

INTRASPECIFIC MORPHOLOGICAL AND GENETIC VARIABILITY IN *RADIX BALTHICA* (LINNAEUS 1758) (GASTROPODA: BASOMMATOPHORA: LYMNÆIDAE) WITH MORPHOLOGICAL COMPARISON TO OTHER EUROPEAN *RADIX* SPECIES

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Abstract *Radix balthica* is a morphologically very variable species that is often very difficult to determine on the basis of the shell characters. Since DNA-taxonomy is still expensive and requires much more time than anatomical determination, and the methodology is hardly available for most colleagues that work faunistically, this work aims to broaden knowledge of the intraspecific variability in the more important systematic characters used for determination of *Radix balthica*. To find reliable distinguishing characters from other *Radix*-species, an integrative approach was sought and data from three different sources were acquired for analysis. Molecular sequence data of the gene fragments ITS-2 and/or *cyt-b* was obtained for 58 individuals in order to study the species identity of *Radix balthica* (Linnaeus 1758). Of the subsample of 24 individuals for which both gene fragments were available, the variability of several characters that are commonly used for determination was documented. These include shell morphology, mantle pigmentation, shape and position of the bursa copulatrix, length and position of the bursa duct, and length ratio of praeputium to penis sheath. Morphological distinguishing characters from *R. auricularia*, *R. labiata*, *R. lagotis* and *R. ampla* are discussed and summarised in a table. Analysis of the network of haplotypes (*cyt-b*) shows that there is no distinct correlation to the geographic distribution pattern.

Key words *Radix balthica*, *R. auricularia*, *R. labiata*, *R. lagotis*, *R. ampla*, morphology, molecular genetics, variation

INTRODUCTION

The common pond snail *Radix balthica* (Linnaeus 1758) is a Palaearctic species widely distributed from Iceland (Mandahl-Barth, 1938) and Norway (Økland, 1990) in the north, Ireland and Great Britain (Kerney, 1999) as well as Spain in the west, up to Southern Siberia in the east (Kruglov, 2005), and N-Africa in the south (Brown, 1994; van Damme 1984).

This euryoecious species prefers low-altitude running and standing freshwater bodies such as lakes, ponds, drainage ditches and lentic zones of rivers, rich in nutrients and submerged vegetation (Glöer & Diercking, 2010). In the brackish water areas of the Baltic Sea it can tolerate a salinity of 10–15 psu (Zettler *et al.*, 2006).

Reliable identification of this species is not only important for malacologists working on faunistics. The exact determination of *Radix* species is

important because some act as vectors of human fascioliasis (e.g. Bargues & Mas-Coma, 2005). On the base of his laboratory studies, Kruglov (2005) reported *R. balthica* as a possible vector of fascioliasis. Under artificial conditions the species is susceptible to *Fasciola hepatica* invasion. It is also essential to have accurate identification when using them in climate modelling predictions (Cordellier & Pfenninger, 2009).

The shells of *Radix* species show an enormous variation in Europe that may be influenced by ecological (Pfenninger *et al.*, 2006) and other conditions. For instance, the growth of *Radix* shells (including *R. balthica*) can increase by 10% (Ward *et al.*, 1997) through infection of the snail by trematodes. Lakowitz *et al.* (2008) identified a predator-induced phenotypic plasticity in *R. balthica* shells. These facts make the traditional determination based only on shell morphology very difficult. Also Pfenninger *et al.* (2006) concluded that taxonomic distinction of species in

this genus cannot be based on shell morphology and proposed DNA-taxonomy for species identification.

In the past years it has become common practice for determination to take additional anatomical characters into account, as well as mantle pigmentation (e.g. Jackiewicz, 1993, 1998, 2000; Gittenberger *et al.*, 1998; Glöer, 2002; Glöer & Meier-Brook, 2003; Stadnichenko, 2004; Kruglov, 2005). Nevertheless, determination often remained unreliable, since the variability of each of the characters had not been studied and documented sufficiently. Furthermore, the different species concepts of authors can lead to difficulties and confusion.

To analyse the variability of the most important distinguishing characters of *Radix balthica* (shell morphology, mantle pigmentation, shape and position of the bursa copulatrix, length and position of the bursa duct, and length ratio of praeputium to penis sheath), 58 individuals of several European localities, as well as from Kazakhstan and Siberia were studied anatomically. Additionally, we attempted to obtain sequence data of the complete nuclear ITS-2 spacer and a 370 bp fragment of the *cyt-b* gene as mitochondrial markers for these individuals. The variability of several characters that are commonly used for determination was documented in the 24 individuals for which both gene fragments were available.

MATERIAL AND METHODS

Since anatomical material that could be used for genetic analyses of the type specimen of *Radix balthica* is not available, our investigations are based on "topotypes" from Øland, which lies geographically close to the original type locality i.e. the beach of Gotland. Since the neotype of *R. balthica* defined by Kruglov & Starobogatov (1983) was collected in the vicinity of Stockholm, the validity of that can be questioned because of its relative distance from the type locality (ICZN 75.3.6). All Swedish *R. balthica* used in the present study was collected in Øland (Fig. 8. 1, 2). Nevertheless, the shell is almost identical with that of the neotype of Kruglov & Starobogatov 1983 and the anatomy of specimens from Øland is identical with that of *R. balthica* sensu Kruglov & Starobogatov.

For outgroup comparison in the molecular genetic analyses we used Palaearctic specimens of the species *Planorbarius corneus* (Linnaeus 1758) and *Aplexa hypnorum* (Linnaeus 1758). As the ingroup we used *Lymnaea stagnalis* (Linnaeus 1758), *Radix labiata* (Rossmässler 1835), *R. ampla* (Hartmann 1821), *R. auricularia* (Linnaeus 1758) and *R. lagotis* (Schrank 1803).

Morphology Snails were fixed in 70–80% ethanol. Shell morphology, mantle pigmentation and anatomy were documented from the specimens studied. The dissections and measurements of the genital organs and shells were carried out using stereo microscopes (Zeiss and Olympus). Photographs were taken using a digital camera system (Leica R8).

All specimens used for molecular examination are listed in Table 1. They were collected or donated for this study and stored in the Molluscan Collection of the Senckenberg Natural History Collections Dresden, Museum of Zoology (SNSD) (see under <http://sesam.senckenberg.de/> for information additional to that in Table 1).

Molecular techniques – DNA extraction Tissue samples were taken under a microscope from the soles of the snails and fixed in 100% ethanol. The samples were registered in the tissue collection of the SNSD with a new collection number (additional to the collection number of the specimen in the molluscan collection) of SNSD and stored at –80°C.

DNA was extracted using DTAB (dodecyl trimethyl ammonium bromide) buffer (Gustincich *et al.*, 1991). The tissue samples were washed with 100 µl TE (Tris-EDTA) buffer and subsequently incubated with 500 µl of preheated DTAB for 30 min at 65°C. The incubation was continued after adding 10 µl Proteinase K (50mg/ml) for 20–24 hours, followed by a short incubation with 10 µl RNase (10 mg/ml) for 30 min at 37°C. Remaining tissue fragments disintegrated after vortexing. For cleaning, 550 µl chloroform/isoamyl alcohol (24:1) was used. The samples were vortexed for 20 s and the phases subsequently separated again at 12,000 g for 3 min. With the upper aqueous phase the procedure was repeated. 100 µl 4M LiCl and 400 µl isopropanol were added to the aqueous phase for precipitation. The samples were cooled at –20°C for 30 min and subsequently the DNA was pelleted

Table 1 Material used in the molecular genetic studies.

