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PHENOTYPIC STUDY OF POPULATION AND DISTRIBUTION OF THE *POECILIA RETICULATA* (CYPRINODONTIFORMES, POECILIIDAE) FROM KYIV SEWAGE SYSTEM (UKRAINE)

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Phenotypic Study of Population and Distribution of the *Poecilia reticulata* (Cyprinodontiformes, Poeciliidae) from Kyiv Sewage System (Ukraine). Nekrasova, O. D., Marushchak, O. Yu., Pupins, M., Bolotova, K. M., Čeirāns, A. & Skute, A. — This paper presents the original data on studies of populations of guppies on the territory of Ukraine on the example of those of them surviving for many years in the drainage system of Kyiv. For 10 years, wild populations of guppies and their morphological features were studied in the warm water flows of the Bortnychi aeration station in Kyiv (2011–2020). During this period, the original “key” was developed to describe the morphology of their coloration peculiarities,

which includes: total length, the number of pattern and coloration elements (4 types, 9 elements), pigmented area (light — orange, dark, pigmentation index), the shape and pigmentation of the tail (6 types) and its asymmetry At present, in the countries of Eastern Europe, the species is not adapted to any waters in the wild due to low winter temperatures, but these fish have become well established in sewage and other warm water bodies in urbanized areas (cities, towns, factories, etc.). We described the places where this invasive species had been found for many years, highlighting its key features. Thanks to GIS modelling, it was revealed that the existence of wild populations of guppies in Ukraine and Latvia is possible only in warm waters (currently mainly sewage systems of big cities) within anthropogenic territories. Such a key and study of relatively isolated populations of invasive species will provide a deeper understanding of micro-evolution of their morphological features (coloration) in isolation, help to track distribution of invasive species in a changing climate and provide material for comparison with possible similar invasions in areas heavily affected by military actions.
Key words: guppies, phenotypic description, invasive fish species, sewage system, aquaculture, Palearctic.

Introduction

In recent decades, the emergence of new exotic animals and their successful existence and adaptation to more unusual (colder) conditions, resulted in widespread discussion and became a matter of concern due to climate change and competition with native species (Pupins & Pupina, 2011; Pupina et al., 2018; Nekrasova et al., 2022; Kuybida et al., 2019; Tytar et al., 2022; Pupins et al., 2023). Import of exotic animals to other countries, mainly, took place not only for the sake of amateurs or accidently, but also for scientific purposes. Thus, interest in the "wild" populations of guppy *Poecilia reticulata* W. Peters, 1859 in the natural biotopes of South America and the islands arose at the beginning of the 20th century, due to the fact that this species is a convenient model object for genetic, phenetic and microevolutionary studies, as well as being a decorative species for aquariums (Seghers, 1973; Endler, 1983). These fish are characterized by well-seen sexual dimorphic coloration characteristics, which make it possible to clearly distinguish between individuals of different sexes. The genetic determinancy of various morphological manifestations in guppies has been investigated and widely highlighted in the literature (Winge & Ditlevsen, 1947; Brooks & Endler, 2001; Karino & Haijima, 2001; Hughes et al., 2005) noting a high level of genetic and phenetic variability. There is a lot of data in the literature on different coloration and pattern elements of male guppies, from unstable and variable areas of blue, green, purple to discrete elements of the body pattern such as location, position, number and size of orange and black spots or stripes (Winge & Ditlevsen, 1947; Hughes et al., 2005). Sometimes different parts of the body, for example tails, were used for such studies, highlighting variations of pigmentation (colored, colorless, etc.) and shape (flag-looking, drop-looking, etc.) (Olendorf et al., 2006). In studies of behavior and sexual selection in guppies it was noted that females prefer those males having bigger area of their body colored, with orange spots or those who are the brightest (UV). In other works, it was discussed that males of rare (new) color variants were selected. Moreover, with the age of females, the selective vector changes. However, in water bodies where there are more predators, there are fewer "orange" and bright males, as they are the most visible (Endler, 1983; Kodric-Brown & Nicoletto, 2001; Smith et al., 2002; Millar et al., 2006; Tripathi et al., 2009). Bright coloration, unpretentiousness, small size, easy breeding methodology — all this made it possible to consider it the most common species for aquariumistics and the breeding of many decorative lines, which were introduced in many countries, both randomly and for special purposes. As a result, guppies were introduced in 32 countries in America, Europe, Asia, Australia and Africa (Froese & Pauly, 2000; FAO, 2004). For example, they arrived in Australia in 1910 and were widely used to control the mosquitos' population (McKay, 1987). They also came to Singapore at the beginning of the 20th century. Later on, this country became the world leader in the export of this aquarium fish after mass breeding and *P. reticulata* was also found there in local "wild" populations (Herre, 1940).

