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COLD HARDINESS AND RANGE OF THE MYRIAPOD *Angarozonium amurense* (POLYZONIIDAE, DIPLOPODA, ARTHROPODA) IN PERMAFROST ENVIRONMENTS

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Abstract

BACKGROUND: *Angarozonium amurense* (Gerstfeldt, 1859) is the only one out of more than a hundred diplopod species described in Siberia and the Far East that inhabits regions with solid permafrost. **OBJECTIVE:** To evaluate the cold hardiness of *A. amurense* that allows this species to inhabit permafrost regions. **METHODS:** The survival temperature thresholds and supercooling points (SCP) were measured. **RESULTS:** The temperature thresholds for adult animals' survival are -8.5°C in summer and -27°C in winter. Average *SCP* decreases from -7.7±0.3°C in summer to -16.9±0.5°C in winter. Water content decreases from 55.7±1.9°% in summer to 49.4±1.6% in winter. **CONCLUSION:** The cold hardiness of *A. amurense* sets the record in this class of animals. It allows it to overwinter in the upper 15 centimeters layer of soil in most biotopes of the coldest permafrost regions in North Asia.

Keywords: Diplopoda, *Angarozonium amurense*, cold hardiness, permafrost.

INTRODUCTION

The depletion of invertebrate fauna in the cold regions is a well-known fact, though its cause is not sufficiently studied. More obvious reasons are common for polyfagous species: consequences of the region's paleogeographic history (such as results of ice-sheet glacier), lack of summer warmth needed for development and cold hardiness of at least one overwintering ontogenetic stage that is insufficient under modern conditions. The occurrence of land invertebrates in Siberia is not linked to paleogeography as a rule because most of its territory was ice-free during Pleistocene epoch. On the contrary, it is thought that the Laurentide Ice Sheet has destroyed all life in the North of the North American continent and certain groups of animals didn't have enough time to colonize the available territory since (18).

The species depletion has not been well researched in the remarkable group of diplopods. More than a hundred species are described in Siberia and the Far East (16), but only one is present in the continuous permafrost region, *Angarozonium amurense* (Gerstfeldt, 1859) (17).

"It seems that the presence of impermeable surface or near-surface permafrost is the most obvious ecological factor limiting the occurrence of *Diplopoda*in any boreal ecosystem (Minelli, Golovatch, 2001). The northern borders of the diplopod areas coincide very distinctively with the permafrost limits" (9, p. 55). In this context, the ecology of *A. amurense* is of interest. This species has "broken through" the permafrost barrier in a region of the Paleo-Arctic that is abnormally cold in winter (Yakutia) and has been found there at 5 sites which are quite far away from each other (Figure 1). The Yana river valley (67° 40' N) is the northernmost location for any diplopods to have been observed in the Holarctic (17).

Verkhoyansk is one of the coldest regions in the Northern Hemisphere. The presence of *A. amurense* can only be due to either the species' significant tolerance to low temperature or inhabiting exclusively warm biotopes. It can be talik zones – territories along rivers and lake banks where the upper horizons of soil are kept warm by never-freezing flow of river or lake water. The soil temperature there can be rather high (-4 to -8°C) even in the middle of winter when the minimal air temperature is much lower than -50°C. It determines the possible role of talik zones as survival stations (1). To resolve the above alternative the cold hardiness of *A. amurense* has been studied. The results of the study form the subject of the current paper.

MATERIALS AND METHODS

Animals

According to the objective, the samples of *A. amurense* should be collected in Yakutia, the coldest region of its habitat. But their numbers there were unknown. Just 1 to 3 specimens of this myriapod were found later while sorting the invertebrate survey materials from certain regions of the republic (15, 17). In addition, a visit to the locations of Yakutia (especially the Yana River) would require significant expenses. Therefore it was deemed appropriate to evaluate first the cold hardiness of these invertebrates from a more accessible site in the upper course of the Bureya river basin. This river is a left tributary of the Amur River. The severity of its winter conditions is similar to Yakutia (3). *A. amurense* specimens of different ages are quite numerous near the towns of Chegdomyn and Sredniy Urgal, in secondary birch and conifer forests with herbaceous and shrubby understory and a well-developed sod formed of dwarf shrub roots and plant litter at different stages of decomposition. In some plots their numbers reached 20 specimens per 1 dm^2 which allowed us to collect more than 400 animals in the period September 7th through 13th 2013.

