On the ecology and range of *Anopheles beklemishevi* (Diptera: Culicidae) with reference to the taxonomy of *An. lewisi*

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ABSTRACT: The ecological features and geographic distribution of *Anopheles beklemishevi* have not been studied extensively. These studies are important in connection with the validity of the '*Anopheles lewisi*' taxon. The materials were collected in Russia and Kazakhstan from 1973 to 2012, and species identity was defined by cytogenetic analysis of polytene chromosomes of larvae and adult females. A total of 7,896 specimens from 34 geographic locations was included in the analysis. It was established that *An. beklemishevi* is distributed from the east coast of the Baltic Sea to the basin of the Lena River, and from the forest-tundra zone to the Altai and Sayan Mountain systems. This species is exophilic and is confined to high and/or swampy terrains found in the zone of conifer and mixed forests. The frequency of *An. beklemishevi* in the southwestern area, where it is sympatric with *An. messeae* s.l., has significantly decreased over the past decades. The results of the study indirectly suggest that *An. beklemishevi* does not play a significant role as a vector of malaria. It is highly improbable that *An. beklemishevi* and *An. lewisi* are the same species. Changes in the proportions of the species of the Maculipennis complex, as well as a shift of their ranges, will significantly impact the epidemiology of malaria over large areas of northern Eurasia. ***Journal of Vector Ecology* 41 (2): 204-214. 2016.**

Keyword Index: *Anopheles beklemishevi*, *Anopheles lewisi*, cytogenetics, ecology, Maculipennis complex, range shift, vector.

INTRODUCTION

Anopheles mosquitoes of the Maculipennis complex are competent vectors of *Plasmodium* and other parasites over vast areas of Eurasia and North America. The species of the complex differ in ecology, geographical distribution, and vector capacity (Beklemishev and Zhelokhovtsev 1937, 1945, Beklemishev 1944, Kitzmiller et al. 1967, Ramsdale and Snow 2000, Takken et al. 2002, Brugman et al. 2015). *Anopheles beklemishevi*, a member of the Palearctic species group, was initially described by Stegnii and Kabanova using materials collected in the vicinity of Tomsk, Russia, in 1976 (Stegnii and Kabanova 1976, 1978). This new species was differentiated from other members of the Maculipennis complex based on egg morphology and the structure of larval salivary gland chromosomes. Analysis of *Anopheles* specimens from various geographical locations within Eurasia demonstrated the presence of *An. beklemishevi* across northern Europe, in the Urals, and in Western Siberia all the way up to Krasnoyarsk in Central Siberia (Stegnii et al. 1978, Saura et al. 1979, Korvenkontio et al. 1979, Utrio 1979, Jaenson et al. 1986). Identification of a novel species belonging to the Maculipennis complex led to the phylogenetic relationships between the species within the complex being revisited, as well as attempts to predict the distribution of *An. beklemishevi* and its identity with *An. lewisi* and *An. selengensis* (White 1978). Both *An. lewisi* and *An. selengensis* were identified by Ludlow (1919, 1920) using specimens collected by surgeons from the U.S. Army expeditionary corps on July 10-12, 1919, at two Trans-Baikal railway stations, Selenga and Verkhneudinsk (Ulan-Ude). In his review, White (1978) erroneously placed Verkhneudinsk (Upper Udinsk in his study) at the geographical location of Nizhneudinsk (Cis-Baikal rather than Trans-Baikal region). Relying on the conclusion of Stegnii and Kabanova (1978) that *An. beklemishevi* distribution can extend to the east of Krasnoyarsk, he added Cis-Baikal and Trans-Baikal regions to the range of this species (Figure 1). Despite the collection of additional distribution data for *An. beklemishevi*, the view that the eastern boundary of this species range lay somewhat to the east of Krasnoyark persisted for a few decades. However, *An. beklemishevi* was subsequently found approximately 500 km to the northeast (Boguchany village) (Novikov 2010) and to the east (the town of Tulun) (Perevozkin and Khalzova 2013) of Krasnoyarsk. Therefore, this study aimed to improve our knowledge of the ecology and geographical distribution of *An. beklemishevi,* with the emphasis on the eastern part of North Asia, and to address the problem of *An. lewisi* (syn. *selengensis*).

This problem is complicated by the existence of a cryptic species complex within *An. messeae* Fall. (Falleroni 1926) comprising *An. messeae* A, *An. messeae* B (Novikov 1984) and *An. daciae* (Nicolescu et al. 2004, Linton et al. 2005, Kronefeld et al. 2014). The presence of known chromosomal inversions specific for *An. messeae* A (Novikov 1984, Novikov and Shevchenko 2001) in the locality where *An. messeae* Fall. has been described (Frizzi 1947) is an argument in favor of identity of these two species. The results of molecular genetic analysis (Vaulin and Novikov 2010) argue in favor of the identity of *An. messeae* A and *An. daciae*. Some researchers have questioned the validity of *An. daciae* (Bezzhonova and Goryacheva 2008). *Anopheles messeae* Fall., *An. messeae* A, and *An. daciae,* are thus the three names of one biological species. Since *An. messeae* A have not been found to the east of Central Siberia (Novikov and Shevchenko 2001, Novikov 2010), *An. beklemishevi* or *An. messeae* B may only be identical to *An. lewisi* (syn. *selengensis*). Because the problem of the cryptic species *An. messeae* A and *An. messeae* B and their designations have not been resolved yet, these names will be used in this study along with the name *An. messeae* s.l.

