The "index of the copulatory apparatus" and its application to the systematics of freshwater pulmonates (Mollusca: Gastropoda: Pulmonata)

«Индекс копулятивного аппарата» и его применение в систематике пресноводных легочных моллюсков (Mollusca: Gastropoda: Pulmonata)

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The *index of the copulatory apparatus* (ICA) is calculated as the ratio between lengths of the praeputium and the penis sheath in the copulatory apparatus in freshwater pulmonate mollusks. ICA is often used for species' delimitation and identification in different taxa of pulmonates, however, its variation (in both intra- and interspecific levels) is still poorly studied that may make troubles for systematicists. The variation of ICA in pond snails (family Lymnaeidae Rafinesque, 1815) has been studied in several aspects (intra- and interpopulation, interspecific). It has been shown that the index varies considerably, and it is almost impossible to identify a single specimen on the base of ICA solely. However, mean ICA values are more useful for taxonomic and identification purposes. Closely related species of lymnaeids demonstrate some extent of overlap between their ranges of ICA variation that hampers their identification but is not a cause for synonymisation. The factors of different origin that potentially may influence the ICA values in freshwater pulmonates have been reviewed. It is revealed that ICA manifests significant ontogenic and (in some lymnaeid species) geographical changes. The use of ICA in pulmonate systematics should be accompanied by data on variation in another characters of conchological or anatomical kind.

«Индекс копулятивного аппарата» (ИКА) определяется у пресноводных легочных моллюсков как соотношение длин препуциума и мешка пениса в копулятивном аппарате. Он часто используется как средство разграничения видов и таксономической идентификации особей в различных группах легочных моллюсков, однако его изменчивость (на внутри- и межпопуляционном уровнях) до сих пор слабо изучена, что создает трудности для его использования таксономистами. В работе исследуется изменчивость ИКА прудовиков (семейство Lymnaeidae Rafinesque, 1815) в различных аспектах (внутри- и межпопуляционная, межвидовая). Показано, что данный индекс значительно варьирует, так что надежное определение отдельно взятого экземпляра на его основе является невозможным. Однако средние внутрипопуляционные значения ИКА представляются более пригодными для целей систематики и видовой идентификации. Значения ИКА у близкородственных видов лимнеид перекрываются, что затрудняет идентификацию принадлежащих к ним особей, но это не является достаточным основанием для их синонимизации. Обсуждаются различные факторы, потенциально влияющие на значения ИКА. Показано, что ИКА подвержен онтогенетической и (у некоторых видов) географической изменчивости. Использование ИКА в систематике пресноводных пульмонат должно сопровождаться привлечением данных о других признаках, как конхологических, так и анатомических.

Key words: pulmonates, taxonomy, species delineation, species identification, variation, copulatory apparatus, Lymnaeidae.

Ключевые слова: легочные моллюски, разграничение видов, идентификация видов, изменчивость, совокупительный аппарат, Lymnaeidae.

INTRODUCTION

Representatives of many groups of freshwater pulmonate mollusks have the male copulatory organ consisting of two main parts: (1) praeputium and (2) penis sheath with the penis imbedded inside the latter (Hubendick, 1978). The ratio between lengths of the praeputium and the penis sheath has frequently been used in pulmonate systematics for species delimitation and diagnostics. For example, in studies of lymnaeid snails (family Lymnaeidae Rafinesque, 1815) it has been exploited since the 1910s (Baker, 1911; Roszkowski, 1914; Colton, 1915). In Russian malacological literature, this ratio is referred to as 'the index of the copulatory apparatus" (ICA, hereafter). It has been effectively used for species delimitation and diagnostics in different families of freshwater pulmonates, including Lymnaeidae (Kruglov, 2005; Pointier, 2006), Physidae Fitzinger, 1833 (Starobogatov et al., 1989), and Planorbidae Rafinesque, 1815 (Meier-Brook, 1964, 1983; Soldatenko & Starobogatov, 2000; Stothard et al., 2002). The usefulness of ICA can be explained partially by the fact that shells of freshwater pulmonates are relatively poor by diagnostic characters. Moreover, the variability of the anatomical characters is thought to have much less extent than the variability of shell characters in pulmonates (Hubendick, 1951; Suvorov, 1999). The internal structures seem, thus, to be more attractive for a taxonomist who is trying to find reliable characters for delineation of snail species.

Some authors mentioned, however, that the praeputium: penis sheath ratio is of no importance for taxonomy due to its high degree of variation (Hubendick, 1955; Kilias, 1992). For example, Hubendick (1955: 457–458) states that "it is ... worthless to make measurements of its [copulatory apparatus] different parts and then to base comparisons on details of proportions". The point is that proportions of the copulatory apparatus are highly sensitive to growth and seasonal changes in the male organ. Moreover, "the degree of contraction in the fixed organ affects its topographical morphology to a considerable extent" (Hubendick, 1955: 458). This criticism has been answered by Meier-Brook (1983: 17), and Jackiewicz (1996), who used ICA successfully for resolving certain taxonomic questions.

Despite the practical significance of this index, its intra- and interpopulation variability in freshwater pulmonates is very scarce studied and sometimes different authors give different values of ICA for the same species. For instance, while Jackiewicz (1998) believes that ICA in the lymnaeid species *Lymnaea* (*Omphiscola*) *glabra* (Müller, 1774) is equal to 1.0, Kruglov (2005) gives a fairly another value (0.81). In both cases, the only fixed ICA value is ascribed to a given species as if no variability in proportions of the copulatory organs is observed in the natural populations of *L. glabra*. Nevertheless, it is clearly not the case since any measured character in a population exhibits some extent of variation. Obviously, lack of sufficient data on ICA variability hampers its using in taxonomy of freshwater pulmonates and may cause troubles in species identification. The knowledge about ICA variability in natural populations of snails and those causes that determine it would be of importance for constructing identification keys for freshwater pulmonate snails.

The goal of this study is to review the data on the ICA variability in freshwater pulmonates in order to estimate how much variation is observed on both intra- and interpopulation levels. The question how effective ICA is for distinguishing between closely related species has been studied as well. Mollusks of the family Lymnaeidae have been chosen as a basis for this study since the index of the copulatory apparatus is most frequently used by students of this pulmonate group and ICA values often employed in keys for determination of different lymnaeid snails (see, for example Kruglov, 2005; Andreyeva et al., 2010). Data on another pulmonate families were taken into account as well.

