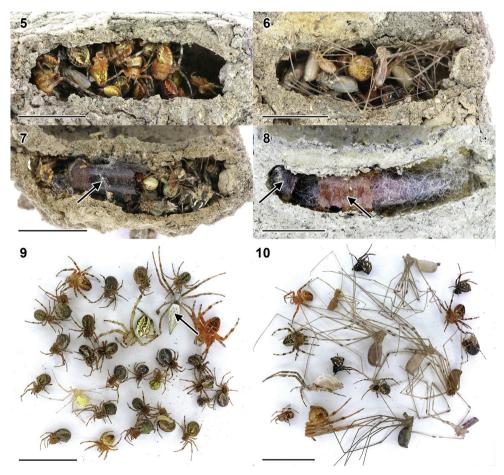
**Figures 1–4.** Nesting of *Chalybion turanicum* (Gussakovskij, 1935) in the Crimea. **I** – The nesting site (arrow indicates the entrance to the room with the nests). **2** – A female with a victim. **3** – A cluster of nests of *Sceliphron destillatorium* (Illiger, 1807) with a female of *Ch. turanicum* arrived with a victim. **4** – Another cluster of nests of *S. destillatorium* with a perching male of *Ch. turanicum*.

Thirty other nests of Ch. turanicum were located inside the cells of the latter species itself. Each nest contained a single cell that corresponded to the host species cell. There were no preliminary plugs; only the final plugs of the cells were made by *Ch*. turanicum. These plugs were made of grayish mud without any additional white material (Fig. 4). It was impossible to establish whether the wasps used exclusively open cells of S. destillatorium (either empty ones or those, from which the host's progeny had emerged), or the cells sealed with mud as well (also either empty or with the dead progeny). Anyway, the females of Ch. turanicum merely filled the cells of S. destillatorium with spiders and then sealed them with the plugs (Figs 5, 6). Thus, the wasps either did not clean the cells prior to provisioning, or could clean them insufficiently, so that the provisioned cells contained the remains of old cocoons of the same species (found in two nests; Fig. 7). In addition, the remains of old cocoons of S. destillatorium (Fig. 8) and its nest parasite Chrysis taczanovskii Radoszkowski, 1876 (Hymenoptera, Chrysididae) were also found in the nests of Ch. turanicum at three occasions each (one cocoon of Ch. taczanovskii was found in the same provisioned cell where one old cocoon of Ch. turanicum was present).

The females of Ch. turanicum hunt for spiders (Figs 2, 3). Eighteen species in five families were identified among the studied prey (Table 1). More than a half of the specimens belonged to the family Theridiidae (eight species) and a third to the Araneidae (seven species). Other three families (about 13% of the caught specimens) were represented by one species each. Among the 211 studied spider specimens, 43 were adults (two males and 41 females) and 168 were juveniles. Most spiders had a total body length of 4–5 mm and a spherical abdomen. Fourteen specimens, however, were different: these were Argiope spp. (one of them is shown in Fig. 9) and Pholcus phalangioides (seven of them are shown in Fig. 10) with an elongated abdomen and the body length clearly exceeding 5 mm. According to a taxonomic composition of the prey, wasps collected spiders from various habitats, from the ground level up to tree crowns, without any visible preferences. A number of the spiders, stored in a cell (Figs 9, 10), varied from five to 31 (mean =  $17.6 \pm 5.4$ , n = 12, p = 0.05). The spiders, stored in freshly sealed nest cells, often retain some of their life activity, particularly, their legs were trembling. One of the five spiders, found in the last-year cell with a dead egg, even shed its cuticle.

Twelve adult wasps (seven females and five males) emerged from the 18 lastyear cells. Males emerged from 25 May to 13 June, females – from 8 June to 30 June. Two fresh cocoons with diapausing prepupae were found in the nests on 12 July, which is evidence of a single generation per year. A reproductive success amounted to 67%, but the data were not representative. The progeny (an egg and a prepupa) died in two cells for uncertain reason. Other four cells were damaged by two species of nest parasites. One was *Acroricnus seductor* (Scopoli, 1786) (Hymenoptera, Ichneumonidae, Cryptinae), with its cocoon being found in one of the last-year cells instead of the host cocoon. The larva of *A. seductor* probably consumed the fifthinstar larva of *Ch. turanicum*, which had already consumed all the provisioned spiders but had not spun its cocoon yet. An adult male of this parasitoid wasp emerged on 7 May. Other three cells contained cocoons of *Chrysis taczanovskii* among the prey remains. It was impossible to establish whether its larvae fed on the prey or host larvae. Two males of *Ch. taczanovskii* emerged on 28 and 30 June and a female emerged on 8 July.

The males of *Ch. turanicum* were observed applying two different mating strategies. Some patrolled several shrubs of *Euonymus japonicus* Thunb. (Celastraceae) and *Juniperus sabina* L. (Cupressaceae) which were cultivated at the nesting site (Fig. 1). Others were perching on the clusters of old nests of *S. destillatorium* inside the room with freezers (Fig. 4). A single male defended each nest cluster. Copulation was not observed.