The myriapods were delivered to the Institute of Biological Problems of the North, Far East Branch, Russian Academy of Sciences, Magadan, in fabric bags containing plant litter and somewhat decomposed plant debris taken from the same habitat, to study their cold hardiness in the laboratory conditions. Animals were transported in thermal containers equipped with DS1922 LiButton Temperature Loggers

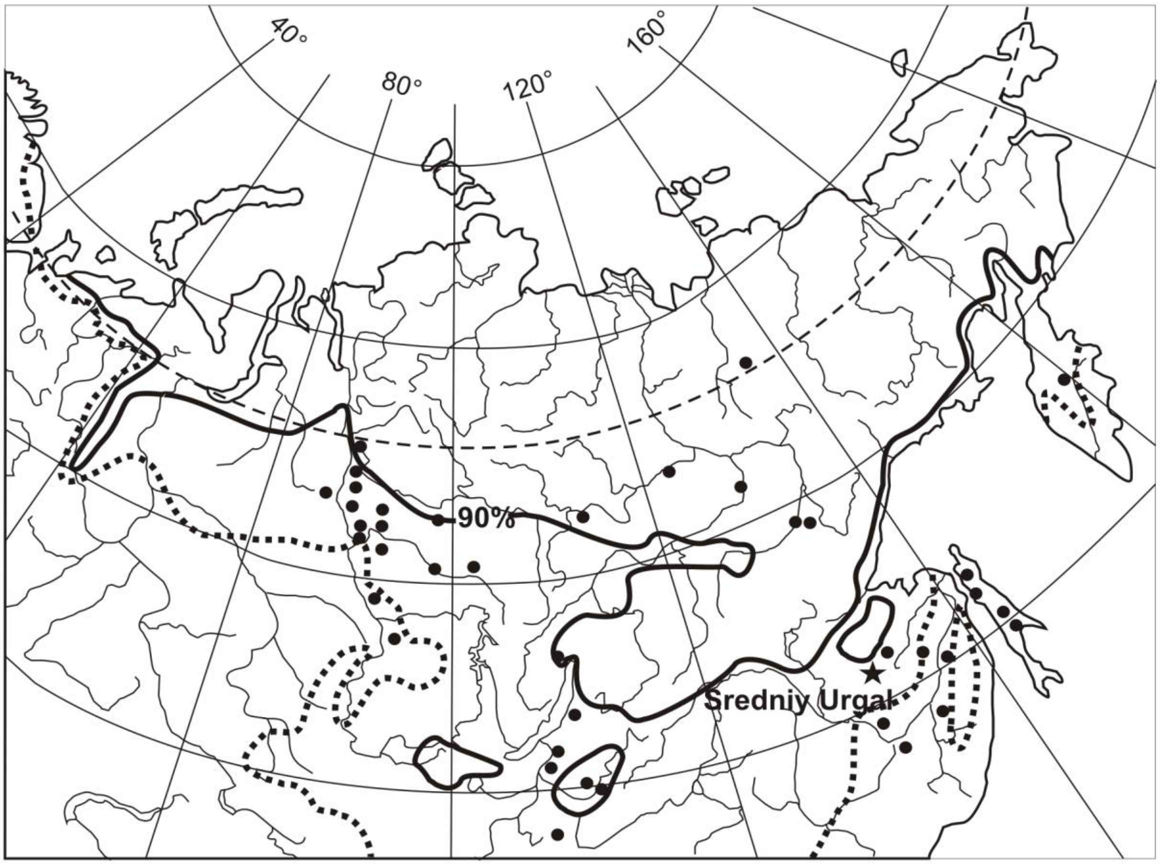

Figure 1. *Angarozonium amurense* distribution (dots are the collection sites). Dotted line shows the southern border of permafrost. Solid line shows the southern border of territory with 10% is covered by talik zones [7]. Asterisk shows the site of myriapod collection for our experiments.

while maintaining temperatures close to those measured in the soil (3°C – 5°C).

The experimental techniques

Upon receiving, animals were placed into 25-ml plastic containers filled with the damp plant substrate up to ¾ of the volume.

The supercooling point (*SCP*) and the threshold of temperature for animal survival (portion of animals that survived exposure to a given temperature) have been measured. The body's water content was also monitored.

