Intraspecific morphological and genetic variability in the European freshwater snail

*Radix labiata* (Rossmaessler, 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 preputium 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 preputium 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* (Rossmaessler, 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 peregra*’, 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 southeast (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*; Huňova 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* Rossmaessler, 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 molusc 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-engeneering.com). Maximum-parsimony (MP) trees were reconstructed using PAUP (version 4.0b10; Swofford, 2002; settings: gapmode = NewState, addseq = closest, max-tree = 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

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

|                  | *Lymnaea stagnalis* | *Radix balthica* | *R. lagotis* | *R. auricularia* | *R. ampla* | *R. labiata* |
|------------------|---------------------|------------------|--------------|------------------|------------|-------------|
| *Lymnaea stagnalis* | 0.282              | -                | -            | -                | -          | -           |
| *Radix balthica*   | 0.308              | 0.225            | 0.086        | 0.156            | -          | -           |
| *R. lagotis*        | 0.281              | 0.199            | 0.146        | 0.090            | 0.185      | -           |
| *R. auricularia*    | 0.301              | 0.251            | 0.112        | 0.123            | 0.174      | 0.109       |
| *R. ampla*          | 0.278              | 0.203            | 0.130        | -                | -          |             |
| *R. labiata*        | 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 (Figs 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-

**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

| 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, 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).
to quantify the intraspecific variability in this species. The individuals of \textit{R. labiata} analysed form a clade, which includes a toptype of \textit{R. labiata} from Tharandt (\textit{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, \textit{R. labiata}, despite their morphological variability and that they clearly differ from the other \textit{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 \textit{R. balthica} groups as the sister of \textit{R. ampla} and these two species form the sister group to \textit{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 \textit{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 \textit{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 geographic 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 \textit{R. balthica} (Schniebs et al., 2011).

\textbf{Morphology}

The variability of the shell of \textit{R. labiata} (Fig. 4) is less than the variation we found in \textit{R. balthica} (Schniebs et al., 2011). Shells of \textit{R. balthica} could be confused with these of \textit{R. auricularia}, \textit{R. ampla}, \textit{R. labiata} and \textit{R. lagotis} because they could be ear-shaped, amploid to nearly conical egg-shaped (Schniebs et al., 2011, Fig. 4). In most specimens of \textit{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 \textit{Stagnicola} (Fig. 4d), similar to those Zettler et al. (2006) mentioned for specimens from Mecklenburg-Vorpommern. Gittenberger et al. (1998) also indicated that \textit{R. labiata} could be confused with dwarf forms of \textit{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. 6. Variability in *Radix labiata* mantle pigmentation: a) Germany, Mecklenburg-Vorpommern, Gressow, alder fen-wood (*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 fen-wood (*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 fen-wood (*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 0.71 to 2.3:1. Kruglov and Starobogatov (1983) and Kruglov (2005) gave a value of 0.66 and 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
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 rep-
resentatives of the genus Stagnicola can be distin-
guished 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 vaginal and
provaginal duct, as in R. labiata.