**Figures 5–10.** The nest content of *Chalybion turanicum* (Gussakovskij, 1935). **5, 6** – Cells with the prey in old nest cells of *Sceliphron destillatorium* (Illiger, 1807). **7** – A cell with the prey and an old cocoon of *Ch. turanicum* (arrow). **8** – A cell with a cocoon of *Ch. turanicum* inside an old cocoon of *S. destillatorium* (arrows). **9, 10** – Spiders from two nest cells, arrow indicates a juvenile specimen of *Argiope bruennichi* (Scopoli, 1772). Scale bars 1 cm.

Family	Species	Number of specimens
Araneidae	Araneus diadematus Clerck, 1757	43 juv. ♀
	Araneus spp.	6 juv.
	Argiope bruennichi (Scopoli, 1772)	1 juv. ∂, 1 juv. ♀
	Argiope lobata (Pallas, 1772)	1 juv. ♀
	Cyclosa sierrae Simon, 1870	1 9
	Mangora acalypha (Walckenaer, 1802)	4 <sup>♀</sup> , 1 juv.
	Neoscona adianta (Walckenaer, 1802)	6 <sup>♀</sup> , 3 juv.
	Neoscona subfusca (C.L. Koch, 1837)	1 ♀, 1 juv. ♀
	Araneidae Gen. sp.	1 juv.
	Subtotal	70 (33.2%)
Mimetidae	Ero aphana (Walckenaer, 1802)	$1 \stackrel{\circ}{\uparrow}$
	Subtotal	1 (0.5%)
Pholcidae	Pholcus phalangioides (Fuesslin, 1775)	1 ♂, 10 juv.
	Subtotal	11 (5.2%)
Theridiidae	Heterotheridion nigrovariegatum (Simon, 1873)	1 9
	Kochiura aulica (C.L. Koch, 1838)	1 ♂, 94 juv. ♀
	Latrodectus tredecimguttatus (Rossi, 1790)	1 juv. ∂, 4 juv. ♀
	Parasteatoda lunata (Clerck, 1758)	3 ♀
	Parasteatoda tepidariorum (C.L. Koch, 1841)	2 9
	Simitidion simile (C.L. Koch, 1836)	1 9
	Steatoda triangulosa (Walckenaer, 1802)	1 ♀, 1 juv. ♀
	Theridion melanurum (Hahn, 1831)	5 9
	Subtotal	114 (54.0%)
Uloboridae	Uloborus walckenaerius Latreille, 1806	15 ♀
	Subtotal	15 (7.1%)
Total		211

**Table 1.** Taxonomic composition of the spiders found in 12 nest cells of *Chalybion turanicum* (Gussakovskij, 1935) in the Crimea.

## Discussion

Nesting in abandoned nest cells of various species of the genus *Sceliphron* was previously known for the majority of the studied *Chalybion* species: viz., *Ch. bengalense*, *Ch. californicum*, and *Ch. zimmermannii* in *S. caementarium* (Drury, 1773) (Rau 1928, Ward 1971, Horner and Klein 1979, Landes et al. 1987, Mei et al. 2012), *Ch. bengalense* and *Ch. japonicum* in *S. madraspatanum* (Fabricius, 1781) (Pham 2018, 2019), *Ch. japonicum* in *S. deforme* (F. Smith, 1856) (Barthélémy 2011), *Ch. tibiale* in *S. spirifex* (Linnaeus, 1758) (Gess and Gess 2014), as well as *Ch. femoratum* (Fabricius, 1781) in *Scelipron* sp. (Bitsch 1988). Yet, most of these species can also nest in other cavities. The exception is *Ch. californicum*, which shows a strong preference to nesting in the *Sceliphron* nests, even in operated ones (Rau 1928). We revealed that *Ch. turanicum* seems to also prefer the *Sceliphron* nest cells, since none of its nests

was found in other types of preexisting cavities (e.g., in trap nests installed every year at the T.I. Vyazemsky Karadag Scientific Station); yet, a nest of this species was earlier found in a wood burrow (Kazenas 2001).

Chalybion turanicum belongs to the Ch. bengalense species group sensu Hensen (1988). Species of this and Ch. tibiale group are renowned for sealing their nests with an additional layer of white material, composed of either lime from whitewashed walls, or uric acid, obtained from bird or reptilian droppings (Javakar and Spurway 1963, Ward 1971, Barthélémy 2011, Gess and Gess 2014, Pham 2018, 2019). According to Bohart and Menke (1976), such behavior correlates to the structure of the female mandible having an inner subapical tooth in Ch. tibiale and Ch. bengalense groups (Hensen 1988). This subapical tooth is absent from Ch. californicum, which does not apply an additional white covering in its nests. In *Ch. turanicum* the tooth is present, but the white nest covering was not observed. Thus, the nesting habits of Ch. turanicum are more similar to those of Ch. californicum than other species of the genus, in particular to Ch. bengalense group. It is noteworthy that Ch. californicum is able to open the freshly sealed nest cells of S. caementarium, remove their contents, and then use them for their own nesting (Rau 1928). The same behavior is also described for Ch. japonicum using the S. deforme nests (Barthélémy 2011). It was impossible to establish whether such peculiar habits exist in Ch. turanicum, because all the nests of S. destillatorium, analysed in our study, were abandoned many years ago, apparently due to the decrease of the population of S. destillatorium in the Crimea, caused by the invasion of such congeners as S. curvatum (F. Smith, 1870) (Fateryga and Kovblyuk 2013, 2014) and S. caementarium.

