Host Range and Recorded Distribution of the Fungal Pathogen Entomophaga Grylli (Entomophthoromycota: Entomophthorales) in Kazakhstan

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Host range and recorded distribution of the fungal pathogen *Entomophaga grylli* (Entomophthoromycota: Entomophthorales) in Kazakhstan

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Abstract

In this paper for the first time we summarized both the existing literature data and our own findings on the occurrence of the infections caused by the fungus *Entomophaga grylli* (Fresenius) A. Batko, 1964 (Entomophthoromycota: Entomophtorales) among short-horned Orthoptera (Caelifera) in Kazakhstan. Almost 800 insect cadavers exhibiting signs of infection by *E. grylli* were collected in 7 out of 14 provinces (oblasts) of Kazakhstan in 2000-2016, with confirmed identification of the fungal pathogen. They belonged to 18 genera and 28 species from two families, Pamphagidae and Acrididae, and from 6 subfamilies within the latter family. A discussion summarizing the current knowledge of the host range of the fungus in Kazakhstan and its potential for the use in biological control programs is provided.

Key words

entomopathogen, grasshoppers, locusts, biological control

Introduction

Locusts and grasshoppers are among the most economically important agricultural pests in Kazakhstan. During outbreaks, they can inflict severe damage to crops and rangeland. For example, in 1999, infestations of the Italian locust *Calliptamus italicus* (Linnaeus) (Orthoptera: Acrididae) in Northern and Central provinces destroyed 220,000 ha of grain crops with an estimated value of USD 15 million (Khasenov 2001). To prevent agricultural losses, every year immense areas of locust and grasshopper infestations in Kazakhstan are treated with broad-spectrum pesticides. In 2000, the area of locust and grasshopper control in the country exceeded 8.1 million ha (Khasenov 2001), which is a world record of anti-acridid treatments per country per year. Such large-scale applications of insecticides have not only very high economic but also tremendous environmental costs (Latchininsky *et al.* 2002). Growing concerns about environmental impacts of locust and grasshopper treatment programs with chemical insecticides in Kazakhstan evoked interest in alternative control strategies involving natural regulators of pest populations (Temreshev & Khasenov 2004). Fungal pathogens are among the most promising natural control agents for locusts and grasshoppers worldwide (Lomer & Prior 1991, Goettel 1992) and in Kazakhstan in particular (Temreshev 2003, Temreshev & Childebaev 2011, Temreshev *et al.* 2012, 2015, 2016).

Despite the fact that there are over 700 known species of entomopathogenic fungi worldwide (Goettel 1992), only very few of them are proven to infect grasshoppers and locusts (Orthoptera: Acridoidea) (Prior & Greathead 1989). Among them, the cosmopolitan fungus *Entomophaga grylli* (Fresenius) A. Batko, 1964 is famous for causing spectacular natural epizootics on different continents: in Africa (Skaife 1925, Lepesme 1938, Chapman & Page 1979), Australia (Milner 1978), North America (Treherne & Buckell 1924, Walton & Fenton 1939, Pickford & Riegert 1964, MacLeod & Müller-Kögler 1973, McDaniel & Bohls 1984, Erlandson *et al.* 1988, Valovage *et al.* 1984, Valovage & Nelson 1990), Central America (Sánchez-Pena 2000) and South America (Marchionatto 1934, Fresa 1971, Méndez-Sanchéz *et al.* 2009, Pelizza *et al.* 2010). The insects infected by *E. grylli* exhibit distinctive and unique signs at the advanced stages of the disease. They climb on top of plants where they die with head pointing upwards and legs tightly clinging around the stems (Fig. 1). Because of such characteristic posturing, the disease caused by *E. grylli* received the term "summit disease."