The myriapods were acclimated for one month at 5°C followed by one month at 1°C in electric dry air cooling thermostats (TCO–1/80 СПУ, manufactured in Smolensk). After that the specimens were transferred to the testing camera WT 64/75 (Weiss Umwelttechnik GmbH) and incubated for another month at -1°C followed by a month at -5°C. The acclimation took 3 months, and the myriapod overwintering at -5°C took another month. The acclimation and wintering conditions were used based on soil temperatures in the habitat of *A. amurense* and on the data from the meteorological station Sredniy Urgal (3), several kilometers from the collection site.

The temperature threshold for survival after acclimation were determined at -15, -20, -24, -27 and -30°C after 36h incubation at each point. WeissWT 64/75 cameras were used for experiments. Temperature was monitored with autonomous registration modules inside the animal containers additionally. The temperature threshold in summer were also determined. Animals were maintained at 18°C for a month. After that, they were cooled down to -4.5°C, -6.5°C, -8.5°C and -11.5°C; the incubation lasted for 36 hours every step. The temperature was lowered at a rate of 0.5°C/hour, and was brought back up to 5°C at the same rate with pausing for 24 hours at -1°C to 1°C; after that the mortality rate was determined. The survival of myriapods was judged over the first few days after de-freezing by their reaction to tactile stimuli, active mobility and the lack of visible damage. Surviving animals were kept in the laboratory for another one and a half months to exclude any unnoticed trauma that could later lead to death.

The *SCP* values were determined in a custom-designed cold camera that cools animals at 0.6°C/min (individual *A. amurense* weight ranges from 3 to 12 mg). Manganin-constantan thermocouples were used, and the signal was recorded to a computer through an analog to digital converter. Thermocouples were mounted inside copper boxes with lid size 5 cm x 8 cm x 8 cm (to ensure even cooling), and put into the cold camera. The experimental specimens were fixed to the thermocouple junction by a thin layer of Vaseline. The *SCP*s were measured both in acclimated (at -5°C) animals and in those that had been kept at 18°C.

Water content was determined by drying animals to constant weight in a drying oven at 60°C. Humidity was calculated as percentage of the weight change. This parameter was also determined both in acclimated and unacclimated animals. A total of 394 adult specimens and 13 immature specimens have been used in the study. Samplings of 10 to 50 specimens were used to determine the temperature thresholds and water contents. Samplings of 20 and 70 were used for *SCP* measurements.

RESULTS

Adult specimens of *A. amurense* are cold hardy in winter. But during the warm season they have little resistance to freezing conditions. In our experiment the average *SCP* of adult animals was -7.7±0.3°C (minimal *SCP* -10.5°C, *n*=20), and 75–77% specimens survived cooling to -6.8°C (*n*=25). Only 23% specimens survived cooling down to -8.5°C (*n*=17). There were no survivor at a temperature <-8.5°C (Figure 2).

Acclimation is accompanied by significant changes in cold hardiness. The average *SCP* in adult animals is reduced from -7.7±0.3°C (*n*=20) to -16.7±0.5°C (*n*=70); the minimal *SCP* is reduced from -10.5°C to -26.4°C. Average *SCP* of immature animals was -18.1±1.0°C (*n*=13) at -5°C, the lowest -26.1°C. The lethality of the myriapods increased from 5.6 to 81.5% after cooling the temperature fell down from -15°C to -27°C. After exposure to -30°C all the animals perished (*n*=28) (Fig 2). Unfortunately the scarcity of material prevented determination of temperature thresholds in immature myriapods or their summer cold hardiness. Water content of animal's body decreases during acclimation from 55.7±1.9% (*n*=21) to 49.4±1.6% (*n*=26) which is typical for animals that overwinter in the supercooled state (4).