The use of old cells without properly cleaning them from old cocoons seems not to be typical in other studied *Chalybion* species. A similar habit was reported for *Ch. femoratum*, which nests in old nest cells of *Sceliphron* sp. (Bitsch 1988).

The diet of Ch. turanicum is typical of the genus Chalybion, consisting of various spiders, with the preference to Theridiidae and Araneidae. Such a composition of prey is most similar to that of Ch. californicum (Rau 1935, Horner and Klein 1979, Landes et al. 1987). Other species are known to hunt for various spiders with the preference to Araneidae: e.g., Ch. bengalense (Chakrabarti 1986, Pham 2019), Ch. japonicum (Barthélémy 2011), and Ch. tibiale (Gess and Gess 2014), or exclusively for Araneidae: Ch. sulawesii Ohl, 2011 (Ohl and Höhn 2011) and Ch. malignum (Kohl, 1906) (Pham and Dang 2017). Only species from the subgenus Hemichalybion hunt exclusively for Theridiidae: e.g., Ch. femoratum (Bitsch 1988) and Ch. spinolae (Gess et al. 1982, Nel et al. 2014); the latter species even specializes in a prey on the single genus Latrodectus, commonly known as Black Widow spiders. Chalybion turanicum was also known as a hunter for Latrodectus (Marikovsky 1990, Kazenas 2001), and our study confirms those observations. Although only five specimens of Latrodectus tredecimguttatus were found in the nest cells of Ch. turanicum, it is worth mentioning that this spider species was indeed rare in the Karadag State Nature Reserve with just five other specimens being earlier known from 14,419 specimens of adult spiders, collected from that site during 12 years (Kovblyuk et al. 2015). Such a preference to the genus *Latrodectus* is also similar to that of *Ch. californicum* having similar nesting habits, as was stated above.

One can assume that the females of *Ch. turanicum* collected specimens of *Pholcus phalangioides* because they occurred near their nests. Nevertheless, it was hardly possible because spider-hunting wasps usually do not attack their victims at their nesting sites (Obin 1982).

Both *Ch. turanicum* and *Ch. californicum* differ in their prey preferences from co-occurring *Sceliphron* species. For example, *S. destillatorium* prefers Araneidae and Oxyopidae (Fateryga and Kovblyuk 2014), *S. caementarium* prefers Araneidae, Salticidae, and Philodromidae (Fateryga and Kovblyuk 2013). At the same time, most of these spider families except Araneidae are absent or only occasionally found in the nests of *Ch. californicum* (Horner and Klein 1979, Landes et al. 1987) and *Ch. turanicum*, although they are sometimes present in the prey of *Ch. bengalense* (Chakrabarti 1986). In its turn, some *Sceliphron* wasps only occasionally collect Theridiidae spiders, e.g., *S. curvatum* (Fateryga and Kovblyuk 2013).

Acroricnus seductor and Chrysis taczanovskii reared from the nests of Ch. turanicum are the typical nest parasites of Sceliphron spp. The former species was already reported as a parasitoid of the invasive Ch. bengalense (inside a nest of S. caementarium) in Europe (Mei et al. 2012). Actually, there are no substantial differences between a Sceliphron cell and that of Chalybion occupying it. Both look the same and contain similar sphecid larvae and spiders as the provision. Thus, it is hardly surprising that Ch. taczanovskii, which was previously known as a nest parasite of S. destillatorium (Fateryga and Kovblyuk 2014), was reared from the studied nests of Ch. turanicum. This species of cuckoo wasps is highly specialized on the Sceliphron nests. Despite it being found in a nest of the eumenine wasp Euodynerus disconotatus (Lichtenstein, 1884) (Hymenoptera, Vespidae, Eumeninae), that nest was also located inside an old S. destillatorium cell (Martynova and Fateryga 2015). Further studies are needed to determine whether the larva of Ch. taczanovskii feeds on different preys (spiders and caterpillars) or on the host larva.

*Chalybion turanicum* is the seventh invasive species of Sphecidae naturalized in Europe after the Nearctic *Isodontia mexicana* (de Saussure, 1867) and *S. caementarium*, the primarily Oriental *S. curvatum*, *S. deforme*, and *Ch. bengalense*, and the Nearctic *Ch. californicum* (Schmid-Egger and Herb 2018). Further observations will certainly reveal its expansion to other territories, influenced by climate change, although such an expansion could be not as rapid as in the case of *S. curvatum*, which has two generation per year (Fateryga and Kovblyuk 2013) and therefore spreads very rapidly.

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