

Intraspecific morphological and genetic variability in the European freshwater snail *Radix labiata* (Rossmassler, 1835) (Gastropoda: Basommatophora: Lymnaeidae)

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Abstract

Radix labiata is a widely distributed Palaearctic freshwater snail. This work aims to improve the knowledge of the intraspecific variability in the most important characters used for its determination. To find out which characters are really suitable to distinguish this species from other similar *Radix* species an integrative approach was applied, involving morphological and molecular data. Molecular sequences of the nuclear spacer fragment ITS-2 and/or the mitochondrial gene fragment cyt-b were obtained from 26 individuals of *R. labiata* from different regions of Europe, including type localities of *Limnaeus pereger labiatus*. From the subsample of 24 specimens of which sequences of both gene fragments were available, the variability of several characters that are commonly used for species identification (shell morphology, mantle pigmentation, shape and position of the bursa copulatrix, length and position of the bursa duct, length ratio of praeputium to penial sheath) were measured or documented. Morphological characters distinguishing *R. labiata* from *R. balthica*, *R. lagotis* and the genus *Stagnicola* are discussed. The best morphological character to differentiate these species is the position of the bursa and the bursa duct. Mantle pigmentation and the ratio of the length of the praeputium to that of the penial sheath are not useful for differentiating *R. labiata* and *R. balthica*. Analysis of the network of haplotypes (cyt-b) revealed no correlation between recognisable clusters and geography amongst the studied specimens.

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Introduction

The pond snail *Radix labiata* (Rossmassler, 1835) is a Palaearctic species that occurs both in lowlands and mountains. In earlier literature this species has commonly been referred to as '*Lymnaea peregra*', '*Radix pereger*', or '*Radix peregra*'. However, the name *R. labiata* has been reinstated for the present species by Falkner *et al.* (2001) while the name *Buccinum peregrum* O. F. Müller 1774 introduced for specimens from Copenhagen (Frederiksberg) is considered to be a junior synonym of *Radix balthica* (Linnaeus, 1758) according to Glöer (2002).

In Europe *R. labiata* is distributed from Norway, Sweden and Finland in the north (Falkner *et al.*, 2001; Welter-Schultes, 2012), to Spain in the west and Italy in the south (Welter-Schultes, 2012), Bulgaria in the south-east (Angelov, 2000; Welter-Schultes, 2012), and the Ukraine in the east (Stadnichenko, 2004; Welter-Schultes, 2012). In Asia it is known from Turkey and the Ural Mountains (Khokhutkin *et al.*, 2009) up to the Irtysh River Basin in the east (Kruglov and Starobogatov, 1983; Kruglov, 2005; Vinarski *et al.*, 2008). In these Russian sources the species is still mentioned under the name *Lymnaea (Peregriana) peregra* (O.F. Müller, 1774).

Radix labiata prefers small, still or slow-running freshwater bodies but also lives in bogs (Glöer, 2002), in springs or water bodies supplied by ground water (Glöer and Diercking, 2010), in floodplains (Khokhutkin *et al.*,

2009) and in shallow parts of high-mountain lakes (Angelov, 2000). In Switzerland this species occurs at altitudes up to 2700 m in small ponds that are free of ice for only three to four months (Turner *et al.*, 1998). The species has also been observed in temporary water bodies (Stadnichenko, 2004; Kruglov, 2005; Vinarski *et al.*, 2008), and in Western Siberia it occurs almost exclusively in such habitats (Andreeva *et al.*, 2010). In lakes and ponds *R. labiata* is mostly confined to areas that temporarily fall dry during dry seasons (Ward *et al.*, 1996 and own observations). In Sylt (N Germany) it was found in a puddle fed by ground water (Reise and Glöer, 2006). This habitat preference for ground water may explain why this species is rare in lowlands and occurs in mountainous regions.

Caron *et al.* (2007) demonstrated that *R. labiata* may act as an incidental intermediate host of *Fasciola hepatica*; Huňová *et al.* (2012) successfully infected it with *Fascioloides magna*. Thus, reliable identification is important not only for malacologists working on faunistics and zoogeography but also for parasitologists. Because morphological and anatomical examination is still the fastest method of determination, even in the era of molecular genetics – a method not yet accessible to all malacologists – we consider the study of intraspecific variability to be very important in order to improve the reliability of determination keys. As already established, the shell of *R. labiata* is relatively conserved in its shape (Glöer, 2002; Andreeva *et al.*, 2010) and does not vary as considerably as in *R. balthica* (Schniebs *et al.*, 2011). However, during morphological examinations we found specimens differing in characters, such as mantle pigmentation, length of the bursa duct and length ratio of praeputium to penial sheath from what has been described in literature. The amount of morphological variation found in the present study raised doubts whether some of the studied specimens really belonged to this taxon. The present work continues our analyses of intraspecific variability that we started with *R. balthica* (Schniebs *et al.*, 2011).

To analyse the variability of the most important distinguishing characters of *R. labiata* (shell morphology, mantle pigmentation, shape and position of the bursa copulatrix, length and position of the bursa duct, and length ratio of praeputium to penial sheath), 26 individuals from several European localities from which we could sequence a 361 bp fragment of the cyt-b gene as a mitochondrial marker were examined. Additionally, we attempted to obtain sequence data of the nuclear ITS-2 spacer for these individuals; this was not successful for every specimen. The variability

of several characters that are commonly used for determination was calculated for the 24 individuals of which both gene fragments were available.

Material and methods

As type specimens of *R. labiata* for anatomical and genetic analyses are not available, our molecular genetic investigations are based on a specimen from the locus typicus of *Limnaeus pereger labiatus* Rossmässler, 1835: Tharandt (Rossmässler, 1835), Saxony (Germany).

All specimens used for molecular and morphological examination are listed in the Appendix. Most were collected or donated for this study and stored in the mollusc collection of the Senckenberg Natural History Collections Dresden, Museum of Zoology (SNSD). Two samples are stored in the collection of Dr Ulrich Bößneck (Vieselbach, Germany).

For outgroup comparison in the molecular genetic analyses we used sequences of specimens of other freshwater molluscs. The outgroup comprised *Planorbarius corneus* (Linnaeus, 1758), family Planorbidae. We also included sequences of *Lymnaea stagnalis* (Linnaeus, 1758), *R. auricularia* (Linnaeus, 1758), *R. ampla* (Hartmann, 1821), *Radix balthica*, and *R. lagotis* (Schrank, 1803) in the ingroup.

Molecular techniques

Tissue samples taken from the foot were fixed in 100% ethanol. All these were registered in the tissue collection of the SNSD with both a new collection number and the collection number of the specimen in the mollusc collection of SNSD and stored at -80°C.

For molecular genetic analyses we obtained sequence data of the complete nuclear ITS-2 spacer (280-495 bp) and a 361 bp fragment of the cyt-b gene as mitochondrial marker. For primers and protocols of DNA extraction, Polymerase Chain Reaction (PCR), purification of PCR products and DNA sequencing see Vinarski *et al.* (2011). Alignments were performed using the sequence alignment editor BioEdit (Hall, 1999). The ITS-2 alignment was obtained using the Clustal algorithm of MEGA4 (Tamura *et al.*, 2007) and improved by eye. Genetic distances for cyt-b were calculated using MEGA4 (Tamura *et al.*, 2007). The data set consisted of 361 bp of 26 individuals. This program was also used to produce the dataset of cyt-b sequences (positions with missing data were excluded: 318 bp) for network analyses (median joining; Bandelt *et al.*, 1999)

with the program 'Network' (www.fluxus-engineering.com). Maximum-parsimony (MP) trees were reconstructed using PAUP (version 4.0b10; Swofford, 2002; settings: gapmode = NewState, addseq = closest, maxtree = 100; number of bootstrap replicates = 10000). For maximum-likelihood analyses, including bootstrap support, we used RAxML (raxmlGUI 0.9 beta 2, Stamatakis *et al.*, 2005; Silvestro and Michalak, 2010). The settings were 'ML+thorough bootstrap' with 100 (replicate) runs and 1000 (bootstrap) repetitions.

