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# KARYOTYPES OF EUROPEAN SPECIES OF *RADIX* (GASTROPODA: PULMONATA: LYMNAEIDAE) AND THEIR RELEVANCE TO SPECIES DISTINCTION IN THE GENUS

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## ABSTRACT

Karyotypes of *Radix auricularia* (Linnaeus, 1758) and three disputable taxa considered by different authors as distinct species or assigned as forms of *Radix peregra* (Muller, 1774), *sensu lato* - *R. labiata* (Rossmassler, 1835), *R. balthica* (Linnaeus, 1758), and *R. ampla* (Hartmann, 1821) - were studied with preparations obtained from gonad tissues by the air-drying method. The studied taxa have the same diploid number (2n = 34), but are characterized by different morphology of some chromosome pairs. In particular, *R. labiata* (traditionally identified as *R. peregra, s.* s.) and *R. balthica (= R. ovata* in traditional understanding) differ in the number of subtelocentric chromosomes (1 and 5, respectively), species status of these taxa being also supported by pronounced differences in centomeric indexes of chromosome pairs 4 and 16. Species distinctness of *R. ampla* is supported by differences in three chromosome pairs, and karyological similarity between this taxon and *R. balthica* is also noted. FN values varied among the studied taxa from 56 in *R. ampla* to 66 in *R. labiata.* The known karyological characters are traced on phylogenetic trees suggested by recent molecular reconstructions. This study demonstrates that karyology can be an effective tool for aiding taxonomic distinctions of historically problematic groups of molluscs.

Key words: *Radix,* karyotypes, taxonomy, species distinctions.

## INTRODUCTION

The group of lymnaeid species bearing the name *Radix* Montfort, 1810, is defined mainly by its thin-walled fragile shell with a relatively large aperture (Falkner, 1990; Gloer & Meier-Brook, 1998; Jackiewicz, 1998; Gloer, 2002). The distinctive karyological character of *Radix,* namely its chromosome number (n = 17) deviating from the other members of the family (typically n = 18, or in some taxa n = 16or19),has also been known for a long time (Inaba, 1969; Choudharyetal., 1992).

Despite intensive research by different methods (Hubendick, 1951; Inaba, 1969; Patterson & Burch, 1978; Kruglov & Starobogatov, 1983, 1993; Remigio & Blair, 1997; Jackiewicz, 1998; Bargues et al., 2001), many taxonomic problems of *Radix* remain unresolved. In particular, the rank of the group is alternatively defined as subgeneric within Lymnaea (Hubendick, 1951; Kruglov & Starobogatov, 1983, 1993; Jackiewicz, 1998; Kerney, 1999) or generic (Patterson & Burch, 1978; Falkner, 1990; Gloer & Meier-Brook, 1998; Bargues et al., 2001; Falkner et al., 2002). A distinct subgenus *Peregriana* was recognized in *Lymnaea* alongside *Radix* by Kruglov & Starobogatov (1983). Still uncertain also is the number of species within this group. Stressing the lack of distinctive anatomical characters and existence of intermediate shell morphotypes, British (Kerney, 1999) and Polish (Jackiewicz, 1998) authors recognized only two European species, namely *Lymnaea (Radix) auricularia* (Linnaeus, 1758) and *L. (R.) peregra* (Muller, 1774), distinguishing in the latter up to four ecological forms: *L peregra s. s.* (= f. *typica, sensu* Jackiewiecz, 1998), f. *ovata* (Drapamaud, 1805), f. *lagotis* (Schrank, 1803), and f. *ampla* (Hartmann, 1821). Evidence for species distinctness of *Radix ovata* was provided by Gloer & Meier-Brook (1998), whereas Falkner (1990) also recognized *R. ampla* as a full species. Five European taxa are supported by recent molecular studies (Bargues et al., 2001) and have been

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TABLE 1. Material for karyological investigation.

