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TAXONOMY OF EUROPEAN LYMNAEIDAE (GASTROPODA: PULMONATA) IN STUDIES WITH THE USE OF MOLECULAR BIOLOGY TECHNIQUES. I. PRELIMINARY VIEW ON THE SUBGENUS *STAGNICOLA* LEACH, 1830 ON THE BASIS OF RAPD ANALYSIS

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ABSTRACT: The random amplified polymorphic DNA (RAPD) technique was used to study genomic relationships of five lymnaeid species: *Lymnaea* (*Stagnicola*) *palustris* (O.F. Müller, 1774), *L.* (*S.*) *turricula* (Held, 1836), *L.* (*S.*) *occulta* (Jackiewicz, 1959), *Lymnaea* (*Lymnaea*) *corvus* (Gmelin, 1791) and *L.* (*L.*) *stagnalis* (Linnaeus, 1758). Altogether 253 characters (253 DNA fragments obtained in PCR) were scored and characters were used to create a matrix of pairwise distances between all the pairs of taxa. Distance data UPGMA cluster and Camin-Sokal maximum parsimony analyses were applied for dendrogram construction. The results support the discrimination of all taxa as separate species which was suggested by the structure of their reproductive organs. The taxonomic status of *L. occulta* (Jack.) as a member of the subgenus *Stagnicola* Leach, 1830 is discussed.

KEY WORDS: Gastropoda, Pulmonata, Lymnaeidae, *Stagnicola*, *Lymnaea corvus*, *L. occulta*, *L. palustris*, *L. stagnalis*, *L. turricula*, taxonomy, RAPD

INTRODUCTION

Lymnaeids are a common freshwater gastropod family of a wide geographical range. Nearly 1,800 species and varieties were described on the basis of their shell, which is characterised by a great morphological variability (PIECHOCKI 1979, JACKIEWICZ 1998). Investigations carried out by ROSZKOWSKI (1923, 1926), HUBENDICK (1951) and JACKIEWICZ (1959, 1988, 1993) proved that only the analysis of anatomical characters, especially of the reproductive system, allowed for correct identification of different species of Lymnaeidae. As a result, the number of the world's lymnaeid species was limited to about 40, placed in several genera. Eleven species occur in Europe (JACKIEWICZ 1998).

JACKIEWICZ (1959) distinguished three separate species in the species complex *Galba palustris* (O. F. Müller, 1774): *G. corvus* (Gmelin, 1791), *G. turricula* (Held, 1836) and *G. occulta* Jackiewicz, 1959. This classification was revised in the following years. For the species treated by JACKIEWICZ (1959) as *Galba turricula*, its former name *Lymnaea palustris* (O.F. Müller, 1774) was reintroduced (FALKNER 1984, JACKIEWICZ 1989). At the same time, *Lymnaea turricula* (Held, 1836) was redescribed as a separate fourth taxon (FALKNER

1985). According to the current classification (JACKIEWICZ 1998), three of the above species are usually assigned to the subgenus *Stagnicola* Leach, 1830, i.e. *Lymnaea* (*Stagnicola*) *palustris* (O.F. Müller, 1774), *L.* (*S.*) *turricula* (Held, 1836) and *L.* (*S.*) *occulta* (Jackiewicz, 1959). The fourth is included in the subgenus *Lymnaea s. str.* as *Lymnaea* (*Lymnaea*) *corvus* (Gmelin, 1791). Their taxonomic position seemed to be finally fixed on the basis of anatomical characters, mainly of their reproductive system (JACKIEWICZ 1993, 1998). However, the discrimination of *L.* (*S.*) *turricula* (Held), *L.* (*S.*) *occulta* (Jack.), *L.* (*L.*) *corvus* (Gmel.) and also *L.* (*L.*) *vulnerata* (Küst.) has been recently questioned again (KILIAS 1992). It has been proposed that some specimens of Hungarian populations of "*Galba palustris*" reveal mixed taxonomic characters of their shell and reproductive system. On that basis, KILIAS (1992) claimed that the above names pertain to the ecological varieties of *L. palustris* (O. F. Müll.). Although JACKIEWICZ (1996) showed that the Hungarian populations of "*Galba palustris*" examined by KILIAS (1992) comprised two different species (*L. palustris* and *L. turricula*), this discussion indicates that some new distinctive characters have to be found in order to determine whether or not the mentioned taxa are distinct species.