Morphology

The snails were fixed in 70-80% ethanol. The shell morphology, mantle pigmentation and anatomy of the specimens studied were recorded. The dissections and measurements of the genital organs and shells were carried out using stereo microscopes (Zeiss and Olympus); photographs were taken with a digital camera (Leica R8). For the taxonomy we followed the current European checklists (Falkner *et al.*, 2001; Bank, 2011).

Results

Molecular genetics

Uncorrected genetic distances (p-distance) from pairwise comparisons of cyt-b sequences (fragment of 361 bp) are shown in Table 1. Pair-wise distances between species of the families Planorbidae and Lymnaeidae (outgroup comparison) ranged between 31% and 28%. Distances between the two genera analysed in the family Lymnaeidae (only sequences of *Lymnaea stagnalis* and all *Radix* species) ranged from 25% to 20%. Among the five *Radix* species analysed, the highest values are between *R. auricularia* and the other species (15%-18%). *R. labiata*, *R. balthica*, *R. ampla* and *R. lagotis* exhibit smaller pair-wise distances between 9% and 16%.

As for molecular phylogeny, the maximum-parsimony (MP) tree of the nuclear marker ITS-2 (tree length = 996, consistency index = 0.9046, retention index = 0.9718) basal branches received high or full bootstrap support by means between 98% and 100% (Fig. 1). Species-clades are generally fully supported. *Radix auricularia* is sister group to all other *Radix* species. *Radix balthica* is the sister group of *R. ampla*; together they form the sister group of *R. lagotis*. All specimens identified as *R. labiata* or *R. cf. labiata* by means of anatomy fall into one cluster with bootstrap support of 100%.

The RAxML tree of the ITS-2 spacer (not shown) differs in two main aspects from the MP tree: 1) The bootstrap supports of the basal branches and of the species-clades are lower in most cases and the tree shows a polytomy between *R. balthica*, *R. ampla* and *R. lagotis*. 2) *Radix auricularia* groups as a sister to *R. labiata* with bootstrap support of 78%. All specimens identified as *R. labiata* or *R. cf. labiata* on the basis of anatomy still fall into one cluster with full bootstrap support.

The maximum-parsimony (MP) tree of the cyt-b sequences (tree length = 336, consistency index = 0.6845, retention index = 0.8997) (Fig. 2) has low or very low support for most of its basal branches. The very low support is also expressed by a polytomy of *R. auricularia* and the other *Radix* species. The clades of the species themselves have high, often full support except for *R. ampla* (70%). All specimens identified as *R. labiata* or *R. cf. labiata* by means of anatomy fall into one cluster with high bootstrap support (91%).

In the RAxML tree of the cyt-b sequences (not shown) most of the basal branches show very low support. This very low support is again expressed by a polytomy of *L. stagnalis*, *R. auricularia*, *R. balthica*, *R. lagotis*, and *R. ampla*, to which the cluster of *R. labiata* groups as a sister with support of 77%.

The network representation of cyt-b mitochondrial

Table 1. Uncorrected pair-wise p-distances of the cyt-b gene fragment (361 bp) calculated using MEGA version 4 (Tamura *et al.*, 2007).

	<i>P. corneus</i>	<i>L. stagnalis</i>	<i>R. balthica</i>	<i>R. lagotis</i>	<i>R. auricularia</i>	<i>R. ampla</i>	<i>R. labiata</i>
<i>Lymnaea stagnalis</i>	0.282	–	–	–	–	–	–
<i>Radix balthica</i>	0.308	0.225	–	–	–	–	–
<i>R. lagotis</i>	0.281	0.199	0.086	–	–	–	–
<i>R. auricularia</i>	0.301	0.251	0.146	0.156	–	–	–
<i>R. ampla</i>	0.278	0.203	0.112	0.090	0.185	–	–
<i>R. labiata</i>	0.281	0.225	0.130	0.123	0.174	0.109	–

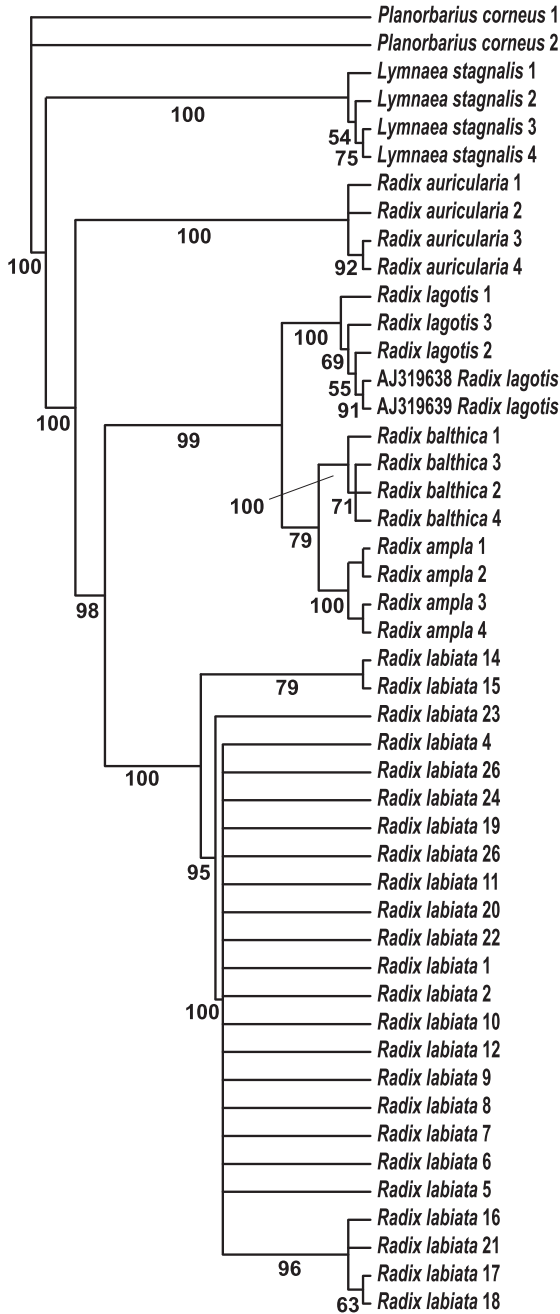


Fig. 1. Hypothesis for the phylogenetic relationships of *Radix labiata*: on the strict consensus of 100 maximum-parsimony trees of the nuclear marker ITS-2 (tree length = 996, consistency index = 0.9046, retention index = 0.9718). Branch lengths are proportional to the number of substitutions and the overall topology corresponds to that of the strict consensus tree. Bootstrap support values above 50% are reported below nodes.

