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Дисертація містить результати власних досліджень. Використання ідей, результатів і текстів інших авторів мають посилання на відповідне джерело

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ANNOTATION

O. Yu. Marushchak. Current state of amphibians of Rightcoastal Polissia. – Qualifying scientific work on manuscript rights.

The dissertation for a scientific degree of the doctor of philosophy on a specialty 091 – «Biology». I. I. Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, Kyiv, 2022.

The dissertation is dedicated to the study of the current state of amphibians (Amphibia, Anura; Caudata) of the Rightcoastal Polissia fauna, the collection of current data on the distribution of all recorded species, the description of previously unstudied aspects of their morphology (including the use of an extended list of morphometric measurements), the study of the tendency to population changes in comparison with studies undertaken almost 40 years ago, research on the species composition of hybridogenic complexes of green frogs of the genus *Pelophylax* and the characteristics of their helminthic invasions, creation of new nature conservation areas valuable for the preservation of amphibian populations. In particular, an important part of the work is devoted to conducting bioclimatic modeling of territories favorable for the life of amphibians in terms of their climatic parameters, as well as predicting changes of such territories for the purpose of more effective and targeted protection of certain species of amphibians. An important component of the work is also the analysis of cases of detection of (background and massive) external morphological anomalies, as well as investigating of the possibilities of their use as bioindicators.

The research was conducted on material consisting of own collections (more than 70 expedition trips, including 21 objects of the nature reserve fund), records from other researchers and citizens within the framework of so-called "citizen science" data collection with personal communication or in social networks, data on records of amphibians in open Internet resources iNaturalist, UkrBIN and GBIF, analysis of materials from the collections of the National Museum of Natural History at the National Academy of Sciences of Ukraine (Kyiv), I. I. Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine (Kyiv), the Zoological Museum of the Ivan Franko National University of Lviv (Lviv), the Museum of Nature of the V.

N. Karazin Kharkiv National University (Kharkiv) and the Chernivtsi Regional Museum of Local Lore (Chernivtsi). Geocoding of the points was done using MAPS.ME and Google Earth Pro spatial orientation software followed by visualization in the geographic information system QGIS. Standard measurements were used for morphological analysis (Shcherbak, 1989; Pysanets, 2007): 8 – for Caudata, 19 measurements and 16 indices for Anura. Data processing was performed in Statistica v. 10.0.228.8 and MS Excel 2013. Geoinformation modeling of climatically favorable areas for 13 identified amphibian species was performed using Maxent (v.3.4.0) software and 19 bioclimatic factors (bio01-bio19) available on the WorldClim open source. Climate changes used to model future changes in climatically favorable areas were used for the MIROC-6 ssp126 climate development model for the years 2000, 2030, 2050 and 2070. The simulation was carried out using the "Bootstrap" replication type. Preprocessing of geocoded data for modeling was performed using the 'ntbox' package in the R environment (v.4.0.5) and MS Excel 2013. The quantitative assessment of climatically favorable areas was performed using the "colordistance" package in the R environment. The probability of differences in the constructed models was investigated using the software SAGA GIS (v.2.14) (Scatterplot function) and Adobe Photoshop CS 2018. The study of helminth fauna was carried out by an invasive method with the dissection of amphibians and the subsequent calculation of the intensity (average, minimum and maximum number of helminths in one individual of the host (II)) and the extensity of infestation (the proportion of infected individuals in the sample (EI)). The species richness of helminths was described using the Sørensen index.

The calculation of the percentage of coloration of the abdomen of representatives of *B. bombina* was carried out using the program Vidana 1.0.1beta (available at the link <http://www.ex.ac.uk/msel>) according to the standard method (Walker, 2011). The classifications of O. D. Nekrasova (Nekrasova, 2008), K. Henle and A. Dubois (Henle et al., 2017b; 2017c; Dubois and Ohler, 2018) were used to define and describe the anomalies. Existing external morphological anomalies were described by calculating the frequency of occurrence of individuals with anomalies (P_{as} , %), the total spectrum of

detected anomalies (S_{ap}) and the partial frequency of occurrence of anomalies (A_{as} , %). To study the species composition of populations of the complex of green frogs of the genus *Pelophylax*, the species affiliation of individuals was determined by electrophoretic analysis of a species-specific genetic marker - the Ldh-B gene. The Standard Data Form Manager (SDM Manager, v.4.2.3) program was used to create new territories of the Emerald Network and their subsequent inclusion in the official Emerald Network of Ukraine. The official part of the application for the inclusion of these objects took place with the mediation and direct participation of the non-governmental public organization "Ukrainian Nature Conservation Group" (UNCG).

