

Mercury in soil, earthworms and organs of voles *Myodes glareolus* and shrew *Sorex araneus* in the vicinity of an industrial complex in Northwest Russia (Cherepovets)

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Received: 17 July 2016 / Accepted: 23 January 2017
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Abstract The characteristic properties of uptake and distribution of mercury in terrestrial ecosystems have received much lesser attention compared to aquatic particularly in Russia. Terrestrial ecosystems adjacent to large industrial manufactures—potential sources of mercury inflow into the environment frequently remain unstudied. This is the first report on mercury (Hg) levels in the basic elements of terrestrial ecosystems situated close to a large metallurgical complex.

Mean values of mercury concentration (mg Hg/kg dry weight) in the vicinity of city of Cherepovets were the following: 0.056 ± 0.033 —in the humus layer of soil; 0.556 ± 0.159 —in earthworms; in the organs of voles *Myodes glareolus* (kidneys— 0.021 ± 0.001 ; liver— 0.014 ± 0.003 ; muscle— 0.014 ± 0.001 ; brain— 0.008 ± 0.002); in the organs of shrew *Sorex araneus* (kidneys— 0.191 ± 0.016 ; liver— 0.124 ± 0.011 ; muscle— 0.108 ± 0.009 ; brain— 0.065 ± 0.000). Correlation dependences between Hg content in soil and earthworms ($r_s = 0.85$, $p < 0.01$) as well as soil and all studied shrews' organs ($r_s = 0.44$ – 0.58 ; $p \leq 0.01$) were found.

Electronic supplementary material The online version of this article (doi:10.1007/s10661-017-5799-4) contains supplementary material, which is available to authorized users.

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The results obtained evidence for a strong trophic link in the bioaccumulation of Hg in terrestrial food webs. Despite the vicinity to a large metallurgical complex, mercury content in the studied objects was significantly lower than values of corresponding parameters in the soils and biota from industrial (polluted) areas of Great Britain, the USA, and China.

Keywords Mercury · Soil · Earthworms · Shrew · Vole

Introduction

Mercury and its compounds are one of the most dangerous toxic substances for living organisms, able to cause a wide range of negative effects in animals (Scheuhammer et al. 2007). Elemental mercury and its inorganic compounds are very poorly absorbed in the gastrointestinal tract (<10%), and additionally, their harmful effects on warm-blooded vertebrates, including man, is much lower than methylmercury (MeHg) which is widespread in the environment, especially in water ecosystems (Wolfe et al. 1988; Wiener et al. 2003). Despite the found toxic effect in predatory animals at the top of the trophic pyramid, their food objects are not as well studied.

At present, most studies on the migration and accumulation of mercury in living organisms were conducted in aquatic ecosystems with relative few studies being done in terrestrial ecosystems. The total amount of anthropogenic metal mercury emission at present time in Russia is about 4% of global releases (Wilson et al.

2006; UNEP. 2008). The main anthropogenic sources of mercury are metallurgy, chlorine-alkaline industry, combustion of natural organic fuel, and solid domestic waste (UNEP, 2008). Soils are an integral compartment in the mercury biogeochemical cycle. Estimates of mercury turnover have shown that a large proportion (ca 60%) precipitates on land before migrating to aquatic ecosystems (Stein et al. 1996; Cristol et al. 2008). Earthworms, as consumers of soil, comprise much of the biomass of edaphic invertebrates with previous research showing them to have a high capacity for accumulating heavy metals relative to insects and plants (Morgan et al. 1992; Reinecke and Reinecke 1998). Earthworms are also an important trophic link in the food webs of terrestrial ecosystems (Giliarov and Striganova 1978). For instance, small mammals often consume large quantities of soil biota such as earthworms (Taylor et al. 1981; Anderson et al. 1982; Hunter et al. 1987; Brewer and Barrett 1995) and thus are suitable for mercury contamination assessment due to their short lifespan and inability to migrate over large distances (Talmage and Walton 1991). In this respect, these different compartments of terrestrial food webs allow the possibility for temporal and spatial monitoring of mercury distribution in different terrestrial biotopes (Vucetich et al. 2001).

