INTRASPECIFIC MORPHOLOGICAL AND GENETIC VARIABILITY IN THE PALAEARCTIC FRESHWATER SNAIL *RADIX AMPLA* (HARTMANN, 1821) (GASTROPODA: BASOMMATOPHORA: LYMNAEIDAE)

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Abstract   Radix ampla *(Hartmann, 1821) is a widely distributed Palaearctic freshwater snail with a still insufficiently known distribution. 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 (including sequences from six outgroup* Radix *species). Molecular sequences of the nuclear spacer fragment ITS-2 and the mitochondrial gene fragment cyt–b were obtained from 30 individuals of* R. ampla *as well as from eight individuals of* R. monnardi *(Hartmann, 1841) from different regions of Europe and Western Siberia. From the subsample of 36 specimens 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 found to be broader than recognised in current literature. The differentiation of atypical* R. ampla *from* R. balthica *(Linnaeus, 1758) and* R. lagotis *(Schrank, 1803) using morphological characters only, was very difficult.* R. monnardi *and* R. hartmanni *(Hartmann, 1821) proved to be junior synonyms of* R. ampla.

Key words   Freshwater gastropods, molecular genetics, morphology, Radix ampla, *variation*

INTRODUCTION

The pond snail *Radix ampla* is a Palaearctic freshwater snail species recorded from Scandinavia, Central and Eastern Europe up to Kazakhstan and Siberia in the East (Falkner, 1990; Falkner, Bank & von Proschwitz, 2001; Kruglov, 2005; Vinarski, 2009; Vinarski & Serbina, 2012) but its distribution is still insufficiently known. In the past, it had often been confused with conchologically similar specimens of other *Radix* species, e.g. amploid forms of *Radix auricularia* (Linnaeus, 1758), *R. balthica*, casting some earlier recordings into doubt.

Reliable records of this rather rare species are known from Germany (Glöer, 2002), Austria (Vinarski & Glöer, 2007), Switzerland (Boschi, 2011), the Czech Republic and Slovakia (Horsák, Čejka, Juřičková, Beran, Horáčková, Hláváč, Dvořák, Hájek, Divíšek, Maňas & Ložek, 2016), Poland (Falniowski 1981; Piechocki & Wawrzyniak-Wydrowska, 2016), Estonia (Bank, 2011b), Lithuania (Zettler, Zettler & Daunys, 2005), Romania (Glöer & Sîrbu, 2005), Greece (Bank, 2006), Belarus (Akimova, Khrisanfova, Tolstenkov, Bychkova, Zhukova, Ryskov & Semenova, 2012; Laenko, 2012), Ukraine (Stadnichenko 2004; Anistratenko, Vinarski, Anistratenko, Furyk & Degtyarenko, 2018), European Russia (Kruglov, 2005), the Urals (Khokhutkin, Erokhin & Grebennikov, 2003; Khokhutkin, Vinarski & Grebennikov, 2009), and southern part of Western Siberia (Andreyeva, Andreyev & Vinarski, 2010). Its alleged presence in Eastern Siberia (Vinarski, 2009) needs confirmation, since the records were based on empty shells.

In earlier literature, this species has been referred to as '*Limnaea auricularia* var. *ampla*', '*Lymnaea pereger* f. *ampla*', '*Lymnaea peregra* f. *ampla*', '*Lymnaea peregra ampla*', '*Lymnaea balthica* f. *ampla*', '*Radix ovata* f. *ampla*', *R. auricularia* f. *subampla*, and '*Lymnaea patula*', however different opinions exist about whether *Turbo patulus* Da Costa, 1778 is a senior synonym

of *Limneus ampla* Hartmann, 1821 (Kruglov, 2005; Vinarski & Glöer, 2007; Prozorova, 2009). Furthermore, Russian malacologists distinguish, in addition to *L. patula* and *R. auricularia* plus related species, seven *Radix* species with ear-shaped shells (Kruglov, 2005; Prozorova, 2009): *Lymnaea monnardi* (Hartmann, 1841), *L. ampullacea* (Rossmässler, 1835), *L. torquilla* (Westerlund, 1877), *L. hartmanni* (Studer, 1820), *L. mucronata* Held, 1936, *L. novikovi* Kruglov & Starobogatov, 1983, *L. tumida* Held, 1836, and *L. mongolitumida* Kruglov & Starobogatov, 1983.

R. *ampla* lives in lake littoral zones and in slow-flowing rivers (Glöer 2002; Piechocki & Wawrzyniak-Wydrowska, 2016) as well as in small and medium sized ditches (Zettler, Jueg, Menzel-Harloff, Göllnitz, Petrick, Weber & Seemann, 2006) and small rivers and the surrounding standing waters (Beran, 2002; Stadnichenko, 2004). In the Transcarpathian this species was found only in rivers (Anistratenko *et al*. 2018). In lakes, it occurs mainly on sandy bottoms, often close to the shore (Piechocki & Wawrzyniak-Wydrowska, 2016) in the area exposed to waves (Zettler *et al*., 2006). There are also observations of this species on muddy bottoms (Zettler *et al*., 2006) as well as on water plants (Zettler *et al*., 2006). Andreyeva *et al*. (2010) and Khokhutkin *et al*. (2009) mention R. *ampla* as typical dweller of water plants in the shallow water of permanent waterbodies with standing and slow flowing water. Findings in the Oder lagoon show tolerance of brackish water (Zettler *et al*., 2006).