During the research, a database of amphibian finds was collected, which includes 1911 geocoded points, of which 32,75% are own finds. 48,61% of all amphibian records on the territory of the Rightcoastal Polissia of Ukraine are new for this territory. The points of amphibian records collected during the research are available in the format of several datasets in the international database of information on the distribution of species of fauna and flora (free access rights) GBIF.

In the course of research, it was found that the composition of the batrachofauna of the Rightcoastal Polissia includes 13 taxonomic units (12 species and 1 hybrid) according to modern taxonomy (62% of the species composition of the batrachofauna of Ukraine; 10 species and 1 hybrid of Anura and 2 species of Caudata). For the first time in the last 20 years, the presence of a reproductive population of the natterjack toad has been confirmed, and it is obvious that this rare species of fauna of Ukraine is still preserved in sufficient numbers at least in the territory of the Volyn Polissia.

Over a 40-year period, the amphibian populations of the Rightcoastal Polissia underwent changes in spatial (places of residence, number, composition of populations) and morphological structure (increase in cases of external morphological anomalies, including anomalous manifestations of coloration), as well as phenological features (shifting of spawning period, interrupting of hibernation), which in most cases is associated with anthropogenic influence on the studied territory (fragmentation of nature ranges, destruction of habitats, pollution, emergence of invasive species, etc.) and global climate changes.

During the study of the biotopic distribution of amphibians, it was determined that the most valuable refugia territories are the valleys of small rivers and water bodies. Lists of characteristic types of meadow habitats (according to the EUNIS classification) that are important for the survival of the amphibian species found in Polissia, and for which these species are the most typical, have also been prepared.

Morphological descriptions of the studied species were made. In particular, on the basis of morphometric measurements, morphometric indicators were specified for all 12 registered species and 1 hybrid form, intersex differences were specified for individual measurements and indices.

During the study of the ventral coloring phenomorphs of *B. bombina*, the average degree of coloring was calculated, which is 45,8%. All 5 clusters of known phenomorphs were found in the studied individuals. Among them, hieroglyphic spots occurred most often (58,7%), pebble-like spots (0,9%) were of the smallest number. A middle correlation was found between the percentage of coloration of the *B. bombina* ventral part and the coordinate of the record, which confirms the assumptions published for this species from the territory of Latvia (Pupins and Pupina, 2008) and demonstrates the so-called clinal variability.

During the study of manifestations of external morphological anomalies, 12 cases of their mass manifestation were revealed (provided the sample size is 25+ individuals). In 11 of them, the frequency of occurrence of abnormal individuals did not exceed the 5% threshold by more than 2 times, and the spectrum of detected anomalies was heterogeneous. The highest rate of occurrence of abnormal individuals was 72.2% for the marsh frog sample from Nyvky Park in Kyiv. During studies of skin anomalies (research covering the entire territory of Ukraine and a much wider time period) it was found out that the percentage of individuals with this type of anomalies tends (a weak correlation) to be higher in regions with a greater integrated index of human influence (Hooman footprint). The largest number of abnormal individuals was found for amphibians of the green frogs' complex ("Pelophylax esculentus" complex - 57.5%), which can be explained by the fact that this group of amphibian fauna of Ukraine spends almost its entire life cycle in an aquatic environment that is an excellent solvent

and transporter of various types of pollutants, which can influence the ontogenesis of these amphibians for a longer time than for those forms that leave the aquatic environment after metamorphosis and return to it exclusively for spawning. The highest partial frequency of occurrence of anomalies was found for skin anomalies – 52,8%. The second partial frequency of occurrence is ectromelia – 5,6%. Other types of anomalies occurred singly.

It was confirmed that *P. ridibundus* and the hybrid form (*P. ridibundus* x *P. lessonae*) have similar ecological preferences based on the identified features of helminth invasions (in similarity - species composition of helminth fauna, in parameters of infection, at the level of helminth infragroups). The difference was that the number of helminths in infragroups and the intensity of invasion of individual species (nematode *I. neglecta*, trematodes *O. ranae*, *C. urniger*) was higher in *P. ridibundus*.

For the first time, bioclimatic modeling of favorable and the most favorable climatically territories as of today was carried out. They make up (in % of the research area) for *P. ridibundus* – 20.73%, *H. orientalis* – 15.89%, *B. viridis* – 9.31%, *B. bombina* – 29.97%, *P. fuscus* – 9.98%, *L. vulgaris* – 16.77%, *E. calamita* – 4.63%, *R. temporaria* – 12.93%, *B. bufo* – 17.97%, *T. cristatus* – 18.88%, *P. ridibundus* x *P. lessonae* hybrid – 11.84%, *P. lessonae* – 5.91%, *R. arvalis* – 28.68%), as well as for the further survival of amphibians of the Rightcoastal Polissia (2050). It was revealed that the territory of the Rightcoastal Polissia will remain favorable for most species in terms of climate until 2050. Thus, such species as *P. ridibundus*, *H. orientalis*, *B. viridis*, *B. bombina*, *P. fuscus*, *L. vulgaris*, *E. calamita* will have a significant increase in climatically favorable territories for their existence; *R. temporaria*, *B. bufo*, *T. cristatus*, *P. ridibundus* x *P. lessonae* hybrid will have only a slight increase in climatically favorable areas. At the same time, the species that are most characteristic precisely on the territory of Polissia and already in the forest-steppe zone almost do not occur, namely *P. lessonae* and *R. arvalis*, will have much less favorable territories from the point of view of climate, and therefore need in this region taking the necessary measures for their protection and preservation.