| Taxon | Synonymy | Locality | No. of specimens studied | No. of metaphases studied* | Remarks |
|---|---|---|---|---|---|
| *Radix auricularia* (Linnaeus, 1758) | *Lymnaea auricularia* | Zhitomyr, River Teterev | 50 | 16 | first studied by Garbar (1998) |
| | | Vinnitsa Region, Shyroka Greblya, River Yuzhny Bug (South Bug) | 14 | 9 | |
| *R. labiata* (Rossmässler, 1835) | *R. peregra, auctt.*<br>*L. peregra, auctt.* | Zhitomyr, small pond | 45 | 29 | first studied by Garbar (2000) |
| | *L. peregra* f. *typica*, *sensu* Jackiewicz, 1998 | Vorokhta, Ivano-Frankivsk Region, small pond | 20 | 11 | |
| *R. balthica* (Linnaeus, 1758) | *R. ovata* (Draparnaud, 1805) | Olevsk, Zhitomyr Region, River Ubort | 30 | 15 | first studied by Garbar (2000) |
| | *L. ovata*<br>*L. peregra* f. *ovata*, *sensu* Jackiewicz, 1998 | Kiev, River Dnieper | 9 | 6 | |
| *R. ampla* (Hartmann, 1821) | *L. peregra* f. *ampla*, *sensu* Jackiewicz, 1998 | Zhitomyr, River Teterev | 65 | 17 | |
| | | Kharkiv, River Udy | 5 | 9 | |

* In some specimens no metaphases could be observed.

included as species in the latest European checklists (Falkner et al., 2002; Gloer, 2002) with the following names: *Radix auricularia, R. labiata* (Rossmassler, 1835) (substituting *R. peregra, s. s.* of previous authors), *R. balthica* (Linnaeus, 1758) (= *R. peregra* of Muller, = *R. ovata, aucti.), R. ampla,* and *R. lagotis.* An even more profound subdivision was suggested by Kruglov & Starobogatov (1983, 1993) but not supported by any of the later studies.

Until now, chromosome numbers were mainly involved in discussions about taxonomy and relationships among freshwater gastropods. However, interspecific differences in chromosome morphology were recently found in *Viviparus* (Barsiene et al., 2000). Furthermore, preliminary investigations of Garbar (1998, 2000) on *Radix* and on *Stagnicola* (Garbar & Korniushin, 2002) have shown that morphological characters of chromosomes may be also helpful by species distinction in lymnaeids. This paper summarizes results of karyological investigation in four European taxa of *Radix,* designated in modern reviews as *R. auricularia, R. labiata, R. balthica* and *R. ampla.*

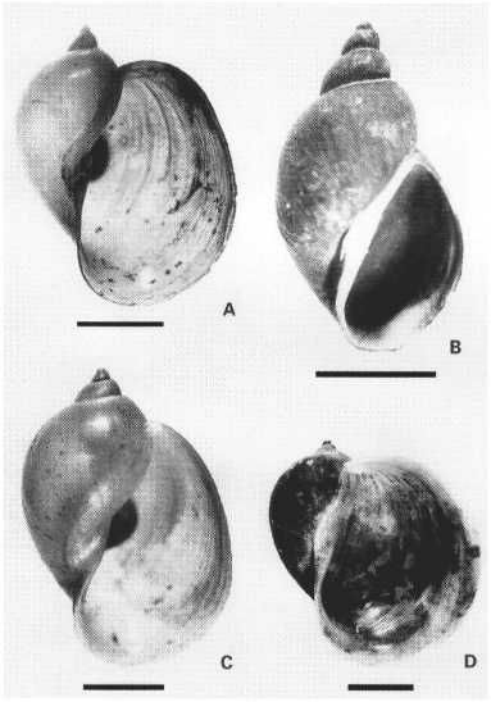


FIG. 1. Shells of the studied species: A. *Radix auricularia* (Linnaeus, 1758); B. *R. labiata* (Rossmassler, 1835); C. *R. balthica* (Linnaeus, 1758); D. *R. ampla* (Hartmann, 1821). Scale bar, 5 mm.

## MATERIALAND METHODS

Material was collected by the first author in 1997-2000 in western and central Ukraine (Table 1). In order to minimize the influence of local factors, each species was sampled in two remote (at a distance of at least 150 km) localities; the sampled populations inhabit different river systems (of the Danube, Yuzhny Bug, and Dnieper drainages) and live under somewhat different climatic conditions. Some populations included in the earlier karyological studies (Garbar, 1998,2000) were re-sampled. Species identification was based on traditional conchological and anatomical characters (Gloer & Meier-Brook, 1998; Jackiewicz, 1998). The studied group is treated in our work as a genus and the studied forms as species, following the latest systematic and phylogenetic works (Remigio & Blair, 1997; Bargues et al., 2001; Falkner et al., 2002). Nomenclature of the latest European monographic review (Gloer, 2002) is used herein; in order to avoid misunderstanding, we provide the list of the studied taxa with synonyms used in the cited publications (Table 1).