The city of Cherepovets houses one of Europe's largest metallurgical industries using huge amounts of coal in the technological processing of metals; some 412.5 million tons of coal were processed from 1956 to 2006 at Cherepovets. The burning of coal releases mercury into local environments. The mercury concentration in coal varies from 20 to 56 mg/kg (Mukherjee 1999), and annual consumption of coal by metallurgical plants at Cherepovets (Cherepovets Metallurgical Kombinate, i.e., ChMK) is 8.5 million tons. Thus, around 1000 tons of mercury have entered the environment over the period of operation.

For example, high concentrations of mercury in perch muscle (>0.5 mg Hg/kg) were repeatedly found in fish from lakes of the Darwin Natural Reserve 100 km from Cherepovets (Haines et al. 1992; Stepanova and Komov 1996, 1997).

Studies on mercury content in organs of predatory mammals, representing the highest trophic level in terrestrial biomes of the Vologda region, have documented mercury concentrations comparable to mammal organs from known contaminated areas of Europe (Komov

et al. 2012; Stepina 2010). However, mercury content in other compartments (or trophic levels) of terrestrial food webs, e.g., soils, soil biota, and small mammals has yet to be determined. Therefore, the aim of the present study was to determine the mercury concentration in soils, earthworms, and organs of small mammals (vole *Myodes glareolus*, shrew *Sorex araneus*) from different terrestrial biotopes in the vicinity of Cherepovets. The results will enable an estimate of the trophic transfer of mercury through the examined terrestrial food webs.

Materials and methods

The collection of soils, earthworms, and small mammals was carried out using standard methods in different biotopes near the ChMK at Cherepovets (Vologodskaya oblast, $59^{\circ} 04' 25''$ – $59^{\circ} 10' 53''$ N, $37^{\circ} 39' 37''$ – $38^{\circ} 00' 40''$ E): 1—near-river willow bush; 2—alder forest; 3—mixed birch–alder forest; 4—dry meadow (Fig. 1). A soil transect was made through the middle of each biotope, and samples were taken from each soil horizon in May 2009.

Earthworms (Lumbricidae, Oligochaeta) were collected in May and September 2009 at soil depths <20 cm. Earthworms were not identified to species in the present study, but simply used to represent a trophic group that consumes soils in the food web. Mercury concentration was measured without prior removal of soil in the earthworm esophagus in order to model natural conditions in which soils can be consumed by organisms of higher trophic levels, in this case voles and shrews.

Common shrews (*Sorex araneus*) were caught in July–August 2009–2010 and bank voles (*Myodes glareolus*) in July–August 2010. Collected animals were weighed and the sex determined, and then samples of their organs (liver, kidneys, muscles, brain) were frozen in polyethylene bags for transport to the laboratory where they were kept at 4 – 16°C prior to analysis. During the study period (2008–2010), 858 samples were collected of the different trophic groups, and a subset was analyzed for mercury content: soils—60, earthworms—305 ($n = 56$ analyzed), bank vole—153 ($n = 50$ analyzed), and common shrew—330 ($n = 87$ analyzed).

Samples of soils, earthworms and mammal organs were dried until constant weight at 39°C prior to Hg analysis. Discrepancy in the number of samples of

