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KARYOTYPES AND MORPHOLOGICAL VARIABILITY OF CRAYFISH *PONTASTACUS LEPTODACTYLUS* AND *P. ANGULOSUS* (MALACOSTRACA, DECAPODA)

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Karyotypes and Morphological Variability of Crayfish *Pontastacus leptodactylus* and *P. angulosus* (Malacostraca, Decapoda). Kostyuk V. S., Garbar A. V., Mezhzherin S. V. — The existence on the territory of Ukraine of two sympatric widespread species definitely different by their chromosome number was proved based on the meiotic chromosome preparations. Besides the nominal species *Pontastacus leptodactylus* (Dana, 1852) with modal haploid chromosome number n = 93 we prove the existence of *P. angulosus* (Rathke, 1837) with n = 88.

Key words: crayfish, Astacidae, *Pontastacus leptodactylus*, *Pontastacus angulosus*, karyotype, morphometry.

Кариотипы и морфологическая изменчивость длиннопалых раков *Pontastacus leptodactylus* и *P. angulosus* (Malacostraca, Decapoda). Костюк В. С., Гарбар А. В., Межжерин С. В. — На основании анализа мейотических хромосомных препаратов доказано существование в Украине двух симпатрических широко распространённых видов, которые чётко отличаются по числу хромосом. Кроме номинативного длиннопалого рака, *Pontastacus leptodactylus* (Dana, 1852), с модальным числом хромосом в гаплоидном наборе на уровне n = 93 доказано присутствие и угластого рака, *P. angulosus* (Rathke, 1837), с модальным значением n = 88.

Ключевые слова: речные раки, Astacidae, *Pontastacus leptodactylus*, *Pontastacus angulosus*, кариотип, морфометрия.

Introduction

The taxonomy of European freshwater crayfish of Astacidae family has been remaining the disputable subject among the scientists. One of the most intricate situations is the one concerning the long clawed crayfish complex where two alternative points of view were formed. On the one hand a number of authors (Brodskiy, 1983; Starobogatov, 1995) assigned the long clawed crayfish to the single genus *Pontastacus* Bott, 1950 containing considerable number of species and subspecies, on the other hand the only one *Astacus leptodactylus* Eschscholtz, 1823 species was considered (Gherardi and Holdich, 1999; Taylor, 2001; Souty-Grosset, 2006), and it could be the species complex as some authors suggested (Holdich, 2002).

Such a disputable situation was caused mostly by strong morphometric variability of crayfish. As a result there were some attempts of using more stable characteristics in forming this group system, for instance, karyological and molecular genetic ones.

Crayfish (and Crustaceans in general) are considered to be the group difficult for cytogenetic studies as most of its species have relatively large diploid chromosome number (for example *Pacifastacus leniskulus* (Dana, 1852) has 2 n = 376 (Niiyama, 1962)). As a result different authors' data concerning the crayfish karyotypes differ significantly. In this connection the recent issue of Croatian scientists (Mlinarec et al., 2011) notes 2n = 180 diploid chromosome number for long clawed crayfish. Consequently, the haploid number of this species may come to n = 90 — the fact that does not correspond to the results of earlier studies (Silver and Cukerzis, 1964) showing this species haploid number n = 184.

The recent molecular-genetic research results prove the idea of long clawed crayfish being a species complex (Sinclair et al., 2004; Crandall and Buhay, 2007), though the taxonomic conclusions are not mentioned in these studies.

Electrophoretic and morphological studies of long clawed crayfish on the territory of Ukraine revealed two groups clearly different from each other on the basis of a number of parameters. As to morphological features these forms correspond to the diagnosis of nominal species *Pontastacus leptodactylus* (Eschscholtz, 1823) and *P. angulosus* (Rathke, 1837), the taxonomic status of which is quite questionable. The allozyme analysis showed these two forms significantly different on the basis of the trait frequency of three polymorphic loci while their areals are widely overlapped (Mezhzherin et al., 2012). Thus these forms are reproductively isolated enough.

The aim of our study was to carry out the morphological and karyological analysis of two studied species of crayfish on the territory of Ukraine.

Material and methods

The analyzed material was gathered in spring-to-autumn periods of 2010–2012 years according to the commonly accepted methods (Brodsky, 1981). Eight samples over the territory of Ukraine were studied (table 1).

The genetically marked adult males indetified according to keys (Starobogatov, 1995) as nominal species *P. leptodactylus* (65 specimens) and *P. angulosus* (40 specimens) were used for kariological studies.

