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Karyotypes of two European species of the genus *Lymnaea* with disputable taxonomic status (Gastropoda: Pulmonata: Lymnaeidae)

With 3 tables and 7 figures

ALEXANDER V. GARBAR & ALEXEI V. KORNIUSHIN

Abstract. Karyotypes of *Lymnaea corvus* and *L. occulta* are studied for the first time and compared to the original data on Ukrainian populations of *L. palustris* and *L. stagnalis.* While all the studied species have the same diploid chromosome number (2n = 36), they differ in chromosome morphology. Karyotypes of *L. occulta* and *L. palustris* include only biarmed chromosomes (number of arms = 72). In contrast, *L. corvus* and *L. stagnalis* have also uniarmed (acrocentric) chromosomes (number of arms = 60 and 62, respectively). Karyological data confirm specific distinctness of *L. occulta* and *L. corvus;* however, similarity in the chromosome morphology of the latter species to *L. stagnalis* is probably based on symplesiomorphies and, thus, cannot support phylogenetic relationship suggested earlier on the basis of anatomical characters.

Kurzfassung. Karyotypen von zwei europдischen Arten der Gattung *Lymnaea* mit umstrittenem taxonomischen Status (Gastropoda: Pulmonata: Lymnaeidae). - Karyotypen von *Lymnaea corvus* und *L. occulta* wurden erstmalig untersucht und mit den originalen Daten fьr ukrainische Populationen von *L. stagnalis* und *L. palustris* verglichen. Alle untersuchten vier Arten weisen dieselbe Anzahl diploider Chromosomen auf (2n = 36), kцnnen aber durch die Morphologie der Chromosomen unterschieden werden. Die Karyotypen von *L. occulta* enthalten nur zweiarmige Chromosomen (Anzahl der Arme = 72). Im Gegensatz hierzu weisen *L. corvus* und *L. stagnalis* auch einarmige (akrozentrische) Chromosomen auf (Anzahl der Arme = 60 beziehungsweise 62). Die karyologischen Befunde bestдtigen die spezifische Verschiedenheit von *L. occulta* und *L. corvus;* jedoch ist die Дhnlichkeit in der Chromosomenmorphologie der letzteren Art gegenьber *L. stagnalis* wahrscheinlich auf Symplesiomorphien zurьckzufьhren, deshalb kann die frьher auf der Basis anatomischer Merkmale behauptete phylogenetische Verwandtschaft von *L. corvus* und *L. stagnalis* nicht unterstьtzt werden.

Key words. Pulmonata, Lymnaeidae, *Lymnaea,* karyotypes, taxonomy, phylogeny, Ukraine.

Introduction

Taxonomy of the European lymnaeids considered earlier a single polymorphic species *Galba palustris* or *Lymnaea palustris* (O.F. MьLLER, 1774) (see KILIAS 1992) became

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Authors' addresses:
Alexander V. Garbar, Department of Natural Sciences, Zhitomyr Pedagogical Institute, Zhitomyr, Ukraine
Dr. Alexei V. Korniushin, Schmalhausen Institute of Zoology, National Academy of Sciences of Ukraine, B. Khmelnitsky str. 15, 01601 Kiev, Ukraine; e-mail: akorn@carrier.kiev.ua

Fig. 1: Localities of karyologically studied samples.

Table 1: Material for karyological investigation.

Species	Locality	Number of specimens studied	Number of metaphases studied
L. stagnalis	Zhitomyr, River Teterev	29	20
	Vinnitsa Region, Shyroka Greblya, River Yuzhny Bug (South Bug)	12	5
	Lviv Region, Kamenka-Bugskaya, River Zakhidny Bug (West Bug)	10	6
	Kharkiv, River Udy	5	3
L. corvus	Zhitomyr, Maryanovka, pond	32	11
	Zhitomyr Region, Olevsk, pond	15	8
	Zhitomyr Region, Dserzhinsk, ditch	39	23
L. palustris	Zhitomyr Region, Perlyavka, temporary pools near the River Teterev	18	15
	Poltava, temporary pools near the River Kolomak	34	20
L. occulta	Zhitomyr Region, Dserzhinsk, temporary pool near the river	30	23

disputable in the last decades. JACKIEWICZ (1959, 1998) separated on the basis of anatomical characters five distinct species of *"Lymnaea palustris"* complex, namely *L. palustris* s.str., *L. turricula* (HELD, 1836), *L. vulnerata* (KЬSTER, 1862), *L. corvus* (GMELIN, 1791), and