MATERIALS AND METHODS

Fourth-instar larvae and adult females of *Anopheles* were collected in Russia and Kazakhstan from 1973 to 2012. Seventy-two unique collections totaling 7,896 specimens from 34 geographic locations were included in the analysis (Tables 1 and 2). Larvae were collected from stagnant or weakly running fresh water and fixed in chilled Clark solution (ethanol: glacial acetic acid, 3:1). Adult females were collected in cattle barns and other buildings in towns and villages. Species identity was determined by analyzing crushed preparations of larval salivary glands and Malpighian tubules of adult females prepared following previously described methods (Kabanova et al. 1972, Novikov and Kabanova 1979), using maps of polytene chromosomes (Stegnii and Kabanova 1976, Stegnii et al. 1976, 1978).

RESULTS AND DISCUSSION

Cytogenetic analysis of specimens collected in 34 locations from the Pskov region in the west to the central Yakutia in the east was performed. The presence of a species in a particular region or locality was recorded based on the identification of at least one individual in at least one collection (regardless of its size) in at least one of the biotopes studied. Similarly, the conclusion regarding the absence of a species in a particular region or locality was based on the absence of specimens in a number of representative collections, at different times in different biotopes. The conclusion of the absence of a species in certain area, in contrast to the conclusion of their presence, is not final. A wide variety of environments where a species can be found would require a detailed analysis, which is discussed below.

Ecological features

Of the total specimens studied, only a small proportion were *An. beklemishevi*, which is characteristic for this species (Tables 1 and 2). The only location that was populated exclusively by *An. beklemishevi* larvae was a pond in the village of Bely Yar, surrounded by swamps. Additionally, *An. beklemishevi* outnumbered other species in collections from the village of Srednii Vasyugan, located in the Great Vasyugan Mire, in a collection from a small water body situated in a forest three kilometers away from Belogorsk (the eastern spurs of the Kuznetsky Alatau), in the two early collections from Cherga (Altai foothills), and in one of the early collections from Kolarovo village. In all other collections, *An. messeae* s.l. was the predominant species. Thus, it can be concluded that the *An. beklemishevi* populations in these regions of Russia are relatively small in size.

In the three geographically distant localities of Krivosheino, Kolarovo, and Cherga, seasonal and temporal changes in the proportions of *An. messeae* s.l. and *An. beklemishevi* were studied. Additionally, in Kolarovo and in Krivosheino the microgeographical distribution of populations of these two taxa was investigated (Table 2). As a result, substantial fluctuations in their proportions were identified. The proportions of the species varied significantly between water reservoirs, and from season to season in the same reservoir, and were different for larvae and adults. *Anopheles beklemishevi* was over-represented at the larval stage in small reservoirs characterized by colder water or by more pronounced daily temperature fluctuations as compared to other reservoirs (Table 3). Two such reservoirs, three and four, from Kolarovo village belonged to this category, and the overall density of larvae in these reservoirs was low (Table 4). The proportion of *An. beklemishevi* larvae both in Krivosheino and in Kolarovo did not depend on the distance between the pond and the village. Therefore, it is the physical properties and chemical composition as well as saprobity of water, rather than reservoir location, that play the major role in their distribution. Thus, *An. messeae* s.l. and *An. beklemishevi* are spatially separated at the water-limiting stages, in accordance with the characteristics of the water reservoirs. This environmental separation of species is likely to be due to the behavior of females with their active choice of the most suitable reservoirs for oviposition. Females of the Maculipennis complex species are guided by their preferences of the odor and physicochemical properties of water (temperature, salinity, pH) in their choice of a reservoir for oviposition (Beklemishev 1944).