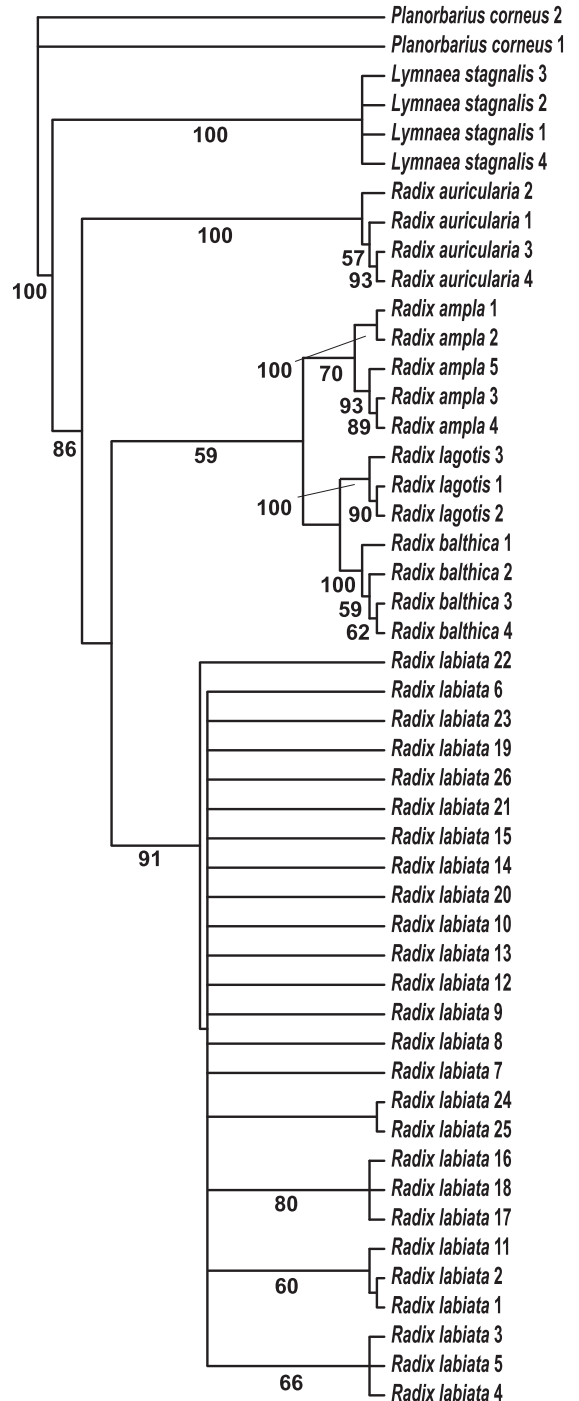


Fig. 2. Hypothesis for the phylogenetic relationships of *R. labiata*: on the strict consensus of 100 maximum-parsimony trees of the fragment sequenced of the mitochondrial marker *cyt-b* (361 bp; tree length = 336, consistency index = 0.6845, retention index = 0.8997). Branch lengths are proportional to the number of substitutions and the overall topology corresponds to that of the strict consensus tree. Bootstrap support values above 50% are reported below nodes.

sequences (318 bp, Fig. 3) of 26 *R. labiata* specimens from different locations in Europe shows scarcely reticulated differentiation of about 16 sites showing variation between individuals. Between individuals from geographically close localities, for example from within Saxony in Germany (blue), differentiation of 9 substitutions could be observed.

Morphology

The shell morphology of the examined specimens ($n=24$) varied from slender, close to the shape of *Stagnicola*, to a conical egg-shape (Fig. 4). They varied from thin-walled and fragile to solid, and from a light horn colour to reddish-brown. The shell height ranged from 7.5 to 16.8 mm and the number of whorls between 3.5 and 4.0 in 9 specimens with preserved first whorls. The shell form varied not only between specimens from different localities but also varied between specimens collected from the same locality (Fig. 5).

The mantle pigmentation of the sequenced specimens of *R. labiata* ($n=24$) is polymorph (Fig. 6). It was however possible to distinguish three main types:

- mantle black or deep blue-black with irregular fine whitish dots of similar size, mantle collar black or blue-black, mantle edge bluish grey or whitish with some irregular patches of black or deep blue-black (Fig. 6a-b, 6d);
- mantle black or grey with numerous, mostly roundish small distinct spots of white, white-grey or rarely grey-green that are very variable in size; mantle collar bluish-grey or whitish-grey with diffuse patches of black; mantle edge and tentacles whitish, blue-grey, brownish-grey or gray-orange (Figs 6c, 6e);
- mantle black or grey-black with roundish patches, mostly of similar size of white or lighter grey-black; mantle collar bluish grey with irregular patches of black; mantle edge blue-grey (Fig. 6f);

The measurements of praeputium and penial sheath of 21 specimens are reported in Table 2 (see also Fig. 7). The ratio of the length of the praeputium to that of the penial sheath varies from 0.58-2.53 ($n=21$). The praeputium is pigmented grey in most specimens. In a few cases it shows a grey-green or whitish-yellow colour.

For the bursa copulatrix, the following three characters were analysed (Fig. 8):

- Position of the bursa copulatrix ($n=20$). The bursa was positioned behind (ventral to) of the provagi-

nal duct and the vagina in most specimens examined ($n=17$). In three specimens the bursa was positioned beside the provaginal duct. But in all cases the bursa duct entered on the ventral side of the provagina above female vent.

- Shape of the bursa. This varied from egg-shaped, to pear-shaped, to elongate pear-shaped, to club-shaped.
- Length of the bursa duct ($n=21$). The length of the bursa duct varied between nearly not visible ($n=7$) up to a length of between 3 and 5 mm ($n=12$), and even to 7 mm ($n=2$).

Discussion

Molecular phylogeny

The main aim of the molecular genetic analyses was to find out whether the specimens with unusual morphology or anatomy belonged to the species *R. labiata* and

Table 2. Measurements of the male genitalia of 21 specimens.

Collection No. SNSD	Length of the praeputium in mm	Length of the penial sheath in mm	Ratio of the length of the praeputium to the length of the penial sheath
Moll 49707	3.5	6	1:1.7
Moll 49708	3.5	3	1.16:1
Moll 51276	2.5	3.5	1:1.4
Moll 51277	2.5	3.5	1:1.4
Moll 51696	2.5	2	1.25:1
Moll 51697	3	2.5	1.2:1
Moll 51698	3.8	1.5	2.53:1
Moll 51699	2	2	1:1
Moll 51864	1.5	1.5	1:1
Moll 51910	3.5	3	1.16:1
Moll 51964	4	4	1:1
Moll 51965	3	2.5	1.2:1
Moll 52416	3	3	1:1
Moll 52427	2.3	2	1.15:1
Moll 52431	2	2.5	1:1.25
Moll 52432	2	3	1:1.5
Moll 52462	2	2	1:1
Moll 52580	3	3	1:1
Moll S284	3.5	4.5	1:1.28
Moll S2779	2.3	2	1.15:1
Moll S2780	2	2	1:1

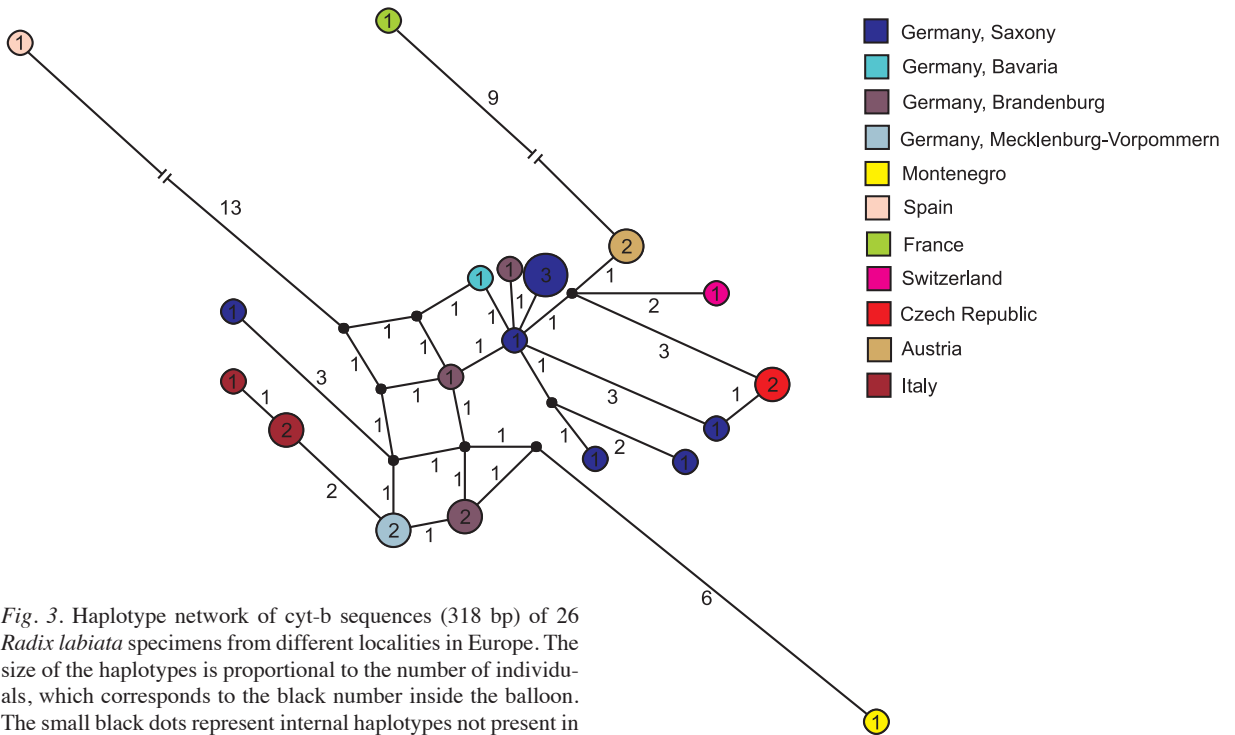


Fig. 3. Haplotype network of *cyt-b* sequences (318 bp) of 26 *Radix labiata* specimens from different localities in Europe. The size of the haplotypes is proportional to the number of individuals, which corresponds to the black number inside the balloon. The small black dots represent internal haplotypes not present in the dataset. The length of the connecting lines is (mostly) proportional to the number of substitutions between haplotypes (reported as black numbers on the lines).