Application of molecular biology methods creates new possibilities of dealing with taxonomic problems (DAVIS 1994). The analysis of nucleotide sequences of nuclear or mitochondrial DNA provides us with new evidence which can serve as the basis for taxonomic and phylogenetic considerations. One of the frequently used molecular methods is RAPD (Random Amplified Polymorphic DNA) technique. RAPDs are generated by the amplification of genomic DNA with a single or combined short primers of arbitrary nucleotide sequence. The reaction of hybridisation of DNA primers with the amplified matrix DNA is carried out at a relatively low temperature. As a result, a set of various, repeatedly copied fragments of DNA is obtained. These fragments are subsequently separated electrophoretically on agarose or polyacrylamide gels. The band patterns obtained after staining the gel with ethidium bromide constitute a complex of molecular characters that can be the basis for taxonomic considerations. The RAPD technique has already been used to establish taxonomic relationships within the gastropod genera *Cochlicopa* (ARMBRUSTER 1997) *Bulinus* (STOTHARD & ROLLISON 1996, STOTHARD et al. 1997) and *Biomphalaria* (VERNON et al. 1995, LANGAND et al. 1999) as well as bivalve genus *Donax* (ADAMKIEWICZ & HARASEWYCH 1996). Molecular biology techniques were used in taxonomic studies on some American species of Lymnaeidae (BARGUES et al. 1997, BARGUES & MAS-COMA 1997, REMIGIO & BLAIR 1997a, b).

In this paper, we present the results of investigations in which the RAPD technique was applied to carry out the comparative analysis of DNA isolated from four species distinguished from the species complex *Galba palustris* (O.F. Müll.). The investigations were aimed at justifying their status as separate taxa. The unquestionably separate species, *Lymnaea stagnalis* (L.) was used for comparison.

MATERIALS AND METHODS

SNAIL MATERIAL

The specimens of the examined lymnaeid species were collected at their Polish localities in 1998–1999. *Lymnaea* (*Stagnicola*) *palustris* (O. F. Müller, 1774) was found in ponds in the vicinity of Morasko (near Poznań, NW Poland). *Lymnaea* (*Stagnicola*) *occulta* (Jackiewicz, 1959) was collected from a drainage ditch in Gorzykowo (near the road connecting Września and Gniezno, NW Poland). *Lymnaea* (*Lymnaea*) *corvus* (Gmelin, 1791) was found in a small reservoir near Ostrów Wlkp. (NW Poland). The specimens of *Lymnaea* (*Stagnicola*) *turricula* (Held, 1836) were found in the reservoir next to the road between Ustrzyki Dolne and Krościenko (Bieszczady Mountains, SE Poland). In addition, specimens of *Lymnaea* (*Lymnaea*) *stagnalis* (Linneaus, 1758) from Budzyńskie Lake near Mosina (NW Poland) were examined. All species were identified on the basis of the reproductive system characters (JACKIEWICZ 1998). Prior to DNA isolation, the specimens had been kept in small aquaria and fed with lettuce leaves.

DNA EXTRACTION

Individual gastropods were used for DNA extraction. Digestive glands were prepared and the remaining parts were preserved in 75% ethyl alcohol for anatomical verification. DNA was isolated using DNeasy Tissue Kit (Qiagen), according to the procedure recommended by Qiagen.