Pictures of shells are provided in Figure 1. Voucher specimens have been deposited in the mollusc collection of the Museum fur Naturkunde, Humboldt Universitat zu Berlin, Germany.

Chromosome preparations were obtained from the gonad tissue according to the recommendations of Barsiene et al. (1996) and Garbar (1998). Molluscs were placed for 17 h in a 0.002% solution of colchicine. Pieces of gonad 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 min in 10% solution of azur-eosine after Romanovski, prepared on 0.01 M phosphate buffer. Stained preparations were placed for short time in xylol and embedded in Canada Balsam. These preparations were studied undera Biolam-L-212 microscope with magnification 10 x 90. The plates with a good dispersion of chromosomes and moderate degree of spiralization were selected for photographing and measuring. The relative length and centromeric index were then calculated for each chromosome. Chromosomes were classified according to Levan et al. (1964). The Fundamental Number (FN) was calculated as the number of autosome arms in haploid complement, with a value of 4 given to metacentric and

submetacentric chromosomes, and a value of 2 to subtelocentric chromosomes (no telocentric chromosomes were found in the studied taxa). Quantitative data from the most numerous samples were processed statistically using standard methods.

## RESULTS

### Descriptions of Karyotypes

*Radix auricularia.* 2n = 34. Chromosomes of adjacent pairs similar in size, their relative length varies between 9.21% and 4.15% (Table 2). Karyotype includes 11 pairs of metacentric, four pairs of submetacentric, and two pairs of subtelocentric chromosomes (Fig. 2, Table 2). FN = 64.

*Radix labiata.* 2n = 34. Chromosomes of adjacent pairs similar in size, with relative length between 9.69% and 3.74% (Table 2). Karyotype includes 12 pairs of metacentric, four pairs of submetacentric, and one pair of subtelocentric chromosomes (Fig. 3, Table 2). FN = 66.

*Radix balthica.* 2n = 34. Chromosomes of adjacent pairs similar in size, with relative length between 9.29% and 3.95% (Table 2). Karyotype includes eight pairs of metacentric, four pairs of submetacentric, and five pairs of subtelocentric chromosomes (Fig. 4, Table 2). FN = 58.

*Radix ampla.* 2n = 34. Chromosomes of adjacent pairs similar in size, with relative length of chromosomes between 9.32% and 4.05% (Table 2). Karyotype includes eight pairs of metacentric, three pairs of submetacentric, and six pairs of subtelocentric chromosomes (Fig. 5, Table 2). FN = 56.

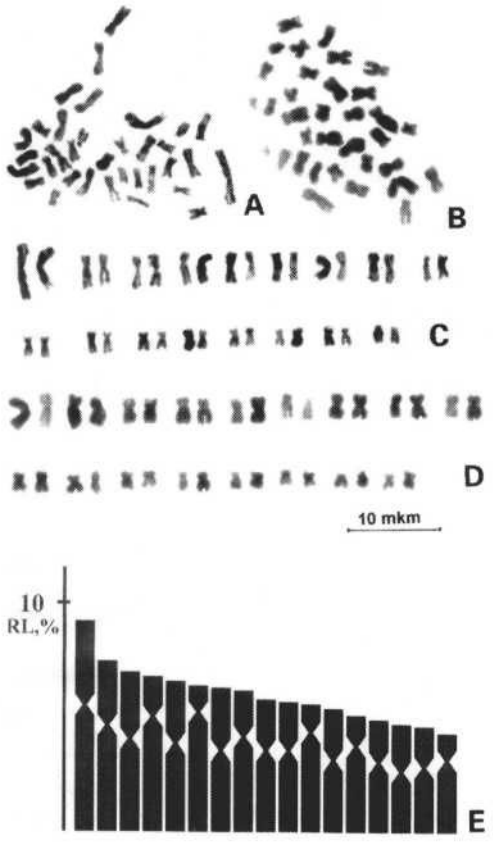


FIG. 2. Chromosomes of *Radix auricularia:* A. Mitotic metaphase of a specimen from Zhitomyr; B. The same of a specimen from Vinnitsa; C, D. Karyotypes of specimens from Zhitomyr and Vinnitsa region, respectively; E. Ideogram (based on data from Table 2). Scale bar, 10 µm.