Chromosome preparations were made from the testis following the technique formerly used for investigations of other astacid species (Mlinarec et al., 2011; Murofushi et al., 1984). The animals were injected with 0.05 % colchicine 5.5–6 hours before the dissection. Small pieces (1–2 mm^3) of male gonads were placed for 30 minutes in KCl (1 M) solution and fixed in the 1 : 3 mixture of glacial acetic acid and ethanol. Chromosome preparations were obtained by the squash method with quick freezing (Sharma and Sharma, 1972). Dried slides were stained with Giemsa-Romanovsky stain in 0. 01M phosphate buffer (pH 6.8). The chromosome spreads were analysed with a "Delta optical genetic pro bino USB" microscope (16 x 100).

Twenty-five standard quantitative indexes for morphological analysis were used (Sint et al., 2007). Statistical processing of obtained data was carried out by means of a package of applied statistical programs PAST 1.18.

Results and discussion

Karyological analysis showed that studied groups (defined by the morphological characteristics as *P. leptodactylus* and *P. angulosus*; fig. 1) differed clearly by chromosome number (fig. 2).

So it was established that the haploid chromosome number of *P. leptodactylus* was n = 93 chromosomes (37 meiosis diakinesis stage plates were analyzed; fig. 2, *b*), while *P. angulosus* was characterized by n = 88 haploid chromosome number (35 meiosis diakinesis stage plates were analyzed; fig. 2, *a*).

The obtained results come close to the Hungarian scientists' data (Mlinarec et al., 2011) showing the diploid chromosome number 2n = 180 for *A. leptodactylus* sensu lato. At the same time the studied members of this complex are characterized by different haploid chromosome number. Thus the further studies of the genetically marked material appear to be important as they may allow avoiding the mistakes in studying the modal chromosome number of the individual members of this complex.

We showed on the example of the previous analysis of the combined samples that *P. leptodactylus* and *P. angulosus* have sufficiently clear differences in a number of mor-

Table 1. Material used in the study
Таблица 1. Материал, использованный в исследовании

Species	Localities	Coordinates	Number of specimens
P. leptodactylus	r. Styr, Lutsk	50.72026° N, 25.36348° E	20
	Pond, Dolyna, Ivano-Frankivsk region	48.97740° N, 23.98616° E	15
	r. Pivdennyi Bug, Vinnytsa	49.23116° N, 28.48223° E	16
	r. Goryn, Zbuzh, Rivne region	50.98210° N, 26.32479° E	14
P. angulosus	Pond, Mali-Zagaitsi, Ternopil region	50.01337° N, 26.02979° E	21
	Water reservoir, Burchtyn, Ivano-Frankivsk region	49.23032° N, 24.67375° E	26
	Pond, Dubivtsi, Ivano-Frankivsk region	49.08016° N, 24.79717° E	19
	Pond, Ikva, Ternopil region	50.10880° N, 25.63917° E	17

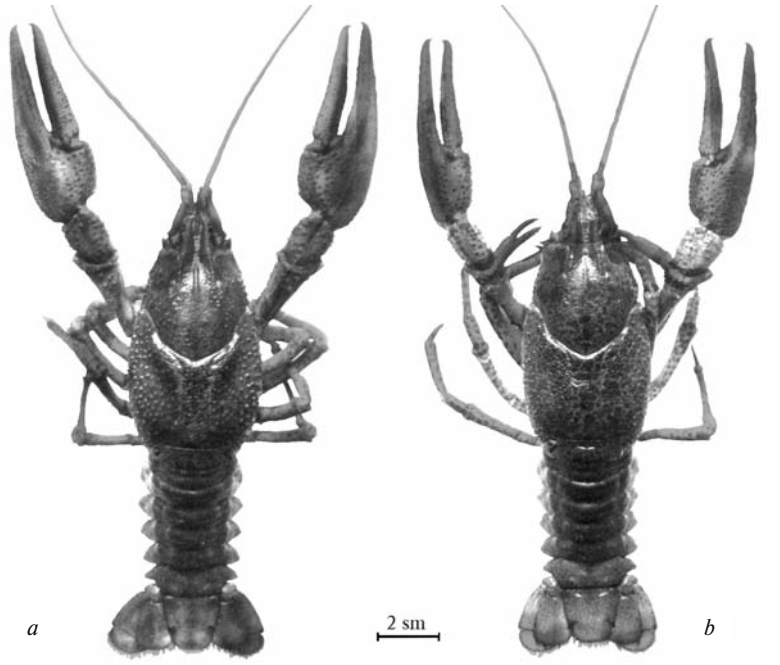
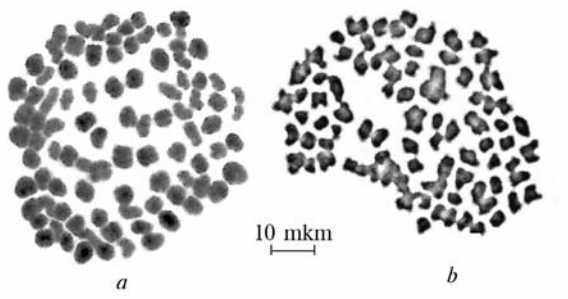