It is important to note that the preference of *An. beklemishevi* for reservoirs with low larval density is in agreement with its reduced ability to compete with other species at the larval stage, a fact revealed while co-rearing *An. beklemishevi* and *An. messeae* s.l. under laboratory conditions at high population density (Novikov et al. 1983). It is probable that it is the preference of *An. beklemishevi* females for the waters typically ignored by *An. messeae* s.l. that allows persistence of this species in the areas where the species' ranges overlap. Remarkably, the average frequency of *An. beklemishevi* in Kolarovo decreased from 1975 to 1989, thereafter its numbers stabilized at 1-3% (Table 2). This coincided with the desiccation of the Tom River floodplain, where all reservoirs surveyed were located. As a result, reservoirs three and four, where the *An. beklemishevi* larval development mainly occurred, dried out. The frequency of *An. beklemishevi* decreased towards the end of the larval period and from early to later years, in Krivosheino, Cherga, and Teguldet (Tables 1 and 2). Although in Cherga and Teguldet, only one collection per season was studied, the unidirectional character of changes in species frequencies for both locations is in agreement with the findings for the regions for which systematic observations were carried out. This supports an overall trend of directional changes in the proportions of related species with a concomitant decrease for *An. beklemishevi*. It is likely that the southern and southwestern borders of the species range gradually shifted to the north and northeast, and in the mountainous areas inhabited by the species, to higher elevations. The spatio-ecological distributions of *An. messeae* s.l. and *An. beklemishevi* populations and rapid changes in their proportions over the breeding season in the areas where the species are sympatric make it extremely difficult to determine their relative abundance in a particular locality. For this, one should take into account the relative contributions of all larval habitats at several time points throughout the whole breeding season. The second approach is to analyze adults whose subpopulation is the natural reflection of the contributions of all subpopulations from different larvae biotopes. This should be done at several time points each season, as the proportions of adults of different species undergo considerable changes with time. Both of these approaches are time and labor-consuming. With other approaches, although adopted by some researchers (Stegnii 1991, Perevozkin et al. 2012, Perevozkin and Khalzova 2013), it is not possible to determine the precise ratio of species inhabiting the same region. Due to variation of species frequencies from season to season and from year to year, as well as the spatio-ecological distribution of their populations, analysis of a single collection from any locality collected during the larval season could allow conclusions about the presence of individuals of a certain species in the collection but not on their relative proportions in the locality. However, there is one stage in the life cycle of malaria mosquitos when the proportions of species at a particular locality can be accurately determined with relative ease: 10-15 days after females leave their wintering shelters. We analyzed collection data for 1982-1983 (Table 2) to determine the species composition of females in diapause and overwintering at Kolarovo. The study of *Anopheles* females in diapause, collected in September and October, 1982 in the cattle barns from the territory of the settlement, showed the absence of *An. beklemishevi* specimens in a representative collection. A thorough search for diapausing females in abandoned cellars, ruined saunas, and deep-buried winter bee huts allowed finding some *Anopheles* females which were all identified as either *An. messeae* B or *An. beklemishevi*. In the spring of the following year, we analyzed females collected in the cattle barns in the territory of the village after overwintering from April 20 to May 5. *Anopheles beklemishevi* and *An. messeae* B females were absent from the collection (Table 2). However, both species appeared in the cattle barns after May 10 and their frequencies increased over a 10-12-day period. There is only one interpretation of this finding: females of *An. beklemishevi* and *An. messeae* B overwinter mainly outside the settlements, whereas for wintering they use only shelters in the territory of the settlements which are similar to those found in the wild. In the wild, females hibernate in many locations, including animal burrows, pits, caves, root networks, tree hollows, bark cracks at the base tree of trunks, in the cavities of old tree stumps and fallen trees, piles of straw on a stubble field, and piles of firewood (Beklemishev 1944, Netzkii 1957). Consequently, as previously mentioned, both of these species are exophilic (Novikov 1984, 1997). These refuges are most often located covered with snow in the forest and heat up later than do buildings within settlements. This explains the later appearance of females of exophilic species in cattle barns and the gradual increase in their density as a result of the extended time of their departure from the refuges. In the surroundings of Tomsk city, the period from 15 to 25 May is optimal to assess the species proportions, since females have already left their wintering shelters and the vast majority have accumulated in the locations where the farm animals are concentrated, while a new generation has not appeared yet. A comparison of these proportions in the collections of females in the spring of 1976, 1978, and 1983 showed that the relative numbers of *An. beklemishevi* in this locality decreased significantly ($p<0.01$) from the early collections to the later ones (Table 2). This means that the proportion of this species has indeed been decreasing over past decades in different regions, likely a reaction of the species to the warming climate.

Importantly, as noted by Markovich (2003), the analysis of natural populations at different time points does not really allow trends to be established in the changes of proportions of species and their ranges in response to warming. However, simultaneous analysis of multiple single collections from geographically distant populations would not allow studying the changes in the proportions of species; moreover, it is hard to carry out. For these reasons, this approach is justified when changes are examined in detail in a few geographic checkpoints and the analysis is based on all available data. Although it is likely that the southwestern border of *An. beklemishevi*'s range shifted to the northeast during the period when the material for the current study was collected, the facts and trends revealed would still be valid.

Ecological features identified for *An. beklemishevi* make possible the analysis and discussion of the geographic distribution of this species. The locations examined in the numerical order from Table 1 are shown in Figure 2. Together with the data available from the literature (Stegnii and Kabanova 1976, Stegnii et al. 1978, Saura et al. 1979, Korvenkontio et al. 1979, Jaenson et al. 1986, Moskaev 2012, Perevozkin et al. 2012), our results allow for a detailed description of the area of *An. beklemishevi* distribution. In Northern Eurasia, its range stretches from west to east for over 6,000 km from Scandinavia to the middle reaches of the Lena River, and from north to south for approximately 1,400 km in the European part of Russia, 1,800 km in Western and 1,100 km in Eastern Siberia. The species range, occupying a part of the forest-tundra zone, is mainly confined to the zone of lowland taiga and mixed forests of Eurasia, as well as to the mountain taiga forests of the Altai and Sayan Mountain systems, the mountains of Central and Eastern Siberia, and swampy regions of middle and northern parts of the West Siberian Plain (Figure 2).