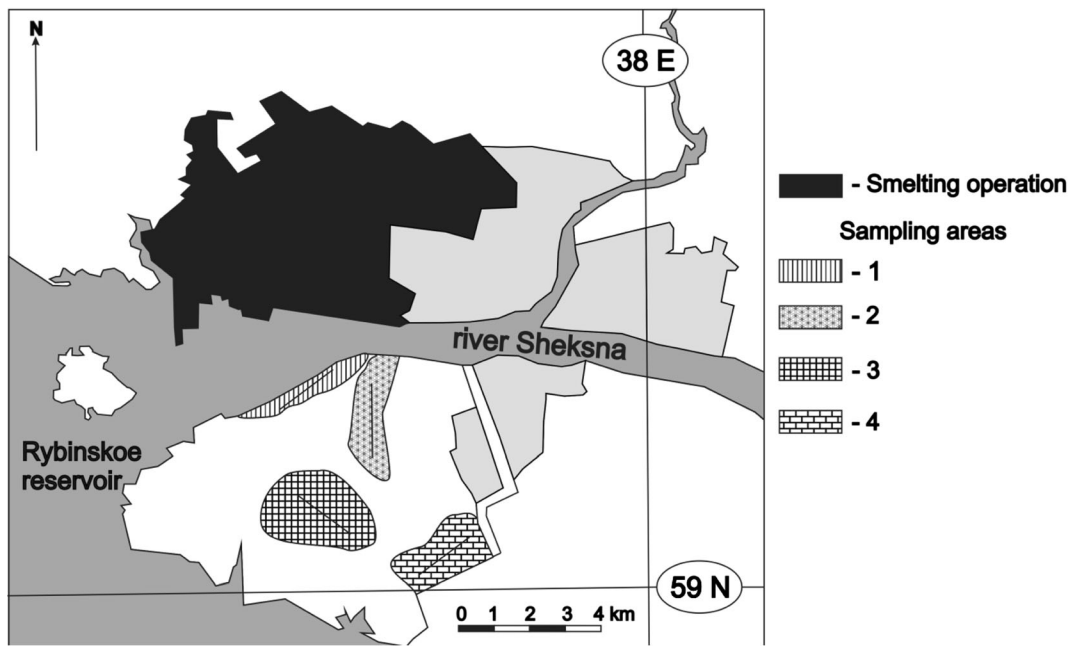


Fig. 1 Schematic map of soil, invertebrates, and small mammals sampling area denoting biotopes: 1—near-river willow bush; 2—alder forest; 3—mixed birch–alder forest; 4—dry meadow; *black color* denotes the territory of metallurgical plant

different organs (n) is due to different degree of preservation of animal carcasses obtained; in some cases, it was impossible to collect all organs needed for analysis. It is important to note that drying sample at 39°C does not lead to a significant loss of mercury in it (see [Supplementary Material](#)).

Mercury concentration for all samples (soil, earthworms, mammal organs) was determined using a PA-915 Hg analyzer equipped with a PIRO attachment. This instrument uses the atom-absorption method with cold steam without preliminary sample preparation. Accuracy of the analytical method was controlled using standard soil samples (GSO SDPS 2498–83, 2499–83, 2500–83) and certified biological material DORM-2 and DOLM-2 (Institute of Environmental Chemistry, Ottawa, Canada).

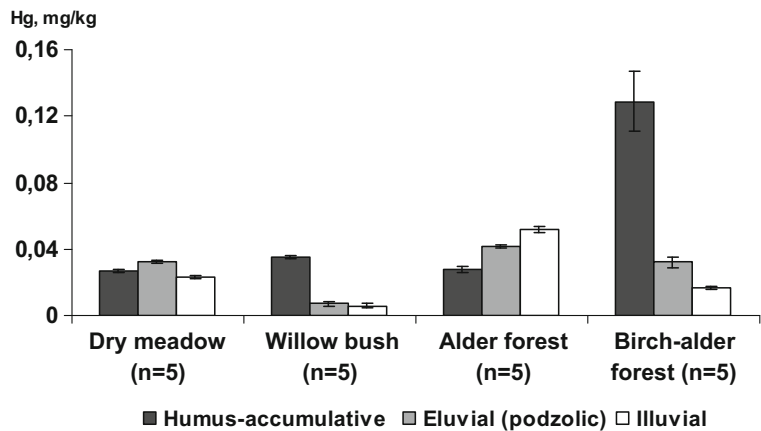
Significant differences between trophic compartments, organ types, biotopes, and year (various analyses) were tested using dispersion analysis (ANOVA, LSD-test) (Sokal and Rohlf 1995). Spearman's nonparametric test was used to test for correlations in metal content between different types of organs as well as between the metal content in organs with the mercury concentration in soils, earthworms, and other environmental-climatic features of the different biotopes. A Shapiro–Wilk test confirmed that the data were not normally distributed.

Results

The average Hg content in soil horizons of the study area was 0.03 mg/kg and varied from 0.014 to 0.047 mg/kg dry mass. The concentration of Hg in soils decreased with increasing soil depth in most biotopes, except alder forest where it increased. Except for alder forest, maximum values were found in the upper (up to 15 cm) humus-cumulative horizon, ranging from 0.027 to 0.149 mg/kg dry mass, and minimal concentrations (0.006–0.032 mg/kg dry mass) were found in the podzolic (down to 25 cm) and illuvial (down to 50 cm) horizons. Birch–alder forest had the highest Hg concentration in the upper layer (0.129 mg/kg dry mass) ($p < 0.05$) (Fig. 2).