Fig. 1. Studied astacids species: *a* — *P. angulosus*; *b* — *P. leptodactylus*.

Рис. 1. Исследованные виды: *a* — *P. angulosus*; *b* — *P. leptodactylus*.



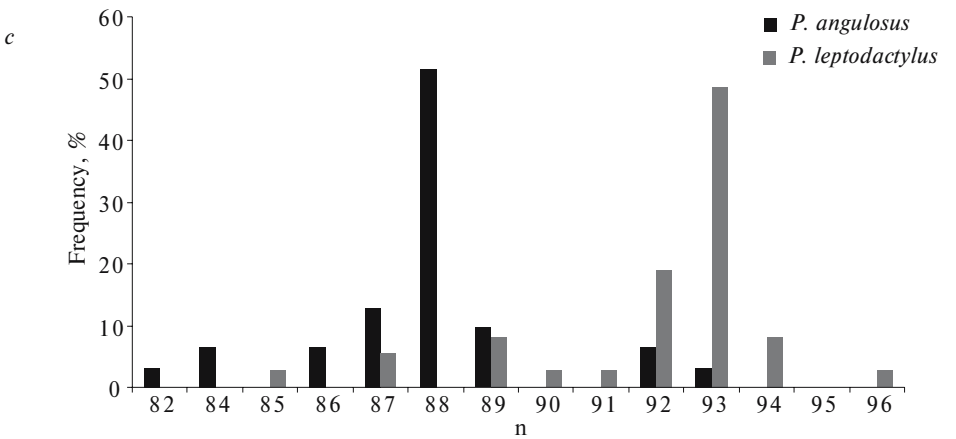


Fig. 2. Crayfish karyotypes: *a* — diakinesis (n = 88) of *P. angulosus*; *b* — diakinesis (n = 93) of *P. leptodactylus*; *c* — haploid chromosome number distribution.

Рис. 2. Кариотипы длиннопалых раков: *a* — диакинез (n = 88) *P. angulosus*; *b* — диакинез (n = 93) *P. leptodactylus*; *c* — распределение количества гаплоидных хромосом.

Table 2. The reliability of the studied crayfish population discrimination on morphological parameters
Таблица 2. Достоверность различий исследованной популяции длиннопалых раков по морфологическим параметрам

Population	%	P. a. 1	P. a. 2	P. a. 3	P. a. 4	P. l. 1	P. l. 2	P. l. 3	P. l. 4
P. a. 1	95.24	20	0	0	0	0	0	1	0
P. a. 2	92.31	0	24	2	0	0	0	0	0
P. a. 3	94.74	0	1	18	0	0	0	0	0
P. a. 4	88.24	2	0	0	15	0	0	0	0
P. l. 1	90.00	0	0	0	0	18	1	0	1
P. l. 2	86.67	0	0	0	1	0	13	1	0
P. l. 3	100.00	0	0	0	0	0	0	16	0
P. l. 4	100.00	0	0	0	0	0	0	0	14
Total	93.24	22	25	20	16	18	14	18	15

Note. P. a. 1 — P. a. 4 — population of *P. angulosus*; P. l. 1 — P. l. 4 — population of *P. leptodactylus*.