The nearest countries of the Palearctic, such as Poland and Germany, where guppies were released by amateur aquarists, are not an exception. The first mention in the literature of guppy breeding in Poland dates back to the 2nd half of the XX century (Courtenay & Stauffer, 1990). The established populations of guppies in Poland seem to be one of the northernmost populations in the world, recorded in the thermally polluted waters of Nowa Huta heating channel in Krakow and thermally polluted waters of Żerań CHP plant in Warsaw (Witkowski, 1989; Nowak et al., 2008; Maciaszek et al., 2019). Until 2018 only one established population has been described for Germany, residing in the thermally altered Gillbach-Erft river system near Cologne (Lukas et al., 2017). As thermal power plants use water as a cooling medium, thus increasing the probability that more thermally influenced freshwater systems (TIFs) exist across Germany, two new established populations of guppies were found in heated water garden near Reden (Lower Saxony) and thermal springs near Bad Sauglau (Baden-Württemberg) (Lukas et al., 2017). The "wild" guppies' populations in Kyiv at the Bortnychi aeration station in warm drains are also of the same age as those registered in more northern countries (Kutsokon et al., 2012; Nekrasova et al., 2021, 2022).

The features of such urban fish settlements are of great interest, since they are subject of constant anthropogenic influence (Afanasyev, 2003) and have specific morphological features that have not yet been described in the literature.

Therefore, the purpose of our work was not only long-term monitoring of unique populations for about 10 years, but also the study of the morphological characteristics of the "wild" forms of guppies that have adapted to urban conditions, as well as the development of a unique formula ("key"), which will make the morphological description of these exotic fish.

Material and Methods

Coloration peculiarities

The peculiarities of guppies' population were studied in 2011–2012 and 2020 in the Bortnychi sewage water system: 50.3837 N, 30.6642 E, Kyiv, Ukraine (fig. 1).

During this time, we studied 130 *P. reticulata* individuals in vivo, of which 110 individuals appeared to be adult males. We developed an original "key" to study the phenotypic peculiarities of *P. reticulata* population within Bortnychi sewage system. Describing the morphological features of brightly colored males, this key includes: (I) exterior features (morphometric features) — body length, maximum tail length; (II) quantitative discrete signs of color and body patterns and their manifestation — 9 elements (fig. 2); (III) intensity of pigmentation (pigmentation index) — the area occupied by orange and black spots on the lateral parts body (%); (IV) the shape and pigmentation of the tail — the presence and color of pigmentation (fig. 2, signs 10–15; fig. 3), the location and presence of sword-like tips on the tail (asymmetry, upper, lower, double, and their complete absence), pigmentation of the tail. To measure the spot area, we used the following software: Vidana 1.0 (University of Exeter Marine Spatial Ecology Lab, Exeter, Devon, United Kingdom; Available from http://www.ex.ac.uk/msel). At the same time, we took the available literature data into account and with observance of all genetic and breeding directions of *P. reticulata* (Tripathi et al., 2009). We studied recently caught individuals of *P. reticulata* in vivo as some features of pigmentation change (fade, destruct etc.) after fixation. Such coloration features as yellow-orange-red and black pigmentation were studied (fig. 2). We took photos of all individuals using an Olympus SP570UZ digital camera. Some of them were preserved for a collection afterwards (I. I. Shmalhausen Institute of Zoology NAS Ukraine, Kyiv). We carried out statistical processing of the obtained data using Statistica for Windows v.8.0. We used multivariate statistics methods (multiple regression analysis) to compare body and tail colorations patterns with other physical characteristics of the body of studied species.