Fig. 4. Variability in *Radix labiata* shells: a) Germany, Mecklenburg-Vorpommern, Gressow, alder fenwood (*Radix labiata* 12); b) Switzerland, Basel City, Riehen, well Nollenbrunnen (*Radix labiata* 23); c) Italy, Lombardy, Province of Brescia, Ponte di Legno, Bleis Lake (*Radix labiata* 17); d) Germany, Saxony, Ammels-hain, quarry Haselberg (*Radix labiata* 24); e) Austria, Carinthia, Hermagor (*Radix labiata* 14); f) Germany, Bavaria, spring of the river Strogn near Buchrain (*Radix labiata* 19).

to quantify the intraspecific variability in this species. The individuals of *R. labiata* analysed form a clade, which includes a topotype of *R. labiata* from Tharandt (*Radix labiata* 26) in the MP trees (Figs 1-2) of both the nuclear marker ITS-2 (with full support) and of the mitochondrial *cyt-b* gene fragment (361 bp) (with very high support). The RAxML trees (not shown) of both markers are also similar in this respect, but whereas the tree based on the ITS-2 sequences yields full support for this clade, support is only 77% in that based on *cyt-b*. We conclude that these individuals all belong to one species, *R. labiata*, despite their morphological variability and that they clearly differ from the other *Radix* species observed. The latter conclusion is confirmed by the large genetic distances based on the *cyt-b* fragment (Table 1). In the ITS-2 MP tree *R. balthica* groups as the sister of *R. ampla* and these two species form the sister group to *R. lagotis*, as Schniebs *et al.* (2011, Fig. 2) already observed based on an ITS-2 parsimony tree. Together these three species form the sister group to *R. labiata*. We confirm the opinion given in Schniebs *et al.* (2011) that the topology of the ITS-2 tree provides a fair reflection of the morphological and anatomical characteristics of the European species of *Radix* that have been investigated. An explanation of the low support of the basal branches in the RAxML tree could be the incomplete taxon sampling.

The analysis of the network relationship of the mitochondrial haplotypes compared with their geo-

graphic origin (Fig. 3) shows that there is no distinct correlation of genetic variability with geography in this relatively small sample of specimens. This result agrees with that found in *R. balthica* (Schniebs *et al.*, 2011).

Morphology

The variability of the shell of *R. labiata* (Fig. 4) is less than the variation we found in *R. balthica* (Schniebs *et al.*, 2011). Shells of *R. balthica* could be confused with these of *R. auricularia*, *R. ampla*, *R. labiata* and *R. lagotis* because they could be ear-shaped, amploid to nearly conical egg-shaped (Schniebs *et al.*, 2011, Fig. 4). In most specimens of *R. labiata* the shell is more or less a conical egg-shape, with relatively high first whorls (Fig. 4a), but we also found slender shells shaped nearly like a *Stagnicola* (Fig. 4d), similar to those Zettler *et al.* (2006) mentioned for specimens from Mecklenburg-Vorpommern. Gittenberger *et al.* (1998) also indicated that *R. labiata* could be confused with dwarf forms of *Stagnicola palustris* (O.F. Müller, 1774). We found considerable variation in shell morphology, even between specimens collected at the same locality (Fig. 5). In all specimens examined the shape of the line tangential to the whorls in adult shells was straight or slightly convex, as already stated in the literature (Glöer, 2002; Stadnichenko, 2004; Kruglov, 2005; Khokhutkin *et al.*, 2009).

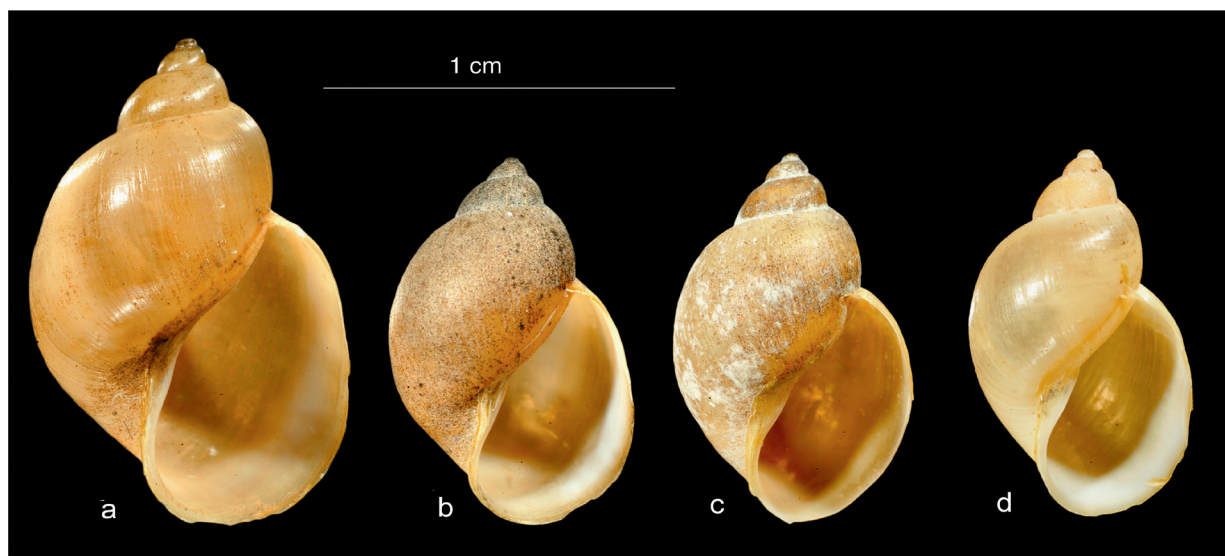


Fig. 5. Variability in *Radix labiata* shells from specimens collected in the same locality (Germany, Brandenburg, small lake near Wachow, specimens a) and c) identified only by anatomy as *R. labiata*, specimens b) (*Radix labiata* 7) and d) (*Radix labiata* 6) are sequenced and identified by anatomy as well).