L. occulta (JACKIEWICZ, 1959). This classification was accepted by some later reviewers (FALKNER 1985, 1990; GLЦER & MEIER-BROOK 1998), but criticised by the other (KILIAS 1992). Further species splitting in the group was suggested by KRUGLOV & STAROBOGATOV (1986, 1993). There are also different views concerning the subgeneric and even generic placement of the mentioned species. While GLЦER & MEIER-BROOK (1998) included all the mentioned species in the genus *Stagnicola,* JACKIEWICZ (1998) puts *L. corvus* and *L. vulnerata* (= *L. fusca* sensu GLЦER & MEIER-BROOK, 1998) in the subgenus *Lymnaea* s.str., restricting *Stagnicola* (treated as another subgenus of *Lymnaea)* to only three species (*L. palustris, L. turricula* and *L. occulta);* KRUGLOV & STAROBOGATOV (1986, 1993) distinguish in *Lymnaea* also a subgenus *Corvusiana* (alongside *Lymnaea* s.str. and *Stagnicola).*

Isozyme and immunological data are involved in taxonomic discussions about the group under consideration in addition to the traditional morphological characters since BURCH et al. (1971). The first data on DNA are reported quite recently (REMIGIO & BLAIR 1997; RYBSKA et al. 2000).

Karyological characters also proved to be rather informative for taxonomic studies in freshwater snails (BARSIENE et al. 2000). Chromosome numbers for pulmonate gastropods including some lymnaeids (mainly North American species) were provided by BURCH (1965), INABA (1969) andPATTERsoN & BURCH (1978); chromosome morphology of several European species was studied recently by BARSIENE et al. (1996) and GARBAR (1999). However, most of the species of the *Lymnaea palustris* complex are still not studied karyologically. This study deals with two such species, namely *L. corvus* and *L. occulta.* Their karyotypes are described for the first time and compared to those of *L. stagnalis* and *L. palustris.* More or less pronounced differences in chromosome morphology are shown for all these species, and their affinities are discussed.

Material and methods

Material for this study was collected by the first author in several regions of Ukraine (fig. 1, table 1) in 1997-2000. Species identification was based on conchological and anatomical characters suggested by JACKIEWICZ (1998). Pictures of shells are provided in the fig. 2. Voucher specimens will be deposited at the mollusc collection of the Staatliches Museum fьr Tierkunde Dresden.

Chromosome preparations were obtained from the gonad tissue according to the recommendations of BARSIENE et al. (1996) and GARBAR (1999). Molluscs were placed for 19 h in a 0.002 % solution of colchicine. Pieces of gonade were fixed in a mixture of ethanol and acetic acid (3:1). The cell suspension was prepared by maceration in a mixture of concentrated acetic and 60 % lactic acids (30:1) and dispersed with a capillar pipette on microscopic slides heated at 50° C. Dried preparations were stained 10-15 minutes in 10 % solution of azur-eosine after Romanovski, prepared on 0.01M phosphate buffer. Then they were placed for short time in xylole and embedded in Canada Balsam. Preparations were studied under a Biolam-L-212 microscope with magnification 10x90. The plates with the good dispersion of chromosomes and approximatly the same degree of spiralisation were selected for photographing and measuring. The relative length and centromeric index were calculated then for each chromosome, and the basic number (number of arms, NF) was determined for each karyotype. Chromosomes were classified according to THIRIOT-QUIEVREUX (1988), acrocentric chromosomes being considered uniarmed. Quantitative data were statistically processed by means of Microsoft Excel 97 package.