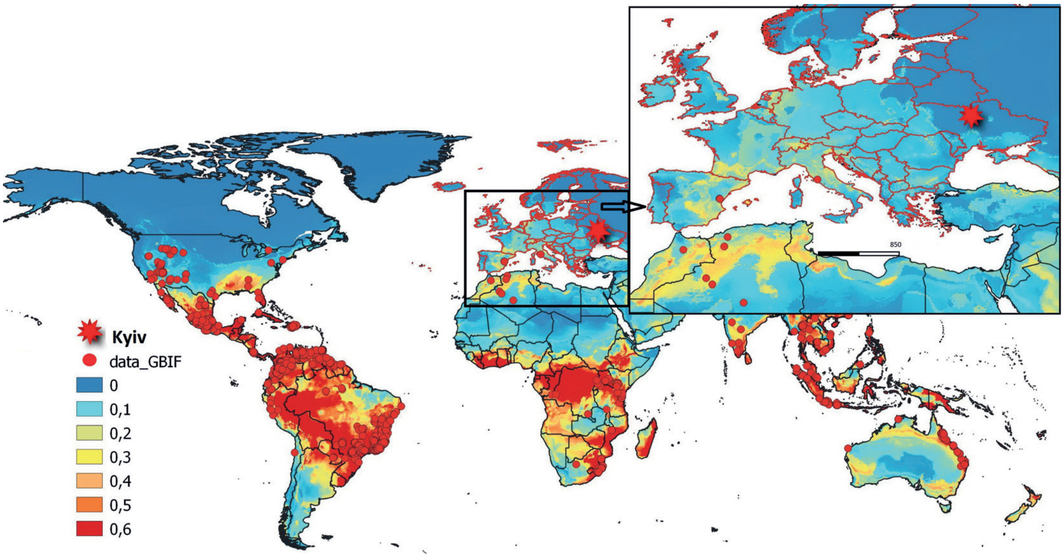

Fig. 1. Potential (probabilistic) model of *P. reticulata* current world expansion built in the Maxent program based on the CliMond climatic data and GBIF data (2021). Areas of highest habitat suitability (> 0.5) are colored in red and areas of lowest (< 0.1) — in blue.

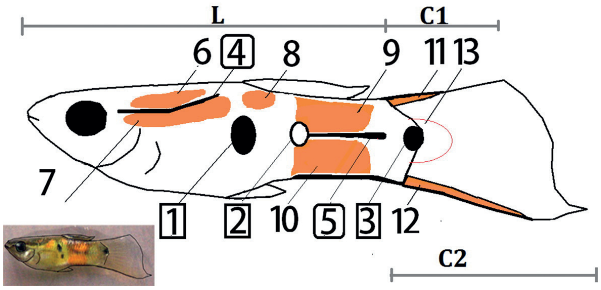

Fig. 2. The main elements of the body coloration pattern of the "wild" form of the male guppy: 1 — lateral dark spot; 2 — lateral dark spot; 3 — dark tail spot; 4 — postorbital dark stripe; 5 — middle caudal dark stripe; 6 —superciliar orange spot; 7 — underbrow orange spot; 8 — central orange spot; 9 — upper tail orange spot; 10 — lower tail orange spot; 11— upper orange spot on caudal fin; 12 — lower orange spot on caudal fin; 13 — coloration of the tail base (# 3); L — body length, C1 — length of the shortest ray of caudal fin, C2 — length of the longest ray of caudal fin.

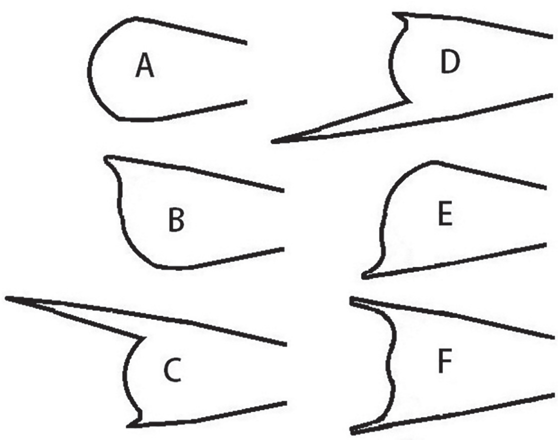

Fig. 3. The main forms of *P. reticulata* tail: A — rounded or triangular; B — asymmetric with an upper outgrowth; C — asymmetrical with the upper sword; D — asymmetrical with the lower sword; E — asymmetric with a lower outgrowth; F — fork-shaped.