The Hg content in earthworms varied between 0.012–5.511 mg/kg dry mass and differed significantly between biotopes (Fig. 3). Maximum mercury concentrations were found in earthworms from birch–alder forest and alder forest, medium concentrations in willow bush, and minimum concentrations in the dry meadow biotope. Although not statistically significant, the average Hg concentration in earthworms overall and in each biotope was higher in spring than in autumn (except for willow bush). The average Hg content in earthworms across all biotopes (0.551 mg/kg dry mass) was tenfold higher than average Hg values in the upper soil layer in

Fig. 2 The Hg content (mg/kg dry mass) of soils in different horizons from different biotopes sampled in the study. Data are given as means and their errors ($\bar{x} \pm m\bar{x}$). Detailed experimental data is given in the [Supplementary Materials](#)



each biotope. There was a significant relationship between mercury content in earthworms and in respective soils ($r_s = 0.85$, $p \leq 0.01$).

Note: *a*, *b*, *c*—values with different literal superscript indices differ significantly between separate biotopes (lines), at $p < 0.05$ (ANOVA).

The Hg concentration in all examined shrew organs was significantly higher (6–10 fold higher) than that in bank vole organs (Figs. 4 and 5). The mercury content in vole organs was comparable to that of soils in the respective biotopes (Fig. 4). The Hg content in organs decreased in the following order: kidneys > liver > muscles > brain. The Hg concentration of vole muscle and liver did not significantly differ among biotopes. However, the Hg content in kidneys of voles captured in the alder forest and in the brain of voles from the birch-alder forest exceeded values of voles inhabiting dry meadow (Fig. 4). *Note:* *a*, *b*—values with different literal superscript indices differ significantly between separate

biotopes (lines), at $p < 0.05$ (ANOVA). Detailed experimental data is given in the [Supplementary Materials](#).

Note: *a*, *b* – values with different literal superscript indices differ significantly between separate

biotopes (lines), at $p < 0.05$ (ANOVA). Detailed experimental data is given in the [Supplementary Materials](#).

The average value of mercury content between organ pairs of voles across the whole sample was correlated: liver—muscle ($r_s = 0.43$, $p \leq 0.01$, $n = 40$), liver—brain ($r_s = 0.42$, $p \leq 0.02$, $n = 29$). There was a significant correlation between mercury content in the kidneys or brain and body mass for the whole sample ($r_s = 0.41$, $p \leq 0.01$, $n = 42$; $r_s = 0.52$, $p \leq 0.01$, $n = 37$, respectively) as well as with Hg concentration in the upper soil layer ($r_s = 0.54$, $p \leq 0.01$; $r_s = 0.38$, $p \leq 0.01$, respectively; $n = 48$ for both). There was no relationship between the mercury content in vole organs and vole sex.

Fig. 3 The Hg content (mg/kg dry mass) in earthworms from different biotopes sampled in the study. Data are given as means and their errors ($\bar{x} \pm m\bar{x}$). Detailed experimental data is given in the [Supplementary Materials](#)

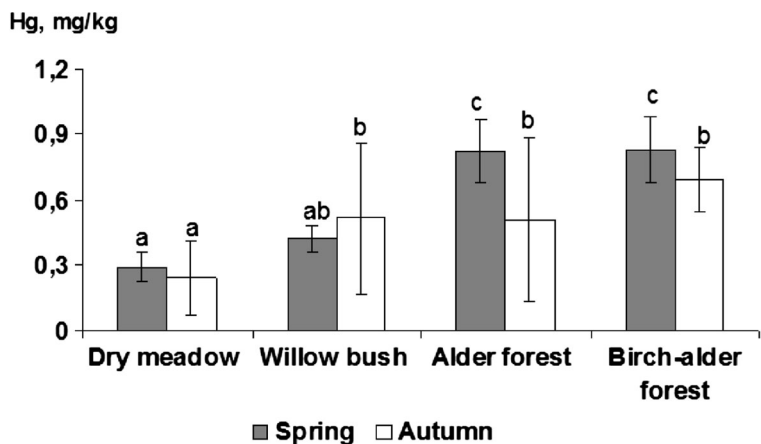
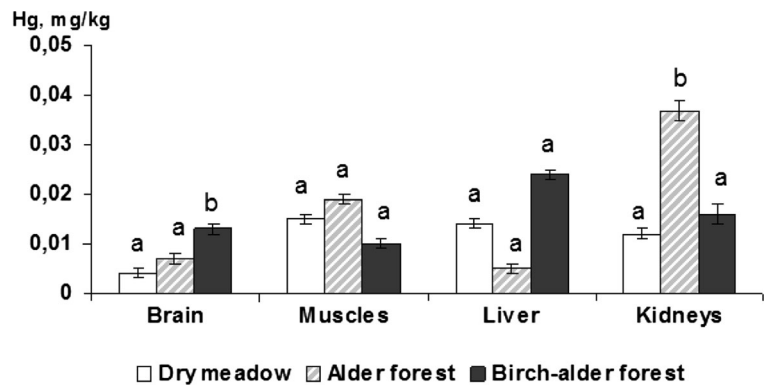