MATERIAL AND METHODS

Materials studied. For study of the intrapopulation ICA variability samples of ten species of lymnaeids belonging to six subgenera of the genus *Lymnaea* Lamarck, 1799 have been used: *Lymnaea* (*Lymnaea*) *fragilis* (L., 1758), *L.* (*L.*) *stagnalis* (L., 1758), *L.* (*Corvusiana*) *kazakensis* Mozley, 1934, *L.* (*Ladislavella*) *terebra* (Westerlund, 1885), *L.* (*Peregriana*) *ampla* (Hartmann, 1821), *L.* (*P.*) *dolgini* Gundrizer & Starobogatov, 1979, *L.* (*P.*) *tumida* (Held, 1836), *L.* (*P.*) *zazurnensis* Mozley, 1934, *L.* (*Radix*) *auricularia* (L., 1758), *L.* (*R.*) *parapsilia* (Vinarski & Glöer, 2009), and *L.* (*Stagnicola*) *saridalensis* Mozley, 1934 (Tables 1, 2). The taxonomic position and nomenclature of species are given according to Kruglov (2005) with changes given by Andreyeva et al. (2010). All the samples were collected by the author in waterbodies of different regions of Russian Federation (mostly situated in Western Siberia). Given the fact that the mode of fixation of the soft body may influence the proportions measurements in pulmonate mollusks (Hubendick, 1954, 1955; Emberton, 1989), all the snails examined have been fixed uniformly in 96% ethanol. Only samples containing not less than 20 individuals were chosen for the study. In total, 1131 specimens of lymnaeid snails taken from 37 samples have been dissected.

Interpopulation variability in ICA values is studied by mean of comparison between lymnaeid samples from geographically remote regions. 10 samples containing 72 specimens of *Lymnaea* (*Peregriana*) *balthica* (Linnaeus, 1758) collected in different habitats of Germany, European part of Russia and Siberia have been examined in order to reveal whether there is any sign of longitudinal variation of the index discussed. The habitats of *L. balthica* used in the analyses are situated between 10° and 109°E and between 51° and 58°N.

The study of the ICA variability on the interspecific level has been conducted by using two pairs of closely allied lymnaeid species. (1) *L. fragilis* and *L. stagnalis*. These species are similar in their conchological characters, however their taxonomic independence is evidenced by the fact that the two species are reproductively isolated from each other (Kruglov & Starobogatov, 1985). The mean values of ICA of these species differs significantly (Davydov et al., 1981), albeit no information on limits of the intraspecific variation of ICA values in *L. fragilis* and *L. stagnalis* is given in the literature (Kruglov & Starobogatov, 1985; Kruglov, 2005). Thus, it is still not clear if there is a hiatus between these species in their ICA values. In this study, 389 specimens of *L. fragilis* and 57 specimens of *L. stagnalis* from different waterbodies of Russia, Germany, and Kazakhstan were collected and fixed in accordance with the standard methods (see above). (2) *Lymnaea auricularia* and *L. parapsilia*. The two species of the subgenus *Radix* Montfort, 1810 are conchologically similar and these often dwells in the same habitat, i.e. syntopically. The differences in their ICA values were previously used by Vinarski & Glöer (2009) to corroborate the species status of *L. parapsilia*. In this study, 117 specimens of *L. auricularia* and 53 specimens of *L. parapsilia* from different water bodies of Russia and Kazakhstan were collected and fixed in accordance with the standard methods (see above). Before calculations, ICA values of all conspecific speciemens collected from different populations were merged into one large massive of data in order to estimate the scope of possible overlap of intraspecific limits of ICA variation.

All voucher specimens of lymnaeid snails studied are kept in the Museum of Siberian Aquatic Mollusks (MSAM, Omsk State Pedagogical University, Russian Federation).

Measurements and statistics. All the snails dissected were full-grown with completely developed reproductive system. Specimens that exhibited signs of trematode invasion have not been used for measurements as it is known that parasites may alter

Table 1. Index of copulatory apparatus (ICA) values in the species studied (from literature sources).

Species	ICA	Source
L. ampla	0.76	Kruglov, 2005*
L. auricularia	1.10	Kruglov, 2005
L. dolgini	1.15–1.25	Kruglov, 2005
L. fragilis	4.37±0.31	Davydov et al., 1981
L. kazakensis	2.70±0.10	Lazareva, 1967
L. parapsilia	0.82–0.85	Kruglov, 2005**
L. saridalensis	0.10– 0.15	Kruglov, 2005
L. stagnalis	3.08±0.21	Davydov et al., 1981
L. terebra	1.13	Kruglov, 2005
L. tumida	1.70	Kruglov, 2005
L. zazurnensis	1.20–1.30	Kruglov, 2005

Notes: *Kruglov (2005) refers this species as to *Lymnaea* (*Peregriana*) *patula* (Da Costa, 1778). **Kruglov (2005) refers this species as to *Lymnaea* (*Radix*) *psilia* (Bourguignat, 1862).

parameters of reproductive organs of their snail hosts down to reduction of the copulatory apparatus (Ginetsinskaya, 1968). The praeputium and the penis sheath were measured in a straightened state (Fig. 1) avoiding an excessive stretching that could distort the results of measurements and consequently influence the ICA calculations. All measurements were taken by using the binocular microscope MBS-10 with the calibrated eyepiece. Total lengths of the praeputium and the penis sheath were determined to the nearest 0.1 mm. For multivariate analyses, shells of lymnaeids of two species (*L. auricularia*, *L. zazurnensis*) have been measured in accordance with a standard scheme of measurements (see Kruglov, 2005; Andreyeva et al., 2010). Six standard measurements (shell height and width, aperture height and width, spire height, body whorl height) were taken from each shell with accuracy to the nearest 0.1 mm. The measurements were made by means of caliper or binocular microscope.

In the intrapopulation study of variation, the mean value of ICA and standard deviation (σ) have been calculated for each sample as well as the coefficient of variability (Cv, %) that is a normalised measure of dispersion of a probability distribution. It is calculated as the ratio of the standard deviation to the mean value of ICA. Moreover, an additional simplest metrics designated here as *R* has been calculated. It represents the ratio of the maximum to the minimum ICA values in a given sample, V_{MAX} / V_{MIN} (see Yablokov, 1966). It serves as a subsidiary means for representation of intraspecific variation of a given character. In order to determine if there is significant correlation between ICA values and certain characters of shell, the Spearman rank correlation coefficient (r_S) has been used. Canonical and discriminative analyses have been performed in order to find differences between shells of snails from the same sample having drastically different ICA values (ICA polymorphism, see below).