DNA AMPLIFICATION

Isolated DNA was amplified by PCR technique (thermal cycler PTC-200 MJ Research). Amplification was performed in a total reaction volume of 10 µl composed of 0.75 ng/µl of total template DNA, 0.5 µM of each primer, 200 µM of each dNTP, 1 mM

spermidine (FIEDOROW & SZWEYKOWSKA-KULIŃSKA 1997), buffer PCR (Qiagen, 1.5 mM $MgCl_2$, TRIS/HCl, KCl, $(NH_4)_2SO_4$) and 0.5 U Taq DNA polymerase (Qiagen). Thermal cycling was performed using a programme consisting of: initial denaturation step (95°C, 5 min), 50 cycles (each composed of three stages: denaturation at 92°C, 1 min; annealing at 35°C, 2 min; extension at 72°C, 2 min), one extension after 50 cycles (75°C, 5 min). Five different primers were used for RAPD analysis with sequences listed below:

P2 – 5'-TGCACACTGA-3' (KLEIN-LANKHORST et al. 1991)
P3 – 5'-TGGTGACTGA-3'(KLEIN-LANKHORST et al. 1991)
P4 – 5'-TGGTCACTGT-3'(KLEIN-LANKHORST et al. 1991)
P5 – 5'-GTCCCGACGA-3' (HOSAKA & HANNEMAN 1994)
P6 – 5'-CTCGTTTGGG-3' (received from Dr. M. JAKUBOWICZ, not published)

Total genomic DNAs isolated from particular lymnaeid species were used as templates for PCR reactions with the following pairs of primers: P2+P5, P3+P6, P6+P6, P4+P6, P2+P6 and P3+P5.

ELECTROPHORESIS

DNA amplification products were separated using 8% PAGE in 1 × TEB (buffer 90 mM TRIS- 2 mM EDTA- boric acid, pH 8.3) for 3 hrs. Gels were stained with ethidium bromide (0.5 µg/ml) and photographed under UV light using Polaroid film.

DATA ANALYSIS

Programmes of PHYLIP v. 3.57c package (FELSENSTEIN 1995) were used to construct and test dendrograms. Two different algorithms were used for the tree construction. One of them was a distance data UPGMA cluster analysis. The pairwise distances were calculated by RAPDistance v.104 package (ARMSTRONG et al. 1994) using the Yule and Kendall algorithm (YULE & KENDALL 1950). The second applied analysis of raw data was performed by Camin-Sokal maximum parsimony algorithm. The number of RAPD fragments for each individual tested was used to construct the data matrix. The characters were coded in a binary system (0, 1): 0 – means no particular DNA fragment (no band on the gel in that position), 1 – means the presence of the particular DNA fragment.

RESULTS AND DISCUSSION

Figure 1 presents an example of PCR amplification products derived from the P3+P5 primers. Table 1 presents the matrix of binary coded characters obtained after the electrophoresis of PCR amplification products for *L.* (*S.*) *palustris, L.* (*S.*) *turricula, L.* (*S.*) *occulta, L.* (*L.*) *corvus* and *L.* (*L.*) *stagnalis*. Each species is represented by the DNA samples isolated from two individuals. A total of 253 DNA fragments were scored. RAPD fragments originating from the same pair of primers and migrating identically during the electrophoresis were assumed as homologous.

The data from Table 1 were used to create a matrix of pairwise distances between all the pairs of taxa. The distances are listed in Table 2. The data compared in this table show that the distances between individuals treated as separate species on the basis of their genital characters range from 0.11 to 0.69, while the distances between the individuals included anatomically in the same species range from 0.0002 to 0.04 which may suggest little variation among them. The range of differences observed among individuals classified as *L.* (*L.*) *corvus, L.* (*L.*) *stagnalis* and *L.* (*S.*) *occulta* seems to confirm their separate taxonomic status. Moreover, the distances separate each individual of *L.* (*S.*) *palustris* and *L.* (*S.*) *turricula* from these three species. On the other hand, the distances between the individuals identified as *L.* (*S.*) *palustris* and *L.* (*S.*) *turricula* may suggest that they are closely related. Therefore, the problem of their taxonomic separateness requires further investigations.

All the dendrograms also confirm the phyletic separateness of the investigated species (Figs 2 and 3). The taxonomic position of *L.* (*S.*) *occulta* remains disputable. The species may be classified either within the subgenus *Lymnaea* s. str. (Fig. 2) or with *Stagnicola* (Fig. 3), depending on the method used for the tree construction.