Range border in the west and north

The extreme southwestern point where *An. beklemishevi* has been found in the current study is the backwater of the Obdeh River in the vicinity of Sokolovo village, Pechora district of Pskov region. This location lies approximately 250 km south-southwest of Tatianino village, Leningrad region, the extreme southwestern point where the species was found by Moskaev (2012). The similarity of landscape and ecological features of the western part of Pskov region and

Table 1. Number of *Anopheles* specimens and the proportion of *An. beklemishevi* in the samples from geographically remote locations of Russia and Kazakhstan (L – larvae, F – females).

Collection localities (coordinates)	Collection date	N	Proportion of *An. beklemishevi* (%)
1 Sokolovo (57º37´N; 27º28´E)	12.08.1989	48 L	4.17 ± 2.88
2 Solnechnogorsk (56º11´N; 36º59´E) *	02.06.1979	78 L	1.28 ± 1.27
3 Suzdal (56º26´N; 40º26´E) *	02.06.1979	30 L	6.67 ± 4.56
4 Verbovsky (55º31´N; 41º59´E) *	27.08.1986	192 L	1.04 ± 0.73
5 Zelenodolsk (55º51´N; 48º31´E) *	07.08.1973	119 L	0.84 ± 0.84
6 Koltashi (57º24´N; 60º52´E) †	11.07.2012	103 L	11.65 ± 3.16
7 Sredniy Vasyugan (59º13´N; 78º14´E)	09.08.1982	74 L	89.19 ± 3.61
8 Chainsk (57º55´N; 82º36´E)	21.08.2010	159 L	43.40 ± 3.93
9 Kolpashevo (58º19´N, 82º55´E)	04.08.1982	104 L	25.96 ± 4.30
10 Krivosheino (57º21´N; 83º56´E) average	2001-2002	882 LF	12.44 ± 3.08
11 Bely Yar (58º26´N; 85º03´E)	02.07.2000	26 L	100
12 Kireevsk (56º21´N; 84º05´E)	27.07.1999	138 L	7.25 ± 2.21
13 Kolarovo (56º20´N; 84º56´E) average	1975-2008	2307 LF	13.31 ± 2.50
14 Itatka (56º49´N; 85º36´E)	25.08.1982	26 L	11.54 ± 6.27
15 Teguldet (57º18´N; 88º10´E)	22.07.1975	109 L	43.12 ± 4.74
Teguldet	08.07.2000	450 L	11.56 ± 1.51
16 Kutonovo (53º63´N; 86º42´E)	06.06.1981	85 L	8.24 ± 2.98
17 Belogorsk (55º01´N; 88º29´E)	15.07.2006	41 L	97.56 ± 2.41
18 Achinsk (56º16´N; 90º30´E)	15.07.1990	67 L	2.98 ± 2.08
19 Ust-Chul (52º58´N; 89º52´E)	08.08.2000	4 L	25.00 ± 21.65
20 Bugach (56º02´N; 92º46´E)	15.08.1989	118 L	2.54 ± 1.45
21 Yeniseisk (58º28´N; 92º08´E)	18.07.2010	55 L	1.82 ± 1.80
22 Boguchany (58º22´N; 97º26´E)	25.07.1991	125 L	1.60 ± 1.12
23 Abakan (53º43´N; 91º25´E)	23.07.2005	96 L	1.04 ± 1.03
24 Voevodskoe (52º47´N; 85º36´E)	18.08.2001	135 L	1.48 ± 1.04
25 Cherga (51º34´N; 85º34´E) average	1984-2008	493 L	40.61 ± 3.22
26 Ziryanovsk (49º44´N; 84º16´E)	21.08.2012	72 L	0
27 Sanaga (50º44´N; 102º48´E)	10.08.2009	97 L	0
28 Kabansk (52º06´N; 106º65´E)	12.08.1998	124 L	0
Kabansk	25.08.2000	143 L	0
29 Ulan-Ude (51º48´N; 107º30´E)	24.08.2008	60 L	0
30 Onokhoy (51º55´N; 108º02´E)	22.07.1987	51 L	0
31 Chelutay (51º47´N; 108º05´E)	25.07.1987	59 L	0
32 Ust-Barguzin (53º24´N; 109º01´E)	14.07.2005	107 L	0.93 ± 0.93
Ust-Barguzin	25.08.2009	64 L	0
33 Lensk (60º43´N; 114º54´E)	02.08.2011	159 L	1.26 ± 0.88
34 Yakutsk (62º01´N; 129º44´E)	12.07.1990	380 L	0
Yakutsk	11.08.1998	215 L	0
35 Igarka (67º28´N; 86º34´E)‡	03.08.2010	1 L	100

Note: *, † – published previously (Novikov and Alexeev 1989, Novikov and Vaulin 2014); ‡ – Artemov (personal communication); N – sample size; standard deviation is given as a measure of statistical error.

Table 2. Number of *Anopheles* specimens and the proportion of *An. beklemishevi* in samples from three West Siberian checkpoints.