DISCUSSION

Range and some details of ecology

The main range of *A. amurense* is located in the central and southern areas of Siberia and

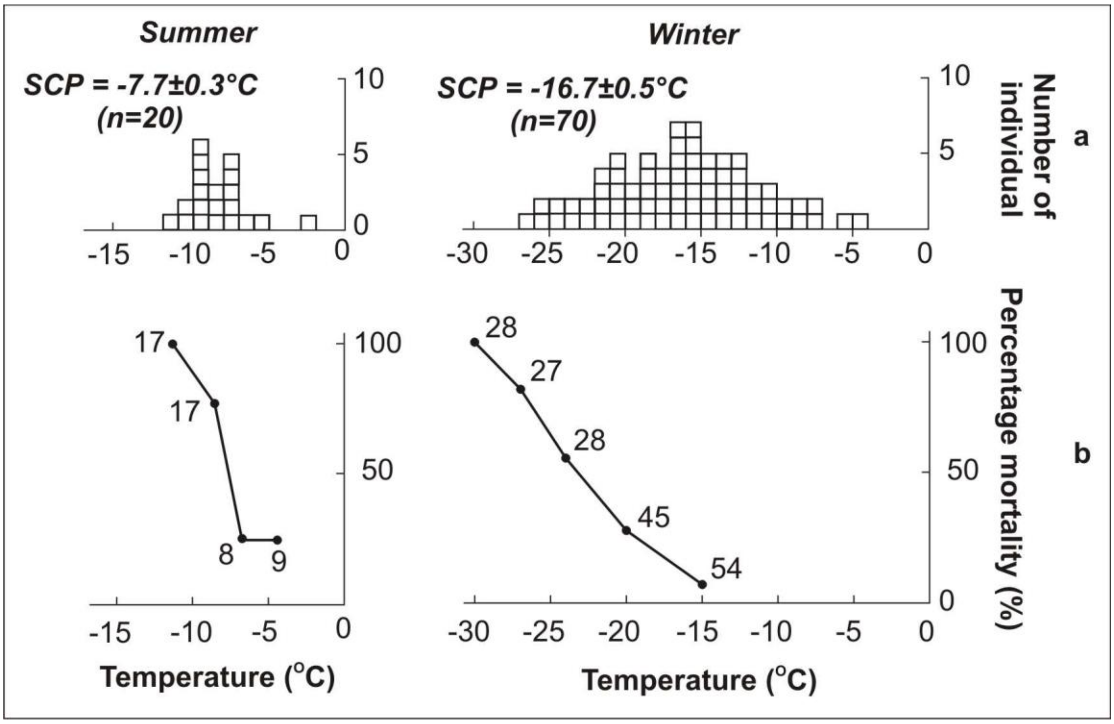

Figure 2. The distribution of supercooling temperatures (a) and relation between the temperature and portion of perishing *Angarozonium amurense* in summer and winter (b). Numbers next to the curves show the sample sizes.

Far East; the species is also found in Southeastern China and Northern Mongolia (Figure 1). It inhabits all forest subzones starting with the northern taiga through most forest biotopes in the south Yenisei River, including the Nizhnyaya Tunguska and Podkamennaya Tunguska rivers. It is found in all soil types but prefers well-drained ones. In these habitats it is dominant (up to 46 specimens per m^2) and often the only diplopod present [20]. It inhabits diverse biotopes of conifer-broadleaf forests in the Amur Region. It is not in prevailing numbers compared to other diplopods. It is a dweller of forest floor and the upper layers of soil and a detritus feeder, little dependent on debris decomposition stage.

The biotope associations of *A. amurense* in Yakutia have not been studied. It is only known that it has been collected in the Yana river basin in an open larch woodland near a brook (N.K. Potapova, personal statement) and in the vicinities of town of Pokrovsk (61°30’ N, 129°09’ E) and Lyampeshka river (64°40’ N, 123°30’ E) in a bog and on moss in a larch forest as well as on leaf litter [14, 15]. The descriptions of the collection sites in the Yenisei basin and in Yakutia regrettably lack any mention of how deep the active layer of permafrost is.

Cold hardiness

The proximity of low-temperature survival thresholds to the lowest *SCP* shows that the supercooled state is highly stable. In invertebrates that cannot survive freezing, these values normally differ by 5°C to 7°C (13). However the obtained ratios of values can characterize *A. amurense* as insignificant freeze tolerants, i.e. a small percentage of specimens survive being cooled below *SCP*. Similar ability was shown previously for the chilopod *Lithobius forficatus* (Linné, 1758) (19) and for part of the selection (3 out of 19 specimens) in the experiments on *Tachypodoiulus niger* (Leach, 1815) (6). The mechanism of cold hardiness in *A. amurense* will be further clarified after the content of ice in the body of overwintering specimens is established.