GIS analysis

GIS modeling was used to explore the potential distribution of *P. reticulata* in the world. For this purpose, GBIF database (*Poecilia reticulata*, GBIF.org, 2021) was used. To account for sampling bias, we used the nearest neighbor distance ('ntbox' package in R (Osorio-Olvera et al., 2020)) method to thin the data, where occurrence points that were > 0.1 units away from each other. Then the 'random selection' module in QGIS (QGIS Development Team 2020; Nekrasova et al., 2019) was applied. All points that were too close to each other were removed to avoid errors due to spatial autocorrelation. As a result, the number of points has significantly decreased; from 4200 *P. reticulata* registration points to only 1586 (Nekrasova et al., 2021).

Modeling and calculations were carried out using Maxent v3.3.3k software with 25 replicates, 1586 record (Phillips, 2005) and employing 5 bioclimatic covariates (CliMond; Kriticos et al., 2014) — Bio36–40 (Bio36 — First principal component of the first 35 Bioclim variables; Bio37 — Second principal component of the first 35 Bioclim variables, Bio38 — Third principal component of the first 35 Bioclim variables, Bio39 — Fourth principal component of the first 35 Bioclim variables, Bio40 — Fifth principal component of the first 35 Bioclim variables). We used two evaluation metrics for SDMs performance, namely binomial tests and the area under the receiver operating characteristic (ROC) curve (AUC) for assessing the discriminatory capacity of the models. ROC is a visual graphical representation of comparison of the amount of true positive versus false positive pixels through an incremental binary classification of the factors used in building distribution maps. AUC > 0.85 is considered excellent (Nekrasova et al., 2019 b; Pupins et al., 2023).

Results

GIS-modelling of the probalistic distribution of *P. reticulata* outside natural range

As a result of our research in winter in Bortnychi aeration station, we identified populations of fish of different ages. These populations, despite the severe frosts in 2012 (air temperature of up to –30 °C) and other unstable conditions, continue to survive successfully. In the end of December 2020 existence of the Kyiv population was confirmed. Up to 50 individuals of *P. reticulata* (subadult and juveniles) were caught in 3 places along the Bortnychi drain. This confirms that these fish breed here in multiple generations. Guppies choose small creeks with a weak course near the water edge (fig. 4). Water temperature measured in all 3 places was +16 — +17 °C. According to local residents for the last 3 years such exotic fish species as *Poecilia* sp. Bloch & Schneider, 1801, *Pterophyllum* sp. Heckel, 1840, *Barbus* sp. Cuvier et Cloquet, 1816 and *Trachemys scripta* (Thunberg in Schoepff, 1792) (Nekrasova et al., 2019 b; Pupins et al., 2019) were occasionally caught in the drain. Additionally, we observed numerous mates of *Pistia* Linnaeus, 1753 alongside the drain. Using Maxent program based on the CliMond climatic data and GBIF data (2021) we built potential (probabilistic) model of *P. reticulata* current world expansion and defined areas of the highest and the lowest habitat suitability for the species in foreign countries outside its natural range (fig. 1).

Fig. 4. Biotope occupied by *P. reticulata* along the Bortnychi sewage drain; areas, where guppies were found have very weak current are marked with red circles (Nekrasova et al., 2021).