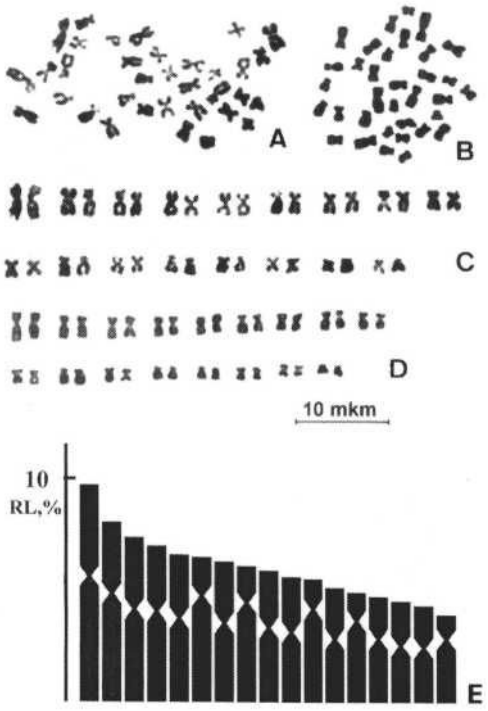


FIG. 3. Chromosomes of *Radix labiata:* A, B. Mitotic metaphases of specimens from Zhitomyr and Vorokhta, respectively; C, D. Karyotypes of specimens from Zhitomyr and Vorokhta, respectively; E. Ideogram (based on data from Table 2). Scale bar, 10 µm.

Comparisons

All studied taxa are characterized by the same chromosome number (2n = 34). Morphological similarity is demonstrative also in some individual chromosome pairs, that is, pairs 1, 3, 5, 7, 9, 11, 12 and 15, belonging to one and the same type in all these taxa. On the other hand, distinctive features of chromosome morphology were noted not only for the doubtless species *Radix auricularia,* but also for three taxa of disputable status - *R. labiata, R. balthica,* and *R. ampla.* The karyotype of *R. labiata* (= *R. peregra, auctt.)* differs from that of *R. balthica,* and *R. ampla* in morphological type in seven to nine pairs. The higher rate of subtelocentric chromosomes in two latter species (five to six out of 17 pairs) is also reflected in the lower values of FN (58 and 56, respectively), compared to FN of *R. labiata* (66). In some cases, such as in the pairs 2, 6, and 13, mean values of centromeric indexes in the compared species were close (Table 2), and assignment of chromosomes to different types might be influenced by individual variation. However, that was not the case in chromosome pairs 4 and 16, for which interspecific differences were the most pronounced. Taking into account that chromosomes adjacent to the mentioned pairs in the ideograms (Figs. 2-5) were morphologically similar among the studied taxa, we conclude that observed differences could not be caused by errors in identification of individual chromosomes. Therefore, they are further referred to as taxonomic characters.

Similarity in chromosome morphology between *R. labiata* and *R. auricularia* is noteworthy: only two pairs (4 and 6) were assigned to