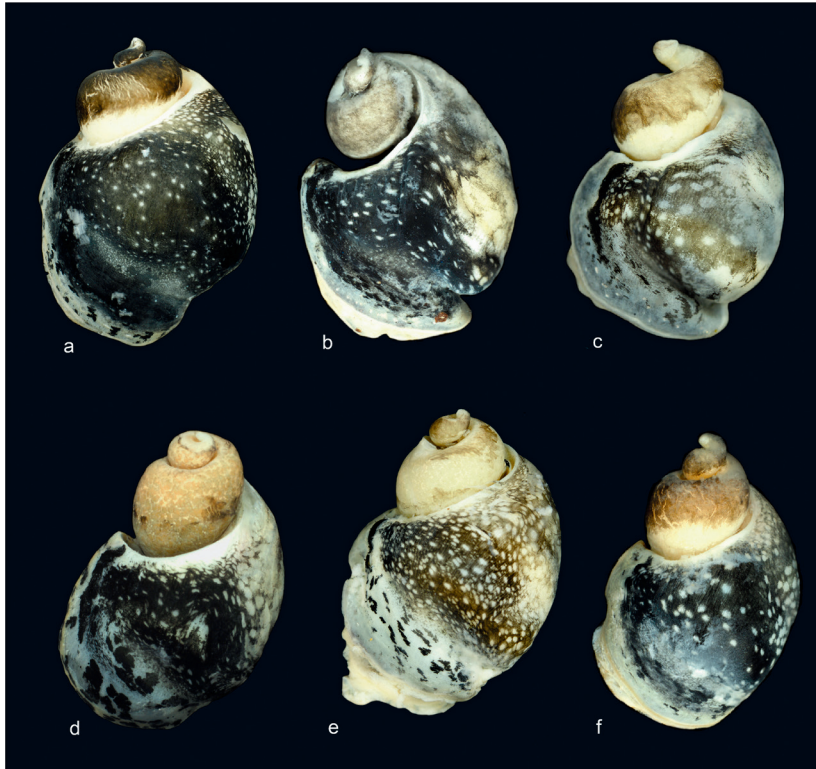


Fig. 6. Variability in *Radix labiata* mantle pigmentation: a) Germany, Mecklenburg-Vorpommern, Gressow, alder fenwood (*Radix labiata* 12); b) Switzerland, Basel City, Riehen, well Nollenbrunnen (*Radix labiata* 23); c) Italy, Lombardy, Province of Brescia, Ponte di Legno, Bleis Lake (*Radix labiata* 17); d) Germany, Saxony, Ammelshain, quarry Haselberg (*Radix labiata* 24); e) Austria, Carinthia, Hermagor (*Radix labiata* 14); f) Germany, Bavaria, spring of the river Strogn near Buchrain (*Radix labiata* 19).

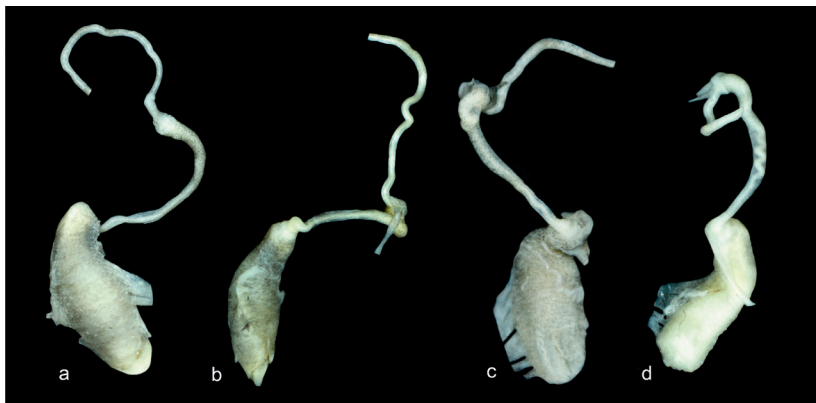


Fig. 7. Variability in *Radix labiata* male genitalia: a) Germany, Mecklenburg-Vorpommern, Gressow, alder fenwood (*Radix labiata* 12); b) Germany, Bavaria, spring of the river Strogn near Buchrain (*Radix labiata* 19); c) Italy, Lombardy, Province of Brescia, Ponte di Legno, Bleis Lake (*Radix labiata* 17); d) Germany, Saxony, Ammelshain, quarry Haselberg (*Radix labiata* 24).

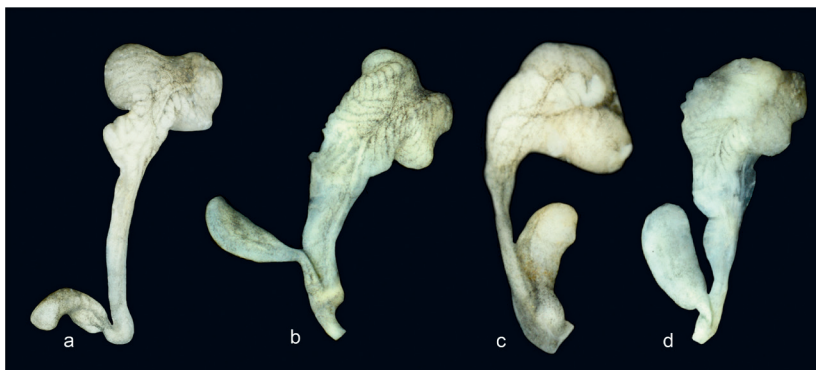


Fig. 8. Variability of the form of the bursa copulatrix and the length of the bursa duct in *Radix labiata*: a) Germany, Mecklenburg-Vorpommern, Gressow, alder fenwood (*Radix labiata* 12); b) Germany, Bavaria, spring of the river Strogn near Buchrain (*Radix labiata* 19) c) Italy, Lombardy, Province of Brescia, Ponte di Legno, Bleis Lake (*Radix labiata* 17); d) Germany, Saxony, Ammelshain, quarry Haselberg (*Radix labiata* 24).

The maximum shell height reported here is exceeded in *R. labiata* from Ukraine (up to 21.4 mm; see Stadnichenko, 2004). In Western Siberia shells of this species can reach a height of 18.4 mm (Andreeva *et al.*, 2010). Possibly, *R. labiata* has the smallest shell height amongst European species of *Radix*.

Typical mantle pigmentation in *R. labiata* is irregular, fine, whitish, blurred dots of similar size on a black, or deep blue-black, mantle, as already mentioned in the literature (Glöer, 2002; Stadnichenko, 2004, see also Fig. 5a). Normally this mantle pigmentation renders this species distinguishable from the other European *Radix* species examined because only a few large white spots on dark background are typical for *R. auricularia* and *R. ampla* whereas the typical mantle pigmentation of *R. balthica* and *R. lagotis* shows medium-sized spots on dark background (see Schniebs *et al.*, 2011; Table 2). But we found also *R. labiata* with larger dots (Fig. 6c), which means that it would be difficult to distinguish this species from *R. balthica* on the basis of the mantle pigmentation alone.

In our specimens examined ($n=21$) the length of the praeputium varied from 1.5 to 4 mm and that of penial sheath from 1.5 to 6 mm (Table 2). The ratio of the length of the praeputium to the length of the penial sheath varied from 0.58 to 2.53 (Fig. 7, Table 2). In six of these the praeputium was longer than the penial sheath (1.15:1, 1.16:1, 1.2:1, 1.25:1 (2x), 2.53:1) (Table 2). We thus found more variability in this characteristic than is recognised in the literature. Meier-Brook in Glöer (2002, p. 217) reported that the length ratio of praeputium to penial sheath can vary from 0.7:1 to 2.3:1. Kruglov and Starobogatov (1983) and Kruglov (2005) gave a value of 0.66 and Stadnichenko (2004) a ratio of 1:1.5. Khokhutkin *et al.* (2009) mentioned that in four specimens from the Urals the ratio of the length of the praeputium to the length of the penial sheath varied from 0.71 to 0.84. The enormous variability of this character confirms our statement (Schniebs *et al.*, 2011) that it is not useful for differentiating *R. labiata* from *R. balthica*. The latter has a ratio of the length of the praeputium to the length of the penial sheath from 0.7 to 1.3 (Schniebs *et al.*, 2011). It should also be noted that in certain *Radix* species this ratio may be polymorphic within a population, and conchologically indistinguishable individuals may differ drastically from each other in penial sheath length (Vinarski, 2011). In the present study the length of the praeputium varies from 2 to 3.8 mm within four specimens of one population in Brandenburg (Germany) (collection No. SNSD Moll 51696-51699) (Table 2). This may consti-

tute another cause of high variation in the proportions of the copulatory organ.

To the usual uniform grey or grey greenish pigmentation of the praeputium already described (Schniebs *et al.*, 2011) we can add a whitish-yellow colour in two of the 21 examined specimens.

We could confirm that the position of the bursa copulatrix behind (ventral to) the provaginal duct and vagina is indeed typical for *R. labiata* (Stadnichenko, 2004; Kruglov, 2005; Schniebs *et al.*, 2011). The variability is similar to that illustrated in Hubendick (1953: p. 28, Figs 22-24). Of all examined European *Radix*, we found this position of the bursa only in *R. labiata*, thus we can suppose that the specimens shown by Hubendick, 1953 belong to *R. labiata*. Only in three specimens out of the 20 examined the bursa was positioned beside the provaginal duct. But in these cases the bursa duct also entered on the ventral side of the provaginal duct.