The number of electrophoretic bands in common for each pair of species (i.e. the presence of DNA fragments identical in length) is shown in Table 3. *L.* (*S.*) *palustris* and *L.* (*S.*) *turricula* share as many as 33 common characters, which may additionally suggest their close relationship and justify their classification within the same subgenus. *L.* (*L.*) *stagnalis* and *L.* (*L.*) *corvus* share 20 characters. Again, the taxonomic position of *L. occulta* remains problematic. The number of characters that *L. occulta* shares with the members of the subgenera *Stagnicola* and *Lymnaea* s. str. is extraordinary.

Although the results of RAPD studies should be used with a great caution in taxonomical analyses (BACKELJAU et al. 1995), it may be stated that the results of this paper confirm JACKIEWICZ's (1993, 1998) discrimination of *L. palustris* (O.F. Müll.), *L. turricula* (Held), *L. occulta* (Jack.) and *L. corvus* (Gmel.) as separate species on the basis of conchological and genital

Table 1. 253 DNA fragments obtained using RAPD technique present in ten lymnaeid individuals

Primers P2+P6

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
A – *L. (L.) corvus* 1	0	1	0	0	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0
B – *L. (L.) corvus* 2	0	1	0	0	1	0	0	1	1	1	0	1	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0
C – *L. (L.) stagnalis* 1	1	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	1	0	0	0	1	0	0	0
D – *L. (L.) stagnalis* 2	1	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0
E – *L. (S.) occulta* 1	0	0	0	0	0	1	0	0	0	1	0	1	1	0	0	0	1	1	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0
F – *L. (S.) occulta* 2	0	0	0	0	0	1	0	0	0	1	0	1	1	0	0	0	1	1	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0
G – *L. (S.) palustris* 1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0
H – *L. (S.) palustris* 2	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0
I – *L. (S.) turricula* 1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	1	1
J – *L. (S.) turricula* 2	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	1

Primers P2+P5

	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79
A – *L. (L.) corvus* 1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B – *L. (L.) corvus* 2	1	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
C – *L. (L.) stagnalis* 1	1	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	1	1	0	1	0	1	0	0	0	1	0	1	1	1	1	0	0	0
D – *L. (L.) stagnalis* 2	1	1	0	0	0	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	1	1	0	1	0	1	0	0	0	1	0	1	1	1	1	0	0	0
E – *L. (S.) occulta* 1	1	0	0	0	0	1	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0
F – *L. (S.) occulta* 2	1	0	0	0	0	1	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0
G – *L. (S.) palustris* 1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	1	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
H – *L. (S.) palustris* 2	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	1	0	1	1	0	0	0	0	0	1	1	0	0	1	0	0	1	0	0	0	1
I – *L. (S.) turricula* 1	1	0	1	1	1	0	0	1	0	0	1	1	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1
J – *L. (S.) turricula* 2	1	0	1	1	1	0	0	1	0	0	1	1	0	1	0	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1

Primers P3+P6

	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	121	123
A – *L. (L.) corvus* 1	1	0	1	1	0	0	0	0	1	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0
B – *L. (L.) corvus* 2	1	0	1	1	1	0	0	0	1	0	0	0	1	0	0	0	1	1	0	0	0	1	0	0	1	1	1	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0
C – *L. (L.) stagnalis* 1	1	1	0	0	0	0	0	0	1	1	0	0	0	1	0	0	1	1	0	1	1	1	1	0	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1	1
D – *L. (L.) stagnalis* 2	1	1	0	0	0	0	0	0	1	1	0	0	0	1	0	0	1	1	0	1	1	1	1	0	1	1	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	1	1
E – *L. (S.) occulta* 1	1	0	0	0	0	1	1	0	0	0	1	0	0	0	0	1	0	1	0	1	0	1	0	1	0	0	1	0	0	0	1	1	0	0	1	0	0	1	0	1	1	0	0	0
F – *L. (S.) occulta* 2	1	0	0	0	0	1	1	0	0	0	1	0	0	0	1	1	0	1	0	1	0	1	0	1	0	0	1	0	0	0	1	1	0	0	1	0	0	1	0	1	1	0	0	0
G – *L. (S.) palustris* 1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	1	1	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
H – *L. (S.) palustris* 2	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	1	1	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0
I – *L. (S.) turricula* 1	1	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	1	1	0	0	1	0	0	0	0	0	0	0
J – *L. (S.) turricula* 2	1	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	1	1	0	0	1	0	0	0	0	0	0	0

