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Some materials of the 1st International Earthworm Congress

The scientific works of the 1st International Earthworm Congress in Shanghai (China, 2018) are relevant now. We analyzed case studies about functional roles of earthworm and vermicompost on the functional guilds of soil micro-food web, crop yield and plant resistance against pests. We have selected the most important scientific trends, presented modern methods of gathering and accounting, taxonomy and the molecular-genetic analysis of earthworms. We have described the problems of systematisation of group. The article is also devoted to the problems of new methods of phylogenetic constructions. The Congress gave the new basic of ecological researches and biodiversity of oligochaetes in various regions of Kazakhstan and the world. The information is given in the aspect of environment protection.

Keywords: International Earthworm Congress, earthworms fauna, vermicompost, taxonomy, molecular-genetic analysis, lumbricidae of Kazakhstan.

Introduction

Lumbricidae is an important taxonomic group in aquatic and terrestrial ecosystems. Now earthworm breeding and use with different purposes: some kinds of earthworm species was widely used to treat and prevent cardiopulmonary and cerebrovascular diseases; vermicomposting is popularized in green agriculture and environmental protection. However of the importance of group, there are presently some serious deficiencies in the knowledge about their taxonomy, biology and ecology. The basic ideas behind the organization of the 1st International Earthworm Congress in Shanghai (China, 2018) were to bring together cutting edge knowledge and the best minds in all fields of science and industry involving earthworms. This event incorporated the 11th International Symposium on Earthworm Ecology (ISEE 11), the 8th International Oligochaeta Taxonomy Meeting (IOTM 8) and the 1st International Earthworm Industry Forum (IEIF 1).

The first International Oligochaete Taxonomy Meeting (1st IOTM) was held in Madrid, Spain (2003), and 2nd IOTM in Cluj Napoca, Romania (2005), 3rd IOTM in Platres, Cyprus (2007), 4th IOTM in Diyarbakir, Turkey (2009), 5th IOTM in Beatenberg, Switzerland (2011), 6th IOTM in Palmeira de Faro, Portugal (2013), 7th IOTM in Paimpont, France (2016). Since 2009 we have presented materials on earthworms of Kazakhstan at 4 international meetings [1–5]. We studied earthworm species diversity, biomass, density and spatial distribution of the steppe zone in Kazakh uplands.

The theme of the congress was Earthworms and Humanity. The Congress will include sessions on the following fields: 1. Earthworms and agriculture; 2. Earthworm ecotoxicology; 3. Global environmental change and earthworms; 4. Earthworm Taxonomy and phylogenetics; 5. Earthworms and medicine; 6. Earthworm Industry Summit Forum. We took part in the 3rd and the 4th sections of Congress.

In the section «Earthworm taxonomy and phylogenetics» we presented material about distribution of earthworms in natural zones in Kazakhstan. The species composition of earthworms of steppe, forest and mountains ecosystems in Kazakhstan was assessed according to modern researches. Earthworm fauna of Ka-

Kazakhstan is poorly studied compared to the contiguous countries. For example, in last studies the majority of earthworms were recorded for the regions of Soviet Union (97 species and 4 subspecies) and than studies focused on the fauna of Russia (56 species and 5 subspecies). Mountains are in the Central, East and South-East Kazakhstan, and North of the country is mainly in the steppe and forest steppe zones. Semi-desert and desert natural zones cross the territory in West, Centre, South, East and South. The first inventory of earthworm species in Tien Shan Mountains carried out by Malevich in 1945. During the last years many studies were baseline on the taxonomic structure of the earthworms of mountain ecosystems too [6, 7]. Malevich [8] gave a list of 26 species for the forest-steppe and steppe zone, later refined by Perel. Dimo [9], Grib [10] and Valiakhmedov [11, 12] explored the fauna of the irrigated lands of the desert. Earthworms were not recorded in Kazakh virgin motley grass-feather grass and dry fescue grass-feather grass steppes and in agricultural soils here [13, 14]. Other local studies in Kazakhstan were conducted to determine species composition and biotopical distribution of earthworms in different anthropogenic regions and some landscape areas. Therefore, the goal of this study is to continue the earthworm faunistic research in Kazakhstan and generalisation of modern data.