Code	Collection No. SNSD	Locality	Genbank No. CytB	ITS-2
<i>Planorbarius corneus</i> (Linnaeus, 1758)				
Linz-1	Moll 52556	Germany, Saxony, Linz, pond Goldgrubenteich, 13°43'09"E 51°19'45"N	ENA FR797880	ENA FR797830
Linz-2	Moll 52557	Germany, Saxony, Linz, pond Goldgrubenteich, 13°43'09"E 51°19'45"N	ENA FR797881	ENA FR797831
<i>Aplexa hypnorum</i> (Linnaeus, 1758)				
Ne-1	Moll S348	Germany, Mecklenburg-Vorpommern, lake Nebel, 12°42'02"E 53°15'32"N	ENA FR797882	ENA FR797832
Ne-2	Moll S350	Germany, Mecklenburg-Vorpommern, lake Nebel, 12°42'02"E 53°15'32"N	ENA FR797883	ENA FR797833
<i>Lymnaea stagnalis</i> (Linnaeus, 1758)				
Kon-1	Moll 53108	Germany, Baden-Württemberg, Konstanz-Egg, ditch Hockgraben, 9°11'34.2"E 47°40'57.3"N	ENA FR797894	ENA FR797834
Kon-2	Moll 53109	Germany, Baden-Württemberg, Konstanz-Egg, ditch Hockgraben, 9°11'34.2"E 47°40'57.3" N	ENA FR797895	ENA FR797835
DD	Moll 49239	Germany, Saxony, Dresden-Zschieeren, old branch of river Elbe, 13°52'28"E 50°59'50"N	ENA HE573102	ENA HE573064
NS	Moll 49835	Germany, Saxony, Niederspree, small pond, 14°54'03"E 51°24'28"N	ENA HE573103	ENA HE573065
<i>Radix auricularia</i> (Linnaeus, 1758)				
Wei-1	Moll 53071	Germany, Bavaria, Weichering near Ingolstadt, pond in the riverside forest of Danube 11°19'23.6"E 48°43'34.1"N	ENA FR797903	ENA FR797843
Wei-2	Moll 53072	Germany, Bavaria, Weichering near Ingolstadt, pond in the riverside forest of Danube 11°19'23.6"E 48°43'34.1"N	ENA FR797904	ENA FR797844
Nie	Moll 50005	Germany, Saxony, Niederspree, pond Neuwiesenteich, 14°52'57"E 51°24'19"	ENA HE573104	ENA HE573066
Frei	Moll 50079	Germany, Saxony, pond Vierteich near Freitelsdorf, 13°41'57"E 51°15'43"	ENA HE573105	ENA HE573067
<i>Radix labiata</i> (Rossmässler, 1835)				
Lan-1	Moll 51275	Germany, Saxony, pond near Langenberg, 12°51'21"E 50°33'09"N	ENA HE573106	ENA HE573068
Lan-2	Moll 51276	Germany, Saxony, pond near Langenberg, 12°51'21"E 50°33'09"N	ENA HE573107	ENA HE573069
Wach-1	Moll 51697	Germany, Brandenburg, small lake near Wachow, 12°43'05"E 52°32'05"N	ENA HE573108	ENA HE573070
Wach-2	Moll 51698	Germany, Brandenburg, small lake near Wachow, 12°43'05"E 52°32'05"N	ENA HE573109	ENA HE573071
<i>Radix ampla</i> (Hartmann, 1821)				
Am-1	Moll 53098	Germany, Bavaria, lake Ammersee, Stegen, 11°08'07"E 48°04'32"N	ENA HE573110	ENA HE573072

Code	Collection No. SNSD	Locality	Genbank No. CytB	ITS-2
Am-2	Moll 53099	Germany, Bavaria, lake Ammersee, Stegen, 11°08'07"E 48°04'32"N	ENA HE573111	ENA HE573073
Lueb	Moll S2193	Mecklenburg-Vorpommern, lake Luebkowsee 2 km east of Schwichtenberg, 13°44.567'E 53°40.967'N	ENA HE573112	ENA HE573074
<i>Radix lagotis</i> (Schrank, 1803)				
AJ319638		Czech Republic, Kadov, Vasi and Podkadovsky pond	–	GenBank AJ319638
AJ319639		Austria, Schoenau, southeast of Vienna	–	GenBank AJ319639
Doe	Moll 53239	Saxony, dam Doellnitzsee near Mutzschen, 12°55'18"E 51°15'45"N	ENA HE573113	ENA HE573075
Fr	Moll 49868	Saxony, pond Vierteich near Freitelsdorf, 13°41'59"E 51°15'39"N	ENA HE573114	ENA HE573076
Linz	Moll 52563	Saxony, pond Goldgrubenteich near Linz, 13°43'09"E 51°19'46"N	ENA HE573115	ENA HE573077
<i>Radix balthica</i> (Linnaeus, 1758)				
Kon-1	Moll 53111	Germany, Baden-Württemberg, Konstanz-Egg, pond near University, 09°11'29"E 47°41'09" N	ENA HE573116	ENA HE573078
Kon-2	Moll 53112	Germany, Baden-Württemberg, Konstanz-Egg, pond near University, 09°11'29"E 47°41'09" N	ENA HE573117	–
DD-1	Moll 51833	Germany, Saxony, Dresden-Kleizschachwitz, river Elbe, 13°52'21"E 51°00'03"N	ENA HE573118	–
DD-2	Moll 51834	Germany, Saxony, Dresden-Kleizschachwitz, river Elbe, 13°52'21"E 51°00'03"N	ENA HE573119	ENA HE573079
Sig-1	Moll 52663	Germany, Baden-Württemberg, river Danube near Sigmaringendorf, 09°15'49.36"E 48°03'45.54" N	ENA HE573120	ENA HE573080
Sig-2	Moll 52665	Germany, Baden-Württemberg, river Danube near Sigmaringendorf, 09°15'49.36"E 48°03'45.54" N	ENA HE573121	–
Wald	Moll 52685	Germany, Baden-Württemberg, Waldbeuren, small creek in a meadow, 09°21'02.39"E 47°54'34.32" N	ENA HE573122	–
Phil-1	Moll S2202	Germany, Baden-Württemberg, Philippsburg, 49°12'16.39" N ditch Geißböckelgraben, 08°27'06.12"E	ENA HE573123	–
Phil-2	Moll S2203	Germany, Baden-Württemberg, Philippsburg, ditch Geißböckelgraben, 08°27'06.12"E 49°12'16.39" N	ENA HE573124	–
Ihl	Moll S2198	Germany, Schleswig-Holstein, lake Ihlsee North of Bad Segeberg, 10°17.533'E 53°57.633'N	ENA HE573125	–
Zsch-1	Moll S369	Germany, Saxony, Dresden, Zschoner Grund, pond Mühlteich, 13°38'26"E 51°03'30"E	ENA HE573126	–

Code	Collection No. SNSD	Locality	Genbank No. CytB	ITS-2
Zsch-2	Moll S370	Germany, Saxony, Dresden, Zschoner Grund pond Mühlteich, 13°38'26"E 51°03'30"E	ENA HE573127	–
Zsch-3	Moll S371	Germany, Saxony, Dresden, Zschoner Grund pond Mühlteich, 13°38'26"E 51°03'30"E	ENA HE573128	–
Zsch-4	Moll S374	Germany, Saxony, Dresden, Zschoner Grund pond Mühlteich, 13°38'26"E 51°03'30"E	ENA HE573129	–
Th-1	Moll S133	France, Region Centre, Thenay, small creek 01°17'31"E 47°23'22"N	ENA HE573130	–
Th-2	Moll S135	France, Region Centre, Thenay, small creek 01°17'31"E 47°23'22"N	ENA HE573131	–
Lie-1	Moll 51282	Switzerland, canton Basel-Landschaft, Liestal, Orishof, 07°43'03"E 47°28'22"N	ENA HE573132	ENA HE573081
Lie-2	Moll 51283	Switzerland, canton Basel-Landschaft, Liestal, Orishof, 07°43'03"E 47°28'22"N	ENA HE573133	ENA HE573082
Rie-1	Moll 51292	Switzerland, canton Basel City, Riehen, Wiesengriener, 07°38'32"E 47°35'21"N	ENA HE573134	ENA HE573083
Rie-2	Moll 51293	Switzerland, canton Basel City, Riehen, Wiesengriener, 07°38'32"E 47°35'21"N	ENA HE573135	ENA HE573084
Mu-1	Moll 52736	Germany, Saxony, Mutzschen, river Mutzschener Wasser, 12°53'24"E 51°15'32"N	ENA HE573136	ENA HE573085
Mu-2	Moll 52737	Germany, Saxony, Mutzschen, river Mutzschener Wasser, 12°53'24"E 51°15'32"N	ENA HE573137	ENA HE573086
Ko-1	Moll 51118	Kazakhstan, Akmolinsk region, lake Kopa near Kokshetau, 69°22'14"E 53°17'22"N	–	ENA HE573087
Ko-2	Moll 51119	Kazakhstan, Akmolinsk region, lake Kopa near Kokshetau, 69°22'14"E 53°17'22"N	ENA HE573138	ENA HE573088
Mi-1	Moll 52412	Croatia, lake Milanovac near Plitvica, 15°36'34"E 44°53'45"N	ENA HE573139	ENA HE573089
Mi-2	Moll 52411	Croatia, lake Milanovac near Plitvica, 15°36'34"E 44°53'45"N	ENA HE573140	–
Oe	Moll 51860	Sweden, Øland, east shore near Lille Seby, 16.565°E 56.345°N		ENA HE573141
Hi-1	Moll 51894	Germany, North Rhine-Westphalia, Hillegossen, creek Meyerbach, 08°36'04"E 51°59'11"N	ENA HE573142	ENA HE573091
Hi-2	Moll 51895	Germany, North Rhine-Westphalia, Hillegossen, creek Meyerbach, 08°36'04"E 51°59'11"N	ENA HE573143	–
Wil	Moll 51865	Germany, North Rhine-Westphalia, Wilkenhoeher, clay pit, 08°34'15.87"E 52°05'42.59"N	ENA HE573144	–