Fig. 4 Mercury content in the organs of voles *Myodes glareolus* from different biotopes (mg/kg dry mass). Data are given as means and their errors ($\bar{x} \pm m\bar{x}$)



There were no significant differences in average Hg concentrations in different organs of shrews among years across all samples, although significant differences among years were found within specific biotopes. For instance, shrew kidneys collected in 2009 in mixed birch–alder forest had higher Hg concentration than shrews collected in 2010, and shrews collected in 2010 in dry meadow contained more Hg than those in 2009. Similar to that of voles, the mercury content in shrew organs decreased in the following order: kidneys > liver \geq muscles \geq brain.

The Hg concentration in shrew organs differed significantly among biotopes: maximum values were found in shrews from birch–alder forest, intermediate values from alder forest, and minimal values from dry meadow and willow bush (Fig. 5). There was a

significant correlation between mercury content in all examined pairs of shrew organs ($r_s = 0.76–0.85$, $p < 0.01$). There was no relationship between mercury content in shrew organs and weight or sex. There was a significant relationship between mercury content in all shrew organs and the Hg content of soils ($r_s = 0.41–0.58$, $p < 0.01$) and earthworms ($r_s = 0.59–0.67$, $p \leq 0.01$).

Discussion

The average Hg concentrations in soils of the different biotopes in the study area adjacent to the industrial center ChMK were 1.4–4.6 times higher than Clark soils (0.01 mg/kg) and 2–3 times lower than Clark

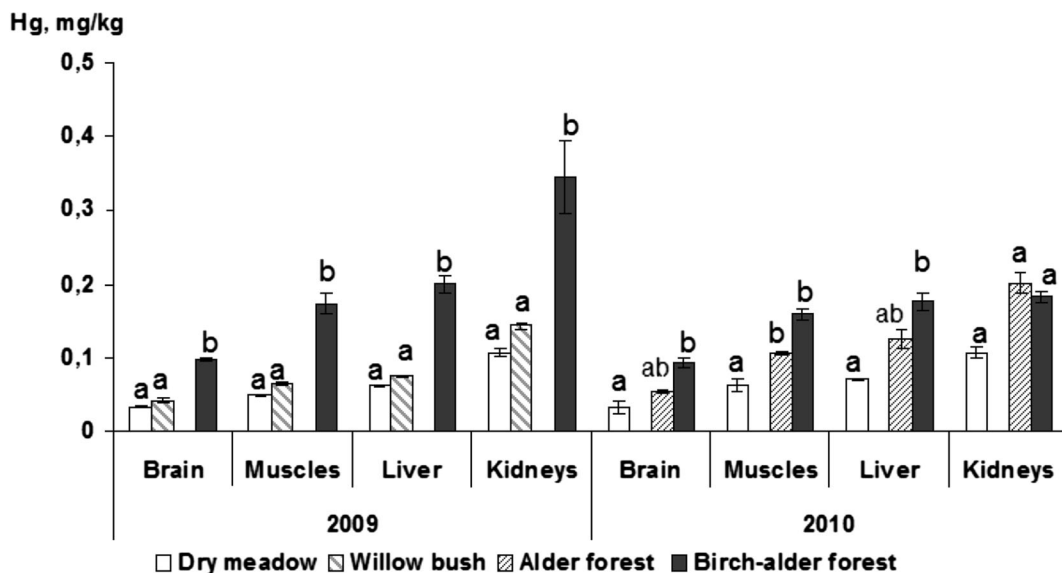


Fig. 5 Mercury content in the organs of shrew *Sorex araneus* from different biotopes (mg/kg dry mass). Data are given as means and their errors ($\bar{x} \pm m\bar{x}$)