In the course of the work, a number of new objects (n=6) of the Emerald Network of Ukraine with a total area of 98804.56 hectares were created on the territory of the Rightcoastal Polissia, which at the time of the protection of this work are officially included in the Emerald Network of Ukraine. Another 2 territories are currently under consideration by the Secretariat of the Bern Convention. Also, for the purpose of protection, including amphibians, projects for the creation of 5 new objects of the nature reserve fund of local importance (43078 hectares) were prepared and submitted for consideration to the Ministry of Environmental Protection and Natural Resources of Ukraine.

The scientific novelty of the work consists in obtaining up-to-date data on the state of amphibian populations of the Rightcoastal Polissia, namely data on the number and trend of its change, conducting an analysis of the presence of territories climatically favorable for the existence of amphibians within the framework of today, and the predicted probabilistic model of climate change. For the first time, the analysis of morphological anomalies of amphibians for this territory was carried out, as well as the data on the morphological descriptions of this group of animals from the studied territory were clarified. For the first time, helminthic infestations of the complex of green frogs ("*Pelophylax esculentus*" complex) were compared, and on the basis of this, the differences in the nutrition ecology of the two species of the complex were revealed. Additional data on the species composition of the specified hybridogenic complex were obtained, which complement the knowledge of the geography of these complexes, the distribution of various variants of hybridogenic populations in the basin of the Dnipro River, the Pripyat River, and the Western Bug River. For the first time in the last 20 years, the presence of a reproductive population of the natterjack toad, a species listed in the Red Book of Ukraine, has been confirmed.

The practical value of the work is that its results can be used to create new and improve the existing methods of amphibian conservation, create new and expand existing objects of the nature reserve fund and the Emerald Network of Ukraine, conduct future monitoring and bioindicative studies. The results of the work were included in the national report under Resolution 8 of the Bern Convention. The

registration points of amphibians are available for use by the international scientific community on the GBIF resource. The work provides important up-to-date data on the number of amphibians at the time of research, as well as fluctuations in climatically favorable territories for amphibians of the Rightcoastal Polissia in the context of global climate changes, which will increase the effectiveness of preserving the country's biodiversity. The results of the research were also used to write up-to-date sketches of two species of amphibians listed in the Red Book of Ukraine (one of them, namely the crested newt, was included in the Red Book of Ukraine which is also an important result of the work).

The results of the work became part of the implementation of the following grant programs and planned themes:

1) section 2. "Distribution of rare and vulnerable species of amphibians and reptiles of the Pripyat River basin and identification of the most valuable territories for their protection" of the planned theme of the Department of Animal monitoring and conservation III-48-20 "Study of taxonomic groups of vertebrate animals within model territories of the nature reserve fund of Ukraine and areas with high anthropogenic impact in order to improve their protection and management" (state registration number 0120U101270);

2) DFFD grant theme No. 76-2018 "Animals of the Red Book of Ukraine under conditions of climate change: current state and prediction in the context of the development of the national nature reserve network";

3) competitive topic of the National Academy of Sciences of Ukraine II-10-20 "Improvement of criteria and data for assessing the state of populations of animal species protected by the Bern Convention and directives 2009/147 EU and 92/43/EU" (state registration number 0120U002105);

4) competitive project of the National Research Fund No. 2020.02/0171 "Development of scientific principles of comprehensive monitoring and threats of the spread of invasive fish species in the river network and transitional waters of Ukraine (based on parasitic, population and genetic markers)";

5) grant project of the National Academy of Sciences of Ukraine No. 29/02-2022(4) "Creation of a genetic database of parasites of background species of amphibians and reptiles of the territory of Ukraine".

The materials of the work can be used in such educational courses as "Herpetology", "Herpetoculture", "Fundamentals of bioclimatic modeling", "Practical nature conservation", etc. The material collected by the author was added to the herpetological collection of the Department of Animal monitoring and conservation of the I.I. Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine.

Key words: amphibians, Rightcoastal Polissia, batrachofauna, bioclimatic modeling, abundance, morphometry, nature conservation, distribution of species, hybridogenic complexes, morphological anomalies.