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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   |
| **Planorbarius corneus** (Linnaeus, 1758) | | | | |
| P. corneus 1 | Moll 52556 | | Germany, Saxony, Linz, pond Goldgrubenteich, 13°43'09"E 51°19'45"N | FR979880 | FR979830 |
| P. corneus 2 | Moll 52557 | | Germany, Saxony, Linz, pond Goldgrubenteich, 13°43'09"E 51°19'45"N | FR979881 | FR979831 |
| **Lymnaea stagnalis** (Linnaeus, 1758) | | | | |
| L. stagnalis 1 | Moll 49239 | | Germany, Saxony, Dresden-Zschieren, old branch of river Elbe, 13°52'28"E 50°59'50"N | HE573102 | HE573064 |
| L. stagnalis 2 | Moll 49835 | | Germany, Saxony, Niederspee, small pond, 14°54'03"E 51°24'28"N | HE573103 | HE573065 |
| L. stagnalis 3 | Moll 53108 | | Germany, Baden-Württemberg, Konstanz-Egg, ditch Hockgraben, 9°11'34.2"E 47°40'57.3"N | FR979894 | FR979834 |
| L. stagnalis 4 | Moll 53109 | | Germany, Baden-Württemberg, Konstanz-Egg, ditch Hockgraben, 9°11'34.2"E 47°40'57.3"N | FR979895 | FR979835 |
| **Radix auricularia** (Linnaeus, 1758) | | | | |
| R. auricularia 1 | Moll 50005 | | Germany, Saxony, Niederspee, pond Neuwiesenteich, 14°52'57"E 51°24'19"N | HE573104 | HE573066 |
| R. auricularia 2 | Moll 50079 | | Germany, Saxony, pond Vierteich near Freiteilsdorf, 13°4'15"E 51°15'43"N | HE573105 | HE573067 |
| R. auricularia 3 | Moll 53070 | | Germany, Bavaria, Weichering, pond in riverside forest, 11°19'23.6"E 48°43'34.1"N | FR979902 | FR979842 |
| R. auricularia 4 | Moll 53071 | | Germany, Bavaria, Weichering, pond in riverside forest, 11°19'23.6"E 48°43'34.1"N | FR979903 | FR979843 |
| **Radix balthica** (Linnaeus, 1758) | | | | |
| R. balthica 1 | Moll 51283 | | Switzerland, canton Basel-Landschaft, Liestal, Orishof, 07°43'03"E 47°28'22"N | HE573133 | HE573082 |
| R. balthica 2 | Moll 51834 | | Germany, Saxony, Dresden-Kleisschachwitz, river Elbe, 13°52'21"E 51°00'03"N | HE573119 | HE573079 |
| R. balthica 3 | Moll 53111 | | Germany, Baden-Württemberg, Konstanz-Egg, pond near University, 09°11'29"E 47°41'09"N | HE573116 | HE573078 |
| R. balthica 4 | Moll 53112 | | Germany, Baden-Württemberg, Konstanz-Egg, pond near University, 09°11'29"E 47°41'09"N | HE573117 | HE577649 |
| **Radix lagotis** (Schrank, 1803) | | | | |
| AJ319638 | — | | Czech Republic, Kadov, Vasi and Podkadowsky pond | — | AJ319638 |
| AJ319639 | — | | Austria, Schoenau, southeast of Vienna | — | AJ319639 |
| R. lagotis 1 | Moll 49868 | | Germany, Saxony, pond Vierteich near Freiteilsdorf, 13°4'15"E 51°15'43" | HE573114 | HE573076 |
| R. lagotis 2 | Moll 52563 | | Saxony, pond Goldgrubenteich near Linz, 13°43'09"E 51°19'46"N | HE573115 | HE573077 |
| R. lagotis 3 | Moll 53239 | | Saxony, dam Doellnitzsee near Mutzschen, 12°55'18"E 51°15'45"N | HE573113 | HE573075 |
| **Radix ampla** (Hartmann, 1821) | | | | |
| R. ampla 1 | Moll 51112 | | Russia, Chelyabinsk Region, river Miass near Dynamo village, 60°02'46.1"E 53°15'45"N | HE798470 | HE798448 |
| R. ampla 2 | Moll 51113 | | Russia, Chelyabinsk Region, river Miass near Dynamo village, 60°02'46.1"E 53°15'45"N | HE798471 | HE798449 |
| R. ampla 3 | Moll 53098 | | Germany, Bavaria, lake Ammersee, Stegen, 11°08'07"E 48°04'32"N | HE573110 | HE573072 |
| R. ampla 4 | Moll 53099 | | Germany, Bavaria, lake Ammersee, Stegen, 11°08'07"E 48°04'32"N | HE573111 | HE573073 |
| R. ampla 5 | Moll S2193 | | Germany, Mecklenburg-Western Pomerania, lake Luebkowsee 2 km E of Schwichtenberg, 13°44.567°E 47°40.567"N | HE573112 | — |