lithosphere (0.080–0.083 mg/kg) (Li et al. 2010). To note, the observed soil Hg concentrations in this study are comparable with those of the European part of Russia (0.02–0.5 mg/kg dry mass) (Bespamiatnov and Krotov 1985) and for forest soils of European areas not affected by industry (0.007–0.55 mg/kg dry mass) (Rieder et al. 2011). In fact, they are 20–40 times lower than the Hg content of soils from contaminated regions in the UK, USA, and China, where concentrations range from 0.25 to 21.5 mg/kg (Bull et al. 1977; Zhang 2009).

Mercury concentrations in the organs of shrews and voles in the study area (0.001–0.886 mg/kg dry mass) were comparable with literature data on mercury content in organs of rodents and insectivores (0.002–0.05 mg/kg dry mass) inhabiting sites distant from contaminant sources and 3–10 times lower than average values in animals from industrial areas of Europe and North America (0.51–1.24 mg/kg dry mass) (Bull et al. 1977; Sanchez-Chardi et al. 2009).

Importantly, the mercury content in shrew organs were significantly higher than those of voles organs. These data suggest that mercury enters these mammals via different food sources, predominantly invertebrates in the common shrew diet and vegetation in the bank vole diet (Ivanter 2008). These patterns of mercury bioaccumulation, e.g., an increase in Hg concentration along the trophic chain as well as more intensive bioaccumulation in carnivores and omnivores relative to phytophages, confirm the previously described process of metal biomagnification and the relatively insignificant role of plants in mercury transfer across trophic levels (Ma 1994; Wiener et al. 2003). There also was a statistically significant correlation between mercury concentrations in all organ pairs for shrews, while these relations were weaker or non-significant for voles, possibly indicating a lower metal intake by voles compared with shrews.

The Hg concentration of shrew organs captured in birch–alder forest with wet acidified soils was significantly higher than that of shrews in dry meadow with dry neutral soils. This result suggests that the amount of Hg accumulation in shrews depends on the soil moisture in a particular biotope. An analogous trend was observed in voles, but only in brain tissue. Inorganic and organic compounds of mercury also possess different degrees of bioaccumulation (Ulfvarson 1970). Thus, observed differences in Hg accumulation in the organs of shrews and voles from different biotopes may be caused by an unevenness in Hg inorganic and organic compounds ratios.

Results of medical and toxicological studies on small mammals (mice, rats, guinea pigs) have shown that metal concentrations of 3–5 mg/kg dry mass in the cerebrum may cause visual, cognitive or neuro-behavioral deficiency in animals (Burbacher et al. 1990). Laboratory studies with shrews have shown that mercury content of over 20 mg/kg wet mass in kidneys caused a nephrotoxic effect manifested in organ lesion, i.e., a change in tissue histology (Talmage and Walton 1990). Deterioration in visual acuity and trainability is detrimental for wild animals as it may strongly affect the ability to hunt, leading to starvation, increased susceptibility to diseases, and lower reproduction success. Mercury concentrations in the organs of the small mammals captured in the study area are significantly lower than those experimentally found to be acutely toxic (lethal) doses of metals for vertebrates and do not exceed the concentration 1.1 mg/kg dry mass that is potentially dangerous for terrestrial ecosystem health (Gerstenberger et al. 2006).

In summary, the mercury content in soils decreased with depth of the soil horizon, depending on the character of vegetation and soil conditions of the respective biotope. The Hg content in earthworms was 10-fold higher than that in soils and a strong correlation was found between Hg concentration in soils and earthworms. The Hg content in shrew organs was 5–10 times higher than that in corresponding vole organs. Positive correlations between mercury content in shrew organs and that of soils and earthworms were found, suggesting a strong trophic link in the bioaccumulation of Hg in terrestrial food webs.

Acknowledgements Support is from the Program on Biological Resources of Russian Federation RAS. Dr. Christopher Robinson and Dmitri D. Pavlov have helped with the English version of the manuscript.

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