Materials and methods

The species richness and the abundance of earthworms in the ecosystem were assessed on the basis of own and literary source. The specimens had been collected from soils by digging and hand-sorted onto a plastic sheet [15]. Earthworms were identified using available keys [7, 232–258].

Field collections of earthworms were carried out during the 2015 year twice per all vegetation season. In each studied biocenosis 12 regular plots were chosen for earthworm sampling. Then we collected earthworms from the soils by hand sorting. The worms were counted, preserved in ethanol and weighed with electronic scales. Earthworm identification was carried out according to recommendation of Vsevolodova-Perel [16]. We calculated index of density to characterize earthworm assemblages: $P = \sqrt{NB}$. Where N is number and B is the biomass of individuals. Study of variability of grassy cover was conducted by method of description of permanent transects on the plots with different degree of water content. It were determined such parameters as constancy, average projective cover, phytocenotic value of grassy plants.

Results and discussion

Seven species in a collection of 46.3 ind./m² earthworms were recorded on the banks of rivers, in cultivated soils of the southern forest-steppe: *Aporrectodea caliginosa*, *Lumbricus rubellus*, *Octolasion lacteum*, *Dendrobaena octaedra*, *Eiseniella tetraedra*. *Dendrodriulus rubidus*, *Eisenia nordenskioldi*. *Ap. caliginosa* comprises the majority of the abundance in birch forests. *D. octaedra* (4 ind./m²) was registered in gray forest soils of lichen pine forest (Kokshetau group of intra-zone forests). Two earthworm species *D. octaedra* and *Eisenia fetida* occurred on the dark-chestnut soils in birch forests (42 ind./m²) and in wet pine forests (14 ind./m²). *D. octaedra* prevails in the sandy soils of intrazonal steppe pine forests, and in more acid, water-logged soils of birch forests of the Naurzum. There are widespread *Ap. caliginosa* and *Ap. rosea* are found in the litter of willow-poplar thickets of region [17]. Earthworms from Kazakh upland are widespread too: *E. fetida*; *L. rubellus*; *L. terrestris*; *Dd. rubidus tenuis*; *Dd. rubidus subrubicundus*; *El. tetraedra*; *D. octaedra*; *Ap. caliginosa caliginosa*, *Allolobophora parva*. Asian species and subspecies are here: *E. nordenskioldi pallida*; *E. n. nordenskioldi*; *Ap. c. trapezoides*.

Only in the mountains of Kazakhstan lumbricids live in soils of natural unchanged biotopes. In the valleys of the Karatau ridge were found: *Al. microtheca*. *Al. albicauda*, *Al. longoclitellata*, *Aporrectodea rosea*, *Ap. caliginosa trapezoides*, *E. nordenskioldi f. acystis*, *El. tetraedra*. Cosmopolitan species tend to anthropogenic landscapes. The epigeic and endogeic species prevailed in highlands soils. *E. nordenskioldi f. acystis* is found in alpine meadows, birch and juniper forests and canyons of the rivers of alass Alatau. In juniper woodlands live only representatives of the ancient genus *Allolobophora*: *Al. kaznakovi*, *Al. ferganae* (rare), *Al. sokolovi*, *Al. arnoldiana*, *Al. microtheca*, *Al. bouchei* (often). Mountain soils in the gorges of the Ugam range inhabit: *Al. kaznakovi*, *Al. ferganae*, *Al. microtheca*, *Al. graciosa*. Eight earthworms species have been revealed in foothill and mountainous zones of Zailiyskiy Alatau: *Octolasion lacteum*, *Ap. rosea*, *Ap. caliginosa*, *Ap. longa*, *L. rubellus*, *Eisenia fetida*, *E. nordenskioldi*, *D. octaedra*. *Al. ophiomorpha* is one of the largest species of earthworms in the post Soviet regions. It occurs on the southern slope of the ridge of Tarbagatay, as well as in the North-West Altai. *Al. ophiomorpha* and *E. magnifica* (endemic of the Western Tien Shan) were listed in the red book of Kazakhstan.