In Eurasia, the infections caused by *E. grylli* attracted special attention of the Father of Modern Acridology, Sir Boris Uvarov, who published his detailed observations on fungal epidemics of grasshoppers and locusts in southern Russia a century ago (Uvarov 1913). However, cases of acridid fungal diseases attributable to *E. grylli* were reported from Russia even earlier (Koeppen 1870, Porchinsky 1894) and probably as early as in the 18th century (Pallas 1771-1801). The fungus is known to attack several economic species of locusts and grasshoppers on a vast geographic scale of the former Soviet Union: in northern Caucasus (Zhdanov 1934), Volga region (Batko 1957), Siberia (Vinokurov 1949, Karelina 1961, Latchininsky 1995, Ogarkov & Ogarkova 2000), Turkmenistan (Tokgaev 1973), Uzbekistan (Gapparov 1988), and Georgia (Abashidze *et al.* 1998). Besides the ex-USSR, other reports of *E. grylli* in Eurasia come from Thailand (Roffey 1968), China (Chen & Liu 1995, Jia 2011), Vietnam (Weiser *et al.* 1985), India (Gupta *et al.* 2011), Palestine (Ali-Shtayeh *et al.* 2003) and Iran (Ghazavi *et al.* 2003). Regarding Kazakhstan, the records of *E. grylli* infections were fragmentary (Vasil'ev 1962; Evlakhova & Shvetsova 1965, Evlakhova 1974, Nasyrova 1992, 1995) until recently, when regulatory potential of fungal diseases of locusts and grasshoppers was re-examined (Temreshev 2003). Yet the relevant information appeared only partially in conference proceedings or unpublished reports which are not available in English (Temreshev & Khasenov 2004, Temreshev & Childebaev 2011, 2012, Temreshev *et al.* 2012). In this paper for the first time we summarized both the existing literature data and our own findings on the occurrence of the infections caused by *E. grylli* among short-horned Orthoptera (Caelifera) in Kazakhstan. A discussion summarizing the current knowledge of the host range of the fungus in Kazakhstan and its potential for the use in biological control programs is provided.

Materials and methods

Data were collected in 7 out of 14 provinces (oblasts) of Kazakhstan in 2000-2012. No systematic effort was made to sample

Fig 1. *Chorthippus angulatus* killed by *Entomophaga grylli* in Kazakhstan. Photo V.L. Kazenas. Reproduced by permission.

all of Kazakhstan. In most cases the dead insect collections were made in the areas of high-density grasshopper and locust populations and known disease occurrence. Dead insects exhibiting the "summit disease" signs were collected mostly from the stems of various plants, placed into plastic or glass vials sterilized with 70% EtOH, and stored at +4°C for subsequent examination. In case there was no visible mycelium on the insect body, the specimens were placed into a "wet chamber" until the sporulation started. The "wet chamber" represented a Petri dish bottom with a round piece of filter paper soaked with distilled water and closed with a Petri dish lid (Evlakhova & Shvetsova 1965, Issi *et al.* 1993). Identification of the pathogen was done by examining the conidia under the microscope MBI-15 (magnification 40× and 90×), measuring the spore size by an ocular micrometer MOB-1-15×, and following the keys of the "*Guide to entomophilous fungi of the USSR*" (Koval 1974).

To ensure the proper identification of Caelifera host species of the fungus, sweep-net samples of live grasshoppers were collected using the standard entomological net of 30 cm diameter. The insects were then killed in a killing jar using ethyl acetate and subsequently identified in the lab using the keys from "*Grasshoppers and Locusts of Kazakhstan, Central Asia and Adjacent Territories*" by Latchininsky *et al.* (2002). Acridoidea classification from the above publication was followed for families and subfamilies.