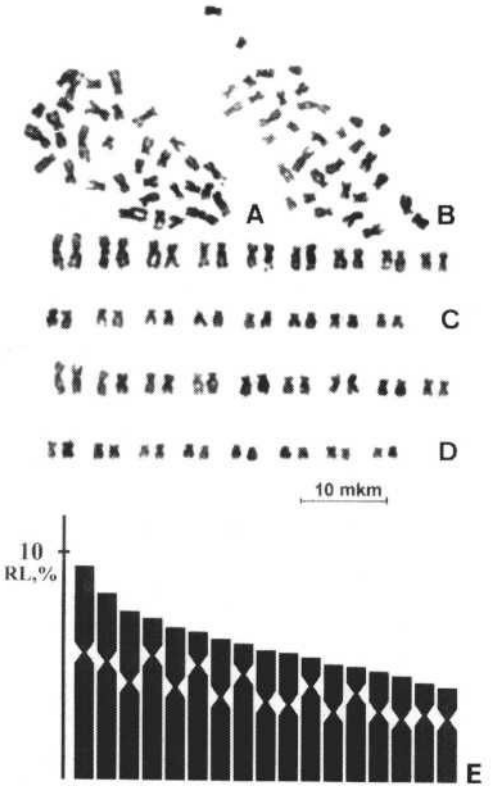


FIG. 4. Chromosomes of *Radix balthica:* A, B. Mitotic metaphases of specimens from Olevsk and Kiev, respectively; C, D. Karyotypes of specimens from Olevsk and Kiev, respectively; E. Ideogram (based on data from Table 2). Scale bar, 10 µm.

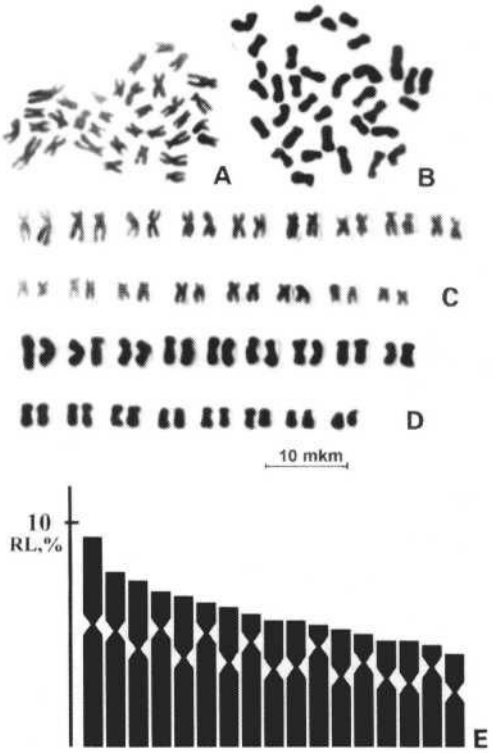


FIG. 5. Chromosomes of *Radix ampla:* A, B. Mitotic metaphases of specimens from Zhitomyr and Kharkiv, respectively; C, D. Karyotypes of specimens from Zhytomyr and Kharkiv, respectively; E. Ideogram (based on data from Table 2). Scale bar, 10 µm.

TABLE 2. Measurements (RL - relative length, Ci - centromeric index, SD - Standard Deviation) and classification of chromosomes (m - metacentric, sm - submetacentric, st - subtelocentric chromosome) of *Radix auricularia* from Zhitomyr, River Teterev, *R. labiata* from Zhitomyr, and *R. balthica* from Zhitomyr Region, Olevsk, and *R. ampla* from Zhitomyr, River Teterev.