Code	Collection No. SNSD	Locality	Genbank No. CytB	ITS-2
Del-1	Moll 51897	Germany, North Rhine-Westphalia, Joellenbeck, Deliusiek, small pond, 08°31'22.16"E 52°05'20.80"N	ENA HE573145	–
Del-2	Moll 51898	Germany, North Rhine-Westphalia, Joellenbeck, Deliusiek, small pond, 08°31'22.16"E 52°05'20.80"N	ENA HE573146	–
Bu	Moll 53279	Germany, Brandenburg, river Havel near Buetzer, 12.3051°E 52.5375°N	ENA HE573147	ENA HE573092
Kau	Moll 51024	Germany, Saxony, creek Geberbach near dam Kauscha, 13°46'42"E 50°59'26"N	ENA HE573148	ENA HE573093
Am-1	Moll 53105	Germany, Bavaria, river Amper near lake Ammersee, 11°07'43"E 48°04'43"N		ENA HE573149
Am-2	Moll 53106	Germany, Bavaria, river Amper near lake Ammersee, 11°07'43"E 48°04'43"N		ENA HE573150
Am-3	Moll 53107	Germany, Bavaria, river Amper near lake Ammersee, 11°07'43"E 48°04'43"N		ENA HE573151
Schw-1	Moll 52467	Germany, Bavaria, Schwillach-Quelle between Hörlkofen and Erding, 11°55'E 48°15'N	ENA HE573152	–
Schw-2	Moll 52468	Germany, Bavaria, Schwillach-Quelle between Hörlkofen and Erding, 11°55'E 48°15'N	ENA HE573153	–
Toll	Moll 53293	Germany, Mecklenburg-Vorpommern, lake Tollensesee near Klein Nemerow, 13.2146° E 53.4909°N	ENA HE573154	–
Flee	Moll S334	Germany, Mecklenburg-Vorpommern, Oberbek south of Fleeth, 12°50'59.76"E 53°12'42.41"N	ENA HE573155	–
Rue-1	Moll S1299	Germany, Saxony, Ruesdorf, ditch, 12°40'56"E 50°46'59"N	ENA HE573156	–
Rue-2	Moll S1300	Germany, Saxony, Ruesdorf, ditch, 12°40'56"E 50°46'59"N	ENA HE573157	–
Tor-1	Moll S2150	Germany, Mecklenburg-Vorpommern, lake Torgelower See, 12°46.622'E 53°34.252'N	ENA HE573158	ENA HE573095
Tor-2	Moll S2151	Germany, Mecklenburg-Vorpommern, lake Torgelower See, 12°46.622'E 53°34.252'N	ENA HE573159	ENA HE573096
Tie-1	Moll S2174	Germany, Mecklenburg-Vorpommern, lake Tiefwareensee, 12°41.258'E 53°32.332'N	ENA HE573160	ENA HE573097
Tie-2	Moll S2175	Germany, Mecklenburg-Vorpommern, lake Tiefwareensee, 12°41.258'E 53°32.332'N	ENA HE573161	ENA HE573098
Ma	Moll S1743	Spain, Mallorca, Tramuntana Mountains, La Granja Manor near Esporles, spring streams, 02°33'33"E 39°40'08"N	ENA HE573162	ENA HE573099

Code	Collection No. SNSD	Locality	Genbank No. CytB	ITS-2
Gre-1	Moll S2099	Germany, Saxony, nature reserve "Alte See Grethen", muddy ditch, 12°40'19"E 51°13'44"N	ENA HE573163	ENA HE573100
Gre-2	Moll S2100	Germany, Saxony, nature reserve "Alte See Grethen", muddy ditch, 12°40'19"E 51°13'44"N	ENA HE573164	–
HH-1	Moll S965	Germany, Hamburg, Ruschort, temporary ditch, 10°03'32.27"E 53°30'04.46"N	ENA HE573165	–
HH-2	Moll S966	Germany, Hamburg, Ruschort, temporary ditch, 10°03'32.27"E 53°30'04.46"N	ENA HE573166	–
Mer-1	Moll S144	France, Mérignac near Bordeaux, 01°10'04.79"E 45°47'20.96"N	ENA HE573167	–
Mer-2	Moll S147	France, Mérignac near Bordeaux, 01°10'04.79"E 45°47'20.96"N	ENA HE573168	–
Gro	Moll 48597	Germany, Saxony, Großbardau, creek Springbach, 12°40'08"E 51°12'32"N	ENA HE573169	–
Moos-1	Moll S251	Switzerland, Basel, Mooswäldchen, 07°39'43"E 47°34'55"N	ENA HE573170	–
Moos-2	Moll S252	Switzerland, Basel, Mooswäldchen, 07°39'43"E 47°34'55"N	ENA HE573171	–
Mot	Moll 51139	Russia, lake Motshishtshe near Tomsk, 84.91333°E 56.219496°N	ENA HE573172	–

by centrifugation at 11,200 g for 20 min at 4°C. The liquid was disposed of and the pellets were dried by inverting the tubes on a paper towel. The pellets were cleaned twice with 200 µl ice-cold 70% ethanol. The DNA pellets were dried 10 min at 50°C and subsequently redissolved in 50 µl of TE buffer.

Molecular techniques – Polymerase Chain Reaction (PCR) and purification of PCR products The PCRs were carried out in a final volume of 20 µl with quantities of DNA from 0.5–5.0 µl depending on the concentration estimated by gel electrophoresis, 2 µl 10× PCR buffer (Bioron, incomplete), 1 µl MgCl₂ (Bioron, 0.055 µS/cm), 1 µl of each primer (10 pmol/µl), 0.5 µl dNTP (10 mM), 0.2 µl Taq DNA polymerase (DFS-Taq, Bioron) and the corresponding volume of sterile water.

From the *cyt-b* gene a region of about 370 bp was amplified with the primers UCytb151F and UCytb270R (Merritt *et al.*, 1998) and a temperature profile of 94°C 4 min (94°C 40 s, 48°C 40 s, 72°C 1.15 min)×40, 72°C 6 min, 8°C hold.

The primers used for ITS-2 were LT1 (Bargues *et al.*, 2001) and ITS2-Rixo (Almeyda-Artigas *et al.*, 2000). The temperature profile used was the following: 94°C 4 min (94°C 30 s, 50°C 30 s, 72°C 1 min)×40, 72°C 7 min, 8°C hold.

PCR products were purified with 0.1 µl Exo Sap-It plus 4 µl double-distilled water and incubation for 30 min at 37°C, and deactivation for 15 min at 80°C.

Molecular techniques – DNA sequencing The primers used for the cycle sequencing were UCytb151F for *cyt-b* and LT1 for ITS-2. Samples were sequenced in both directions if necessary. The quantity of PCR product used for cycle-sequencing ranged from 0.5–5.0 µl depending on the concentration estimated by gel electrophoresis. The following were added were added to make a final volume of 10 µl in sterile water: 0.5 µl BigDye T-Mix (ABI, Applied Biosystems); 2.25 µl BigDye buffer (5x); 0.5 µl primer (10 pmol). The following temperature profile was used: (96°C 10 s, 50°C 5 s, 60°C 4 min)×25, 8°C hold. The products were purified by adding

1 µl 3M NaAc (pH 4.6) and 25 µl EtOH (100%), centrifuging at 13 000 g for 15 min., inverting the tubes on a paper towel and washing with 200 µl 70% EtOH. After removing the EtOH the pellets were dried for 10 min at 50°C. Samples were sequenced on an ABI 3130 xl (Applied Biosystems).

Molecular techniques – sequence alignments
Alignment was performed by eye using BioEdit Sequence Alignment Editor (Hall, 1999). It was demanding for ITS-2 sequences, which is why we repeated it three times independently. Since the results were the same in all three trials, we accepted the alignment for analyses.

Phylogenetic analyses of sequences
For maximum-likelihood analyses, including bootstrap support, we used raxmlGUI 0.9 beta 2 (RAxML) (Silvestro & Michalak, 2010; Stamatakis *et al.*, 2005). The settings were “ML+thorough bootstrap” with 100 (replicate) runs and 1000 (bootstrap) repetitions.

Maximum-parsimony (MP) trees were reconstructed using PAUP (version 4.0b10; Swofford, 2002; settings: gapmode=NewState, addseq=closest, maxtree=10000). For presentation of the MP results, one of the best trees was chosen to be able to illustrate branch lengths (one showing the same overall topology as the majority rule consensus tree was chosen).

Genetic distances of the cyt-b were calculated using MEGA version 4 (Tamura *et al.*, 2007). This program was also used to produce the dataset of cyt-b sequences without missing data for network analyses (median joining; Bandelt *et al.*, 1999) with the program “Network” (www.fluxus-engineering.com). This dataset contained 308 bp of 58 individuals.