Earthworms were not recorded or rarely found in natural cenoses of Bayan-Aul and Karkaraly mountains except fringe woods in valleys of streams and small rivers. We aimed to obtain quantitative data on earthworms in plant associations of dominant tree *Alnus glutinosa* and in birch and aspen forests (dominants are *Betula pendula* and *Populus tremula*) replaced alder thickets along channel of streams. The conditions of moistening are regular and stagnant in biotops. The soils in the forests belong to peaty-humus and alluvial turf loamy types.

We identified transient plant complexes with similar conditions of humidity: 1) shore back alder forest with dense layer of grasses includes *Matteusia* species — birch forest with dense layer of grasses includes *Scirpus*, *Festuca* and *Carex* species; 2) shore back alder forest with loose layer of grasses includes *Polygonum* species — aspen forest with *Rosa cinnamomea*, *Cotoneaster melanocarpa* in underground and dense layer of grasses includes *Rubus saxatile*, *Pyrola rotundifolia*, *Filipendula ulmaria* and other species. In the first transitional series density of earthworm population (P) varied from 107.88 to 73.63. *Lumbricidae* belong to six species but *Dendrobaena octaedra* more frequently recorded in shore back alder forest than other species. The autochthonic species *D. octaedra* penetrated to the upland with boreal flora elements from the northern part of the West Siberian Plain, the Altai and the Southern Urals. Birch forests are secondary. Density of earthworm population in these cenoses decrease to 15.84 generally. Dominance is higher for *Dendrodrilus rubidus tenuis*.

Index of density of earthworm population in the second series of transitional biocenoses comprise about 126.95–55.59, respectively plant complexes. *D. octaedra* are present in moist litter of aspen forest. The index of density varied here from 56.0 to 4.3. Distribution of vegetative cover in shore black alder forest is a consequence of inhomogeneous structure and relief of habitats, present of micro sites. So, in shore black alder forest systems of micro sites allows keeping plant species, their seeds and rhizomes in condition of different level of humidity. This is basis for conservation of species composition of different ecological groups of plants. On about stem plots of alder are more stably hygromesophytes, in streams are hydrophytes, on kecks are hygrophytes. The most numerous populations of earthworms were registered in these cenoses with non-uniform structure and a relief of the soil layer.

Conclusion

The earthworms fauna of Kazakhstan include about 25 species and 8 subspecies. The earthworm fauna is more diverse in forests of the northern and in the mountains of the southern parts of Kazakhstan. Steppe and forest-steppe zones of Kazakhstan are populated by widely spread species of earthworms. Dynamic micro communities of grassy plants in black alder forests create the best conditions for reproduction of surface-living species of earthworms and their migrations in other habitats through streams. Furthermore earthworms are the important component of detritus chain of fringe woods in valleys of streams and small rivers in Kazakh upland. The system of soil-plant micro sites of black alder forests is the guaranty of preservation of the dominant role of earthworms among soil invertebrates. Alpine fauna of earthworms is specific. The heterogenetic natural conditions determined the dispersion of earthworms, which have habitat requirements enabling most of them to live only in soils with a regular regime of moistening.

Other posts in this section were very diverse. Neither are our works some studies aimed to clarify earthworm diversity and discuss relationship between diversity and soil variables in different countries. The modern and very important are the results demonstrate the importance of integrative taxonomy in earthworms in order to present reliable taxonomic and biogeographic data [18]. The authors showed that species identification of earthworms using morphology is difficult and inconclusive as homoplasy in many characters is high. The use of DNA barcodes has been demonstrated to improve earthworm taxonomy. According to this reliable identification of earthworm species is crucial for both vermicomposting industry and ecotoxicology testing. DNA barcoding should be employed for taxonomic evaluations and revisions. According to the divergence times estimated by Bayesian method ancestral reconstruction of distribution areas made clear the evolutionary of earthworms in mountains.