For estimation of the transgression level of specific ranges of ICA variation in closely allied species of Lymnaeidae, CD coefficient has been calculated as follows (Zagorodnyuk, 2004):

$$CD = \frac{(\bar{x}_1 - \bar{x}_2)}{\sigma}$$, where

$$\sigma = \frac{\sqrt{\sigma_1^2 + \sigma_2^2}}{2}$$ ($\sigma_{1,2}$ – standard deviation values of the two samples compared);

$x_{1,2}$ – mean ICA values of the two samples compared;

Table 2. Index of copulatory apparatus (ICA) in samples of lymnaeid snails examined in present study.

Sampling site	Number of specimens	ICA			
		Variation limits	Mean ± standard deviation	R	Cv %
	L. ampla				
Chelyabinsk Prov., Miass R.	21	0.68–1.20	0.92±0.15	1.78	8.3
	L. auricularia				
Altai Terr., Kuznetsovo L.	25	0.95–1.29	1.09±0.09	1.36	8.3
Novosibirsk Prov., Novosibirsk Reservoir (form with long praeputium)	18	0.91–1.31	1.06±0.10	1.44	9.4
Novosibirsk Prov., Novosibirsk Reservoir (form with short praeputium)	7	0.34–0.61	0.52±0.09	1.79	17.3
Omsk Prov., Krivoye L.	21	1.02–1.36	1.12±0.09	1.36	8.0
Tomsk Prov., lake at Novomikhailovka	30	0.93–1.39	1.10±0.12	1.49	10.9
	L. dolgini				
Omsk Prov., lake close to Ananyevskoye L.	20	0.75–1.27	1.03±0.13	1.69	12.6
	L. fragilis				
Altai Terr., swamp in floodplain of Kulunda R.	26	3.52–5.91	4.83±0.64	1.67	13.3
Altai Terr., Valovoye L.	30	3.77–5.90	4.73±0.56	1.56	11.8
Altai Terr., Kuznetsovo L.	30	3.48–5.74	4.56±0.57	1.65	12.5
Altai Terr., Noven'koye L.	25	3.83–5.93	4.63±0.61	1.55	13.2
Altai Terr., stream at Uglovskoye	20	3.70–6.33	4.59±0.74	1.71	16.1
Bryansk Prov., drainage channel near Maloye Kozhanovskoye L.	25	4.04–5.62	4.76±0.48	1.39	10.1
Omsk Prov., Atachka R.	22	3.45–5.48	4.37±0.51	1.59	11.7
Omsk Prov., Omsk, lake in Moskovka District	26	4.23–6.64	5.19±0.65	1.57	12.5
Tyumen Prov., floodplain lake of Irtysh R. near Tobol'sk	22	3.95–5.75	4.74±0.45	1.46	9.5
	L. kazakensis				
Altai Terr., floodplain marsh of Kulunda R.	31	2.06–2.88	2.48±0.22	1.39	8.9
	L. parapsilia				
Tomsk Prov., lake at Novomikhailovka	30	0.70–0.96	0.86±0.06	1.37	7.0
	L. saridalensis				
Novosibirsk Prov., Fadikha L.	32	0.08–0.18	0.12±0.03	2.25	25.0
Novosibirsk Prov., floodplain marsh of Kargat R.	40	0.09–0.22	0.12±0.03	2.44	25.0

Table 2 (continued).

Sampling site	Number of specimens	ICA			
		Variation limits	Mean ± standard deviation	R	Cv %
Omsk Prov., floodplain marsh of Irtysh R. in Omsk	78	0.12–0.24	0.17±0.03	2.33	17.7
Omsk Prov., Trauly L.	37	0.10–0.21	0.14±0.02	2.10	14.2
Omsk Prov., Kabankul' L.	54	0.10–0.23	0.16±0.03	2.30	18.8
Sverdlovsk Prov., Murzinka R.	28	0.11–0.24	0.18±0.04	2.18	22.2
Tyumen Prov., vicinity of Labytnangi, Vylposl Channel	35	0.10–0.19	0.14±0.02	1.90	14.3
	L. terebra				
Altai Terr., floodplain marsh of Kulunda R.	31	1.00–1.68	1.29±0.22	1.68	17.1
Altai Republic, Teletskoye L.	30	0.91–1.50	1.10±0.14	1.65	12.7
Novosibirsk Prov., pool near Kuibyshev – Severnoye Road	24	0.82–1.20	1.01±0.13	1.46	12.9
Omsk Prov., marsh at Krivoye L.	29	0.83–1.36	1.05±0.13	1.64	12.4
Omsk Prov., lake at Cherlak	23	1.04–1.84	1.31±0.23	1.72	17.6
Omsk Prov., lake near Malye Bodachi L.	29	0.77–1.38	1.03±0.16	1.79	15.6
Omsk Prov., marsh in Omsk	36	0.95–1.55	1.19±0.12	1.63	10.1
Omsk Prov., Lake at Kirgap	31	0.87–1.35	1.13±0.13	1.55	11.5
Tomsk Prov., floodplain marsh of Ob' R. near Nikolskoye	30	0.88–1.35	1.08±0.13	1.53	12.0
Tyumen Prov., vicinity of Labytnangi, lake at Vylposl Channel	30	0.78–1.32	1.06±0.14	1.69	13.2
Tyumen Prov., marsh near Labytnangi	37	0.81–1.30	1.05±0.13	1.61	12.4
	L. tumida				
Sverdlovsk Prov., Shaytanka R.	20	1.37–2.09	1.75±0.21	1.53	12.0
	L. zazurnensis				
Altai Republic, Teletskoye L. (form with long praeputium)	43	1.17–1.87	1.46±0.19	1.60	13.0
Altai Republic, Teletskoye L. (form with short praeputium)	5	0.78–1.03	0.89±0.09	1.32	10.1

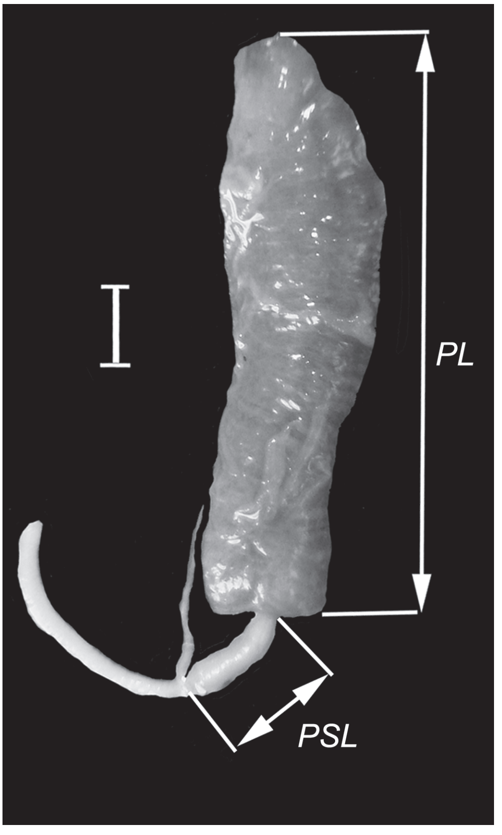

Fig. 1. *Lymnaea fragilis*, structure of the copulatory organ and the scheme of measurement of its parts; *PL* – praeputium length, *PSL* – penis sheath length. Scale bar: 2 mm.