RESULTS

Molecular genetics

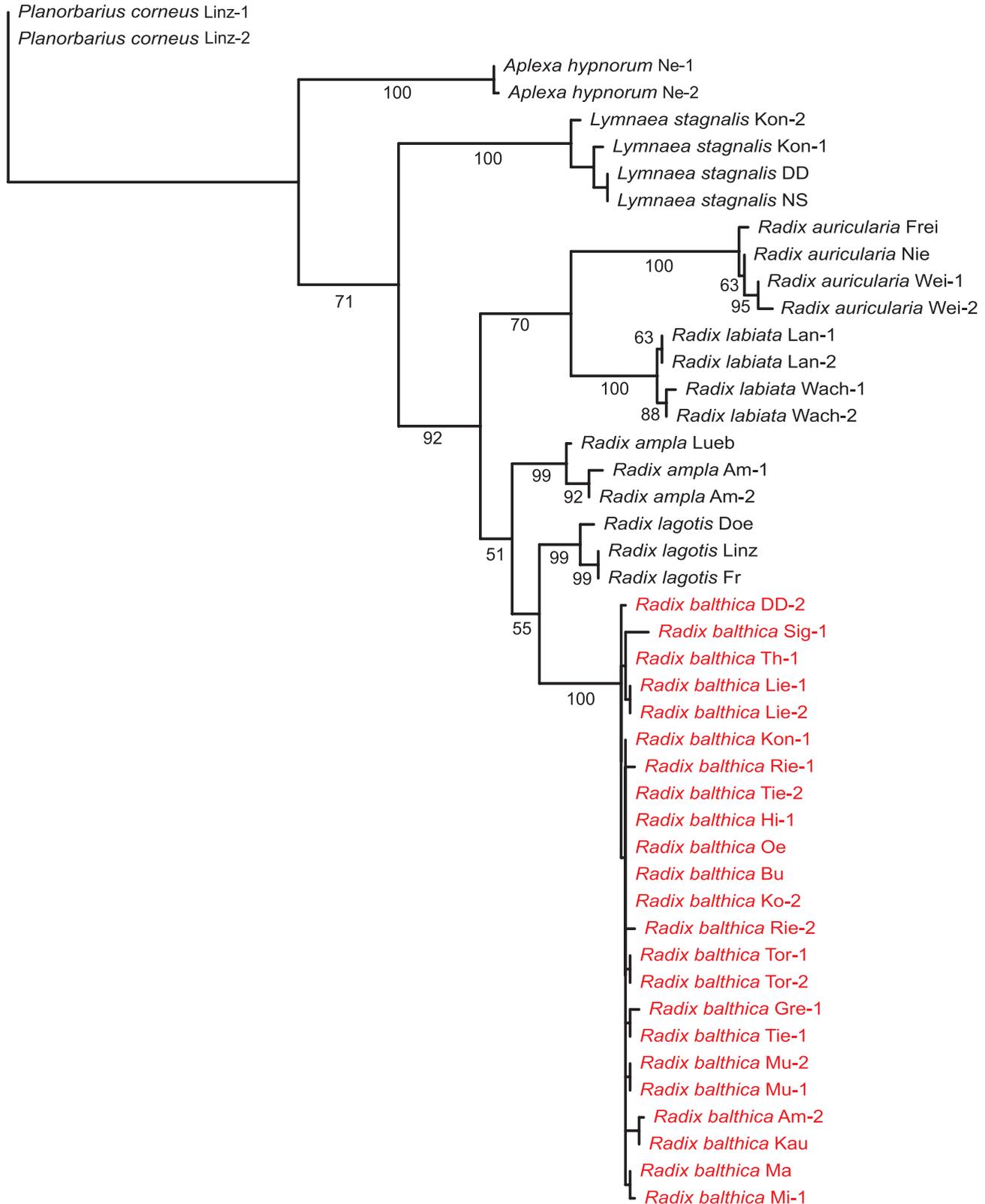
Distance analyses
Genetic distances from pairwise comparisons of cyt-b sequences (fragment of about 370 bp) are shown in Table 2. Differences between species of different families (outgroup comparison) ranged between 31.2% and 25.8%. Distances between *Lymnaea stagnalis* and the *Radix* species ranged from 26.0% to 20.3%. Among the five *Radix* species analysed, the highest values are between *R. auricularia* and the other species (between 19.2% and 15.6%). Lower values (from 13.5% to 9.0%) are between *R. labiata*, *R. balthica*, *R. ampla* and *R. lagotis*.

Molecular phylogeny
The maximum-parsimony (MP) tree of the cyt-b sequences is illustrated in Fig. 1 (tree length = 380, consistency index = 0.7474, retention index = 0.9091). Although basal branches have less than 80% bootstrap support, the clades of the species themselves have high, often full support. The result of the RAxML (not shown) calculation is very similar. In both trees *L. stagnalis* groups are sister to the *Radix*-species. Although a branching pattern between the *Radix*-species is proposed in both trees, very low support speaks in favour of interpretation as a polytomy. With both methods, the specimens of *R. balthica* form a distinct clade that is well separated from those of the other *Radix* species.

The maximum-parsimony (MP) tree of the nuclear marker ITS-2 (tree length = 1414, consistency index = 0.7560, retention index = 0.9290) (Fig. 2) is well-supported within the Lymnaeidae (*Lymnaea* and *Radix*). It shows *R. auricularia* as a well-separated sister group to the other *Radix*-species analysed. Within these, *R. labiata* forms a

Table 2 Evolutionary distances of the cyt-b gene fragment (about 370 bp) calculated using MEGA version 4 (Tamura *et al.*, 2007).

	<i>P. corneus</i>	<i>A. hypnorum</i>	<i>L. stagnalis</i>	<i>R. auricularia</i>	<i>R. labiata</i>	<i>R. ampla</i>	<i>R. balthica</i>	<i>R. lagotis</i>
<i>Planorbarius corneus</i>	–	–	–	–	–	–	–	–
<i>Aplexa hypnorum</i>	0.312	–	–	–	–	–	–	–
<i>Lymnaea stagnalis</i>	0.282	0.290	–	–	–	–	–	–
<i>Radix auricularia</i>	0.303	0.268	0.260	–	–	–	–	–
<i>Radix labiata</i>	0.285	0.265	0.236	0.177	–	–	–	–
<i>Radix ampla</i>	0.285	0.260	0.213	0.192	0.104	–	–	–
<i>Radix balthica</i>	0.310	0.258	0.224	0.156	0.135	0.120	–	–
<i>Radix lagotis</i>	0.280	0.262	0.203	0.163	0.129	0.092	0.090	–



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Figure 1 Hypothesis for the phylogenetic relationships of *R. balthica* based on one of the 10000 best maximum-parsimony trees of the sequenced fragment of the mitochondrial marker *cyt-b* (about 370 bp; tree length = 380, consistency index = 0.7474, retention index = 0.9091). 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.