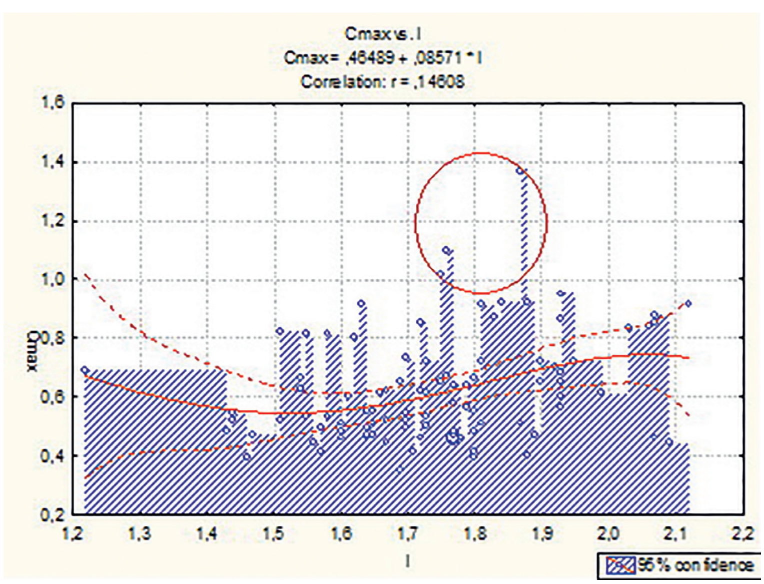

Fig. 5. The ratio of body length and maximum tail length in male guppies.

Morphometric characteristics

Males of *P. reticulata* are considerably smaller than females. Body length of adult males is from 1.22 to 2.27 cm (average value L = 1.75 ± 0.02), while the length of the tail (its longest part, C2) is from 0.35 to 1.37 cm (average value C2 = 0.63 ± 0.02). On average, tail in males occupies more than 1/3 of the body length — from 20.7 % to 73.3 % (mean value 35.9 ± 1.05 %). Moreover, males with a body length of 1.7–1.9 cm tend to have the longest tail (fig. 5). In females, body length ranges from 1.56 to 4.50 cm (average value L = 3.03 ± 0.32) while tail length from 0.4 to 1 cm (average value C = 0.58 ± 0.06). On average, the tail of females occupies less than 1/3 of the body length — from 18.16 % to 28.75 % (mean C = 23.28 ± 1.24 %). Thus, in Kyiv guppies, the total body length in males is 1.85–3.24 cm, and in females — 2.00–5.50 cm. According to the literature adult males reach length of 3 cm, while females — 5–6 cm (Nowak et al., 2008).

Quantitative discrete characteristics of body color and pattern and their manifestation (9 elements)

Using the genetic conditionality of certain traits (Tripathi et al., 2009; Kemp et al., 2009), it is possible to compose a key for describing and processing data obtained in the description of both populations and separate animals — fish individuals used in experiment or when crossing. Elements of coloration and pattern can be divided into dark spots (II.A.) — 3 elements (fig. 2: No. 1–3), stripes (II.V.) — 2 elements (fig. 2: No. 4–5) and orange spots — 5 elements on the body (II.C.) (fig. 2: No. 6–10) and 2–3 on the tail (II.D.) (fig. 2: No. 11–13).

These signs are quite individual and accounting for them can be difficult because they can merge, forming a strip or a single huge spot or have abnormal localization. Therefore, the pigmentation index must also be considered (see III). In general, the number of spots can vary greatly from their complete absence to: II.A. — 4; II.B. — 4; II.C. — 8; II.D. — 4 maximum.

Intensity of body pigmentation of *P. reticulata* (area occupied by a specific trait relative to body area (without tail) %)

The manifestation of pigmentation in fish is a result of many factors: season, age, sex (including sexual selection), health, food, external conditions (hydrochemical, etc.). All these factors form the phenotype of a particular fish population, which can also be used for bioindication. To study the intensity of pigmentation, the most "stable" elements were used — yellow-orange, associated with carotenoids and caused by lutein, astaxanthin and other related pigments (II.C.; fig. 2: No. 6–10) and black spots and stripes caused by melanin (II.A. and II.B.; fig. 2: No. 1–5). Since for the study of other types of pigmentation (for example, green and blue), due in part to iridiophores (guanine crystal, etc.), certain conditions are necessary, due to possible optical effects and variability (seasonal, age, etc.). Thus, it was found that the number of orange spots on the body can vary — from their complete absence to 6 elements. Moreover, on average they occupy 20.7 ± 1.1 % (range — 4–40 %) of the total body area (fig. 6). And dark spots can also appear on the body with different intensities — up to 4 elements (II.A. and II.V.). Moreover, half of the guppy's body can be dark in color (black — nigrocaudatus) and occupy up to half of the body, with dark elements of the pattern still visible (fig. 2), which were measured. In this case, the orange elements of the pattern occupy smaller area. In general, the area of dark spots is 8.8 ± 0.9 % (range — 1–34 %) of the total body area.