The values of CD ≥ 6.0 indicate that there is no overlap between specific ranges, whereas the CD values < 6.0 correspond to some extent of transgression between these (for example, when CD=3 one third of specimens fall into overlapping zone and cannot be identified surely; see for details Zagorodnyuk, 2004).

RESULTS

Intrapopulation variation of ICA. In all lymnaeid populations studied, ICA exhibits rather considerable extent of variability, and its maximum value is in most cases 1.50–2.00 times larger than the minimum one that is apparent from the values of *R* metrics (Table 2). Judging from the values of Cv, the extent of variability in most samples could be estimated as 'modest' (Cv values lie between 10 and 20%) or even as 'low' (Cv values less than 10%). Only three samples out of 37 (about 8%) demonstrate higher level of variability (Cv values exceed 20%). The mean ICA values obtained in this study are more or less close to those reported in the literature for concrete lymnaeid species (compare Tables 1 and 2), however this correspondence is not consistent. For example, mean ICA values in *L. fragilis* in eight cases exceeded the mean given by Kruglov (2005) for this species, and only one sample (Omsk Region, Atachka River) corresponds to the Kruglov's data in this respect (see Table 2). The extent of species' ICA variability observed in this study proved to be generally wider than it is reported in the literature. The scope of intrapopulation variability is not the same in different subgenera. The higher values of *R* and Cv observed in species of the subgenus *Stagnicola*, and the lesser values of these coefficients in representatives of the subgenera *Corvusiana* and *Radix*.

In two populations studied, individuals with abnormal values of ICA were found. In each case, such snails form a separate group of individuals with very long penis sheaths that results in very low (as compared with the species norm) values of their ICA. For example, in a sample of *L. auricularia* from the Novosibirsk Region (n=25), bulk of specimens (n=18) have length of penis sheath comparable with that of praeputium (Fig. 2, b), and their ICA values vary from 0.91 to 1.31 (see Table 2). Seven other individuals collected from the same habitat have ICA values 0.61 and less (see Table 2) and, hence, are characterised by very long penis sheath that is nearly twice long as praeputium (see Fig. 2, d). The same situation has been revealed in a sample of *L. zazurnensis* from the Teletskoye Lake (Altay Mts., south Siberia), where two anatomically distinct morphs, with long and short praeputium correspondingly, are found (Fig. 3). There is a narrow hiatus in ICA values between these forms of *L. zazurnensis* (see Table 2).

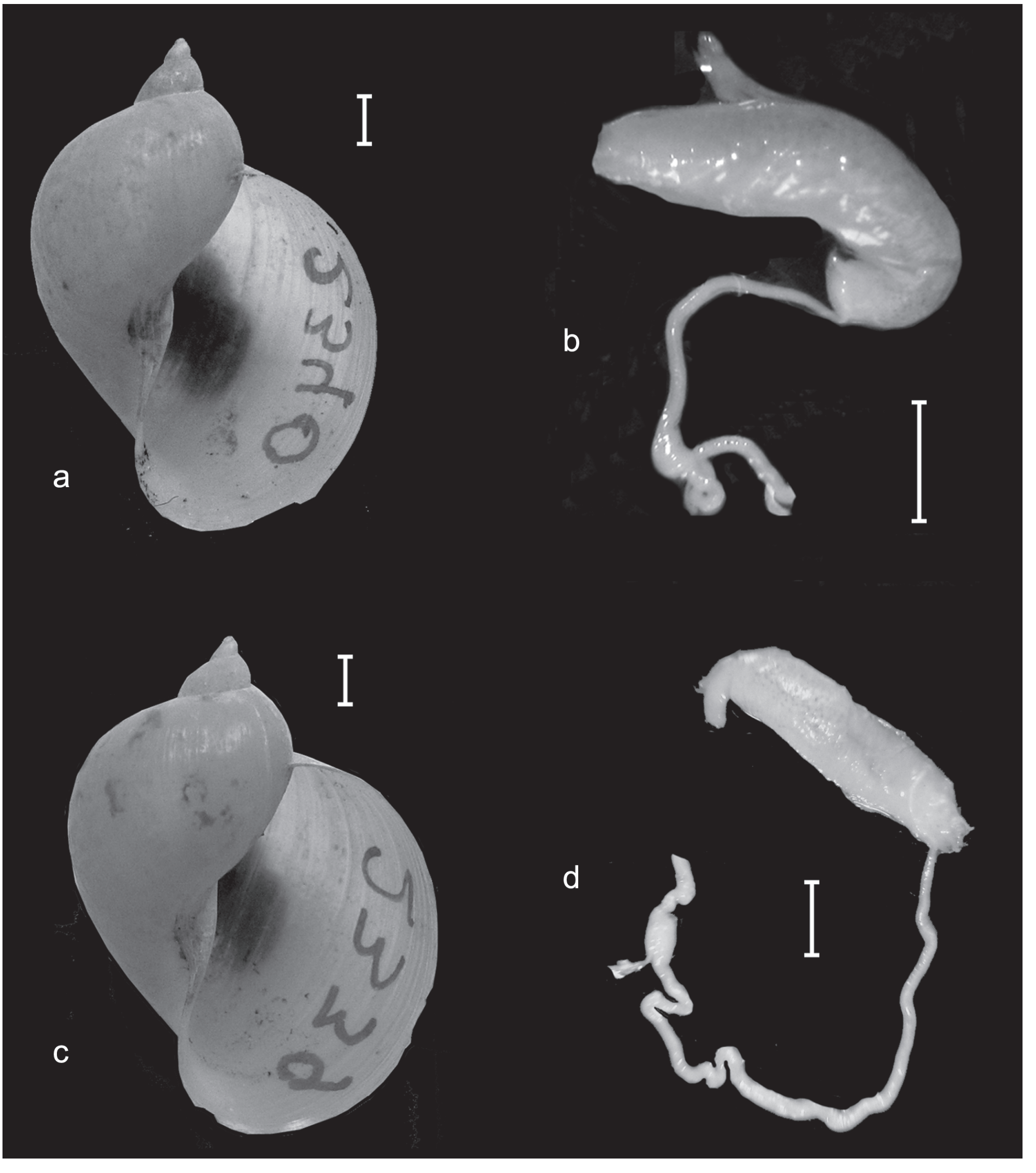

Fig. 2. *Lymnaea auricularia*, shells **(a, c)** and copulatory organs **(b, d)** of two morphs with different ICA values. **a, b** – a typical morph with short penis sheath, **c, d** – a morph with atypically long penis sheath. Scale bars: 1 mm (a, c), 2 mm (b, d).