The earthworms are highly diversified both locally and among sites collected on many regions and isolated mountain. That's why some researches conducted the molecular phylogenetic analysis using Bayesian inference and than biogeographic analysis of the earthworms to provide insight on the species diversification and distribution of these animals in relation to the geological history of the regions [19].

On the other hand, scientists continue to carry out the determination of earthworms traditionally, using morphological characters. The development of modern technologies and its applicability makes it necessary

to implement in taxonomy as well. Therefore, in order to facilitate earthworm determination taxonomists have developed Earthworm Identification Key — as a computer program [20].

The determination keys that can easily and freely be downloaded facilitate the determination of different organism group and make the determination available to everyone interested. This is also a good way to interest people for an earthworm determination and earthworms in general.

Knowledge of the laws of zoomicrobial interactions is important for understanding of trophic networks in the soil, as well as for solving various applied problems: environmental monitoring, optimization of the nutrient regime in agroecosystems, control of parasitic invertebrates (nematodes, etc.), production of vermicompost, and many others.

The thematic of zoomicrobial interactions was continued by Spanish researchers in several microbiological experiments [21, 22]. Aira M. and other researchers aimed to describe in detail the bacterial communities living in the cocoons of the red worm *Eisenia andrei* and the tiger worm *E. fetida*, and to compare them with the bedding material in which cocoons were deposited. Microbiologists then assessed whether cocoon microbiotas consist of bacterial groups of vertically transmitted symbionts plus random environmental bacteria incorporated during cocoon formation or whether there is selective recruitment during cocoon colonization. The experimental design did not allow them to specify whether these ASVs were derived from the environment or the earthworms, consequently longitudinal studies would also help to clarify the dynamics of embryo colonization.

The species *Eisenia andrei* was a participant of the experiment in definition the earthworm gut microbiome and identification whether the composition of ingested microbiomes from animal faeces impact the composition of the earthworm gut microbiome. Scientists applied 16S rRNA pyrosequencing and metagenomic analysis to characterize the taxonomic and phylogenetic composition of bacterial communities in three different types of animal microbiomes (herbivore, ruminant and omnivore) that have divergent bacterial communities before and after passing through the gut of the earthworm. The authors of the article showed that when a detritivorous earthworm digest them, animal gut microbiomes change their taxonomic bacterial composition. In animals, the needs of processing diet in takes modulates gut microbiome composition. However, earthworms build up their microbiome being less dependent on diet by selecting over the pool of ingested bacteria. Such microbiological experiments are important for developing vermicomposting plans, which were discussed in the next section.

In section «Earthworms and agriculture» the some works were focused on interesting theme: vermicomposting of substrate originating from the Greengood Composter. This type of composter is spread and commonly used especially in Asia countries [23]. The new vermicomposting device was developed, verified and patented in the Czech Republic in cooperation with the Research Institute of Agricultural Engineering in Prague and the Czech University of Life Science in Prague. Purpose of this vermireactor was controlled treatment of biowastes in a container with a support of speasies *Eisenia fetida* or *Eisenia andrei*. Advantage of the Greengood Composter is a relatively quick degradation of biowastes within 24 hours. During the time is possible to reduce content and weight of treated wastes up to 90 %.

The researchers from Cornell University and the University of Vermont were scientificated the exploration of vermicompost products and their potential and mechanisms for use in plant production and the suppression of diseases [24]. One of the important goals of this study was the new family of liquid vermicompost extracts.

Some researches provides an overview of the potential of earthworms and vermicomposting for circular economy and sustainable agriculture. There was shown through the development of an integrated cycle that allows convert «in situ» the waste generated in the wine industry in high quality vermicompost with beneficial biofertilizer and plant-defence properties. J. Dominguez in his work presented the results of a case study in which grape marc derived from white and red wine were processed on a pilot-scale vermireactor to yield a high quality organic, polyphenol-free fertilizer, as well as grape seeds [25]. The vermicompost derived from the different wine varieties was applied in different formulations to the grapevines in the same vineyards were the grapes were harvested to make wine and the grape marc obtained. The results of these investigations provide an important advance in the knowledge of the interactions between earthworms and microorganisms during the decomposition processes and they will be fundamental for the elaboration of biofertilizers and bioplaguicides derived from organic wastes and by-products of the wine industry.