	Collection localities, stage	Collection date	N	Proportion of *An. beklemishevi* (%)
Krivosheino	larvae, reservoir 1	31.07.2001	91	28.57 ± 4.74
	larvae, reservoir 1	01.09.2001	87	12.64 ± 3.56
	larvae, reservoir 2	31.07.2001	71	23.94 ± 5.06
	larvae, reservoir 2	01.09.2001	139	15.11 ± 3.04
	larvae, reservoir 2	16.06.2002	105	16.19 ± 3.60
	females	20.06.2002	127	7.09 ± 2.28
	larvae, reservoir 2	10.09.2002	60	3.33 ± 2.32
	larvae, reservoir 3	17.06.2002	93	3.23 ± 1.83
	larvae, reservoir 3	11.09.2002	109	1.83 ± 1.28
Kolarovo	larvae, reservoir 2	17.07.1975	82	4.88 ± 2.38
	larvae, reservoir 3	17.07.1975	197	49.24 ± 3.56
	larvae, reservoir 4	17.07.1975	120	27.50 ± 4.05
	larvae, reservoir 5	17.07.1975	103	0
	larvae, reservoir 2	21.08.1975	102	1.96 ± 1.37
	larvae, reservoir 3	21.08.1975	89	26.97 ± 4.70
	larvae, reservoir 4	21.08.1975	44	63.64 ± 7.25
	larvae, reservoir 5	21.08.1975	100	5.00 ± 2.18
	overwintered females	19.05.1976	126	26.19 ± 3.92
	females	22.06.1976	212	13.21 ± 2.33
	females	11.08.1976	144	1.39 ± 0.98
	overwintered females	11.06.1978	129	26.36 ± 3.88
	females in diapause	10.10.1982	172	0
	overwintered females	30.04.1983	129	0
	overwintered females	20.05.1983	76	6.58 ± 2.84
	females	26.06.1989	108	3.70 ± 1.82
	larvae, reservoir 5	26.06.1989	102	0.98 ± 0.98
	females	21.07.1989	84	14.29 ± 3.82
	larvae, reservoir 5	21.07.1989	75	1.33 ± 1.32
	larvae, reservoir 5	08.07.1994	101	0.99 ± 0.99
	larvae, reservoir 5	18.07.1997	153	0.65 ± 0.65
	larvae, reservoir 5	11.07.2007	102	2.94 ± 1.67
	larvae, reservoir 5	08.07.2008	58	1.72 ± 1.71
Cherga	larvae	30.06.1984	61	96.72 ± 2.28
	larvae	20.08.1998	116	60.34 ± 4.54
	larvae	30.08.2005	135	31.85 ± 4.01
	larvae	01.09.2007	95	9.47 ± 3.00
	larvae	15.08.2008	86	4.65 ± 2.27

Note: N – sample size; standard deviation is given as a measure of statistical error.