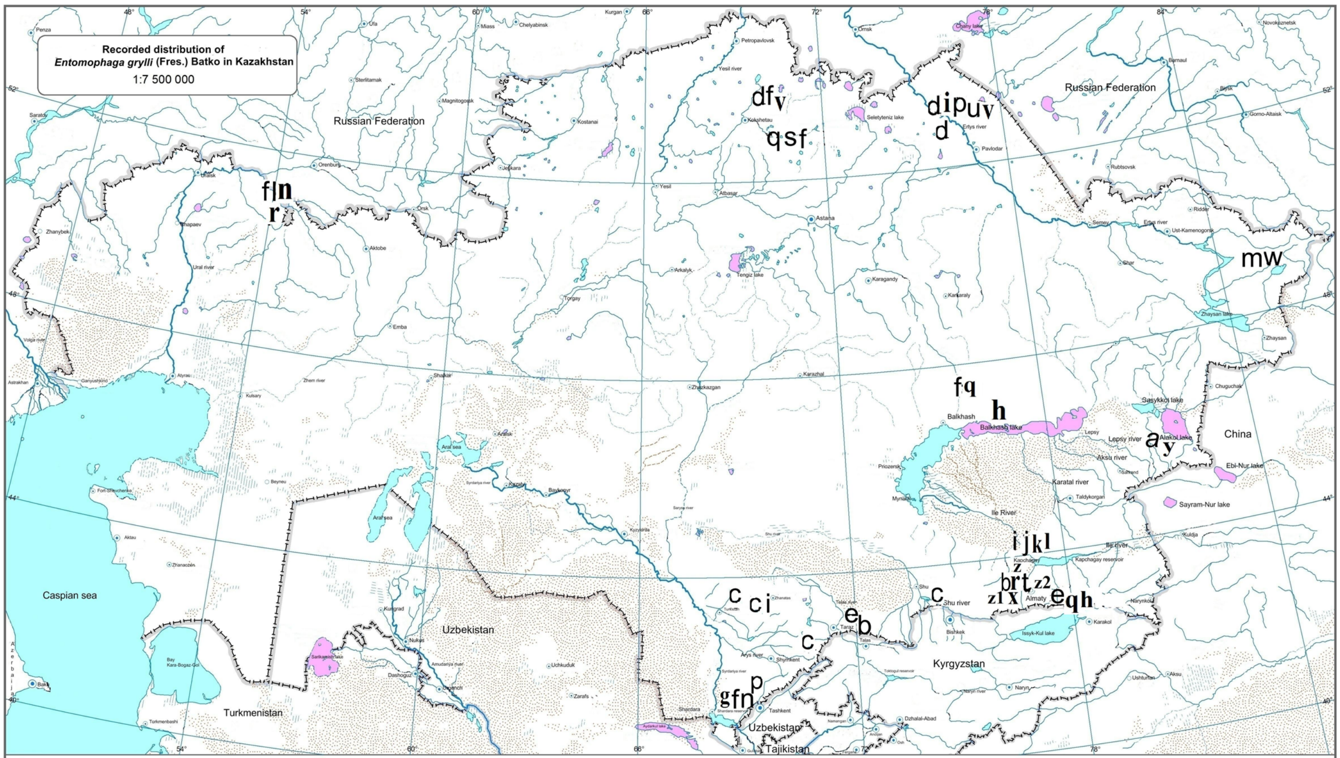
Results and discussion

In total almost 800 cadavers of grasshoppers and locusts (Caelifera) exhibiting signs of infection by *E. grylli* were collected, with confirmed identification of the fungal pathogen. They belonged to 18 genera and 28 species from two families, Pamphagidae and Acrididae, and from 6 subfamilies within the latter family (Table 1). All infected insects were adults or last (5^{th}) instar nymphs except for one 4^{th} instar nymph of *Euchorthippus pulvinatus* (Fischer von Waldheim) (Orthoptera: Acrididae: Gomphocerinae). Data from these collections were used to produce a distribution map of *E. grylli* in Kazakhstan (Fig. 2). In the vast majority of cases the prevalence of the *E. grylli* mycosis in the host population was low except for cases of *Podisma pedestris* (Linnaeus) (Orthoptera: Acrididae: Melanoplinae) and *Gomphocerus sibiricus* (Linnaeus) (Orthoptera: Acrididae: Gomphocerinae) in the Pavlodar province of NE Kazakhstan in June 2004.

The present study is the first attempt to summarize existing literature and original field data on Caelifera host range and geographic distribution of *E. grylli* mycoses in Kazakhstan. The data are by no means exhaustive; undoubtedly, more comprehensive surveys will complement the list of susceptible host species and geographic locations of *E. grylli* occurrences. Yet even this, admittedly fragmentary information provides valuable insights regarding the host range and distribution of this important pathogen in Kazakhstan.

First, it was of interest to find out if *E. grylli* affects pest and/or rare Caelifera species. The fauna of Caelifera in Kazakhstan includes 264 species and subspecies (Latchininsky *et al.* 2002) with about 20 of those being recurrent agricultural pests (Mistchenko 1972, Nurmuratov *et al.* 2000). None of the Kazakhstani Caelifera species are currently designated as threatened or endangered; however, there are 45 species and 16 subspecies endemic to Kazakhstan, some of which are extremely rare (Childebaev 2000). Based on our study, 14 out of 28 species affected by *E. grylli* in the field were agricultural pests (designated by an asterisk in Table 1), including all three locusts species inhabiting Kazakhstan: *Locusta migratoria migratoria* Linnaeus (Orthoptera: Acrididae: Oedipodinae), *Dociostaurus maroccanus* (Thunberg) (Orthoptera: Acrididae: Gomphocerinae), and *C. italicus*. For the latter locust species, *E. grylli* is well known as one of the most important population regulators (Uvarov 1928, Evlakhova & Shvetsova 1965). It is interesting to note though that according to Uvarov (1977), the Italian locust mycoses caused by *E. grylli* were never reported from dry steppes of Kazakhstan because of unfavorable arid conditions for the fungus (p. 459). Our findings from several sites in dry steppes of Central Kazakhstan (Table 1) contradict this assertion. Finally, none of the *E. grylli* hosts from our collections were Kazakhstani endemics, probably because of their lower population densities compared to more common and abundant species.