Cold resistance of the species populations from Yakutia may turn out to be higher than in the animals from the river Bureya studied here since overwintering conditions in Yakutia are harsher and the resistance of supercooled organisms increase in the process of acclimation (2). It is possible that *A. amurense* will be found in other regions of Siberia. So far it has not been found in the thoroughly surveyed locations of the Magadan region (upper course of the

Kolyma River, Magadan vicinity), even though the winter conditions there would allow its survival. For example the average minimal air temperature in January at the Ust-Omchug meteorological station at the upper Kolyma river basin is -39.1°C and the height of the snow layer is 20 to 24 cm, whereas at the station of Sredniy Urgal, in the upper Bureya river basin, they are, respectively, -36.7°C and 20 to 30 cm (3).

The cold hardiness of *A. amurense*is record-breaking so far for this class. The highest resistance to negative temperatures among European myriapods was detected previously in *Polyzonium germanicum* Brandt, 1837. Average winter *SCP* in immature myriapods of this species is -18.5±0.6°C (minimum -23.1°C, *n*=25) while the average adult *SCP* does not exceed -13.5±0.6°C with a minimum at -22.7°C (*n*=150) (5). Nevertheless the *P. germanicum* range does not reach the Trans-Polar Europe or the permafrost regions (12). Another 16 myriapod species studied up to now did not survive temperature below -9°C (6, 10).

Cold hardiness and permafrost

The cold hardiness detected in *A. amurense* allows it to survive winter even in the most extreme Siberian regions, and importantly, the most common biotopes rather than exceptionally warm ones like the talik zones or under blown-up snow cover etc. For comparison, ants (*Formica gagatoides* Ruzsky, 1904, *Myrmica kamtschatica* Kupianskaya, 1986 and others) survive down between -25°C and -28°C in a supercooled state which enables them to overwinter successfully in the upper soil horizons in a broad range of habitats of the upper Kolyma basin (2). Furthermore, the polar ant *F. gagatoides* is one of the most common herpetobiont insect species in the Hypo-Arctic zone in general and in the forested regions of the North East including the Palaearctic Pole of Cold – the Oymyakon valley with omnipresent continuous permafrost.

Since the temperature survived by *A. amurense* are as low as the ones for the polar ant we can conclude that the spread of this myriapod is not limited by its cold hardiness. The fact that it has been found so far mainly to the south of the territory where more than 90% is covered by permafrost (Fig 1) only show that the soil fauna of Northern Siberia is insufficiently studied.

It is thought that the permafrost is limiting the diplopods' spread north specifically by harsh edaphic conditions: "Since diplopods need soil for their egg-laying and further development of larvae and frequently also for shelter from unfavorable conditions the absence of permafrost becomes crucial. It shouldn't be surprising that not only vast territories of tundra in the North of Canada, Alaska and Russia but also boreal forest ecosystems are entirely lacking diplopods" (9, p. 55).

There is no doubt that the south border of the permafrost zone and the north distribution border of the absolute majority of diplopods coincide. However the current *A. amurense* study results just like with many other invertebrates from colder regions demonstrate that the problem is not physically limiting the habitable space because of permafrost. The presence of permafrost in the territory in question only shows that the average year soil temperature is below 0°C. Specific temperature, not to mention edaphic soil conditions can differ even between neighboring biotopes. For example the seasonal soil thawing in different biotopes of the headwaters of the rivers Indigirka and Kolyma varies from 50 cm on northern slopes covered in open larch woodland with moss and small shrub understory, to 250 cm in steep southern slope with steppe vegetation. The entire series of biotopes with intermediate depths of thawing can be observed. Well-developed soils of many types (11) provide for the existence of diverse invertebrates (2), and *A. amurense* is no exception to that.

It is hard to predict specific factors connected with the presence of permafrost that limit the northbound spread of multiple diplpod species known from Primorie, the Amur region and Southern Siberia. It is only clear that it's not the lack of soil. It is quite possible that among these factors are the low winter temperatures (given the diplopods' low cold hardiness) but a lot of other factors can certainly be limiting and not only temperature.

As a possible future project it would be extremely interesting to determine the cold hardiness of the myriapod *Proteroiulus fuscus* (Am Stein, 1857) (8) which reaches further east into the cold regions of Europe to the north of Polar Circle than other diplopods(67°30' N). It is only ten minutes of latitude short of the *A. amurense* record spread to the north (17), although in basically milder climatic conditions.

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