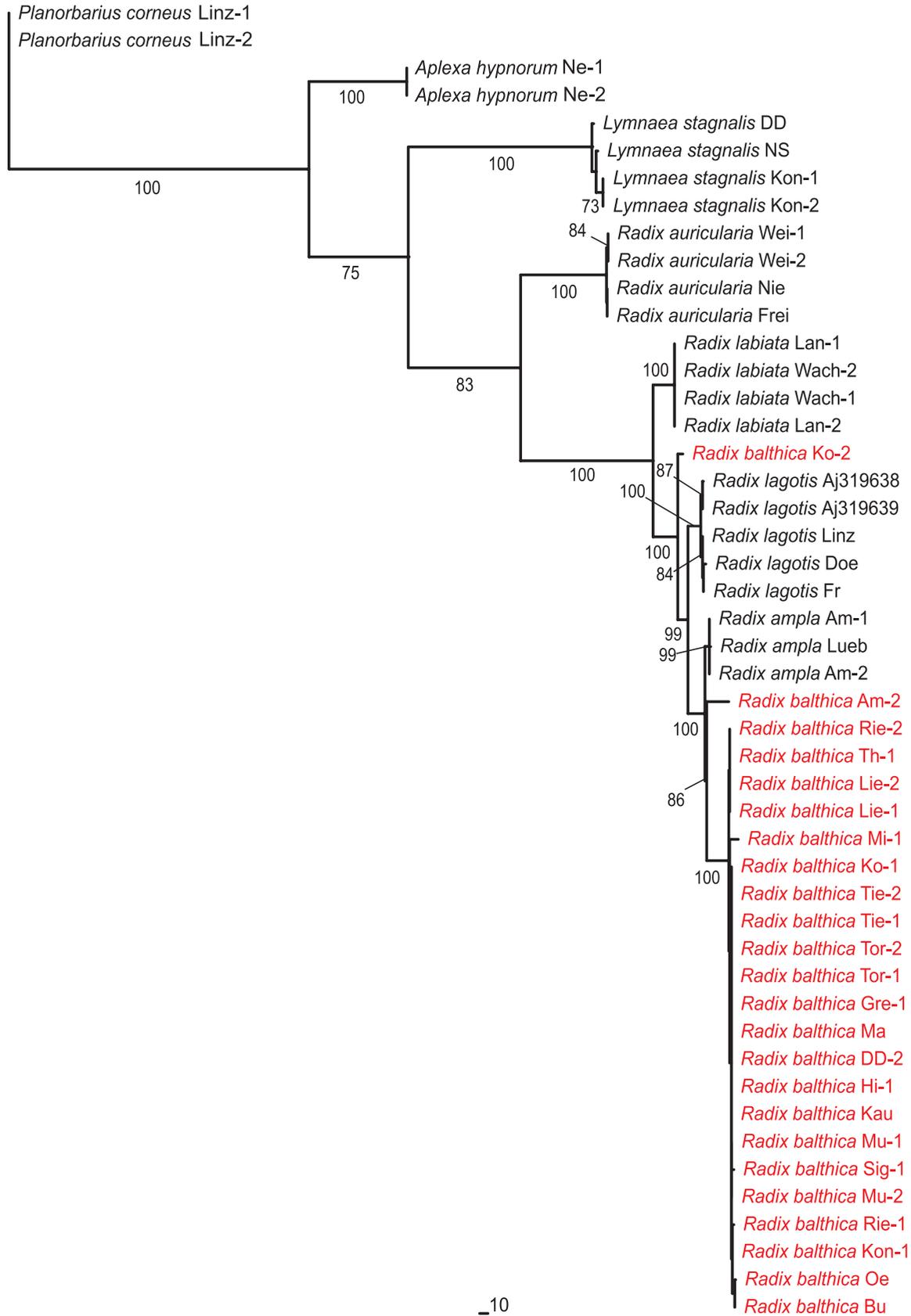


Figure 2 Hypothesis for the phylogenetic relationships of *R. balthica* based on one of the best 118 maximum-parsimony trees of the nuclear marker ITS-2 (tree length = 1414, consistency index = 0.7560, retention index = 0.9290). Branch lengths are proportional to the number of substitutions and the overall topology corresponds to that of the strict consensus tree. Bootstrap support values above 50% are reported below nodes.

distinct sister group to *R. lagotis*, *R. ampla* and *R. balthica*, this sister group relation has full bootstrap support. *Radix lagotis* forms a distinct sister group to *R. ampla* plus *R. balthica* (nearly full support). The Genbank ITS-2-sequences for *R. lagotis* specimens published by Bargues *et al.* (2001) are used in this analysis to show that the species definition of *R. lagotis* is the same as in that study. In contrast to the two trees of the mitochondrial marker, not all individuals of *R. balthica* fall into one distinct cluster. Two specimens (*R. balthica* Am-1 and *R. balthica* Ko-2) lie clearly outside of the main *R. balthica* cluster. While they each form a separate branch within *Radix* in the MP tree they form a poorly supported clade in the RAxML tree (not shown), which forms the sister group to *R. ampla*. A further difference of the RAxML tree (not shown), to the MP tree (Fig. 2) is that there is hardly any differentiation between *R. balthica* and *R. lagotis*. In both reconstructions these two species, together with *R. ampla*, form the sister group to *R. labiata*. *Radix auricularia* forms a clearly differentiated sister group to these four species in both reconstructions.

Network analyses The network representation of *R. balthica* mitochondrial sequences (cyt-b, about 370 bp; Fig. 3) shows hardly reticulated differentiation of up to about 13 mutations between individuals. The greatest differentiation can be observed between individuals from geographically close localities, for example within the federal state of Baden-Württemberg in Germany (light yellow). The same haplotype can occur in individuals from localities situated far from one another, for example in 14 individuals from different federal states of Germany, from Kazakhstan and from the island of Öland in Sweden. Sequences of specimens from Saxony in Germany, from France, Switzerland and Siberia (Russia) only differ by one substitution each.

Morphology

Shell (Fig. 4) The shells of examined specimens vary from conical egg-shaped to egg-shaped to nearly spherical and ear-shaped. They vary from thin-walled and fragile to solid and are of a light horn to reddish-brown in colour. The height of the shells ranges from 9.9 to 28.0 mm and the number of whorls between 3 and 4.

Mantle pigmentation (Fig. 5) Mantle pigmentation of the sequenced specimens of *R. balthica* shows a broad polymorphism. It is however possible to distinguish three types:

- mantle and mantle collar of a deep blue-black with a bluish grey mantle edge (Fig. 5: 8)
- mantle grey-black with roundish patches of lighter grey-black mantle collar bluish grey with numerous irregular patches of black; mantle edge light (Fig. 5: 7)
- mantle black, grey-black, or grey-yellow with few or numerous roundish distinct spots of white or rarely grey-green or grey-yellow, that are very variable in size; mantle collar white or bluish white with numerous irregular small patches of black; mantle edge white or rarely yellowish (Fig. 5.1–6, 9–12).

The third type of mantle pigmentation occurs most frequently and is the one usually described in the literature (Jackiewicz, 1993, 1998, 2000; Gittenberger *et al.*, 1998; Glöer, 2002; Stadnichenko, 2004).

Male genitalia (Fig. 6) The ratio of the length of the preputium to that of the penis sheath varies from 0.7–1.3 in the specimens examined. The preputium is pigmented grey mostly over the entire surface, although in some individuals the distal part (about one third of the total length) is not pigmented.

Bursa copulatrix (Fig. 7) The following three characters were analysed:

Position of bursa copulatrix. The bursa is positioned dorsally to the vagina and rarely (if of a certain size) also dorsally to the provaginal duct.

Shape of the bursa. The shape of the bursa varies from nearly spherical over pear-shaped up to elongate-tubular.

Length of the bursa duct. The length of the bursa duct varies between nearly not visible up to half of the length of the bursa (if bursa is filled).

DISCUSSION

Molecular phylogeny One aim of the molecular genetic analysis was to show that the individuals described morphologically and anatomically belong to the species *R. balthica*. In both the MP (Fig. 1) and the RAxML tree (not shown) based

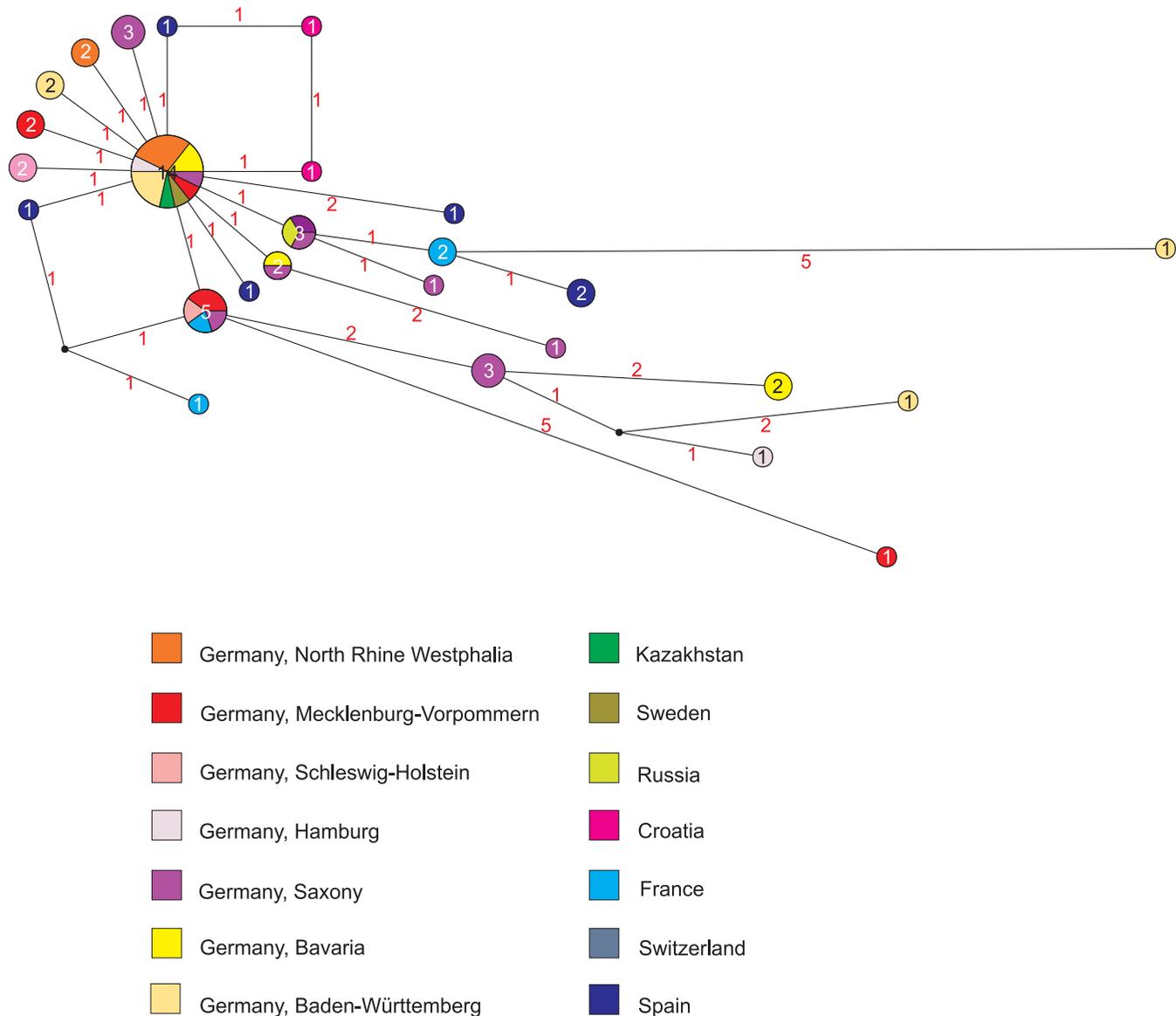


Figure 3 Haplotype network of *cyt-b* sequences of 58 *Radix balthica* specimens from different locations in Europe, Kazakhstan and Russia. The size of the balloons is proportional to the number of individuals having each haplotype, which corresponds to the black number inside the balloon. The small black dots represent internal haplotypes not represented by the dataset. The length of the connecting lines is proportional to the number of substitutions between haplotypes, corresponding to the red number on the line.