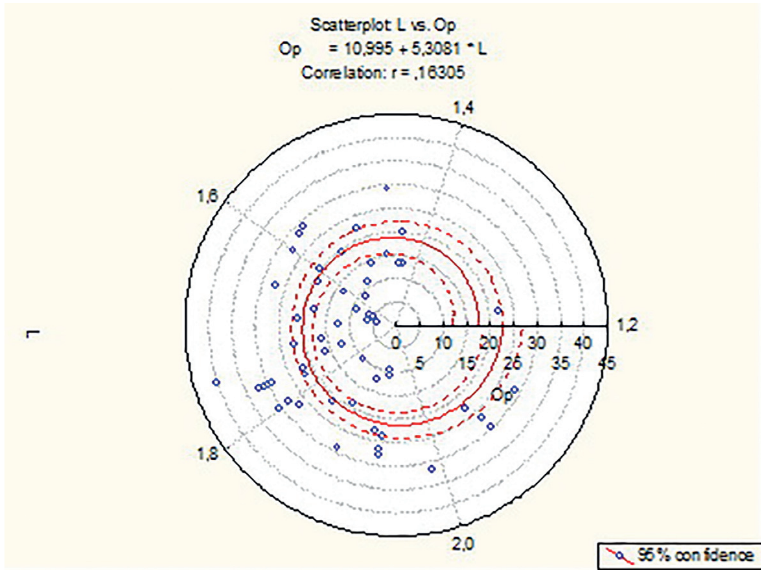

Fig. 6. Dependence of the area of orange spots (stripes, %) on the body length of male guppies (L) *P. reticulata*.

Form and pigmentation of the tail

According to the literature (Endler, 1983), it is known that for decorative purposes, about 13 forms of guppies, differing in the size and shape of the caudal fin, have been bred for eight decades. These are round-tailed, flag-tailed, needle-tailed, shovel-tailed, spear-tailed, "lower sword", "upper sword", "double sword", lyre-tailed, skirt- or veil-tailed, acute-angled fan-tailed, edged fan-tailed and non-edged fan-tailed.

As mentioned earlier, the relative tail length of males is significantly longer than that of females and can occupy up to 73.3 % of total length and can have different shapes. From the material collected, the following three main groups of tail shape variants were identified (fig. 3, table 1): 1) rounded (approximately symmetrical about the body axis, (fig. 3, A); 2) asymmetric (with one outgrowth or the sword — lower or upper, fig. 3, B–E) and 3) fork-shaped (symmetrical with two outgrowths — lower and upper, fig. 3, F). At the same time, males with a tail of the usual rounded shape are the most numerous — 46.5 % of the total sample of males (98 specimens), while the tail occupies about 1/3 of the body. The group with an asymmetric tail and with upper outgrowths is significantly greater — 39.5 % (fig. 3, B, C) than with lower outgrowths — 9 % (fig. 3, D, E). Males with fork-shaped tails (2 swords) are quite rare — 5 % (fig. 3, F; table 1). Of all the above, males with upper swords (fig. 3, C), and males with fork-shaped tails on average tend to have the longest tails — 47 % of the body length (fig. 7).

Table 1. Main types of tail forms in *P. reticulata* (fig. 3)

No	Form	Main tail forms	Form ratios in different groups,%	
1.	A	rounded	46.5 ± 5.23	46.5 ± 5.2
2.	B	asymmetric with an upper outgrowth	27.7 ± 4.69	48.5 ± 3.2
	C	asymmetrical with the upper sword	11.8 ± 3.38	
	D	asymmetrical with the lower sword	5.0 ± 2.28	
	E	asymmetric with a lower outgrowth	4.0 ± 2.05	
3.	F	aork-shaped (or double-sworded)	5.0 ± 2.28	5.0 ± 2.3