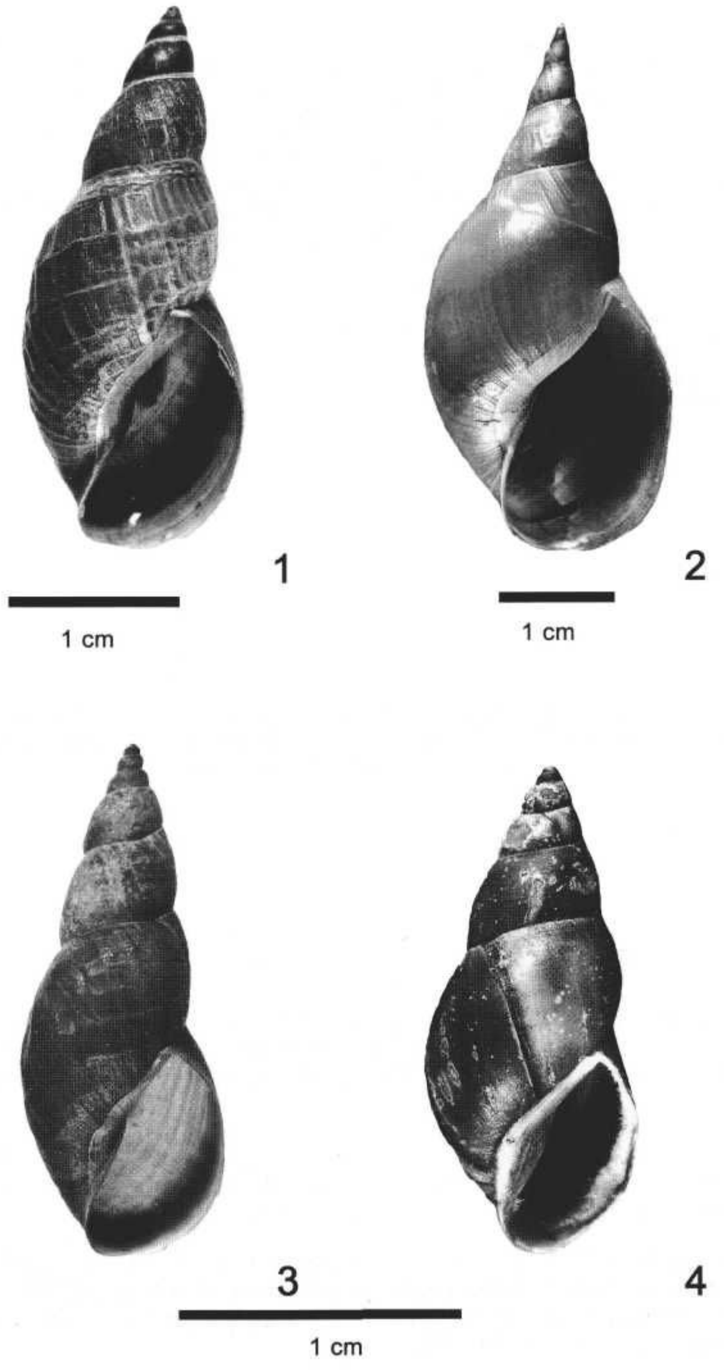

Fig. 2: Shells of the studied species: 1 - *Lymnaea corvus* (GMELIN, 1791); 2 - *Lymnaea stagnalis* (LINNAEUS, 1758); 3 - *Lymnaea palustris* (O.F. MÜLLER, 1774); 4 - *Lymnaea occulta* (JACKIEWICZ, 1959).