on the *cyt-b* gene fragment (about 370 bp) these individuals form a clade including a “topotype” of *Radix balthica* from Øland (*R. balthica* Oe). This allows the conclusion that in spite of the high morphological variability observed, they all appear to belong to one species – *Radix balthica* – that clearly differs from the other *Radix*-species analysed. A different picture appears with the tree result of the nuclear marker ITS-2, based on both MP (Fig. 2) and RAxML (not shown). The two trees differ slightly, probably due to the fact that the alignment contains many indels and the

RAxML-analysis does not interpret these as a fifth character state. The RAxML-analysis of this ITS-2 dataset (not shown) appears not to be able to distinguish closely related species: there is hardly any differentiation between *R. balthica* and *R. lagotis* and the support for differentiation to the cluster containing *R. ampla* is not very high either (70). In our opinion the three species *R. ampla*, *R. lagotis* and *R. balthica* do have species status, supported by sufficient large genetic distances based on the *cyt-b* fragment (Tab. 2). Further support comes from the MP tree of the ITS-2 dataset (Fig.

2), in which these three closely related species appear as well differentiated clades (with the exceptions discussed below). In our opinion the overall topology of this tree reflects the morphological and anatomical specialties and differences between the representatives of the genus *Radix* well.

However, in both the MP and RAxML trees based on ITS-2, individuals *R. balthica* Ko-2 and *R. balthica* Am-1 are clearly different from the remaining individuals of *R. balthica*, and appear to belong to other species. Since their mitochondrial haplotype based on the *cyt-b* fragment is clearly part of the *R. balthica* cluster (Fig. 1), these individuals may represent hybrids with other species of *Radix*. However, since they do not appear to group as part of the clusters formed by the other species included in this analysis, the hybridisation appears to have been with *Radix* species that have not been sampled. Alternatively, the results could reflect incomplete lineage sorting of the nuclear ITS-2 marker since the time when *R. balthica*, *R. labiata*, *R. ampla* and possibly even *R. lagotis* separated. In our molecular studies we have detected several such cases in which the mitochondrial genes (*cyt-B*, *COI*) indicate different species affiliation than the nuclear marker ITS-2 (e.g. also in the genus *Stagnicola*, unpublished results).

The analysis of the network relationship of the mitochondrial haplotypes illustrated with respect to their geographic origin (Fig. 3) shows that there is no distinct correlation of genetic variability to the geographic distribution pattern.

Morphology The anatomy of *Radix balthica* is much more plastic than usually believed. Glöer & Beckmann (2007) described *Radix lilli* from Majorca, mainly separated by the bursa duct which was longer than in typical *Radix balthica*, as depicted in Glöer (2002: 212, fig. 240b). But the DNA sequences indicate to us that this species (*R. balthica* Ma) belongs to the same clade as *R. balthica*. On the other hand the sequenced species had a sitting bursa copulatrix, which might be explained in a single population if the bursa duct varies or if two species occur at a particular sampling site.

The shells of the examined specimens of *R. balthica* vary considerably, not only in size and colour, but also in form and the number of whorls, which partly also leads to strong devia-

tion from the egg-shaped shell of the neotype which has a relatively low spire (Kruglov & Starobogatov, 1983). The heights of the shells lie above that of the neotype, at 9.8 mm (Kruglov & Starobogatov, 1983). Usually the shell has a weak columellar fold and a regular convex last whorl.

The differentiation of the shells of amploid forms of *R. balthica* (Fig. 4.10) from shells of *R. ampla* can be difficult. *R. ampla* usually has no columellar fold (Fig. 9.3), however in rare cases this can also be missing in *R. balthica* f. *subampla*. In these cases the anatomy may also not provide certainty, since the anatomical character states of *R. balthica* and *R. ampla* overlap. Juvenile specimens may possibly be of help, since the shells of juvenile *R. ampla* already show the typical amploid shape, which is not the case in other *Radix*-species, such as *R. balthica* and *R. auricularia*. Mantle pigmentation can however be useful as a source of morphological differentiation: the white spots on dark background are larger in *R. ampla* (Fig. 9.3) than in *R. balthica*.

In addition, the shell shape of *R. balthica* (Fig. 4.11) can be similar to that of *R. auricularia* (see Fig. 9.2). In this case the anatomical differentiation is relatively easy, as *R. auricularia* has a very long bursa duct with a spherical bursa (see Fig. 9.2), in contrast to *R. balthica* (Fig. 7). Furthermore, *R. auricularia* is the only European species with a pigmentation of "freckles" on the tentacles, head and foot (see Fig. 9.2).

Whether a clear differentiation from *R. lagotis* is possible with characters of the shell only, has not been studied sufficiently.

A typical morphology for *R. balthica* is a shell with a regular, convex last whorl and a weak columellar fold (see shell of the neotype Fig. 8.3).

The mantle pigmentation of specimens analysed shows more variation than commonly described in the literature (Jackiewicz, 1993, 1998, 2000; Glöer, 2002). Only Hubendick (1945) and Falniowski (1980a, b) mentioned specimens with a completely black mantle pigmentation in *Radix peregra*. Unfortunately, it is unclear from Falniowski (1980a,b) which *Radix*-species is referred to, since *R. lagotis* and *R. labiata*, which were interpreted as forms of *R. peregra* by the author, can also have completely black mantle pigmentation. Hubendick (1945) mentions that mantle pigmentation may also be absent.

As a distinguishing characteristic to other *Radix* species only the typical mantle pigmentation can



Figure 4 Variability in *Radix balthica* shells: 1 Germany, Baden-Württemberg, river Danube near Sigmaringendorf (Sig-1); 2 France, Region Centre, Thenay (Th-1); 3 Kazakhstan, lake Kopa (Ko-2); 4 Germany, Saxony, creek Geberbach near Kauscha (Kau); 5 Germany, Bavaria, river Amper near lake Ammersee (Am-1); 6 Germany, Saxony, Dresden-Kleitzschachwitz, river Elbe (DD-2); 7 Switzerland, canton Basel City, Riehen, Wiesengriener (Rie-1); 8 Switzerland, canton Basel City, Riehen, Wiesengriener (Rie-2); 9 Switzerland, canton Basel-Landschaft, Liestal, Orishof (Lie-1); 10 Germany, Mecklenburg-Vorpommern, lake Torgelower See (Tor-2); 11 Germany, Mecklenburg-Vorpommern, lake Tiefwareensee (Tie-1); 12 Germany, North Rhine-Westphalia, Hillegossen, creek Meyerbach (Hi-1).