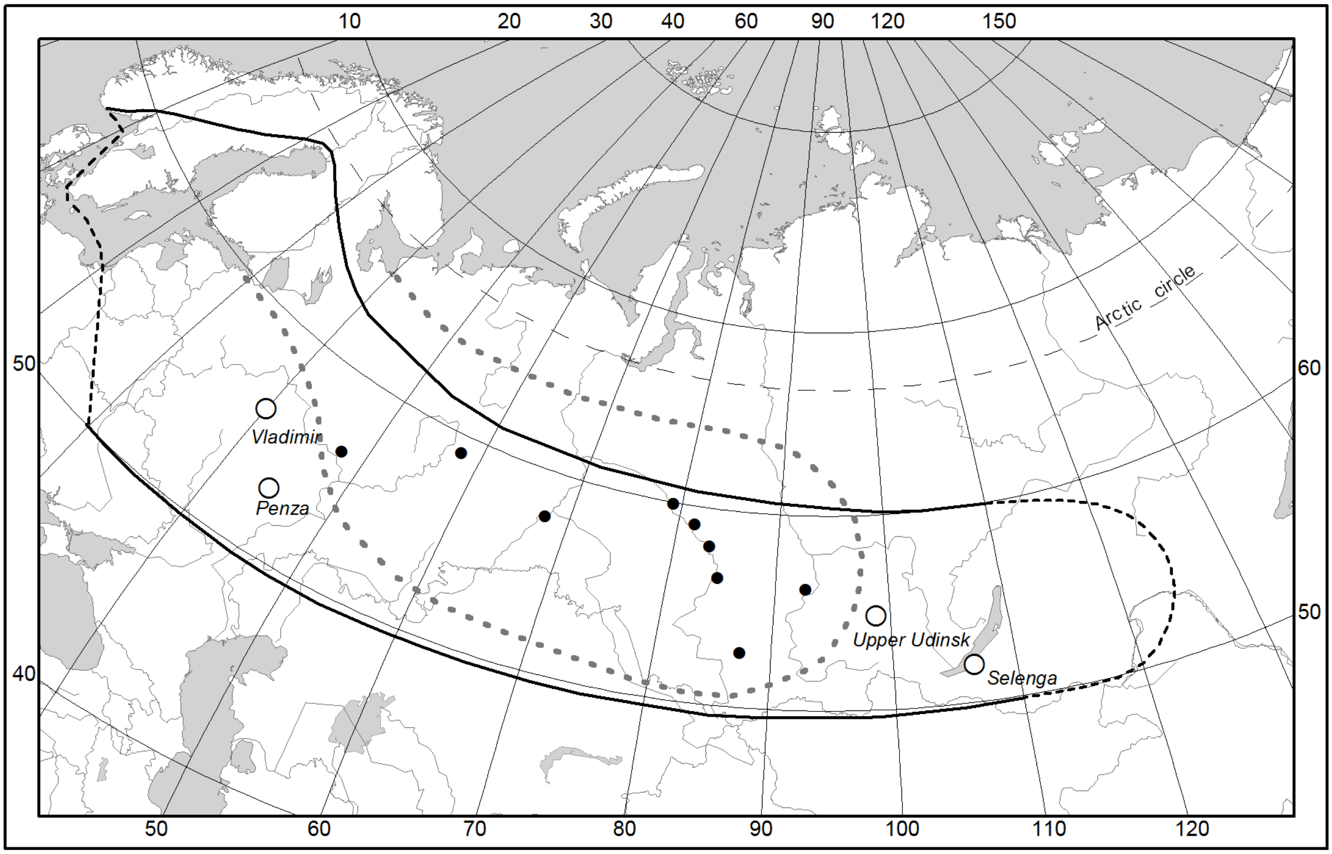

Figure 1. Distribution area of *An. beklemishevi* described by Stegnii et al. (1978) (the boundary of the range is indicated by gray dashed line) and by White (1978) (black solid and dotted line).

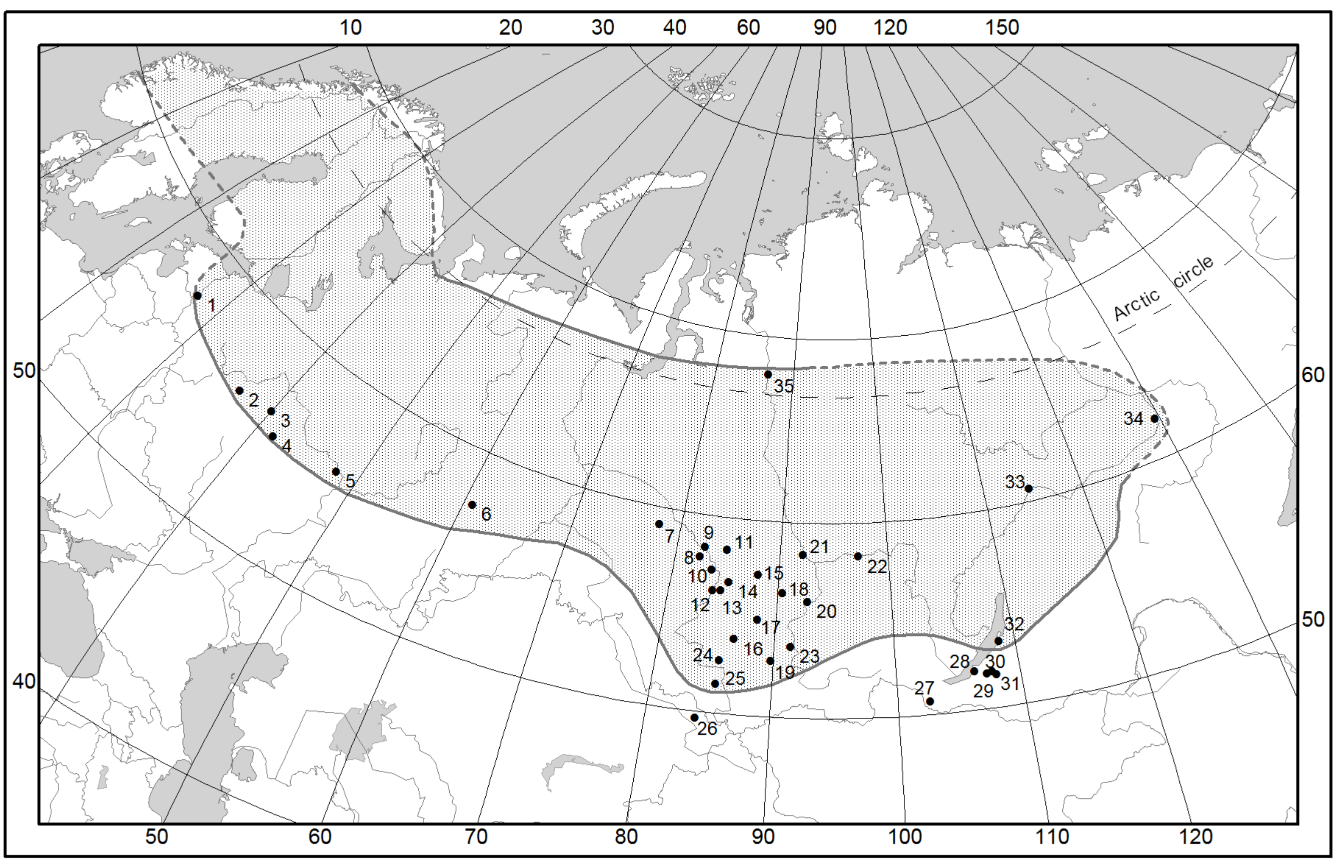

Figure 2. Distribution area of *An. beklemishevi* according to our findings. Locations have the same numbers as in Table 1.

Table 3. Daily fluctuations in surface water temperature (˚C) in Kolarovo village reservoirs on August 12-13, 1975.