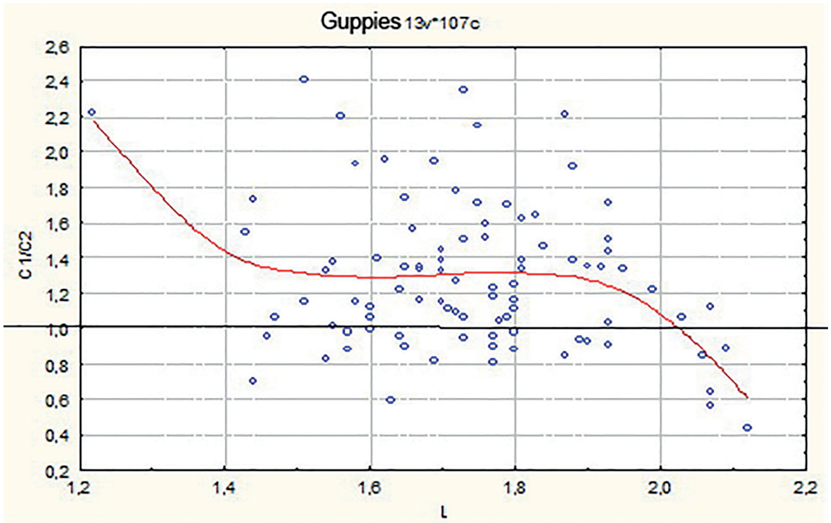

Fig. 7. Dependence of the proportions of the tail (C1/C2) on the body length of *P. reticulata*: C1/C2 = 1 ("symmetrical tail") is shown by the line.

There is a tendency that in large males the number of individuals with a lower tail outgrowth is greater.

Thus, all the above indicated morphological characters can be written in the following formula ("key" by Nekrasova O.), which characterizes the phenotypic peculiarity of studied *P. reticulata* population:

I. Total length: males — 1.9–3.2 cm; females — 2.0–5.5 cm.

II. The number of pattern and coloration elements: A — 0–4; B — 0–4; C — 0–8; D — 0–4.

III. Orange area — 20.7 % (range — 4–40 %); black spots (stripes) — 8.8 % (range — 1–34 %) of the total body area.

IV. Tail shape: A — 46.5 %, B — 27.7 %, C — 11.8 %, D — 5.0 %; E — 4.0 %, F — 5.0 %.

The asymmetry of the tail appears in 48.5 % of cases.

Such cases of asymmetry confirm the insufficiently stable and extreme conditions in which guppies live in the collector of the purification station in Bortnychi. The created model (fig. 1) shows how optimal the conditions can be connected with constantly changing climatic parameters and outside the warm waters of a large metropolis for an exotic species of fish. Therefore, the conditions for the habitation of exotic fish in the South of Ukraine may be quite suitable. And the created "key" can be used to study the adaptations of *P. reticulata* populations as well as for bioindication in different regions of the world.

Conclusions

Based on the example of the morphological characteristics of the Kyiv population of guppies and studying the literature data, we solved the problem associated with the description of invasive populations of this species in Eastern Europe. As a result of GIS-modelling, it was revealed that the guppies' populations introduced (mainly accidently) in Ukraine and Latvia survive only in warm waters presented mainly by sewage systems of big cities) within anthropogenic territories. And in the context of a changing climate, in the nearest future we will face the problem of identifying and studying microevolutionary processes associated with the adaptation of this invasive species to a new environment. The advantages of the original "key" for describing fish allow not only to take into account the coloration, pigmentation and tail structure of males, but also allow taking into account the manifestation of asymmetry, that is very important in the study of bioindication features of animals in the unstable environment of anthropogenic territories. It is especially important for studying the emergence of new hybrid fish populations and identifying new morphological features that give them an advantage in a new environment in comparison with the aboriginal aquatic fauna. In the future, populations of *P. reticulata* can be used for biological methods of combating new invasive insects that are now appearing in Eastern Europe (mosquitoes, flies, etc.).

Additionally in terms of ongoing climate changes and increasing of the number of ways (mainly human-induced) for expansion of invasive species (fish among them) such studies will help to track such distribution ways and terminate their further acting. For example, using such keys it will be possible to detect what population and place the newly registered invasive fish come from.

The example of guppies' population in Bortnychi is one of many unique demonstrations of how even tiny aquarium fish species can survive in extremely polluted and anthropogenically transformed habitat. Following the consequences of war in the nearest future we might see many other similar examples on both free and deoccupied territories, where guppies might also have had an opportunity to get released into the wild. Such study will allow to compare consequences of such introductions and more precisely assess their impact on nature in the territories of military actions and on species micro-evolution in particular cases.

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