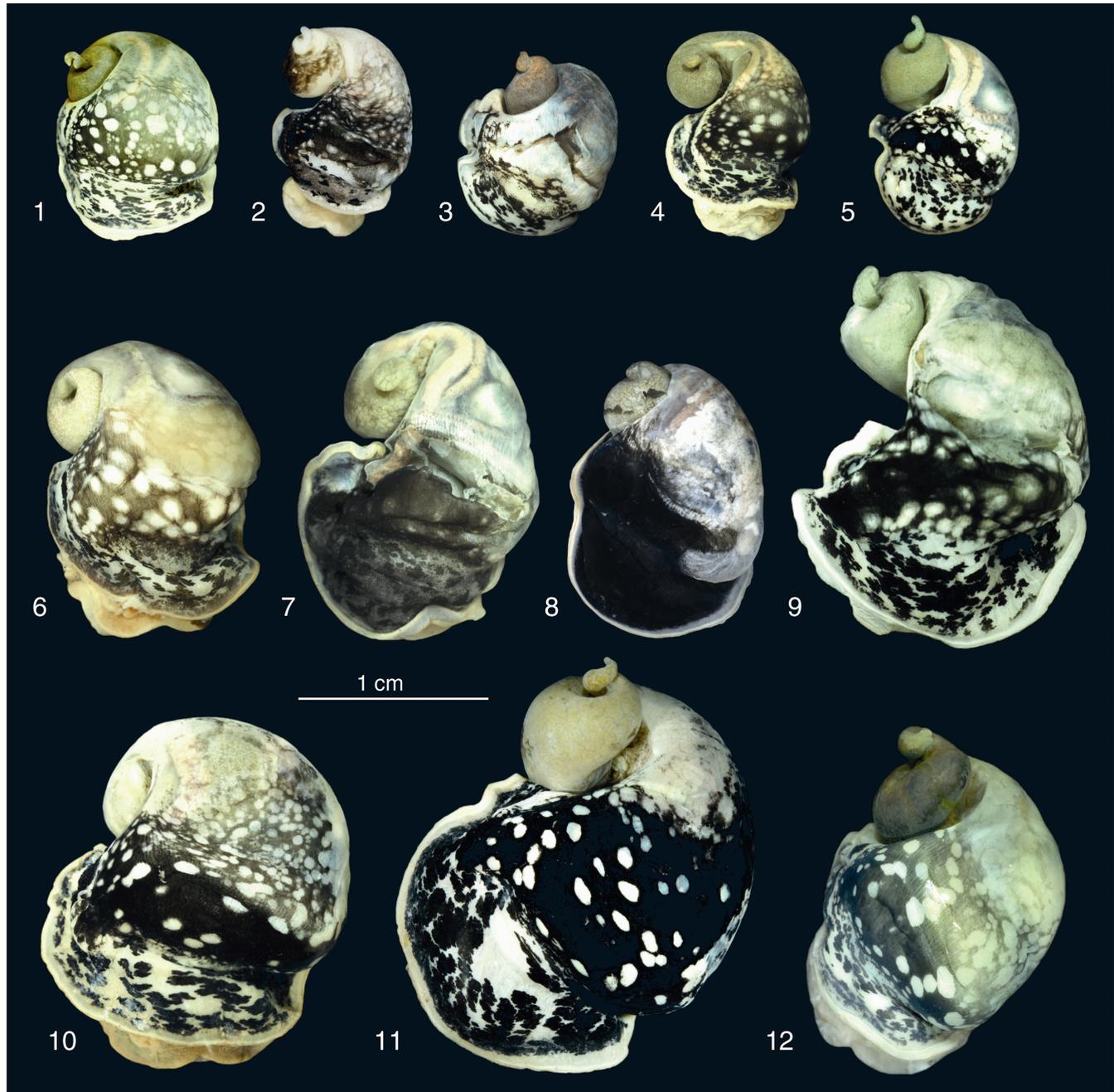


Figure 5 Variability in *Radix balthica* mantle pigmentation: 1 Germany, Baden-Württemberg, river Danube near Sigmaringendorf (Sig-1); 2 France, Region Centre, Thenay (Th-1); 3 Kazakhstan, lake Kopa (Ko-2); 4 Germany, Saxony, creek Geberbach near Kauscha (Kau); 5 Germany, Bavaria, river Amper near lake Ammersee (Am-1); 6 Germany, Saxony, Dresden-Kleitzschachwitz, river Elbe (DD-2); 7 Switzerland, canton Basel City, Riehen, Wiesengriener (Rie-1); 8 Switzerland, canton Basel City, Riehen, Wiesengriener (Rie-2); 9 Switzerland, canton Basel-Landschaft, Liestal, Orishof (Lie-1); 10 Germany, Mecklenburg-Vorpommern, lake Torgelower See (Tor-2); 11 Germany, Mecklenburg-Vorpommern, lake Tiefwareensee (Tie-1); 12 Germany, North Rhine-Westphalia, Hillegossen, creek Meyerbach (Hi-1)

be used (see Fig. 5.1, 5, 10, 11). However, this character should best be used in combination with anatomical criteria for determinations.

A typical morphology for *R. balthica* is a black, grey-black, or grey-yellow mantle with many

medium-sized distinct lighter spots on a dark background.

In the specimens of *R. balthica* studied, the shape of the bursa copulatrix as well as the length of the bursa duct show a similar high variation



Figure 6 Variability in *Radix balthica* male genitalia: 1 Germany, Baden-Württemberg, river Danube near Sigmaringendorf (Sig-1); 2 France, Region Centre, Thenay (Th-1); 3 Germany, Bavaria, river Amper near lake Ammersee (Am-1); 4 Germany, Saxony, Dresden-Kleitzschachwitz, river Elbe (DD-2); 5 Switzerland, canton Basel-Landschaft, Liestal, Orishof (Lie-1); 6 Germany, Mecklenburg-Vorpommern, lake Torgelower See (Tor-2).



Figure 7 Variability of the form of the bursa copulatrix and the length of the bursa duct in *Radix balthica*: 1 France, Region Centre, Thenay (Th-1); 2 Germany, Saxony, creek Geberbach near Kauscha (Kau); 3 Germany, Bavaria, river Amper near lake Ammersee (Am-1); 4 Germany, Saxony, Dresden-Kleitzschachwitz, river Elbe (DD-2); 5 Germany, Mecklenburg-Vorpommern, lake Torgelower See (Tor-2); 6 Germany, Mecklenburg-Vorpommern, lake Tiefwareensee (Tie-1); 7 Switzerland, canton Basel City, Riehen, Wiesengriener (Rie-2).

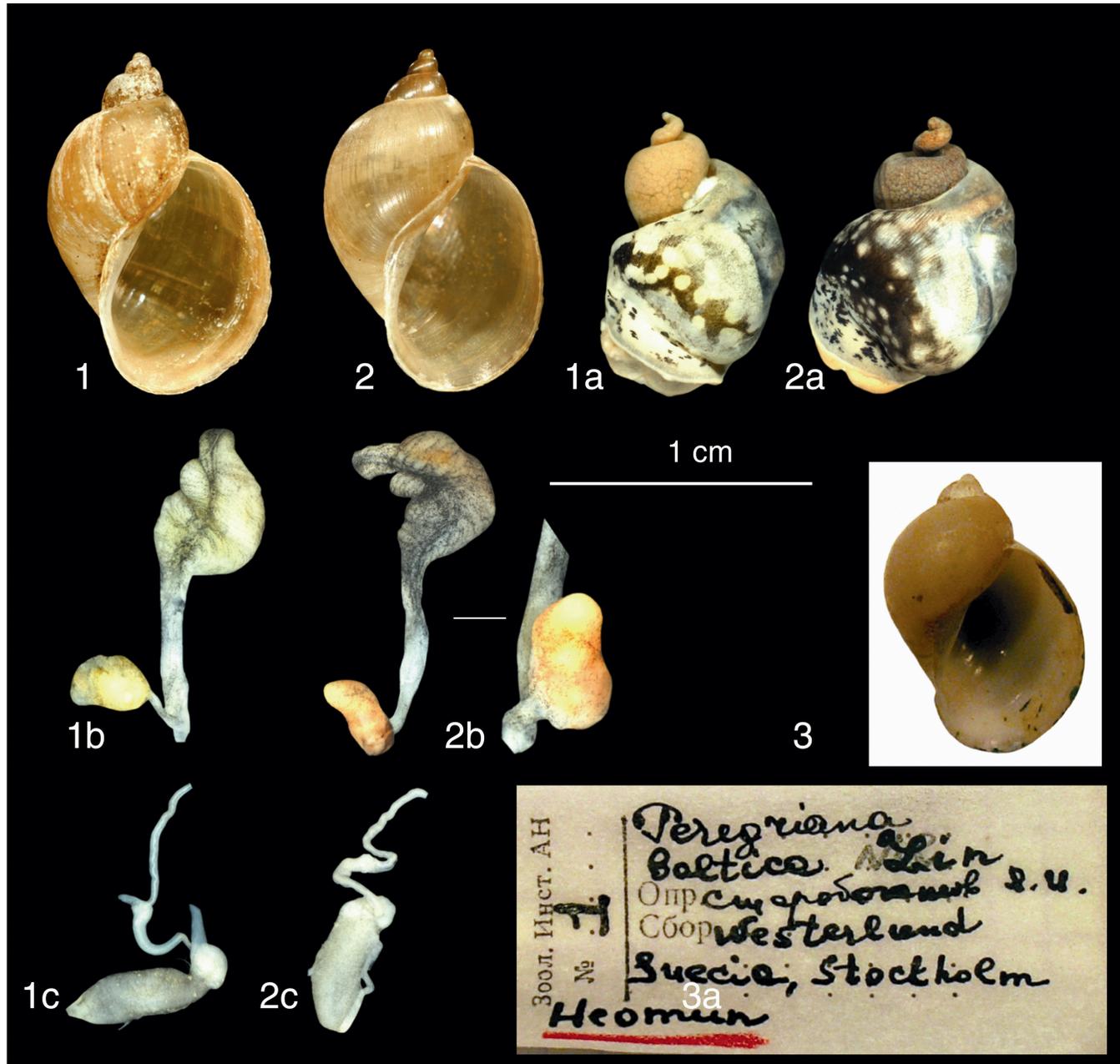


Figure 8 Examined topotypes of *Radix balthica*: 1 Öland, seashore; 2 Öland, moor; 3 Neotype of *Radix balthica* of Kruglov & Starobogatov. The Russian inscriptions and abbreviations on the table say that this shell was collected by Westerlund and determined by Starobogatov. The word underlined red is “Неотип” = neotype. 1a-2a mantle pigmentation, 1b-2b female sex tract, 1c-2c male copulatory organ.