| Code | Collection No. SNSD | Locality | ENA No. |
|------|---------------------|----------|--------|
|      |                     |          |        |
| **Radix labiata** (Rossmässler, 1835) |
| **R. labiata** 1 | Moll 49707 | Czech Republic, České Budějovice, Ponesice, 14°28'60"E 49°06'00"N | HE798472 HE798450 |
| **R. labiata** 2 | Moll 49708 | Czech Republic, České Budějovice, Ponesice, 14°28'60"E 49°06'00"N | HE798473 HE798451 |
| **R. labiata** 3 | Moll 51275 | Germany, Saxony, pond near Langenberg, 12°51'21"E 50°33'09"N | HE798474 — |
| **R. labiata** 4 | Moll 51276 | Germany, Saxony, pond near Langenberg, 12°51'21"E 50°33'09"N | HE798475 HE798459 |
| **R. labiata** 5 | Moll 51277 | Germany, Saxony, pond near Langenberg, 12°51'21"E 50°33'09"N | HE798476 HE798452 |
| **R. labiata** 6 | Moll 51696 | Germany, Brandenburg, small lake near Wachow, 12°43'05"E 52°32'05"N | HE798477 HE798468 |
| **R. labiata** 7 | Moll 51697 | Germany, Brandenburg, small lake near Wachow, 12°43'05"E 52°32'05"N | HE798478 HE798459 |
| **R. labiata** 8 | Moll 51698 | Germany, Brandenburg, small lake near Wachow, 12°43'05"E 52°32'05"N | HE798479 HE798451 |
| **R. labiata** 9 | Moll 51699 | Germany, Brandenburg, small lake near Wachow, 12°43'05"E 52°32'05"N | HE798480 HE798453 |
| **R. labiata** 10 | Moll 51863 | Montenegro, Žabljak, Black Lake, sand pools, 19°05'42"E 43°08'50"N | HE798507 HE798455 |
| **R. labiata** 11 | Moll 51910 | Germany, Saxony, Dehnitz, renaured swimming baths, 12°44'24"E 51°20'56"N | HE798481 HE798456 |
| **R. labiata** 12 | Moll 51964 | Germany, Mecklenburg-Western Pomerania, Gressow, alder fenwed, 11°18.592'E 53°41.898"N | HE798482 HE798454 |
| **R. labiata** 13 | Moll 51965 | Germany, Mecklenburg-Western Pomerania, Gressow, alder fenwed, 11°18.592'E 53°41.898"N | HE798483 — |
| **R. labiata** 14 | Moll 52415 | Austria, Carinthia, Hermagor, 13°22'E 46°37'N | HE798484 HE798457 |
| **R. labiata** 15 | Moll 52416 | Austria, Carinthia, Hermagor, 13°22'E 46°37'N | HE798485 HE798458 |
| **R. labiata** 16 | Moll 52427 | Italy, Lombardy, Province of Brescia, Borno, lower Varicla Lake, 4°15'24"W 45°58'50"N | HE798486 HE798459 |
| **R. labiata** 17 | Moll 52431 | Italy, Lombardy, Province of Brescia, Ponte di Legno, Bleis Lake, 1°53'39"W 46°16'52"N | HE798487 HE798460 |
| **R. labiata** 18 | Moll 52432 | Italy, Lombardy, Province of Brescia, Ponte di Legno, Bleis Lake, 1°53'39"W 46°16'52"N | HE798488 HE798461 |
| **R. labiata** 19 | Moll 52462 | Germany, Bavaria, spring of the river Strogn near Buchrain, 11°58'41"E 48°13'24"N | HE798489 HE798462 |
| **R. labiata** 20 | Moll 52580 | Germany, Saxony, Linz, five year old pond, 13°43'33"E 51°20'51"N | HE798490 HE798463 |
| **R. labiata** 21 | Collection U. Bößneck, Tissuelver 3956 | Spain, Santa Marina del Valdeon, Vega de Liordes, marshy headwater region, 1940 m, 4°50'09"W 43°09'11"N | HE798491 HE798464 |
| **R. labiata** 22 | Moll S172 | Switzerland, Basel City, Riehen, well Nollenbrunnen, 1°18.592'E 53°41.898"N | HE798492 HE798465 |
| **R. labiata** 23 | Moll S284 | Switzerland, Basel City, Riehen, well Nollenbrunnen, 1°18.592'E 53°41.898"N | HE798493 HE798466 |
| **R. labiata** 24 | Moll S2779 | Germany, Saxony, Ammelshain, quarry Haselberg, 12°39'16"E 51°17'49"N | HE798494 HE798467 |
| **R. labiata** 25 | Moll S2780 | Germany, Saxony, Ammelshain, quarry Haselberg, 12°39'16"E 51°17'49"N | HE798495 HE798468 |
| **R. labiata** 26 | Moll S2904 | Germany, Saxony, small brook north of Tharandt, 13°34'19"E 51°00'08"N | HE798496 HE798469 |