| | *R. auricularia* | | | *R. labiata* | | | *R. balthica* | | | *R. ampla* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pair no. | RL % (± SD) | Ci % (± SD) | Type | RL % (± SD) | Ci % (± SD) | Type | RL % (± SD) | Ci % (± SD) | Type | RL % (± SD) | Ci % (± SD) | Type |
| 1 | 9.21 ± 0.27 | 40.23 ± 1.11 | m | 9.69 ± 0.08 | 41.81 ± 0.69 | m | 9.29 ± 0.11 | 41.23 ± 0.73 | m | 9.32 ± 0.19 | 41.60 ± 1.21 | m |
| 2 | 7.38 ± 0.11 | 38.07 ± 1.50 | m | 8.00 ± 0.09 | 39.59 ± 0.96 | m | 8.07 ± 0.08 | 37.38 ± 1.38 | sm | 7.71 ± 0.09 | 34.27 ± 1.50 | sm |
| 3 | 6.88 ± 0.08 | 42.73 ± 1.49 | m | 7.33 ± 0.07 | 43.30 ± 0.80 | m | 7.34 ± 0.06 | 42.45 ± 0.74 | m | 7.28 ± 0.06 | 41.57 ± 1.19 | m |
| 4 | 6.65 ± 0.08 | 25.43 ± 1.10 | sm | 6.89 ± 0.06 | 42.71 ± 0.69 | m | 7.01 ± 0.07 | 22.22 ± 1.16 | st | 6.82 ± 0.11 | 23.03 ± 1.55 | st |
| 5 | 6.45 ± 0.05 | 42.47 ± 1.41 | m | 6.54 ± 0.04 | 43.35 ± 0.69 | m | 6.59 ± 0.05 | 39.07 ± 0.82 | m | 6.57 ± 0.05 | 41.07 ± 1.55 | m |
| 6 | 6.30 ± 0.03 | 16.61 ± 1.43 | st | 6.42 ± 0.05 | 25.88 ± 1.07 | sm | 6.43 ± 0.05 | 21.35 ± 1.12 | st | 6.32 ± 0.08 | 20.56 ± 1.75 | st |
| 7 | 6.15 ± 0.05 | 43.90 ± 0.89 | m | 6.20 ± 0.04 | 43.86 ± 0.74 | m | 6.13 ± 0.05 | 40.97 ± 0.86 | m | 6.05 ± 0.06 | 39.80 ± 1.16 | m |
| 8 | 5.07 ± 0.04 | 33.6 ± 1.05 | sm | 6.02 ± 0.05 | 26.28 ± 0.84 | sm | 5.87 ± 0.04 | 22.65 ± 1.07 | st | 5.81 ± 0.04 | 20.54 ± 1.60 | st |
| 9 | 5.72 ± 0.06 | 37.52 ± 1.46 | m | 5.75 ± 0.04 | 42.94 ± 0.67 | m | 5.61 ± 0.04 | 40.01 ± 0.78 | m | 5.52 ± 0.04 | 38.23 ± 1.81 | m |
| 10 | 5.55 ± 0.05 | 38.13 ± 1.49 | m | 5.52 ± 0.04 | 43.06 ± 0.68 | m | 5.49 ± 0.03 | 40.09 ± 1.01 | m | 5.50 ± 0.04 | 35.72 ± 1.56 | sm |
| 11 | 5.45 ± 0.06 | 22.55 ± 1.63 | st | 5.35 ± 0.05 | 23.52 ± 1.02 | st | 5.27 ± 0.04 | 22.66 ± 1.09 | st | 5.35 ± 0.05 | 19.33 ± 0.89 | st |
| 12 | 5.25 ± 0.07 | 41.24 ± 1.40 | m | 5.01 ± 0.05 | 43.67 ± 0.67 | m | 5.00 ± 0.05 | 40.57 ± 0.98 | m | 5.12 ± 0.06 | 38.60 ± 0.94 | m |
| 13 | 5.00 ± 0.08 | 26.21 ± 1.40 | sm | 4.78 ± 0.05 | 25.27 ± 1.07 | sm | 4.85 ± 0.05 | 23.09 ± 1.12 | st | 4.90 ± 0.04 | 24.81 ± 0.92 | st |
| 14 | 4.79 ± 0.06 | 38.10 ± 1.44 | m | 4.59 ± 0.06 | 39.50 ± 1.22 | m | 4.67 ± 0.05 | 35.75 ± 1.31 | sm | 4.63 ± 0.06 | 34.53 ± 0.97 | sm |
| 15 | 4.58 ± 0.05 | 42.56 ± 1.38 | m | 4.36 ± 0.06 | 42.55 ± 0.78 | m | 4.46 ± 0.04 | 41.11 ± 0.74 | m | 4.56 ± 0.06 | 38.31 ± 1.50 | m |
| 16 | 4.46 ± 0.09 | 37.75 ± 1.28 | m | 4.16 ± 0.05 | 43.40 ± 0.83 | m | 4.24 ± 0.04 | 31.04 ± 1.32 | sm | 4.37 ± 0.08 | 20.74 ± 0.83 | st |
| 17 | 4.15 ± 0.09 | 25.63 ± 1.44 | sm | 3.74 ± 0.04 | 26.99 ± 1.21 | sm | 3.95 ± 0.05 | 33.98 ± 1.11 | sm | 4.05 ± 0.07 | 38.38 ± 1.07 | m |

different types in these two taxa, and values of FN were also close. The karyotype of *R. ampla* is close to that of *R. balthica,* differing in morphological type of three chromosome pairs; presence of one more subtelocentric pair (16) is the most characteristic feature of the former taxon.