Primers P6+P6

	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156
A – *L. (L.) corvus* 1	0	0	1	1	1	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0
B – *L. (L.) corvus* 2	0	0	0	1	1	0	0	0	0	1	0	0	0	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0
C – *L. (L.) stagnalis* 1	0	1	0	1	1	0	1	1	1	1	0	1	0	0	1	1	0	0	1	1	0	1	0	1	0	0	1	0	0	0	1	1	0
D – *L. (L.) stagnalis* 2	0	1	0	1	1	0	1	1	1	1	0	1	0	0	1	1	0	0	1	1	0	1	0	1	0	0	1	0	0	0	1	1	1
E – *L. (S.) occulta* 1	0	0	0	1	1	1	0	0	0	1	1	0	0	1	1	0	1	1	1	1	0	1	0	0	1	1	1	0	0	1	1	0	0
F – *L. (S.) occulta* 2	0	0	0	1	1	1	0	0	0	1	1	0	0	1	1	0	1	1	1	1	0	1	0	0	1	1	1	1	1	0	1	0	0
G – *L. (S.) palustris* 1	1	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0
H – *L. (S.) palustris* 2	1	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
I – *L. (S.) turricula* 1	1	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	1	1	1	0	0	1	0	0	0	0	0	0	0	1	0	0
J – *L. (S.) turricula* 2	1	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	1	1	1	0	1	1	0	0	0	0	0	0	0	1	0	0

Primers P4+P6

	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202
A – *L. (L.) corvus* 1	1	0	1	0	0	0	0	1	0	1	0	0	1	1	1	0	0	0	0	1	1	0	0	0	0	1	0	0	0	1	0	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0
B – *L. (L.) corvus* 2	1	1	1	1	0	0	1	0	0	1	0	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0
C – *L. (L.) stagnalis* 1	0	1	1	0	0	0	0	1	0	0	0	0	0	1	0	1	1	1	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0
D – *L. (L.) stagnalis* 2	0	1	1	0	0	0	0	0	1	0	0	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0
E – *L. (S.) occulta* 1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	1
F – *L. (S.) occulta* 2	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	0	0	0	0	1	0	1	1
G – *L. (S.) palustris* 1	0	0	1	0	0	1	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
H – *L. (S.) palustris* 2	0	0	0	0	0	1	0	0	0	0	1	1	0	1	0	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
I – *L. (S.) turricula* 1	0	0	1	1	1	0	0	0	0	0	0	1	0	1	0	0	0	1	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0
J – *L. (S.) turricula* 2	0	0	1	1	1	0	0	0	0	0	0	1	0	1	0	0	0	1	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0

Primers P3+P5

	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253
A – *L. (L.) corvus* 1	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0
B – *L. (L.) corvus* 2	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0
C – *L. (L.) stagnalis* 1	1	1	0	1	0	1	0	0	1	0	1	1	0	0	1	0	1	0	0	1	1	1	1	0	0	0	1	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0
D – *L. (L.) stagnalis* 2	1	1	0	1	0	1	0	0	1	1	1	0	0	0	1	0	1	0	0	1	1	1	1	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	1	1	0	1	1	0	0	0	1	0	0	0
E – *L. (S.) occulta* 1	1	0	0	0	0	0	1	0	0	1	1	0	1	0	0	1	0	0	0	1	1	0	0	1	0	1	0	0	0	0	0	1	1	0	1	0	1	0	1	0	0	0	1	0	0	1	0	0	0	1	0
F – *L. (S.) occulta* 2	1	0	0	0	0	0	1	0	0	1	1	0	1	0	0	1	0	0	0	1	1	1	0	0	1	0	1	0	0	0	0	0	1	1	0	1	0	1	0	1	0	0	0	0	0	1	0	0	0	1	0
G – *L. (S.) palustris* 1	1	0	1	0	1	0	0	1	1	1	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
H – *L. (S.) palustris* 2	1	0	1	0	1	0	0	1	1	0	1	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
I – *L. (S.) turricula* 1	1	0	1	0	1	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	1	1
J – *L. (S.) turricula* 2	1	0	1	0	1	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1