Another intriguing question is the taxonomic status of *E. grylli* in Kazakhstan. Although the fungus is cosmopolitan, beyond the North American continent its taxonomy is studied insufficiently (Humber 1989). The fungus is known to be represented by several distinctive pathotypes which differ in host ranges, isozyme banding patterns, growth requirements, life cycles, and DNA characteristics (Soper *et al.* 1983, Humber 1989, Bidochka *et al.* 1995). There is a growing molecular biological evidence that these pathotypes are distinctive species (Bidochka *et al.* 1995, Casique-Valdez *et al.* 2012).



Designated by infected host species: a - ***Asiotmethis muricatus*** **(Pallas); b -** ***Conophyma almasyi*** **(Kuthy); c -** ***Conophyma sokolovi decorum*** **Mistshenko; d -** ***Podisma pedestris*** **(Linnaeus); e -** ***Calliptamus barbarus cephalotes*** **Fischer von Waldheim; f -** ***Calliptamus italicus*** **Linnaeus; g -** ***Calliptamus turanicus*** **Tarbinsky; h -** ***Acrida oxycephala*** **(Pallas); i -** ***Gomphocerus sibiricus*** **(Linnaeus); j -** ***Chorthippus angulatus*** **Tarbinsky; k -** ***Chorthippus apricarius*** **(Linnaeus); l -** ***Chorthippus biguttulus*** **(Linnaeus); m -** ***Chrysochraon dispar*** **(Germar); n -** ***Dociostaurus brevicollis*** **Eversmann; o -** ***Dociostaurus kraussi kraussi*** **(Ingenitskij); p -** ***Dociostaurus maroccanus*** **(Thunberg); q -** ***Euchorthippus pulvinatus*** **(Fischer von Waldheim); r -** ***Notostaurus albicornis*** **Eversmann; s -** ***Omocestus haemorrhoidalis*** **(Charpentier); t -** ***Omocestus viridulus*** **(Linnaeus); u -** ***Stauroderus scalaris*** **(Fischer von Waldheim); v -** ***Stenobothrus fischeri*** **(Eversmann); w -** ***Stenobothrus eurasius*** **Zubovski; x -** ***Epacromius tergestinus*** **(Charpentier); y-** ***Locusta migratoria migratoria*** **(Linnaeus); z -** ***Oedaleus decorus*** **Germar; zl -** ***Oedipoda caerulescens*** **(Linnaeus); z2 -** ***Oedipoda miniata*** **(Pallas).**

Fig 2. Map of *Entomophaga grylli* distribution in Kazakhstan.

E. grylli was described by Fresenius from Europe, so some specialists suggest that there exists a European pathotype, or *E. grylli* (*sensu stricto*) (Carruthers *et al.* 1997), which may occur in Kazakhstan (R. Humber, *pers. comm.* 2013). Without further analyses using molecular techniques it is impossible to assert which pathotype(s) occur(s) in Kazakhstan. Since some of the studied pathotypes exhibit differences in host ranges, the question of the pathotype designation has important practical implications for the use of *E. grylli* in biological control of pest acridids. Preliminary evidence from Kazakhstan (unpub. data) suggest that inoculations by *E. grylli* spores obtained from one grasshopper species were successful in species across several Acrididae subfamilies, which reveals a broad host range of the pathogen.

Our investigation showed that many pest species were susceptible to *E. grylli* mycoses, which makes the fungal pathogen a potential candidate for developing a biological product for locust and grasshopper control. However, it is well known that *E. grylli* does not produce reproductive stages on most complex solid or liquid artificial media (Evlakhova & Shvetsova 1965, Ramoska *et al.* 1988, Bidochka *et al.* 1995, Pell *et al.* 2001, Geshtovt 2002), which makes impractical its mass production for biological control purposes (Bidochka & Khachatourians 1991, Carruthers *et al.* 1997). Despite some progress in growing *E. grylli* hyphae *in vitro* (Sánchez-Peña 2005), the production of conidia has not been successful to date. Under such circumstances, it is conceivable to use *E. grylli* in augmentative biological control strategy, which consists in inoculation of insects with fungal spores in the lab and their subsequent releases in the field, in order to create areas of higher-than-average level of pathogen infection (Carruthers *et al.* 1997).