Interestingly, in both cases of anatomical polymorphism mentioned above, no significant differences in size and proportions of mollusk shells have been found. Thus, the two anatomical morphs are indistinguishable by their conchological features. The canonical analysis of shell variation shows that individuals with long penis sheaths form common 'cloud' of points with snails having typical proportions of the copulatory apparatus (Fig. 4).

Interpopulation variation of ICA. In majority of cases, the interpopulation differences of mean ICA values within the same species are not significant (Table 3), and it indicates that intraspecific differentiation is

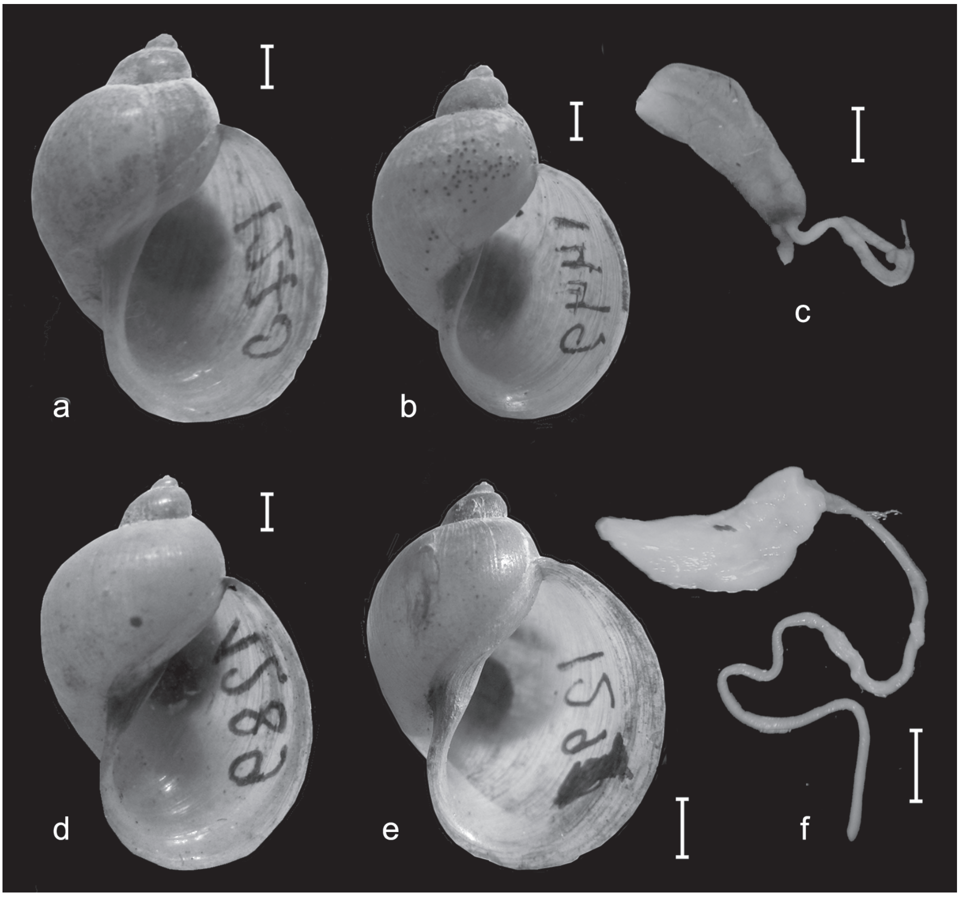

Fig. 3. *Lymnaea zazurnensis*, shells (**a, b, d, e**) and copulatory organs (**c, f**) of two morphs with different ICA values; **a–c** – a typical morph with short penis sheath, **d–f** – a morph with atypically long penis sheath. Scale bars: 1 mm (a, b, d, e), 2 mm (c, f).

not considerable. The only known exclusion is the species *L. terebra* that was found to demonstrate latitudinal variation of ICA in Western Siberia, and the northern population of this species are characterised by significantly lower ICA values as compared to the southern ones (see Discussion). Some signs of longitudinally oriented variation of ICA in *L. balthica* have been found during this study. In this species, mean ICA values increase in west – east direction (Table 4), and there is strong positive correlation between ICA value and longitude (r_S=0.85, p=0.002). It should be noted, although, that most samples of *L. balthica* studied contained less than 10 individuals and it is not sufficient to confirm reality of this variation pattern with certainty.

Interspecific variation of ICA. Closely allied lymnaeid species (species pairs *L. fragilis* – *L. stagnalis* and *L. auricularia* – *L. parapsilia*) could be distinguished by the mean ICA values but there is no hiatus between these, and intraspecific variation limits of ICA are overlapping. For instance, the lowest known ICA value in *L. auricularia* (normal form with short penis sheath) is 0.91, whereas the highest value in *L. parapsilia* is equal to 0.96 (Fig. 5, Table 2). Thus, some proportion (17.6%) of snails having ICA values falling into this zone of overlap could not be determined

Table 3. Statistical significance of interpopulation differences between mean values of ICA (Student's *t*-test; statistically significant differences are marked by bold).