(Fig. 7) as observed by Hubendick (1945, 1953) and Falniowski (1980a,b) for this species. Since the length of the bursa duct varies so strongly and can reach nearly half of the length of the bursa (if bursa is filled), we do not find this a good differentiating character with respect to *R. labiata*. However, the position of the bursa dorsally to the vagina and (depending on the size) to the provaginal duct is a distinct character for differ-

entiating *R. balthica* from *R. labiata*, in which the bursa is positioned ventrally to the vagina (see also Stadnichenko, 2004: 197; Kruglov, 2005: 309).

No differential character states of *R. balthica* could be found with respect to *R. ampla*.

Radix lagotis can clearly be differentiated by the distinctly longer bursa duct, the length reaching half to about 2/3 of the length of the bursa (if the bursa is filled) (Fig. 9.4).



Figure 9 1 *Radix labiata* (Germany, Bavaria, Loiperding), 2 *R. auricularia* (Germany, Hamburg), 3 *R. ampla* (Germany, Mecklenburg-Vorpommern, Tollensesee), 4 *R. lagotis* (Germany, Brandenburg Havelland).

A typical morphology for *R. balthica* is a sitting bursa or a bursa with a very short duct. The colour of the filled bursa is orange in most cases, rarely white.

In the specimens from Europe in which we examined the male genitalia (n=10), the measured

length ratio of praeputium to penis sheath varies from 0.7–1.3, confirming the interval provided by Meier-Brook in Glöer (2002), who reported that this characteristic can vary between 0.4:1 and 1.6:1 in *R. balthica*. In Western Siberia this ratio varies from 1.16:1 to 1.59:1 (n = 10) but falls within

Table 3 Distinguishing characters for *Radix balthica*, *R. labiata*, *R. ampla*, *R. auricularia* and *R. lagotis*.

Character	<i>Radix balthica</i>	<i>Radix labiata</i>	<i>Radix ampla</i>	<i>Radix auricularia</i>	<i>Radix lagotis</i>
shape of the line tangential to the whorls in adult shells	usually convex, rarely concave	usually straight	always concave	always concave	always convex
upper edge of aperture towering above whorls in adult shells	no, or hardly	no	yes	no	no
columella fold in adult shells	weak	weak	absent	distinct	weak
typical mantle pigmentation above lung cavity	many medium-sized light, distinct spots on dark background	many small light blurred dots on dark background	a few large white spots on dark background	a few large white spots on dark background	a few light distinct or blurred medium-sized spots on dark background
presence of pigmentation reminding of "freckles" on tentacles, head and foot	no	no	no	yes	no
length of the bursa duct (if bursa is filled)	between nearly not visible and half of the length of the bursa	from short to about nearly one third of the length of the bursa	between nearly not visible and half of the length of the bursa	very long, usually as long as the provaginal duct	from half to about 2/3 of the length of the bursa
position of bursa and bursa duct	above vagina and provaginal duct	behind vagina and above provaginal duct	above vagina and provaginal duct	near pericardium	above vagina, provaginal duct, uterus and prostata
colour of praeputium	usually uniform dark grey or dark bluish grey	uniform grey greenish or dark grey	light and without pigmentation	with dorsal pigmentation reminding of "freckles"	light to dark bluish grey

Meier-Brook's data. In comparison, Jackiewicz (1998, 2000) quotes the ratio of praeputium to the penis sheath length as 1:1, and Stadnichenko (2004) as 1.2:1 and Kruglov (2005) 1.20:1.

According to Meier-Brook in Glöer (2002) the ratio of praeputium length to penis sheath in *R. labiata* can vary from 0.7:1 to 2.3:1. Thus this character can not always be used for differentiation between *R. balthica* and *R. labiata*. It is also not useful for differentiation between *R.*

balthica, *R. auricularia* and *R. ampla*. In *R. auricularia* the ratio of praeputium to the penis sheath length is 1:1 (Jackiewicz, 1998, 2000; Glöer, 2002), 1.10:1 (Stadnichenko, 2004; Kruglov, 2005), or 0.6–1.3 according to our own measurements (n=6 from different locations). Vinarski & Glöer (2009) reported values of this index in *R. auricularia* between 0.96:1 and 1.41:1 (n = 65).

Falniowski (1980a) mentioned the existence of a dorsal pigmentation of the praeputium in *R.*

auricularia, which is confirmed by our own observations. This pigmentation consists of single very small, dark pigmentation marks ("freckles").

The praeputium of the specimens of *R. balthica* analysed by molecular genetics was regularly dark grey or dark grey-blue over the entire surface. In one case the colouration was regular (without clearly distinguishable single dots of pigmentation) only to about two thirds. The praeputium of the individual from Øland was light yellow. The specimens of *R. balthica* with *ampla*-shaped shell we studied, clearly differ from *R. ampla* by a praeputium with dark pigmentation. It appears to be light and without pigmentation in *R. ampla* of seven different sampling sites. In *R. labiata* the praeputium is grey-greenish or dark grey in colour, in *R. lagotis* it varies from light to dark blue-grey.

CONCLUSION: DIFFERENTIATING FEATURES FROM OTHER EUROPEAN *RADIX*-SPECIES

The most important morphological characters for differentiation of the species *R. balthica*, *R. labiata*, *R. ampla*, *R. lagotis* and *R. auricularia* are summarised in Table 3 taking account of our present knowledge on the morphological variability of these species.

Specimens of *R. balthica* with shells similar to those of *R. auricularia* are differentiated by the length of the bursa duct, which is often nearly as long as the provaginal duct and ends in a rounded or pyriform bursa (see Fig. 9.2). The mantle pigmentation can be used for differentiation in addition. In most cases, the mantle of *R. auricularia* has distinctly larger white spots than that of *R. balthica* (see Fig. 9.2). The tentacles, the head, the surface of the foot and often also the sole of the foot show a pigmentation reminding of "freckles" in *R. auricularia* (see Fig. 9.2), which is absent in all the other European *Radix* species, but is apparent in many Asian *Radix* spp. A reliable differentiation and determination based on shell morphology alone is very difficult.

Specimens of *R. balthica* with amploid shells mostly differ from *R. ampla* in mantle pigmentation, which often shows a few large white spots on dark background in *R. ampla* (Fig. 9.3), that sometimes fuse with one another. A reliable differentiation and determination based on shell morphology alone is very difficult.

Radix balthica differs most clearly from *R. labiata* by the position of the bursa and the bursa duct. They lie dorsal to the vagina and the provaginal duct in *R. balthica*, whereas in *R. labiata* bursa and bursa duct lie ventrally to the vagina and the provaginal duct. *R. labiata* differs from specimens of *R. balthica* in most cases in that the typical mantle pigmentation has many more small light diffuse dots on a dark background (Fig. 9.1).

The best character for distinguishing *Radix lagotis*, which is clearly differentiated from *R. balthica*, *R. labiata*, *R. ampla* and *R. auricularia* based on molecular genetics (see also Bargues *et al.*, 2001), is the length of the bursa duct, which is distinctly longer in *R. lagotis*, reaching half to about 2/3 of the length of the bursa (if filled) (Fig. 9.4).

ACKNOWLEDGEMENTS

We would like to express our thanks to Prof. Dr. Uwe Fritz (SNSD) for financial support of the greater part of the molecular analyses, Anke Müller (SNSD) for some sequences and the instruction of K.S. in lab work, as well as André Reimann (SNSD), Dr. Michael L. Zettler (Leibnitz Institute for Baltic Sea Research Warnemuende), Michael Korn (University of Konstanz, Limnological Institute), Dr. Nicole Schröder-Rogalla (Munich), Susanne Thiel (Munich), Robert Haldemann (Strausberg), Christoph Oberer (Natural History Museum Basel), Eric Gallerne (Leguevin), Dr. Daniel Rondelaud (Faculté de Médecine Limoges Cedex), Holger Menzel-Harloff (Wismar), Uwe Jueg (Ludwigslust), Alfried V. Karimov (Omsk State Pedagogical University), Gerhard Falkner (State Museum of Natural History Stuttgart), Dr. Ira Richling (Kronshagen), Armin Deutsch (Bielefeld), Reinhard Diercking (Hamburg), Hajo Kobialka (Hoexter), Andrea Pohl (Dresden), Gudrun Rutsch (Dresden) and Christa Schniebs (Oelsnitz) for the material collected and provided. Last but not least we also thank two anonymous reviewers for improvements to the manuscript.

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