As elsewhere in the world, grasshoppers and locusts infected by *E. grylli* in Kazakhstan were found clinging to tops of plant stems (Fig. 1). Some specialists consider this behavior as an attempt to thermoregulate and increase the body temperature above the thermal limit of *E. grylli* (Kemp 1986) while others suggest that the fungus causes this behavioral modification to increase the aerial dissemination of spores (Carruthers *et al.* 1997). Grasshopper and locust cadavers exhibiting the "summit disease" signs were most frequently found in damp areas with dense herbaceous vegetation such as roadside ditches, moist meadows and hayfields, intermittent waterways in pastures and weedy perimeters of cropland. This is consistent with findings in other geographic areas indicating that areas with higher humidity create favorable conditions for *E. grylli* mycoses; for the same reason, the higher prevalence of *E. grylli* is usually correlated with wetter than usual years (Evlakhova & Shvetsova 1965, Erlandson *et al.* 1988, Valovage & Nelson 1990, Packham *et al.* 1993, Carruthers *et al.* 1997, Laws *et al.* 2009, Pelizza *et al.* 2010). Interestingly, the two epizootics of *Podisma pedestris* and *Gomphocerus sibiricus* developed in notably drier conditions, namely at the forest borders, *i.e.* the interface between the forest and the steppe (Temreshev & Childebaev 2012). This observation suggests that *E. grylli* in Kazakhstan can infect its acridid hosts across a relatively wide moisture gradient, which is an important trait for a potential biocontrol agent.

To sum up, our investigations showed that: a) *E. grylli* attacks a wide range of Caelifera hosts, including 28 species from 6 subfamilies of the Acrididae family in Kazakhstan; b) the majority of recurrent agricultural and/or rangeland pests, including 3 locusts, are susceptible to *E. grylli*; and c) *E. grylli* mycoses occur not only in humid but also in relatively dry sites. All the above-mentioned traits make the Kazakhstani strain(s) of *E. grylli* a promising candidate for use in grasshopper and locust augmentative biocontrol program through inoculative releases of this native pathogen.

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Table 1. Annotated list of Caelifera hosts of *Entomophaga grylli* in Kazakhstan. Pest species are marked with an asterisk (*).