Samples compared	*t*	p
L. auricularia		
Kuznetsovo L./Krivoye L.	–1.60	0.12
Kuznetsovo L./Novomikhailovka L.	–0.09	0.93
Kuznetsovo L./Novosibirsk Reservoir (only specimens of typical form with short praeputium)	1.01	0.32
Novomikhailovka L./Krivoye L.	–1.60	0.12
Novomikhailovka L./Novosibirsk Reservoir	0.12	0.91
Krivoye L./Novosibirsk Reservoir	–1.71	0.10
L. fragilis		
Maloye Kozhanovskoye L./Atachka R.	2.71	0.01
Maloye Kozhanovskoye L./Moskovka L.	–2.64	0.01
Maloye Kozhanovskoye L./Tobol'sk L.	0.18	0.86
Maloye Kozhanovskoye L./Kulunda R.	0.39	0.70
Maloye Kozhanovskoye L./Valovoye L.	0.22	0.82
Maloye Kozhanovskoye L./Kuznetsovo L.	1.40	0.17
Maloye Kozhanovskoye L./Noven'koye L.	0.90	0.38
Maloye Kozhanovskoye L./stream Uglovskoye	0.97	0.38
Atachka R./Moskovka L.	–4.77	0.00002
Atachka R./Tobol'sk L.	–2.54	0.01
Atachka R./Kulunda R.	–2.69	0.01
Atachka R./Valovoye L.	–2.40	0.02
Atachka R./Kuznetsovo L.	–1.24	0.22
Atachka R./Noven'koye L.	–1.42	0.16
Atachka R./Uglovskoye Stream	–1.12	0.27
Moskovka L./Tobol'sk L.	2.74	0.09
Moskovka L./Kulunda R.	2.02	0.048
Moskovka L./Valovoye L.	2.83	0.01
Moskovka L./Kuznetsovo L.	3.81	0.0004
Moskovka L./Noven'koye L.	3.07	0.004
Moskovka L./Uglovskoye Stream	2.93	0.005
Tobol'sk L./Kulunda R.	–0.53	0.60
Tobol'sk L./Valovoye L.	0.05	0.96
Tobol'sk L./Kuznetsovo L.	1.21	0.23
Tobol'sk L./Noven'koye L.	0.73	0.47
Tobol'sk L./Uglovskoye Stream	0.82	0.42
Kulynda R./Valovoye L.	0.59	0.56
Kulynda R./Kuznetsovo L.	1.62	0.11
Kulynda R./Noven'koye L.	1.13	0.26
Kulynda R./Uglovskoye Stream	1.18	0.25
Valovoye L./Kuznetsovo L.	1.17	0.25
Valovoye L./Noven'koye L.	0.69	0.49
Valovoye L./Uglovskoye Stream	0.79	0.43
Kuznetsovo L./Noven'koye L.	–0.33	0.74
Kuznetsovo L./Uglovskoye Stream	–0.14	0.89
Noven'koye L./Uglovskoye Stream	0.14	0.89
L. saridalensis		
Omsk City/Fadikha L.	7.87	0.00
Omsk City/Kargat R.	7.90	0.00
Omsk City/Trauly L.	5.22	0.00
Omsk City/Kabankul' L.	1.94	0.053
Omsk City/Murzinka R.	–1.71	0.09
Omsk City/Vylposl Channel	3.15	0.002
Fadikha L./Kargat R.	–0.74	0.46
Fadikha L./Trauly L.	**–3.58**	**0.001**
Fadikha L./Kabankul' L.	**–5.10**	**0.00**
Fadikha L./Murzinka R.	**–7.26**	**0.00**
Fadikha L./Vylposl Channel	**–3.48**	**0.001**
Kargat R./Trauly L.	**–2.83**	**0.01**
Kargat R./Kabankul' L.	**–4.92**	**0.00**
Kargat R./Murzinka R.	**–7.07**	**0.00**
Kargat R./Vylposl Channel	**–2.93**	**0.004**
Trauly L./Kabankul' L.	**–2.59**	**0.01**
Trauly L./Murzinka R.	**–5.45**	**0.00**
Trauly L./Vylposl Channel	–0.84	0.40
Kabankul' L./Murzinka R.	**–2.77**	**0.01**
Kabankul' L./Vylposl Channel	1.27	0.21
Murzinka R./Vylposl Channel	3.58	0.001

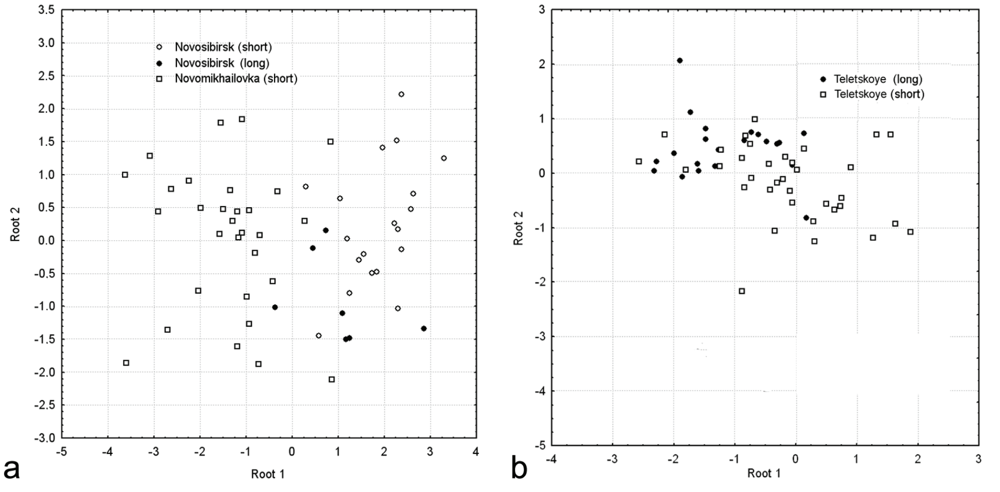

Fig. 4. Canonical analysis of conchological variation of interpopulation morphs of *L. auricularia* (**a**) and *L. zazurnensis* (**b**). Individuals with short (typical) and long (atypical) penis sheaths are compared.

Table 4. Means and variation limits of ICA in *L. balthica* samples collected from longitudinally remote populations.

Country, region, habitat, number of dissected snails	Coordinates	Mean (variation limits)
Germany, Hamburg, ditch Marschlande (10)	53°33´N 10°00´E	1.00 (0.69–1.24)
Germany, Saxony, Herthasee L. at Trebsen (6)	51°17´N 12°44´E	1.17 (0.91–1.51)
Germany, Saxony, Kranichbach brook at Trebsen (5)	51°16´N 12°44´E	1.12 (0.99–1.38)
Russia, Pskov Prov., Plissa R. at Nevel' (3)	56°01´N 29°53´E	1.29 (1.21–1.34)
Russia, Udmurtia, Ubyt' R. (12)	57°43´N 52°28´E	1.27 (1.00–1.67)
Russia, Altai Terr., Burla R. (15)	53°17´N 78°14´E	1.52 (1.18–1.76)
Russia, Novosibirsk Prov., Malaya Chicha L. (12)	54°43´N 78°35´E	1.22 (0.98–1.66)
Russia, Altai Terr., Kabanye L. (4)	53°27´N 78°46´E	1.36 (1.16–1.59)
Russia, Altai Terr., Valovoye L. (2)	51°45´N 80°22´E	1.38 (1.16–1.59)
Russia, Irkutsk Prov., Kirensk, branch of Kirenga R. (3)	57°46´N 108°06´E	1.50 (1.36–1.58)

on the base of proportions of their copulatory apparatus. CD coefficient for this species pair is equal to 4.14, and, according to Zagorodnyuk (2004), it means the species could be classified as morphologically similar species separated by 'significant' (but not complete!) hiatus.