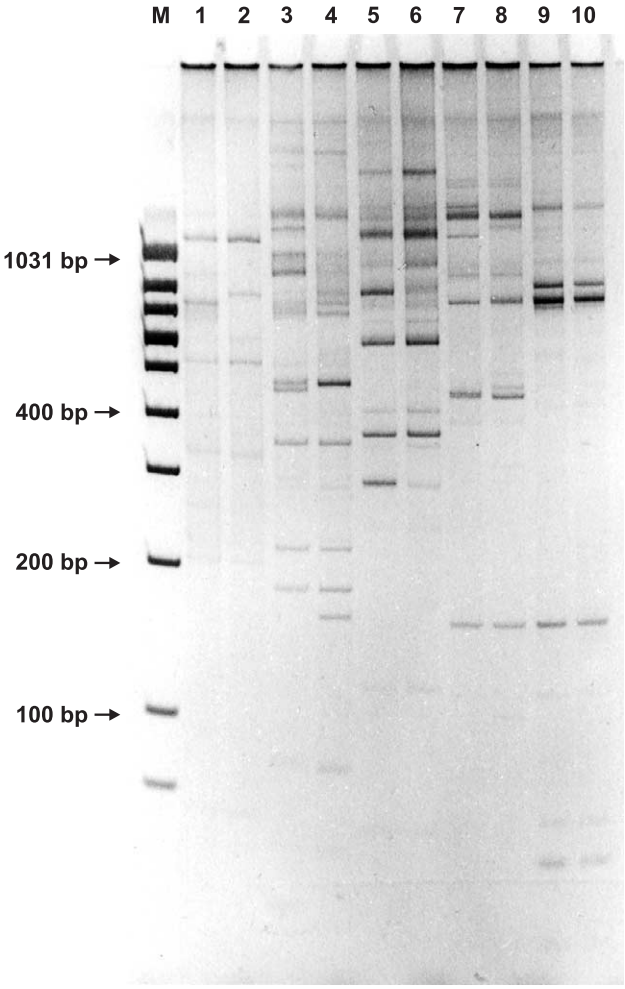

Fig. 1. An example of electrophoretic separation of PCR amplification products derived from P3+P5 primers. M – marker MBI Fermentas GeneRuler 100 bp DNA Ladder; 1, 2 – *Lymnaea* (*Lymnaea*) *corvus*; 3, 4 – *L.* (*L.*) *stagnalis*; 5, 6 – *Lymnaea* (*Stagnicola*) *occulta*; 7, 8 – *L.* (*S.*) *palustris*; 9, 10 – *L.* (*S.*) *turricula*

characters. However, these results call for further investigations with the use of other molecular biology techniques aimed at verification of the subgeneric placement of *L. occulta* (Jack.).