We showed that the length of the bursa duct (Fig. 8) varies more than indicated in the current literature used for determination of freshwater molluscs: Glöer (2002) and Kruglov (2005) mentioned only that *R. labiata* has a short bursa duct, Huňova *et al.* (2012) described the bursa duct as shorter than half-length of bursa copulatrix, whereas Stadnichenko (2004, p. 200) provides the information that the bursa duct could be as long as the diameter of the bursa or the diameter of the bursa can clearly be longer than the length of the bursa duct. In 7 of 21 specimens examined for this study the bursa duct was nearly not visible (Fig. 8a). Hubendick (1953: p. 28, Figs 22-24) found nearly the same extent of the variability of the bursa duct as we did.

In summary, the length of the bursa duct in *R. labiata* varies from nearly not visible to one third of the length of the bursa. This overlaps with the length of the bursa duct we found in *R. balthica* from between nearly not visible to half of the length of the bursa (Schniebs *et al.*, 2011). This is a further reason to confirm our former statement (Schniebs *et al.*, 2011) that the length of the bursa duct is not a good character to differentiate *R. labiata* from *R. balthica* whereas the position of the bursa is.

Conclusions

In Central Europe, *R. labiata* can be confused mainly with specimens of *R. lagotis* and *R. balthica* with slender shells (Fig. 9a-b) and with representatives of the genus *Stagnicola* (Fig. 9c). The results of this present

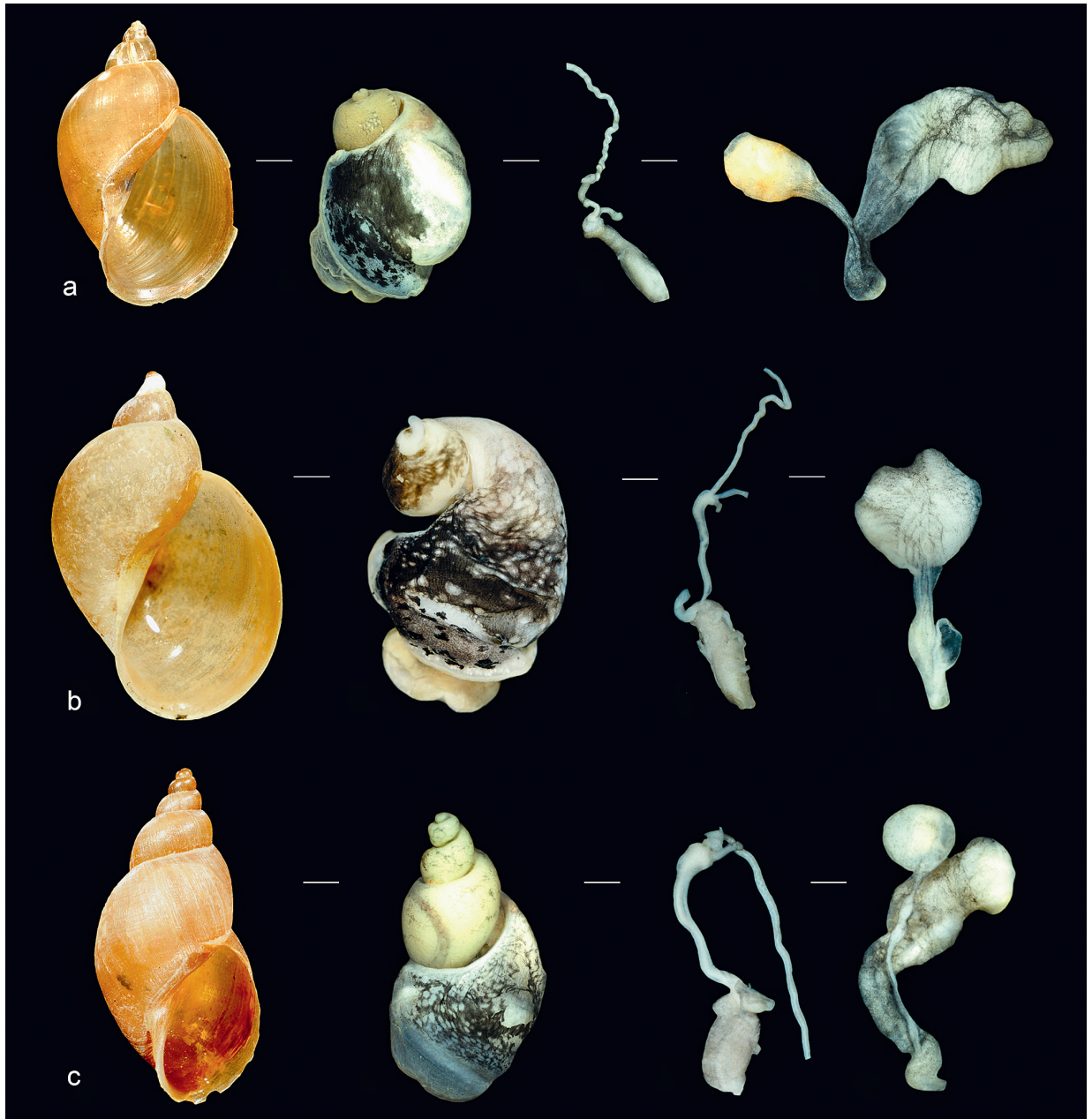


Fig. 9. a) *Radix lagotis* (Germany, Saxony, pond Kleiner Kirchenteich near Grethen); b) *Radix balthica* (France, Region Centre, Thenay); c) *Stagnicola palustris* (Germany, Saxony, Dresden, small pond in the valley of the spring Kaitzbach).

study confirm our earlier conclusions concerning the differentiation of *R. labiata* from *R. balthica* (Schniebs *et al.*, 2011) that the clearest anatomical character is the position of the bursa and the bursa duct: in most specimens of *R. labiata* they lie ventrally to the vagina and the provaginal duct. Additionally, we discovered that sometimes bursa and

bursa duct can lie beside the provaginal duct. The shells of *R. labiata* usually have a straight line tangential to the whorls in adult shells, whereas in *R. balthica* it is usually convex or rarely concave (Schniebs *et al.*, 2011).

Radix lagotis specimens with slender shells and dark mantle pigmentation also differ from *R. labiata*

in the positions of the bursa and the bursa duct and by the length of the bursa duct: in *R. lagotis*, bursa and bursa duct lie above (dorsal to) the provaginal duct, and vagina, uterus and prostate, and the bursa duct is 1/2 to about 2/3 of the length of the bursa (if filled) (Fig. 9a; see also Schniebs *et al.*, 2011).

Specimens of *R. labiata* with a shell similar to representatives of the genus *Stagnicola* can be distinguished by the much shorter bursa duct and by the position of the bursa, which lies near the pericardium in *Stagnicola* (Fig. 9c) and not ventral to vagina and provaginal duct, as in *R. labiata*.

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References

Andreeva SI, Andreev NI, Vinarski MV. 2010. Families Acroloxidae and Lymnaeidae. P. 200. in: *Key to freshwater gastropods of Western Siberia (Mollusca: Gastropoda)*. V. 1. *Gastropoda: Pulmonata*. Fasc. 1. Omsk. [in Russian]

Angelov AM. 2000. Mollusca (Gastropoda et Bivalvia) aquae dulcis. Pp. XIV + 57. In: *Catalogus Faunae Bulgaricae* 4. Sofia, Leiden: Pensoft & Backhuys Publishers.

Bandelt H-J, Forster P, Rohlf A. 1999. Median-joining networks for inferring infraspecific phylogenies. *Molecular Biology and Evolution* 16:37-48.

Bank R. 2011. Fauna Europaea project. Systematical and distributional checklist of species-group taxa of continental Mollusca of Europe, version 2.4. <www.faunaeur.org>

Caron Y, Lasri S, Losson B. 2007. *Fasciola hepatica*: An assessment on the vectorial capacity of *Radix labiata* and *R. balthica* commonly found in Belgium. *Veterinary Parasitology* 149: 95-103.

Falkner G, Bank RA, von Proschwitz T. 2001. Check-list of the non-marine Molluscan Species-group taxa of the States of Northern, Atlantic and Central Europe (CLECOM 1). *Heldia* 4: 1-76.