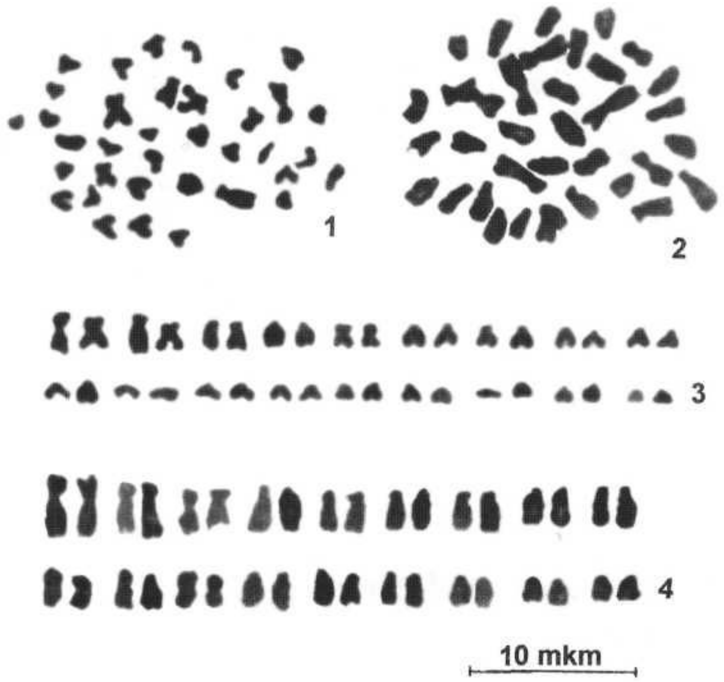

Fig. 3: Chromosomes of *Lymnaea corvus:* 1,3 - mitotic metaphase plate and karyotype of a specimen from Dserzhinsk, Zhitomyr Region; 2,4 - mitotic metaphase plate and karyotype of a specimen from Zhitomyr.

Results

Descriptions of karyotypes

Lymnaea corvus. Diploid number 2n = 36. Chromosomes of adjacent pairs similar in size, relative length between 8.66 % and 3.89 % (table 2). One pair of metacentric, four pairs of submetacentric, seven pairs of subtelocentric and six pairs of acrocentric (uniarmed) chromosomes (fig. 3, table 2). NF = 60.

Lymnaea stagnalis. Diploid number 2n = 36. Relative length of chromosomes between 9.61 % and 3.73 % (table 2). One pair metacentric, seven pairs submetacentric, five pairs subtelocentric and the rest (five pairs) acrocentric (fig. 4, table 2). NF = 62.

Lymnaea occulta. Diploid number 2n = 36. Except first pair (relative length 10.78 %), chromosomes rather similar in size: relative length between 7.74 % and 4.24 % (table 3). All chromosomes biarmed: seven pairs metacentric, nine pairs submetacentric and two pairs subtelocentric (fig. 5, table 3). NF = 72.

Lymnaea palustris. Diploid number 2n = 36. Relative length of chromosomes between 9.47 % and 3.44 %, chromosomes of adjacent pairs very similar in size (table 3). Only biarmed chromosomes: seven pairs metacentric, five pairs submetacentric and six pairs subtelocentric (fig. 6, table 3). NF = 72.

Fig. 4: Chromosomes of *Lymnaea stagnalis:* 1,3 - mitotic metaphase plate and karyotype of a specimen from Zhitomyr; 2,4 - mitotic metaphase plate and karyotype of a specimen from Kamenka-Bugskaya, Lviv Region.

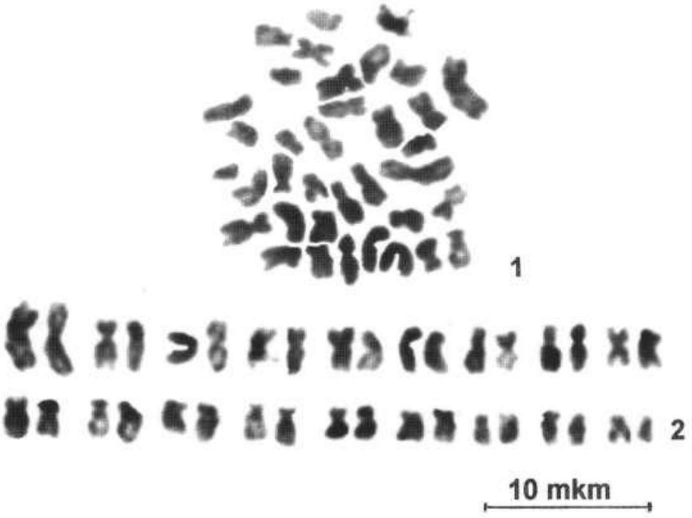

Fig. 5: Chromosomes *of Lymnaea occulta:* 1,2 - mitotic metaphase plate and karyotype of a specimen from Dserzhinsk, Zhitomyr Region.

Fig. 6: Chromosomes of *Lymnaea palustris:* 1,3 - mitotic metaphase plate and karyotype of a specimen from Poltava; 2,4 - mitotic metaphase plate and karyotype of a specimen from Perlyavka, Zhitomyr Region.