#	Caelifera host species	N	Date	Geographic location
	Family Pamphagidae			
	Subfamily Pamphaginae			
1	*Asiotmethis muricatus* (Pallas, 1771)	3	June 06, 2002	SE Kazakhstan, Almaty province, Alakol district
	Family Acrididae			
	Subfamily Conophyminae			
2	*Conophyma sokolovi decorum* Mistshenko, 1951	10	May 14, 2012	S Kazakhstan, Zhambyl province, Kordai pass
				S. Kazakhstan, Zhambyl province, near city Taraz
		20	May 15, 2012	S. Kazakhstan, South Kazakhstan province, town im. T. Ryskulova
		30	May 25, 2012	
				S. Kazakhstan, South Kazakhstan province, near city Kentau
		10	May 17, 2012	S. Kazakhstan, South Kazakhstan province, Karatau Mtns, Tassarai gorge
		15	May 17-18, 2012	
3	*Conophyma almasyi* (Kuthy, 1905)	3	July 8, 2015	SE Kazakhstan, Almaty province, Ile-Alatau months, Big Almaty gorge
	Subfamily Melanoplinae			
4*	*Podisma pedestris* (Linnaeus, 1758)	10	July 7, 2002	N. Kazakhstan, Akmola province, Zerendin district
		350	June 25, 2004	N. Kazakhstan, Pavlodar province, Kachiry district
		30	June 26, 2004	N. Kazakhstan, Pavlodar province, Irtysh district
	Subfamily Calliptaminae			
5*	*Calliptamus barbarus cephalotes* Fischer von Waldheim, 1846	3	May 27, 2000	S. Kazakhstan, Zhambyl province, Zhualin district
		6	June 25, 2000	SE Kazakhstan, Almaty province, Kaskelen district
6*	*Calliptamus italicus* (Linnaeus, 1758)	15	July 7, 2002	N. Kazakhstan, Akmola province, Zerendin district
		12	July 12, 2002	N. Kazakhstan, Akmola province, Enbekshilder district
		3	July 13, 2004	Central Kazakhstan, Karaganda province, Bektau-Ata Mtn
		?	1991	W Kazakhstan, West Kazakhstan province, Aksuy district
7*	*Calliptamus turanicus* Tarbinsky, 1930	5	May 09, 2002	S Kazakhstan, South Kazakhstan province, Saryagash district
	Subfamily Acridinae			
8	*Acrida oxycephala* (Pallas, 1771)	1	June 25, 2000	SE Kazakhstan, Almaty province, Kaskelen district
		5	July 08, 2004	Central Kazakhstan, Karaganda province, Aktogai district
9	***Subfamily Gomphocerinae***			
	Chorthippus angulatus Tarbinsky, 1927 (Fig. 1)	9	June 17, 2011	SE Kazakhstan, Almaty province, Kerbulak district, foothills of Katutau Mtns., 135 km from Saryozek town.
		4	June 24, 2011	S Kazakhstan, South Kazakhstan province, Karatau Mtns., River Ikansu valley
10	*Chorthippus apricarius* (Linnaeus, 1758)	6	June 17, 2011	SE Kazakhstan, Almaty ptovince, Kerbulak district, foothills of Katutau Mtns., 135 km from Saryozek town
11*	*Chorthippus biguttulus* (Linnaeus, 1758)	?	1991	W Kazakhstan, West Kazakhstan province, Aksu district
		12	June 17, 2011	SE Kazakhstan, Almaty province, Kerbulak district, foothills of Katutau Mtns., 135 km from Saryozek town
12*	*Chrysochraon dispar* (Germar, 1835)	3	July 7, 2013	E Kazakhstan, East Kazakhstan province, Abay district
13*	*Dociostaurus brevicollis* Eversmann, 1848	?	1991	W Kazakhstan, West Kazakhstan province, Aksu district
14*	*Dociostaurus kraussi kraussi* (Ingenitskij, 1897)	8	June 25, 2004	N. Kazakhstan, Pavlodar province, Kachiry district
15*	*Dociostaurus maroccanus* (Thunberg, 1815)	8	May 05, 2002	S Kazakhstan, South Kazakhstan province, Saryagash district
16	*Euchorthippus pulvinatus* (Fischer von Waldheim, 1846)	2	July 13, 2004	Central Kazakhstan, Karaganda province, Bektau-Ata Mtn,
		3	June 24, 2001	SE Kazakhstan, Almaty province, Kaskelen district
17*	*Gomphocerus sibiricus* (Linnaeus, 1767)	150	June 25, 2004	N Kazakhstan, Pavlodar province, Kachiry district
18	*Notostaurus albicornis* Eversmann, 1848	10	June 25, 2012	SE Kazakhstan, Almaty province, Ili district
		?	1991	W Kazakhstan, West Kazakhstan province, Aksu district
19	*Omocestus haemorrhoidalis* (Charpentier, 1825)	10	July 7, 2002	N Kazakhstan, Akmola province, Zerendin district
20	*Omocestus viridulus* (Linnaeus, 1758)	6	July 8, 2015	SE Kazakhstan, Almaty province, Ile-Alatau months, Kazachka gorge
21*	*Stauroderus scalaris* (Fischer von Waldheim, 1846)	12	June 25, 2004	N Kazakhstan, Pavlodar province, Kachiry district
22*	*Stenobothrus fischeri* (Eversmann, 1848)	4	July 7, 2002	N Kazakhstan, Akmola province, Zerendin district
		8	June 25, 2004	N Kazakhstan, Pavlodar province, Kachiry district
23	*Stenobothrus eurasius* Zubovski, 1898	1	July 7 2013	E Kazakhstan, East Kazakhstan province, Abay district
	Subfamily Oedipodinae			
24	*Epacromius tergestinus* (Charpentier, 1825)	2	June 24, 2001	SE Kazakhstan, Almaty province, Kaskelen district
25*	*Locusta migratoria migratoria* Linnaeus, 1758	6	July 06, 2002	SE Kazakhstan, Almaty province, Alakol district
26*	*Oedaleus decorus* Germar, 1817	5	June 25, 2012	SE Kazakhstan, Almaty province, Ili district
27	*Oedipoda caerulescens* (Linnaeus, 1758)	3	June 22, 2001	SE Kazakhstan, Almaty province, Kaskelen district
28	*Oedipoda miniata* (Pallas, 1771)	2	September 12, 2014	SE Kazakhstan, Almaty province, Almaty city

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