Time and date		Reservoir No. 2	3	4
15:00	12.08	24.8	25.8	18.5
18:00	12.08	22.8	24.1	19.5
21:00	12.08	20.2	20.8	18.4
24:00	12.08	18.7	19.1	17.9
03:00	13.08	16.8	17.9	16.8
06:00	13.08	16.0	15.5	15.7
09:00	13.08	18.0	17.9	16.3
12:00	13.08	21.2	21.2	18.1
15:00	13.08	24.2	25.8	21.2
	Daily average	20.3	20.9	18.0
	Change (deg/h)	0.709	0.858	0.429

Table 4. The average number of *Anopheles* 1st to 4th instar larvae and pupae collected per m^2 of reservoir surface in Kolarovo village in July and August, 1975.

Reservoir No.	Reservoir area (m^2)	Date 07.07	12.07	17.07	22.07	28.07	03.08	08.08	13.08	Monthly average
2	1200	89	148	123	97	128	479	337	156	194.6
3	280	3	32	52	35	36	26	48	24	32.0
4	35	3	34	24	23	20	20	26	42	24.0
5	25000	18	32	45	33	27	19	33	39	30.8

Estonia allow us to assume that *An. beklemishevi* inhabits the northeast, if not the entire territory, of this country, as well as most of Fennoscandia, not only its northern part as is usually considered (Saura et al. 1979, Utrio 1979, Jaenson et al. 1986). Because *An. beklemishevi* is exophilic, low in number, and is scattered all over the area, it is difficult to register its presence in some areas as compared to other species of the Maculipennis complex. In northern Fennoscandia, the range border is likely to lie in the forest-tundra zone. Solovei and Likhoded (1966) found *Anopheles* to the northwest of Murmansk city (68°58'N; 33°05'E). The exact identity of these species is unknown, however; Saura et al. (1979) and Jaenson et al. (1986) reported *An. beklemishevi* in the far north of Finland and in northern Sweden. Perevozkin et al. (2012) found *An. beklemishevi* near the town of Kem (64°57'N; 34°36'E). The obvious association of its range with certain landscape/climatic zones indicates the important role of temperature and temperature-dependent climatic factors in its geographical distribution, as has been postulated for the entire Maculipennis complex (Beklemishev and Zhelokhovtsev 1937, 1945, Beklemishev 1944). Therefore, the border of *An. beklemishevi* that ranges from the Kola Peninsula further to the east should roughly coincide with the northern boundary of the forest tundra and woodlands, that is, the boundary of the climate zone, where the sum of air temperatures over the period with an average daily temperature over 10° C is higher than 400° C. In other words, the border lies to the north of the Arctic Circle (66°33'N). In spite of the fact that in this segment of the species range the most northern points where *An. beklemishevi* was identified, Ukhta (63°34'N; 53°42'E) (Stegnii 1991) and Berezovo (63°53'N; 65°03'E) (Stegnii et al. 1978), my conclusion is valid because *An. maculipennis* s.l. was found in the area surrounding the Shchuchya River (66°48'N; 68°22'E) on the Yamal Peninsula (Polyakova and Patrusheva 1974), and on Cape Povorotniy (68°67'N; 76°29'E) in the Taz Bay (Kiseleva 1927, 1936), whereas in Igarka town (67°28 'N; 86°34' E) the presence of *An. beklemishevi* was documented (G.N. Artemov, personal communication). An indirect argument for the presence of *An. beklemishevi* in this region is its obvious affinity for the forest-tundra, taiga forests, and upland and wetland areas. In the surrounding area of the Shchuchya River, where the spur of the Polar Urals is partially located, the most northern in the Yamal Peninsula floodplain forests are found. Cape Povorotniy is situated in the tundra, and the mosquito developmental cycle is unlikely to proceed there. The explanation that specimens collected in this area resulted from migration of mosquitoes (Kiseleva 1927) is also unlikely. Maybe they have been moved to this locality by air masses. Apparently, all of the currently published works within the area about the northern border of *Anopheles maculipennis* s.l. (Gutsevich et al. 1970) only refer to *An. beklemishevi* and *An. messeae* B. A segment of the border of *An. beklemishevi*'s range from Igarka to the Lena River has not been characterized yet, however, the association of *An. beklemishevi* populations with certain natural latitudinal and altitudinal zones suggests that it lies to the northeast of the Vilyuy River mouth and Yakutsk. This is contrary to the findings that in the surrounding areas of Yakutsk, *An. beklemishevi* specimens were not recovered (Table 1).

Range border in the east and the problem of *An. lewisi* and *An. selengensis*

Significant numbers of *Anopheles maculipennis* s.l. mosquitoes were collected from many settlements of the Middle Vilyuy to its mouth (Kolpakova 1933) and in other regions of Yakutia (Chebotarev and Ryabykh 1961). It is possible that *An. beklemishevi* was present in those collections, since Kolpakova (1933), among others, noted the dark color of the mosquitoes, which is characteristic of *An. beklemishevi.* Yakutsk city, where our collections were made, is located in the Tuymaada Valley, where the summer is usually very hot and dry. The landscape has obvious signs of steppe formation and was transformed by human activities many decades ago. All this is not appropriate for *An. beklemishevi*. As a result, it is likely that the species has not been detected here. Alternatively, this region might not be inhabited by *An. beklemishevi* or might be characterized by its rare occurrence. However, in accordance with the ecology of the species and the fact that mountain taiga spans to the northeast of Yakutsk, *An. beklemishevi* can inhabit the regions far north and east of the Tuymaada Valley. In more southern regions of Eastern Siberia, *An. beklemishevi* was found inside the town of Lensk, the Shantalyk River floodplain of Ust-Barguzin, as well as in Central Siberia, however, it is absent from the southeastern and southern parts of the Baikal region (Table 1). Our identification of *An. beklemishevi* in Yakutia and its close relationship with an American species *An. earlei* (Stegnii 1991) indirectly supports the hypothesis of its penetration from America into Eurasia via the Bering Land Bridge. Of course, this will require an extensive genetic study of both species to confirm or refute this hypothesis.