In *L. stagnalis*, the intraspecific range of ICA variation (n=57) is between 2.37 and 3.86, whereas in *L. fragilis* (n=389) the same range is between 3.45 and 6.64 (see Table 2). In this case, 56 specimens (or 12.6%) fall into the zone of overlap of intraspecific variation ranges. CD value is 5.10 that indicates significant level of morphological differentiation between the two species.

DISCUSSION

The ICA values have often been used by students of lymnaeid snails as a useful tool for species discrimination and identification. In particular, statistically significant differences between ICA values of two conchological forms are sometimes considered as evidence of their species independence

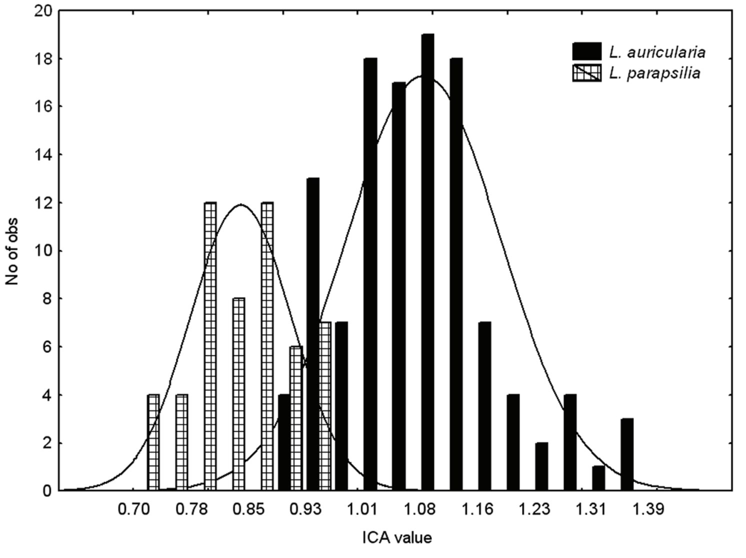

Fig. 5. Distribution of ICA values in two closely related species of Lymnaeidae, *L. auricularia* (n=117) and *L. parapsilia* (n=53).

(Lazareva, 1967; Davydov et al., 1981; Vinarski & Glöer, 2009). However, the data obtained during this study lead me to conclusion that the using of ICA for discrimination and identification of lymnaeid species is limited. The intrapopulation extent of the ICA variation is considerable and intraspecific ranges of it sometimes overlap in closely related species.

About 50 years ago, Stiglingh et al. (1962) studied ICA variation in the African planorbid snail *Bulinus tropicus* (Krauss, 1848). The authors' conclusion was that "the ratio PS/PP [= ICA] varies so much in each of the samples that it is impossible to use the ratio obtained from a single specimen either as a measure to characterise the species, or as a criterion to identify the particular specimen" (Stiglingh et al., 1962: 103). It should be noted, however, that Stiglingh et al. (1962) may have been used a mixture of independent species in their study as the species *B. tropicus* proved to be a complex of several species (Brown, 1994), and now it is split into a series of 'good' taxa of species rank such as *B. depressus* Haas, 1936 (de Kock & Wolmarans, 2005). However, my own data confirms, in general, this statement since intrapopulation variability of ICA in all samples of lymnaeids studied (see Table 2) seems to be wide enough to make rather unreliable any attempts to identify the species identity of a separate snail on the base of proportions of parts of its copulatory organ. In my opinion, more or less reliable conclusions could be drawn from variation ICA values calculated as a result of dissection of a series of conspecific individuals taken from a given sample. In this case, however, possibility of interspecific overlap of ICA limits should be taken into account.

While using ICA for the taxonomic and/or identification purposes, several factors potentially influencing this ratio should be remembered. These are as follows.

1. Ontogeny. Beriozkina and Starobogatov (1988) showed that the ICA values in lymnaeids change drastically with age. For example, in *Lymnaea* (*Stagnicola*) *atra* (Schrank, 1803), mean ICA increases from 0.55 to 0.91 (Fig. 6). The same pattern is found in *L. auricularia* from a natural population (Fig. 7). The cause of the ontogenic variation in the ICA values is the allometric growth of the copulatory organ: the praeputium length increases faster than the penis sheath length. The seasonal changes of ICA values in lymnaeids are not registered (Beriozkina & Starobogatov, 1988).

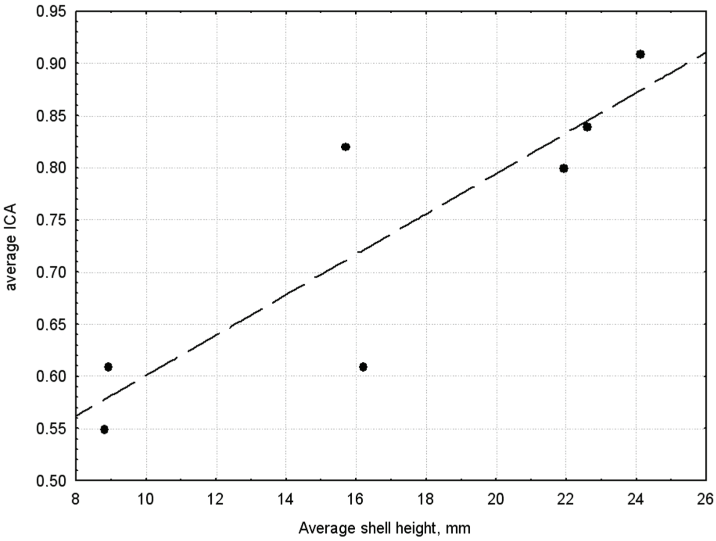

Fig. 6. Relationship between shell height (a proxy for age) and ICA; r_S=0.86 (p=0.01) in *L. atra*. Calculated from Beriozkina and Starobogatov's (1988) data.