Gittenberger E, Janssen AW, Kuijper WJ, Kuiper JGJ, Meijer T, van der Velde G, de Vries JN. 1998. De Nederlandse Zoetwatermollusken. Recente en fossiele weekdieren uit zoet en brak water. P. 288 in: Gittenberger E, Janssen AW, eds, *Nederlandse Fauna* 2. Leiden, Utrecht: Nationaal Natuurhistorisch Museum Naturalis, KNNV Uitgeverij, European Invertebrate Survey – Nederland.

Glöer P. 2002. Die Süßwassergastropoden Nord- und Mitteleuropas. P. 327. In: *Die Tierwelt Deutschlands* 73. Hackenheim: Conchbooks.

Glöer P, Diercking R. 2010. Atlas und Rote Liste der Süßwassermollusken in Hamburg. Behörde für Stadtentwicklung und Umwelt, Freie und Hansestadt Hamburg.

Hall TA. 1999. BioEdit: a user friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* no. 41: 95-98.

Hartmann JDW. 1840-1844. *Erd- und Süßwasser-Gasteropoden der Schweiz. Mit Zugabe einiger merkwürdigen exotischen Arten* I. Pp. XX + 227. St. Gallen: Scheitlin & Zollikofer.

Hubendick B. 1953. Recent Lymnaeidae. Their variation, morphology, taxonomy, nomenclature, and distribution. *Kungliga Svenska Vetenskapsakademiens Handlingar Series* 4, 3(1): 1-223.

Huňová K, Kašný M, Hampl V, Leontovych R, Kuběna A, Mikeš L, Horák P. 2012. *Radix* spp.: Identification of trematode intermediate hosts in the Czech Republic. *Acta Parasitologica* 57: 273-284.

Khokhutkin IM, Vinarski MV, Grebennikov ME. 2009. The family Lymnaeidae (Gastropoda, Pulmonata, Lymnaeiformes). Pp. 1-156. In: *Molluscs of the Urals and the adjacent areas* 1. Yekaterinburg, Goshchitskiy Publishers. [in Russian]

Kruglov ND. 2005. *Lymnaeid snails (Lymnaeidae Gastropoda Pulmonata) of Europe and Northern Asia*. P. 507. Smolensk State Pedagogical University Press. [in Russian]

Kruglov ND, Starobogatov YI. 1983. A contribution to the morphology and taxonomy of European representatives of the subgenus *Peregriana* (*Lymnaea*, Gastropoda, Pulmonata). *Zoologicheskoy Zhurnal* 62: 1462-1473. [in Russian].

Linnaeus C von. 1758. *Systema naturae per regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis*. Tomus I. Editio decima, reformata. Pp. 1-824. Laurentius Salvius: Holmiae.

Müller OF. 1774. *Vermium terrestrium et fluviatilium, seu animalium infusorium, helminthicorum et testaceorum, non marinorum, succincta historia*. Vol. 2. Pp. I-XXVI, 1-214. Heineck et Faber: Havniae et Lipsiae.

Reise K, Glöer P. 2006. Limnische Molluskenfauna einer Nordseeinsel - verändert nach 40 Jahren. *Heldia* 6 (3/4): 153-167.

Rossmässler EA. 1835-1844. *Iconographie der Land- und Süßwasser-Mollusken, mit vorzüglicher Berücksichtigung der europäischen noch nicht abgebildeten Arten* 1(1). Dresden, Leipzig: Arnoldische Buchhandlung [1835].

- Schniebs K, Glöer P, Vinarski MV, Hundsdoerfer AK. 2011. Intraspecific morphological and genetic variability in *Radix balthica* (Linnaeus, 1758) (Gastropoda: Basommatophora: Lymnaeidae) with morphological comparison to other European *Radix* species. *Journal of Conchology* 40: 657-678.
- Schrank FVP. 1803. Durchdachte Geschichte der in Baiern einheimischen und zahmen Tiere. – Nacktschnecken und Schalthiere. *Fauna Boica* 3 (2): 250-301.
- Silvestro D, Michalak I. 2010. RAXMLGUI: a graphical front-end for RAXML. Available at <http://sourceforge.net/projects/raxmlgui/>.
- Stadnichenko AP. 2004. *Lymnaeidae and Acroloxidae of the Ukraine*. P. 327. Kiev: Center of textbooks. [in Russian]
- Stamatakis A, Ludwig T, Meier H. 2005. Raxml-iii: a fast program for maximum likelihood-based inference of large phylogenetic trees. *Bioinformatics* 21: 456-463.
- Swofford DL. 2002. *PAUP. Phylogenetic analysis using parsimony (and other methods), version 4*. Sunderland: Sinauer Associates.
- Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24: 1596-1599.
- Turner H, Kuiper JGJ, Thew N, Bernasconi R, Rüetschi J, Wüthrich M, Gostelli M. 1998. Atlas der Mollusken der Schweiz und Liechtensteins. *Fauna Helvetica* 2.
- Vinarski MV. 2011. The 'index of the copulatory apparatus' and its application to the systematics of freshwater pulmonates (Mollusca: Gastropoda: Pulmonata). *Zoosystematica Rossica* 20: 11-27.
- Vinarski MV, Andreeva SI, Andreev NI, Lazutkina EA, Karimov AV. 2008. Diversity of gastropods in the inland waterbodies of Western Siberia. *Invertebrate Zoology* 4: 173-183.
- Vinarski MV, Schniebs K, Glöer P, Hundsdoerfer AK. 2011. The taxonomic status and phylogenetic relationships of the genus *Aenigmomphiscola* Kruglov et Starobogatov, 1981 (Gastropoda: Pulmonata: Lymnaeidae). *Journal of Natural History* 45: 2049-2068.
- Ward PI, Goater CP, Mikos M. 1996. Shell variation in sympatric freshwater *Lymnaea peregra* and *L. ovata* (Gastropoda: Lymnaeidae). *Biological Journal of the Linnean Society* 61: 139-149.
- Welter-Schultes F. 2012. *European non-marine molluscs, a guide for species identification*. P. 679. Göttingen: Planet Poster Editions.
- Zettler ML, Jueg U, Menzel-Harloff H, Göllnitz U, Petrick S, Weber E, Seemann R. 2006. *Die Land- und Süßwassermollusken Mecklenburg-Vorpommerns*. P. 318. Schwerin: Obotritendruck.

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Appendix

Material used for the molecular genetic and morphological analyses. ENA = European Nucleotide Archive.