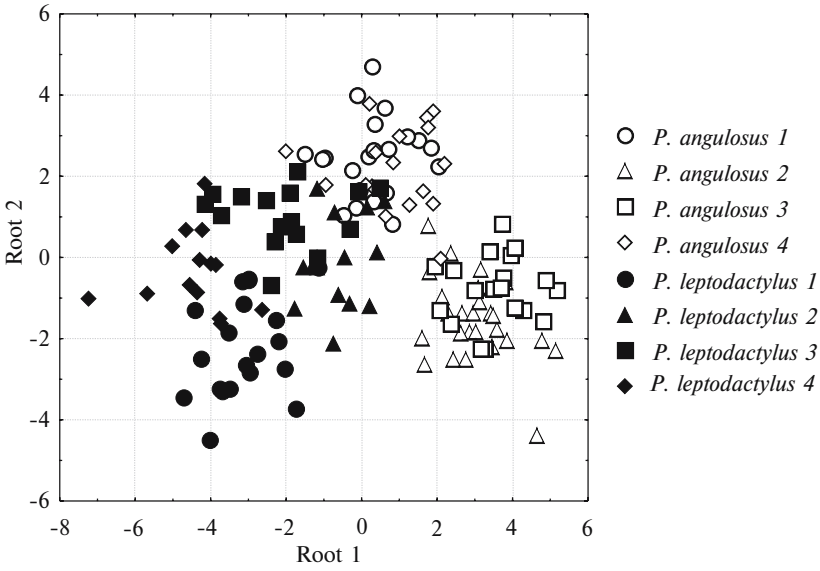


Fig. 3. The distribution of the studied crayfish samples in the field of first two canonical functions.

Рис. 3. Распределение образцов исследованных длиннопалых раков в поле первых двух канонических функций.

phometric parameters (Mezhzherin et al., 2012). However, the nature of this character variability remains unclear. So we carried out the comparative morphological analysis of four largest samples of each species from geographically separated populations.

The results of discriminate analysis of size characteristics and morphometric indexes combination (table 2) prove the high level discrimination of geographically separated populations.

Meanwhile, the populations belonging to one species form the compact groups that are nearly not overlapped (fig. 3). Therefore in case of the analysis of these species united samples their discrimination level naturally comes to 96.62 %.

It is evident from fig. 3 the first canonical function makes the greatest contribution into the discrimination of the studied species and well-correlates with number of size parameters and morphometric indexes (table 3). Most of these parameters are related to the claw, carapace and abdomen proportions.

The allozyme variability analysis of *P. leptodactylus* sensu lato (Mezhzherin et al., 2012) showed that *P. leptodactylus* and *P. angulosus* differed apparently on the basis of alleles' frequencies of three polymorph loci. Here the main differentiated feature is the

Table 3. The most significant correlations (r) of the first canonical function with morphometric characteristics
Таблица 3. Наиболее значительные корреляции (r) первых канонических функций с морфометрическими характеристиками

Parameters	CLWl	ROL	HEL	TEL	ABW
r	−0.29	0.35	−0.23	0.25	0.45
Parameters	ARW	CLLr\CPLr	CFLr/CPLr	CLLl/CLWl	CLLl/CPLl
r	−0.29	−0.35	−0.36	0.33	−0.27
Parameters	CFLl/CPLl	ROL/ROW	HEL/ARL	ABL/ABW	ABL/ABH
r	−0.26	0.46	−0.38	−0.50	0.28

Note. ABH — abdomen height, ABL — abdomen length, ABW — abdomen width, ARW — areolar width, CFL — claw finger length, CLL — claw length, CLW — claw width, CPL — claw palm length, HEL — head length, ROL — rostrum length, ROW — rostrum width, TEL — telson length.

allelic variants of Es-2 locus. *Pontastacus leptodactylus* is characterized by Es-$2^{1\circ\circ}$ type, but *P. angulosus* — by Es-$2^{11\circ}$. The last case allele product is most likely overlapped with Es-3 locus product that is invariant for these species.

As the areals of *P. leptodactylus* and *P. angulosus* widely overlap, the significant differences between two population groups could be observed only in case of their reliable reproductive isolation. Though it is impossible in this case to avoid the limited introgressive hybridization because of the genetic differences peculiarities.

Conclusions

Thus, the karyological, morphometric and biochemical-genetic data prove the existence of two widespread and common species of the genus *Pontastacus* — *P. leptodactylus* and *P. angulosus* — on the territory of Ukraine. The scientists' views on taxonomic status of the last one are disputable. So, S. Brodsky (1983) considered it only as a form of the nominative subspecies — *P. leptodactylus leptodactylus* f. *angulosus*, while Ya. Starobogatov (1995) considered it as the separate species — *P. angulosus*. The results of our study proved the last mentioned point of view. However, the status of the other forms of long-clawed crayfish discussed by Ya. Starobogatov as the separate species remains disputable and requires the further studies by means of modern methods.

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