The other scientific work was implemented to investigate the feasibility of vermicomposting malting sludge and its mixtures with straw pellets on the basis of chemical and biological properties [26].

Earthworms are the soil engineers and play a key role in the degradation of soil organic matter by consuming dead organic matter or stimulating microbial communities in the soil. This function conducts according to the three morpho-ecological groups of earthworms: epigeic, anecic and endogeic. The researchers note that enzymatic activities of worms were significantly stimulated in the presence of epi-anecic compared to strict-anecic species [27]. The main conclusions were about necessary to add straw pellets at a minimum of 50 % (vol.) for successful vermicomposting of malting sludge.

The epigeic earthworm *Eisenia fetida* was the main object of compost production researches. The vermicomposting results showed that the dry grass clippings and rice straw along with cow manure were successfully processed to vermicompost and had a dark color, mull-like soil odor and was homogeneous. The combination of rice straw and grass had the highest production of 105 kg, followed by grass and rice straw with 102.5 kg and 87 kg respectively. The harvested vermicompost had an excellent nutrient status, confirmed by the chemical analyses and had all the essential macro and micro plant nutrients like N, P, K, Ca, Mg, Mn, Cu, Zn and Fe [28].

Further, were found that the microbiomes of the intestine and the composted substrate significantly differ, suggesting the elimination of pathogenic bacteria during the food passage through the *E. fetida* intestine [29].

The other species, *Lumbricus terrestris*, to play an important role in repressing toxigenic phytopathogens, like *Fusarium culmorum* and its mycotoxin deoxynivalenol. Scientists use the importance of earthworms for pest control to conceptualize how farmers' management practices influence soil ecosystem services [30].

The new and interesting method is eDNA metabarcoding to assess tillage system influence on earthworm communities [31]. This study also shows that tillage effects on earthworm DNA relative read abundance do not agree with the tillage effects on total biomass obtained by conventional hand sorting. DNA metabarcoding can supplement the conventional hand-sorting in earthworm ecology studies/

In section «Earthworm ecotoxicology» explorers focused on one question: Do we have the appropriate (e.g., scientifically sound, robust, standardized) test methods with earthworms to cover the new data requirements — and if not, which new methods have to be developed [32]. The outcome there were presented the list of recommendations regarding the further use of earthworms for the environmental risk assessment of pesticides in the European Union (and beyond).

Heavy metal pollution disturbs the soil ecosystem by negatively affecting soil fauna and flora. Earthworms have been used as bioindicators of heavy metals and many other pollutants. One of the researches team's objectives was to explore the effects of different concentrations of mercury on the reproductive toxicity of earthworms [33]. Mercury stress affected the morphology and ultrastructure of the sperm. The DNA damage of the sperm increased with the Hg concentration. These results add our knowledge of mercury ecotoxicity, and provide useful information for environmental monitoring and assessment of Hg contamination in soils.

Earthworms are one of the first organisms affected by heavy metal contamination in soil and as such are good model organisms for assessing soil contamination. In other studies the objective was to identify the proteins involved in the earthworm response to arsenite in order to explore the underlying mechanism of arsenite, cadmium toxicity [34, 35]. This knowledge could provide insight into the underlying mechanisms of potential sub-clinical physiological effects from heavy metal pollution of the soil, thus helping in the biomonitoring and assessment of contamination.

An increasing use of silver nanoparticles has raised concerns about associated health and environmental risks, particularly as a source of ionic silver in soils and sediments. Some studies have been performed using standardized earthworm-based tests to establish the toxicological effects of Ag NP within soil [36]. The use of soil-dwelling earthworms was to assess the influence of Ag NP on a geophagous, earthworm, understanding of how nanomaterials interact with soil fauna. The study also sought to provide further information on the efficacy of Artificial Soil as a test substrate for soil-dwelling earthworms.