Code	Collection No. SNSD	Locality	ENA No.	
			cyt-b	ITS-2
<i>Planorbarius corneus</i> (Linnaeus, 1758)				
<i>P. corneus</i> 1	Moll 52556	Germany, Saxony, Linz, pond Goldgrubenteich, 13°43'09"E 51°19'45"N	FR797880	FR797830
<i>P. corneus</i> 2	Moll 52557	Germany, Saxony, Linz, pond Goldgrubenteich, 13°43'09"E 51°19'45"N	FR797881	FR797831
<i>Lymnaea stagnalis</i> (Linnaeus, 1758)				
<i>L. stagnalis</i> 1	Moll 49239	Germany, Saxony, Dresden-Zschieren, old branch of river Elbe, 13°52'28"E 50°59'50"N	HE573102	HE573064
<i>L. stagnalis</i> 2	Moll 49835	Germany, Saxony, Niederspree, small pond, 14°54'03"E 51°24'28"N	HE573103	HE573065
<i>L. stagnalis</i> 3	Moll 53108	Germany, Baden-Württemberg, Konstanz-Egg, ditch Hockgraben, 9°11'34.2"E 47°40'57.3"N	FR797894	FR797834
<i>L. stagnalis</i> 4	Moll 53109	Germany, Baden-Württemberg, Konstanz-Egg, ditch Hockgraben, 9°11'34.2"E 47°40'57.3"N	FR797895	FR797835
<i>Radix auricularia</i> (Linnaeus, 1758)				
<i>R. auricularia</i> 1	Moll 50005	Germany, Saxony, Niederspree, pond Neuwiesenteich, 14°52'57"E 51°24'19"N	HE573104	HE573066
<i>R. auricularia</i> 2	Moll 50079	Germany, Saxony, pond Vierteich near Freiteilsdorf, 13°41'57"E 51°15'43"N	HE573105	HE573067
<i>R. auricularia</i> 3	Moll 53070	Germany, Bavaria, Weichering, pond in riverside forest, 11°19'23.6"E 48°43'34.1"N	FR797902	FR797842
<i>R. auricularia</i> 4	Moll 53071	Germany, Bavaria, Weichering, pond in riverside forest, 11°19'23.6"E 48°43'34.1"N	FR797903	FR797843
<i>Radix balthica</i> (Linnaeus, 1758)				
<i>R. balthica</i> 1	Moll 51283	Switzerland, canton Basel-Landschaft, Liestal, Orishof, 07°43'03"E 47°28'22"N	HE573133	HE573082
<i>R. balthica</i> 2	Moll 51834	Germany, Saxony, Dresden-Kleizschachwitz, river Elbe, 13°52'21"E 51°00'03"N	HE573119	HE573079
<i>R. balthica</i> 3	Moll 53111	Germany, Baden-Württemberg, Konstanz-Egg, pond near University, 09°11'29"E 47°41'09"N	HE573116	HE573078
<i>R. balthica</i> 4	Moll 53112	Germany, Baden-Württemberg, Konstanz-Egg, pond near University, 09°11'29"E 47°41'09"N	HE573117	HE577649
<i>Radix lagotis</i> (Schränk, 1803)				
AJ319638	—	Czech Republic, Kadov, Vasi and Podkadovsky pond	—	AJ319638
AJ319639	—	Austria, Schoenau, southeast of Vienna	—	AJ319639
<i>R. lagotis</i> 1	Moll 49868	Germany, Saxony, pond Vierteich near Freiteilsdorf, 13°41'57"E 51°15'43"N	HE573114	HE573076
<i>R. lagotis</i> 2	Moll 52563	Saxony, pond Goldgrubenteich near Linz, 13°43'09"E 51°19'46"N	HE573115	HE573077
<i>R. lagotis</i> 3	Moll 53239	Saxony, dam Doellnitzsee near Mutzschen, 12°55'18"E 51°15'45"N	HE573113	HE573075
<i>Radix ampla</i> (Hartmann, 1821)				
<i>R. ampla</i> 1	Moll 51112	Russia, Chelyabinsk Region, river Miass near Dynamo village, 60°02'E 45°57'N	HE798470	HE798448
<i>R. ampla</i> 2	Moll 51113	Russia, Chelyabinsk Region, river Miass near Dynamo village, 60°02'E 45°57'N	HE798471	HE798449
<i>R. ampla</i> 3	Moll 53098	Germany, Bavaria, lake Ammersee, Stegen, 11°08'07"E 48°04'32"N	HE573110	HE573072
<i>R. ampla</i> 4	Moll 53099	Germany, Bavaria, lake Ammersee, Stegen, 11°08'07"E 48°04'32"N	HE573111	HE573073
<i>R. ampla</i> 5	Moll 52193	Germany, Mecklenburg-Western Pomerania, lake Luebkowsee 2 km E of Schwichtenberg, 13°44.567'E 53°40.967'N	HE573112	—

Cont.

Code	Collection No. SNSD	Locality	ENA No.	
			cyt-b	ITS-2
<i>Radix labiata</i> (Rossmässler, 1835)				
<i>R. labiata</i> 1	Moll 49707	Czech Republic, České Budějovice, Ponesice, 14°28'60"E 49°06'00"N	HE798472	HE798450
<i>R. labiata</i> 2	Moll 49708	Czech Republic, České Budějovice, Ponesice, 14°28'60"E 49°06'00"N	HE798473	HE798451
<i>R. labiata</i> 3	Moll 51275	Germany, Saxony, pond near Langenberg, 12°51'21"E 50°33'09"N	HE798474	—
<i>R. labiata</i> 4	Moll 51276	Germany, Saxony, pond near Langenberg, 12°51'21"E 50°33'09"N	HE798475	HE573069
<i>R. labiata</i> 5	Moll 51277	Germany, Saxony, pond near Langenberg, 12°51'21"E 50°33'09"N	HE798476	HE798452
<i>R. labiata</i> 6	Moll 51696	Germany, Brandenburg, small lake near Wachow, 12°43'05"E 52°32'05"N	HE798477	HE577648
<i>R. labiata</i> 7	Moll 51697	Germany, Brandenburg, small lake near Wachow, 12°43'05"E 52°32'05"N	HE798478	HE573070
<i>R. labiata</i> 8	Moll 51698	Germany, Brandenburg, small lake near Wachow, 12°43'05"E 52°32'05"N	HE798479	HE573071
<i>R. labiata</i> 9	Moll 51699	Germany, Brandenburg, small lake near Wachow, 12°43'05"E 52°32'05"N	HE798480	HE798453
<i>R. labiata</i> 10	Moll 51863	Montenegro, Žabljak, Black Lake, sand pools, 19°05'42"E 43°08'50"N	HE798507	HE798455
<i>R. labiata</i> 11	Moll 51910	Germany, Saxony, Dehnitz, renatured swimming baths, 12°44'24"E 51°20'56"N	HE798481	HE798456
<i>R. labiata</i> 12	Moll 51964	Germany, Mecklenburg-Western Pomerania, Gressow, alder fenwod, 11°18.592'E 53°41.898'N	HE798482	HE798454
<i>R. labiata</i> 13	Moll 51965	Germany, Mecklenburg-Western Pomerania, Gressow, alder fenwod, 11°18.592'E 53°41.898'N	HE798483	—
<i>R. labiata</i> 14	Moll 52415	Austria, Carinthia, Hermagor, 13°22'E 46°37'N	HE798484	HE798457
<i>R. labiata</i> 15	Moll 52416	Austria, Carinthia, Hermagor, 13°22'E 46°37'N	HE798485	HE798458
<i>R. labiata</i> 16	Moll 52427	Italy, Lombardy, Province of Brescia, Borno, lower Varicla Lake, 2°15'24"W 45°58'50"N (Monte Mario)	HE798486	HE798459
<i>R. labiata</i> 17	Moll 52431	Italy, Lombardy, Province of Brescia, Ponte di Legno, Bleis Lake, 1°53'39"W 46°16'52"N (Monte Mario)	HE798487	HE798460
<i>R. labiata</i> 18	Moll 52432	Italy, Lombardy, Province of Brescia, Ponte di Legno, Bleis Lake, 1°53'39"W 46°16'52"N (Monte Mario)	HE798488	HE798461
<i>R. labiata</i> 19	Moll 52462	Germany, Bavaria, spring of the river Strogn near Buchrain, 11°58'41"E 48°13'24"N	HE798489	HE798462
<i>R. labiata</i> 20	Moll 52580	Germany, Saxony, Linz, five year old pond, 13°43'33"E 51°20'51"N	HE798490	HE798463
<i>R. labiata</i> 21	Collection U. Böbneck, TissueInver 3956	Spain, Santa Marina del Valdeon, Vega de Liordes, marshy headwater region, 1940 m, 4°50'09"W 43°09'11"N	HE798491	HE798464
<i>R. labiata</i> 22	Moll S172	France, Bourgogne, Département Cote-d'Or	HE798492	HE798465
<i>R. labiata</i> 23	Moll S284	Switzerland, Basel City, Riehen, well Nollenbrunnen, 7°40'17"E 47°34'41"N	HE798493	HE798466
<i>R. labiata</i> 24	Moll S2779	Germany, Saxony, Ammelshain, quarry Haselberg, 12°39'16"E 51°17'49"N	HE798494	HE798467
<i>R. labiata</i> 25	Moll S2780	Germany, Saxony, Ammelshain, quarry Haselberg, 12°39'16"E 51°17'49"N	HE798495	HE798468
<i>R. labiata</i> 26	Moll S2904	Germany, Saxony, small brook north of Tharandt, 13°34'19"E 51°00'08"N	HE798496	HE798469