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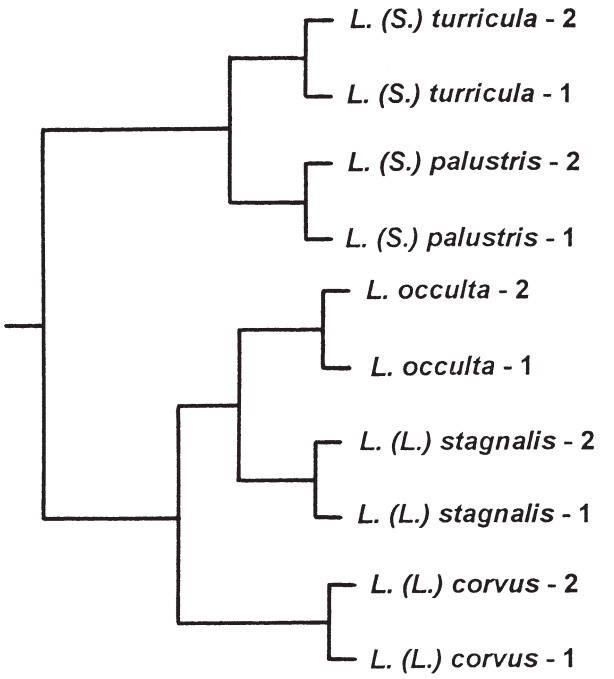

Fig. 2. Dendrogram constructed with the use of Camin-Sokal method (Penny algorithm) for the studied lymnaeid species

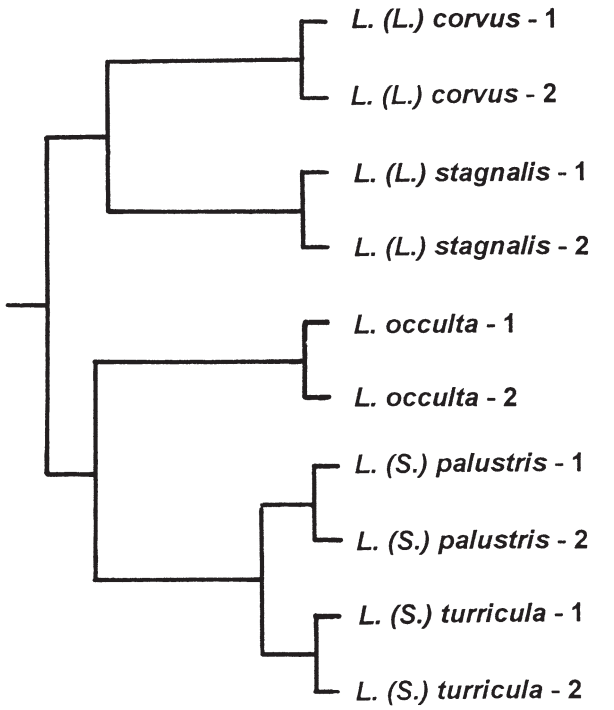

Fig. 3. Dendrogram obtained using UPGMA method for the studied lymnaeid species

Table 2. Pairwise distance matrix calculated by RAPDistance v.1.04 package using the YULE & KENDALL (1950) algorithm

	A	B	C	D	E	F	G	H	I	J
A	x	0,005750	0,427918	0,469570	0,602273	0,575460	0,650181	0,622587	0,596542	0,566502
B		x	0,454128	0,448276	0,554896	0,529412	0,619585	0,666667	0,591024	0,560777
C			x	0,021416	0,536181	0,620237	0,602071	0,693145	0,625000	0,574276
D				x	0,469903	0,574171	0,618988	0,703102	0,587506	0,544910
E					x	0,045137	0,592444	0,670157	0,501558	0,492193
F						x	0,512195	0,536496	0,462537	0,477847
G							x	0,005722	0,118519	0,126374
H								x	0,160161	0,169811
I									x	0,000255
J										x

A, B – *Lymnaea* (*Lymnaea*) *corvus*; C, D – *Lymnaea* (*Lymnaea*) *stagnalis*; E, F – *Lymnaea* (*Stagnicola*) *occulta*; G, H – *Lymnaea* (*Stagnicola*) *palustris*; I, J – *Lymnaea* (*Stagnicola*) *turricula*

Table 3. The number of RAPD fragments shared by the pairs of lymnaeid species (presence of DNA fragments identical in length)

L. (*L.*) *corvus*				
20	*L.* (*L.*) *stagnalis*			
15	23	*L.* (*S.*) *occulta*		
6	14	12	*L.* (*S.*) *palustris*	
11	20	21	33	*L.* (*S.*) *turricula*

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