Comparisons

Since each of the studied karyotypes included several chromosome pairs of similar size and morphology, individual identification of chromosomes was complicated and alignments presented here are to a certain extent approximate. Nevertheless, differences between species in chromosome morphology were evident (fig. 7).

In contrast to that of *L. palustris* and *L. occulta,* metaphase plates of *L. corvus* and *L. stagnalis* included uniarmed (acrocentric) chromosomes alongside biarmed (meta-, submeta- and subtelocentric). Especially characteristic for *L. corvus* were three small acrocentric pairs. In this feature, the latter species could be readily distinguished from *L. palustris* to which it was synonymised for a long time. Noteworthy, that *L. corvus* had even more acrocentric chromosomes than *L. stagnalis.*

Karyotype of *L. occulta* is generally similar to that of *L. palustris.* However, two big subtelocentric pairs (treated here as the second and the fourth pair) could be diagnostic for the latter species (all big chromosomes of *L. occulta* are metacentric or submetacentric).

Table 2: Measurements (RL - relative length, Ci - centromeric index) and classification of chromosomes (m - metacentric, sm - submetacentric, st - subtelocentric, t - acrocentric chromosome) of *Lymnaea corvus* from Zhitomyr Region, Dserzhinsk, and *Lymnaea stagnalis* from Zhitomyr.

Pair no.	*L. corvus*			*L. stagnalis*		
	RL % (±SD)	Ci % (±SD)	Type	RL % (±SD)	Ci % (±SD)	Type
1	8.66±0.15	39.34±1.24	m	9.61±0.22	39.13±1.35	m
2	7.30±0.11	27.49±1.12	sm	7.30±0.12	33.57±2.17	sm
3	6.84±0.10	36.36±2.23	sm	6.79±0.09	35.76±2.22	sm
4	6.63±0.08	13.47±1.88	st	6.41±0.05	12.68±1.11	st
5	6.31±0.09	35.05±2.64	sm	6.09±0.09	33.94±1.82	sm
6	5.95±0.03	9.65±0.72	t	5.89±0.07	9.44±1.87	t
7	5.74±0.08	31.45±2.82	sm	5.63±0.06	27.39±1.81	sm
8	5.50±0.12	9.15±1.20	t	5.51±0.06	8.37±1.53	t
9	5.34±0.12	22.80±1.06	st	5.28±0.05	22.69±1.66	st
10	5.14±0.10	23.35±1.94	st	5.16±0.06	33.57±2.31	sm
11	4.91±0.05	9.76±0.86	t	5.09±0.05	8.77±1.59	t
12	4.79±0.06	22.79±1.55	st	4.89±0.05	28.67±2.19	sm
13	4.70±0.08	8.23±1.08	t	4.85±0.06	7.11±1.02	t
14	4.55±0.07	20.46±1.36	st	4.70±0.05	21.61±2.11	st
15	4.47±0.07	20.05±1.42	st	4.53±0.05	27.40±1.70	sm
16	4.37±0.08	9.05±1.55	t	4.41±0.06	8.80±1.36	t
17	4.13±0.10	10.24±1.13	t	4.14±0.05	20.44±2.68	st
18	3.89±0.10	14.66±2.27	st	3.73±0.08	16.74±2.05	st

Table 3: Measurements (RL - relative length, Ci - centromeric index) and classification of chromosomes (m - metacentric, sm - submetacentric, st - subtelocentric, t - acrocentric chromosome) of *Lymnaea palustris* from Poltava and *L. occulta* from Dserzhinsk, Zhitomyr Region.