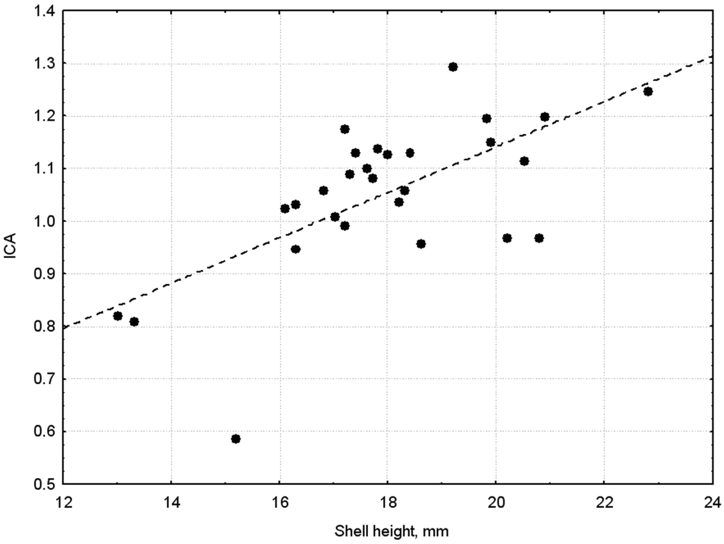

Fig. 7. Relationship between shell height and ICA in *L. auricularia* (Altai Terr., Kuznetsovo L., n=28), r_S=0.57 (p=0.001).

It should be stressed here that the strong intrapopulation differences in the ICA values reported in *L. auricularia* and *L. zazurnensis* (anatomical polymorphism; see Results) are not the product of ontogenic changes. There is no significant difference between shell height of snails with short and long praeputiums in both species mentioned. Therefore mollusks of different anatomical forms unlikely belong to distinct age cohorts.

2. Geography. Vinarski (2009) found one lymnaeid species, *L. terebra*, to demonstrate significant decrease in the ICA value in the south-north (latitudinal) direction in Western Siberia. Another stagnicoline species, *L. saridalensis*, however, does not exhibit any significant latitudinal trend (Vinarski, 2009). The finding of *L. balthica* ICA increase in the longitudinal direction in this study (see Results) suggests that the geographical variation in the copulatory organs may be widespread in lymnaeids and, perhaps, in other groups of freshwater pulmonates. The causes and probable adaptive significance of this variation is not clear,

Table 5. Possible changes in ICA in *L. fragilis* due to measurement errors (see text for explanations).

Praeputium length, mm (fixed value)	Penis sheath length, mm	ICA value
	2.0	5.00
	2.1	4.76
	2.2	4.55
10.0	**2.3**	**4.3**
	2.4	4.17
	2.5	4.00
	2.6	3.85

and only some hypotheses may be discussed (see Vinarski, 2009).

3. Parasitic invasion. The trematode larvae that use snails as intermediate hosts in their life cycles often damage or even destroy completely the reproductive organs of the mollusks (Ginetsinskaya, 1968). It is designated usually as "parasitic castration" (Wilson & Denison, 1980; Sorensen & Minchella, 2001). Apparently, it may cause alterations in proportions of the copulatory apparatus and, hence, in the ICA values.

4. Measurement accuracy. ICA values are sensitive to measurement errors and even slight change of one parameter would alter the ratio value. Consider a hypothetical example taking *Lymnaea fragilis* as a model. Let the praeputium length of a specimen is equal to 10.0 mm, and the penis sheath length is equal to 2.3 (ICA=4.30). Then, even a relatively weak measurement error equal to only ± 0.3 mm leads to considerable alteration of the ICA value (Table 5). The measurement error equal to 0.6 mm changes ICA value by a factor of 1.3 (from 3.85 to 5.00). It may be recommended to repeat each measurement at least three times and to use the arithmetic mean as the most reliable one.

5. Mode of fixation. The changes in proportions of the reproductive organs arising due to different mode of fixation have repeatedly been mentioned in the literature (Hubendick, 1954, 1955; Meier-Brook, 1976; Emberton, 1989). To make ICA values comparable, the using of the uniform fixation protocol is needed. Some authors recommend using different methods of snail anesthetisation before fixing that leads to relaxation of the internal organs and ti reducing of ICA variation (Meier-Brook, 1976, 1983; Barker, 1981; Kunigelis & Saleuddin, 1984). However, even using of any substances for snail narcotisation before killing does not prevent completely irregular retraction of genital organs (Emberton, 1989), and, thus, this method cannot be considered as an ultimate solution of the problem.

6. Abnormalities. In *Lymnaea fragilis* and *L. stagnalis* (and, most probably, in other lymnaeids), some forms of abnormalities in the copulatory apparatus appearance can be observed (Vinarski et al., 2010). For example, the shape of praeputium can change from elongated tube-like (typical for the two species) to shortened club-like (see Vinarski et al., 2010, Fig. 2). It apparently leads to decrease in the praeputium length and, consequently, to decrease of ICA.

7. Anatomical polymorphism. In some lymnaeids, interpopulation polymorphism in the ICA values is observed (see Results). Possibly, such phenomenon will be discovered in another species of freshwater pulmonates in the future. The causes and probable adaptive significance of the anatomical polymorphism in Lymnaeidae are still unknown. In my opinion, these intrapopulation differences in proportions of the copulatory organs do not prevent snails from effective copulation and hence do not constitute an effective isolating barrier for reproduction. This opinion is corroborated by the fact that many cases of interspecific copulation in lymnaeids are registered

(Kruglov, 1980; Beriozkina & Starobogatov, 1988), and sometimes the species that may copulate are characterised by quite different proportions of their genitals.

At last, the lack of complete hiatuses of the ICA values between closely allied species of snails has to be discussed. It has been revealed that the intraspecific ranges of the ICA variation in such species can overlap to some extent (see Fig. 5). In my opinion, this fact troubles the identification of snails but does not give us any grounds to regard these species as conspecific entities. Though the classical concept of hiatus requires that two independent species must be separated by a full morphological gap (Kottelat & Freyhof, 2007), in reality there are many cases when 'good' species are not separated completely. For example, many pairs of closed species of small mammals of Eastern Europe are not separated by a hiatus though the mean values of their diagnostic characters are significantly different (Zagorodnyuk, 2004). The lack of a hiatus in the ICA values requires simultaneous use of other characters, either conchological or molecular, for reliable species identification of lymnaeid snails.

CONCLUSIONS

1. The intra- and interpopulation ICA variability is considerable and, in lymnaeid snails, makes it impossible to reliably identify each single specimen based on its ICA.

2. Mean ICA values seem to be more useful for species delineation and identification in lymnaeid snails and, most probably, in another families of freshwater pulmonates than in other groups of mollusks. However, the data on the ICA variability in these families are still lacking (e.g. Stiglingh et al., 1962; Meier-Brook, 1983). It is therefore desirable to make a special study using large samples of planorbid and physid snails belonging to various genera and subgenera.

3. Various factors potentially influencing the ICA values should be taken into account when attempting to use the proportions of the copulatory apparatuses for taxonomic purposes.

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