The analysis of collections from the Baikal region is of particular importance in relation to the problem of *An. lewisi* and *An. selengensis*. *Anopheles beklemishevi* was absent from the 267 individuals collected in the surroundings of Kabansk village (the lower reaches of the Selenga River); 170 specimens collected in different years in the neighborhood of Ulan-Ude city (former Verkhneudinsk = Upper Udinsk) and Chelutay/Onohoy stations; as well as 97 individuals collected in the Sanaga ulus. It was from this region (Selenga station and Ulan-Ude) that Ludlow (1919, 1920) used collections to describe two new species of *Anopheles*. She also proposed that *An. selengensis* may be a form of *An. lewisi*. Since, as we demonstrated, *An. beklemishevi* specimens were absent among 534 *Anopheles* individuals collected in a number of locations in the southeastern part of the Baikal region, together with the fact that this species was not found in the west of the Baikal region and the surroundings of Chita in an earlier report (Stegnii et al. 1978), the ideas of White (1978) on the identity *An. beklemishevi* and *An. lewisi* (syn. *selengensis*) has no grounds. On the other hand, one of the two cryptic species of *An. messeae* s.l., namely, exophilic *An. messeae* B, is abundant in this region. This circumstance allows identification of *An. lewisi* (syn. *selengensis*) as *An. messeae* B. Even if we assume that at the beginning of the twentieth century the border of the *An. beklemishevi* range was to the south of Ulan-

Ude, it is highly unlikely that individuals of a species whose population size is small throughout its range were present in very limited collections of adults from Selenga station (2♀, both were described as *An. selengensis*) and Verkhneudinsk (8♀ and 14♂, all were described as *An. lewisi*). Conversely, it is almost certain that these individuals belong to *An. messeae* B. It should be noted that Ludlow pointed to the almost black color of the *An. selengensis* body. However, she was almost convinced that *An. selengensis* was a form of *An. lewisi*, because she did not find any other differences between them. According to our observations, body color of *An. messeae* s.l. imago varies widely, from light brown to rather dark. All of the above infers that both groups of specimens studied by Ludlow were conspecific.

Range border in the south

West of Lake Baikal, the range border of *An. beklemishevi* lies to the north of Irkutsk, along the northern slopes of the Western Sayan Mountains, south of Lake Teletskoe and the village of Shebalino (51°17´N; 85°40´E). In the town of Zyryanovsk, *An. beklemishevi* was not found. However, its absence in one collection cannot be evidence for its absence in the entire region. It is possible that *An. beklemishevi* is present in certain areas of southern Altai and in eastern Kazakhstan. Along the border of the forest landscape zone, the range border rises to the north, reaches the latitude of Novosibirsk city and continues further westward, crossing the Ural Mountains just south of Yekaterinburg. We failed to find *An. beklemishevi* in the surroundings of Taldykorgan (45°01'N; 78°22'E), Semipalatinsk (50°24'N; 80°13'E), Pavlodar (52°19'N; 76°57'E), Shchuchinsk (52°56'N; 70°12'E), and Petropavlovsk (54°51'N; 69°08'E). In the European part of Russia, the southern border of the *An. beklemishevi* range was established by Novikov and Alexeev (1989) and it has been refined in this study (Sokolovo, Pskov region, Table 1). In accordance with a trend identified previously (Novikov 1997, Novikov and Vaulin 2014), the border could have shifted further to the north at this time. The species previously described within the collections of adults made in the surroundings of Vladimir and Penza cities (Figure 1) as *An. alexandraeschingarevi* (Shingarev 1928) could be *An. messeae* A (*An. daciae*), *An. messeae* B, *An. beklemishevi,* and with the least likelihood, *An. maculipennis* s.s., with its precise identification no longer possible.

This study facilitates substantial refinement of the borders of *An. beklemishevi*'s range, especially in the eastern areas. Furthermore, it provides additional indirect support to the idea that this species, or its immediate ancestor, penetrated into northern Eurasia via the Bering Land Bridge where the species range underwent substantial expansion. Exophily, the small size of its populations, and its predominant north and northeast distribution in Eurasia indirectly suggest a low epidemiological potential of *An. beklemishevi* in relation to malaria. It is highly unlikely that Ludlow (1919, 1920) described either *An. lewisi* or *An. selengensis* using *An. beklemishevi* specimens and it is highly probable that her collections included only *An. messeae* B. The changes in the proportions of the Maculipennis complex species in the areas where they are sympatric as well as the shift of their ranges will significantly impact the epidemiology of malaria over large areas of northern Eurasia.

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