Pair no.	*L. palustris*			*L. occulta*		
	RL % (±SD)	Ci % (±SD)	Type	RL % (±SD)	Ci % (±SD)	Type
1	9.47±0.19	43.56±1.18	m	10.78±0.14	42.74±1.26	m
2	8.56±0.13	22.30±1.75	st	7.74±0.17	32.14±1.81	sm
3	7.41±0.14	43.29±1.54	m	7.10±0.10	38.96±1.78	m
4	6.88±0.10	24.42±1.14	st	6.82±0.12	32.43±1.12	sm
5	6.26±0.07	47.56±2.09	m	6.54±0.07	39.44±1.93	m
6	6.18±0.08	16.01±1.82	st	6.54±0.05	29.44±1.67	sm
7	5.65±0.07	40.49±1.80	m	5.81±0.08	39.68±1.50	m
8	5.42±0.07	22.54±1.76	st	5.81±0.07	33.33±1.43	sm
9	5.19±0.07	41.22±2.21	m	5.62±0.08	49.18±1.69	m
10	5.12±0.10	34.30±1.88	sm	5.35±0.10	37.36±1.86	sm
11	5.12±0.06	29.92±2.33	sm	5.35±0.07	31.03±1.58	sm
12	4.51±0.07	42.37±2.40	m	5.07±0.06	38.18±2.03	m
13	4.43±0.09	24.13±1.47	st	5.07±0.08	24.09±1.05	st
14	4.28±0.09	42.87±1.62	m	4.88±0.08	37.74±1.98	m
15	4.28±0.10	37.42±1.46	sm	4.79±0.08	37.31±1.64	sm
16	3.97±0.09	36.50±1.52	sm	4.70±0.10	36.10±1.57	sm
17	3.82±0.10	20.00±2.54	st	4.33±0.09	24.66±2.31	st
18	3.44±0.10	33.30±1.98	sm	4.24±0.11	35.65±2.10	sm

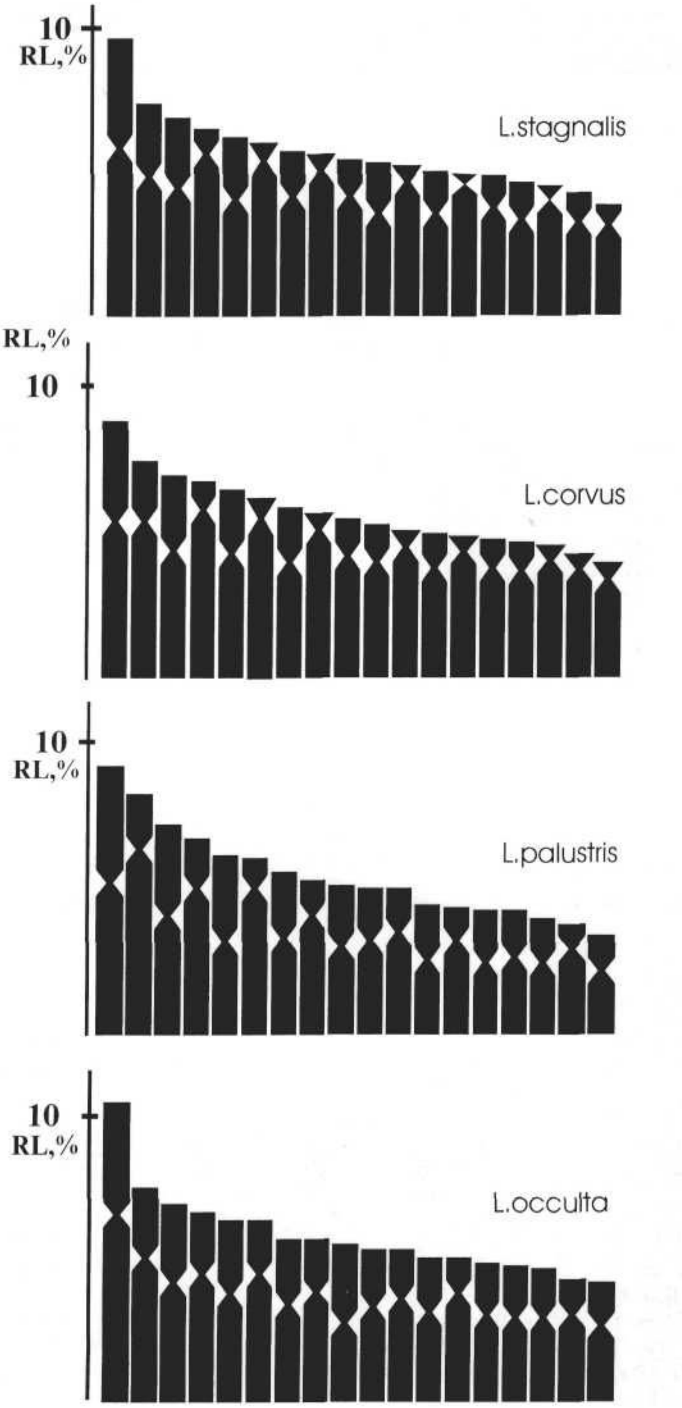

Fig. 7: Idiograms of chromosome sets in the studied species. RL - relative length.