Veterinary drug residues are a new type of environmental pollutants. When veterinary drugs enter the environment along with the dung and urine of livestock, it may have a profound impact on various organisms in the environment. The experts of School of Agriculture and Biology in Shanghai measured the toxicity of representative drugs in order to explore the ecotoxicity of the residues to earthworms *Eisenia fetida* [37]. The methods referred to Chemical Pesticides Environmental Safety Evaluation Test Guidelines of China and OECD-207.

The articles of 6 section «Earthworm Industry Summit Forum» were devoted to the development of modern agricultural technology, inorganic fertilizers have been widely used in agricultural production, which leads to a sharp deterioration of the soil [38]. The earthworms cast is applied to organic aquaculture and fruit and vegetable cultivation. This model not only eliminates the negative impact of livestock manure and solid waste on the environment, but also successfully improves the soil quality. The «planting and breeding» mode and reuse of earthworms cast increase the output of vegetables and farmers' income, and has good ecological, economic and social benefits.

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New wastewater treatment technology was presented at the Congress. Vermifiltration technology is a transformational wastewater treatment technology which utilises the unique characteristics of worms to process organic waste [39]. The worms are the apex species in the vermifilter, without which the performance would not be maintained. Vermifilters are green alternative technology which requires no power or supplementary blowers or pumps. The worm casts are a nutrient rich natural fertilizer, with nutrient levels being determined by the wastewater being treated. Vermifiltration is one solution to counter water scarcity and pollution provides the benefits of a circular economy. The authors believe that versatility vermifiltration provides a treatment solution for many industries including brewing, food processing, agriculture and sewage from residential development.

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Жауынкұрттар бойынша I Халықаралық конгрестің кейбір материалдары

Мақалада Шанхай қаласындағы (Қытай, 2018) I-ші Халықаралық конгресте жауын құрттар бойынша ғылыми жұмыстар тақырыптары берілген. Топырақтың құнарлылығын, ауыл шаруашылығы дақылдарының өнімділігін және өсімдіктердің зиянкестер мен ауруларға төзімділігін арттырудағы жанбырлы құрттардың ролін және вермикомпостың мәнін сипаттайтын баяндамаларға талдау жүргізілген. Мақалаға сәйкес, жауынкұрттарды жинау мен есепке алудың қазіргі әдістері, әртүрлі түрлердің таксономикалық және молекулалық-генетикалық талдауы ұсынылған. Топтарды топтастырудың мәселесі баяндалған. Люмбрицид филогенетикасының жаңа әдістері сипатталған. I Халықаралық конгресс экологиялық зерттеулер және Қазақстан мен әлемнің әр түрлі аймақтарында

олигохеттердің биоалуантүрлілігін зерттеу үшін тың серпін берді. Материалдар қоршаған ортаны қорғауда қызығушылық танытады.

Кілт сөздер: жауынқұрттар бойынша Халықаралық конгресс, жауын құрттар фаунасы, биогумус, таксономия, молекулалық-генетикалық сараптама, Қазақстан лямбрицидтері.

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Некоторые материалы I Международного конгресса по дождевым червям

В статье представлен обзор научных работ I Международного конгресса по дождевым червям в Шанхае (Китай, 2018). Проведен анализ докладов, характеризующих роль дождевых червей и значение вермикомпоста в повышении почвенного плодородия, урожайности сельскохозяйственных культур и устойчивости растений к вредителям и болезням. Приведены современные методы сбора и учета дождевых червей, таксономического и молекулярно-генетического анализа разных видов. Описаны проблемы систематики группы. Охарактеризованы новые методы филогенетики лямбрицид. В целом, I Международный конгресс дал новый стимул для экологических исследований и изучения биоразнообразия олигохет в различных регионах Казахстана и мира.

Ключевые слова: Международный конгресс по дождевым червям, фауна дождевых червей, биогумус, таксономия, молекулярно-генетический анализ, лямбрициды Казахстана.

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