## DISCUSSION

Diploid numbers of all studied species (2n = 34) agree well with the published literature on the genus *Radix* (Inaba, 1969; Patterson & Burch, 1978; Coudhary et al., 1992; Barsiene et al., 1996; Garbar, 1998, 2000). No cases of hypodiploidy or polyploidy were observed by our study, this result being in contrast with data on Spanish populations identified as *R. peregra* (Barsiene et al., 1996). The presence of telocentric (t) chromosomes in the karyotype of *R. auricularia* from the Zhitomyr population reported by Garbar (1998) was also not confirmed by this study. In all probability, this work dealt with an artifact caused by very profound spiralization of chromosomes. At the same time, our present observations on *R. labiata* and *R. balthica* agree with the data of Garbar (2000) on these species (for correspondence of nomenclature: Table 1). The karyotype of *R. ampla* has been studied here for the first time.

Differences in the chromosome morphology (most pronounced in the pairs 4 and 16) support species status of *R. balthica (= R. ovata)* and *R. labiata* (traditionally referred to as *R. peregra)* - two taxa considered conspecific by many taxonomists dealing with shell and anatomical characters (Hubendick, 1951; Jackiewicz, 1998; Kerney, 1999). However, karyological distinction between *R. labiata* and *R. balthica* shown by this study should be checked on the representative material taken throughout their distributions. Noteworthy, chromosome pair 4 is apparently of the same type in the karyotype of Spanish *R. peregra* shown by Barsiene etal. (1996) and Ukrainian specimens of *R. labiata* included in this study, but similarities/differences in other chromosome pairs cannot be evaluated, because centromeric indexes for the Spanish specimens were not provided.

Species status of *R. ampla* is also supported by this study, but the karyological differences between this taxon and *R. balthica* were apparently less pronounced than those reported for *R. balthica* and *R. labiata.* Furthermore, the karyotype of *L. (Peregriana) fontinalis* (Studer, 1820), as described by Garbar (2000), is similar to that of *R. ampla* (especially in having subtelocentric chromosome pair 16), with moderate (about 6%) difference in mean values of centromeric indexes of chromosome pair 2. This result is surprising, because *L fontinalis* (in the understanding of Russian authors) corresponds in its conchological and anatomical characters (Kruglov & Starobogatov, 1983: fig. 2,3; Garbar, 2000: fig. 1) to *R. balthica* of modern western European reviewers (Gloer, 2002) and is apparently different from *R. ampla.* Thus, correlation between chromosome morphology and the other characters in the *R. balthica/R. ampla* complex should be checked by further studies.

The observed pattern of karyological differences in *Radix* is consistent with the phylogenetic trees based on ITS-2 sequences (Bargues et al., 2001), supporting the following topology in the clade of European *Radix* species (nomenclature as used here): *(R. labiata, (R. auricularia, (R. lagotis, R. ampla, R. balthica))).* In particular, the peculiar karyotype of *R. labiata* and the karyological similarity between *R. ampla* and *R. balthica* corroborate this phylogenetic hypothesis. In particular, the overwhelming prevalence of meta- and submetacentric chromosomes characterizes the basal taxa *R. labiata* and *R. auricularia,* whereas the high number of subtelocentric chromosomes is a common feature of *R. balthica* and *R. ampla,* which belong to the terminal clade. Thus, the state of the karyotype of the former may be interpreted as plesiomorphic, and the latter as apomorphic within the analysed group. The results of our investigation are also consistent with the molecular analysis (Bargues et al., 2001) in suggesting, that *R. ampla* is a valid species alongside *R. balthica.* Neither karyological nor molecular characters support the subgenus *Peregriana* of Kruglov & Starobogatov (1983, 1993) as including, among other species, *R. labiata, R. balthica, R. lagotis* and *R. ampla,* but not *R. auricularia.* Broad understanding *of Lymnaea peregra* (Jackiewicz, 1998) also contradicts both data sets.

The results of this work, as well as earlier observations on other freshwater gastropods (Barsiene et al., 2000; Garbar & Korniushin, 2002), show that the study of chromosome morphology may provide additional characters for species diagnosis and phylogenetic analysis. Karyological study of Lymnaeidae should be continued, given the parasitological importance of this group and remaining uncertainty about its species-level taxonomy.

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