Discussion

It is notable that *L. occulta* is reported from Zhitomyr region of Ukraine for the first time. Earlier it was known only from the Western regions (STADNICHENKO 1968; KORNIUSHIN 1999).

Chromosome numbers of *L. stagnalis* and *L. palustris* reported by this study (2n = 36) well agree with the data of INABA (1969) and PATTERSON & BURCH (1978). The same numbers were reported for some other species and subspecies assigned to the subgenus *Stagnicola* (PATTERSON & BURCH 1978).

Karyotypes of at least three North American species of *Stagnicola (L. palustris wyomingensis, L. exilis* and *L. catascopium)* also include only biarmed chromosomes (NF = 72) (INABA 1969) and thus are similar to those of *L. palustris palustris* and *L. occulta.* In contrast, *L. corvus,* with its karyotype including uniarmed (acrocentric) chromosomes and the low value of NF (60), is much more similar to *L. stagnalis* than to the species of *Stagnicola.* If ordinate the studied species according to the rate of metacentric and submetacentric chromosomes in their karyotype, *L. corvus* and *L. occulta* occupy the extreme positions.

Close affinity between *L. corvus* and *L. stagnalis* was first suggested by JACKIEWICZ (1998) on the basis of anatomical characters. In contrast to *L. palustris* and *L. occulta,* penis sheath in these species is much shorter than preputium, and prostate has multiplied inner folds. The mentioned character states are treated by JACKIEWICZ (1998) as synapomorphies, supporting the subgenus *Lymnaea* s.str.

A different pattern of affinities was shown in the study of foot muscle proteins carried out by BURCH et al. (1971): *L. corvus* appeared to be quite distinct from *L. stagnalis* and closely related to the North American *L. palustris elodes.* Anatomically, the latter form is similar to the European *L. palustris* in having only one fold of prostate. Unfortunately, *L. corvus* was the only biochemically studied European species of the *"Lymnaea palustris"* complex, therefore the obtained pattern of relationships was not complete. However, the mentioned authors interpreted the peculiar set of proteins characterising both European and North American *L. stagnalis* as a synapomorphy for *Lymnaea* s.str. (excluding *L. corvus)* and an alternative state as a plesiomorphy.

In the phylogeny based on the 16S mitochondrial ribosomal DNA sequences (REMIGIO & BLAIR 1997), *L. corvus* and *L. palustris* appear as a pair of sister taxa and *L. stagnalis* as an outgroup to this pair. The clade including all three species is a basal lineage in the family. Surprisingly, American species assigned to *Stagnicola* do not belong to this clade. Comparison of the karyological and molecular data shows that presence of acrocentric chromosomes shared by *L. stagnalis* and *L. corvus* is in all probability a plesiomorphic feature, which cannot support close relationship of the latter species and monophyly of *Lymnaea* s.str. sensu JACKIEWICZ (as including *L. corvus).*

The recent analysis of the DNA structure by means of the RAPD technique (RYBSKA et al. 2000) demonstrated transitional position of *L. occulta* between the other species of the subgenus *Stagnicola (L. palustris* and *L. turricula)* and the species of *Lymnaea* s.str. (*L. stagnalis* and *L. corvus).* This result correlate with the distribution of some anatomical characters (both *L. corvus* and *L. occulta* have a particular swelling at the base of the spermathecal duct) but not with the karyological characters (*L. corvus* and *L. occulta* are the most different species in this aspect).

Thus, phylogenetic relationships of lymnaeid snails are still disputable. Given the controversy between the patterns of relationships resulted from the analyses of different character sets, investigation of this intricate group should be continued. In particular, a phylogenetic analysis of the whole set of taxa (including North American ones) based on morphological and karyological characters is desirable, in order to define apomorphic viz. plesiomorphic status of various character states.

No definite mechanisms of karyotype transformations among the species of *Lymnaea* can be suggested on this stage of investigation. It is evident, however, that they included rearrangements within some chromosomes, while the number of chromosomes within the studied group remained constant.

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