Whereas *R. ampla* is classified as 'Least Concern' in the European Red List of non-marine molluscs (Cuttelod, Seddon & Neubert, 2011), in Germany this species is at risk of extinction (Jungbluth & von Knorre, 2010) and listed as 'Endangered' in Austria (Frank & Reischütz, 1994) and Switzerland (Rüetschi, Stucki, Müller, Vincentini & Claude, 2012) as well as 'Vulnerable' in the Czech Republic (Beran, Juřičková & Horsák, 2017).

Not only because of its rarity and its protection status in some Central European countries, it is important to be able to distinguish *R. ampla* from *R. balthica* specimens with nearly spherical and ear-shaped shells (Schniebs, Glöer, Vinarski & Hundsdoerfer, 2011). As shown by Garbar & Korniushin (2003), *R. ampla* has the same diploid number (2n=34) of chromosomes as *R. auricularia*, *R. balthica*, and *R. labiata* (Rossmässler,

1835). However, differences in three chromosome pairs support its species distinctness as do molecular genetic analyses (e.g. Bargues, Vigo, Horak, Dvorak, Patzner, Pointier, Jackiewicz, Meier-Brook & Mas-Coma, 2001; Bargues & Mas-Coma, 2005; Albrecht, Wolff, Glöer & Wilke, 2008; Schniebs *et al*., 2011, 2013, 2015; Vinarski, Aksenova, Bespalaya, Bolotov, Schniebs, Gofarov & Kondakov, 2016). But this methodology is hardly available for most colleagues that work faunistically. That is why it is necessary to broaden the knowledge of the intraspecific variability of this species from which we have still poor data from morphological characters such as shape and position of the bursa copulatrix or length and position of the bursa duct (Glöer 2015; Piechocki & Wawrzyniak-Wydrowska, 2016; Schniebs *et al*., 2011) that appear to be useful for determination of different *Radix* species (Glöer 2015; Kijashko, Soldatenko, Vinarskij, 2016; Piechocki & Wawrzyniak-Wydrowska, 2016; Schniebs *et al*., 2011, 2013, 2015).

The present work continues our analyses of intraspecific variability in Palaearctic *Radix* species that we started with *R. balthica* (Schniebs *et al*., 2011) and *R. labiata* (Schniebs *et al*., 2013).

Material and Methods

All specimens used for molecular genetic studies are listed in Table 1. Specimens are stored either in the mollusc collection of the Senckenberg Natural History Collections, Dresden if used for morphological analyses as well and/or samples of tissue are stored in the tissue collection of the SNSD. For comparative purposes, we examined a series of *Radix ampla* shells and soft bodies from Western Europe and ex-USSR territory kept in the mollusc collections of the Zoological Institute of the Russian Academy of Sciences, Saint-Petersburg and the Laboratory of Macroecology & Biogeography of Invertebrates of the Saint-Petersburg State University, Russia.

The snails were fixed in 70–80% ethanol or isopropyl alcohol. Shell morphology, mantle pigmentation and anatomy were documented from the specimens studied. Genital organs were dissected and measured using stereo microscope (Nikon SMZ18). Photographs were taken with a digital camera system (Nikon DS-Fi2). Samples of tissue taken from the foot were fixed in absolute ethanol for analysis. They were registered

Table 1 Specimens used for molecular genetic studies.

| Code | Collection No. SNSD | Locality | ENA/GenBank No. | | References |
			cyt-b	ITS-2	
Aplexa hypnorum (LINNAEUS 1758)					
Aplexa hypnorum 1	Moll S348	Germany, Mecklenburg-Western Pomerania, lake Nebel, N 53°15'32" E 12°42'02"	FR797882	FR797832	VINARSKI *et al.* 2011
Aplexa hypnorum 2	Moll S350	Germany, Mecklenburg-Western Pomerania, lake Nebel, N 53°15'32" E 12°42'02"	FR797883	FR797833	VINARSKI *et al.* 2011
Lymnaea stagnalis (LINNAEUS 1758)					
Lymnaea stagnalis 1	Moll 49239	Germany, Saxony, Dresden-Zschieren, old branch of river Elbe, N 50°59'50" E 13°52'28"	HE573102	HE573064	SCHNIEBS *et al.* 2011
Lymnaea stagnalis 2	Moll 49835	Germany, Saxony, Niederspree, small pond, N 51°24'28" E 14°54'03"	HE573103	HE573065	SCHNIEBS *et al.* 2011
Lymnaea stagnalis 3	Moll 53108	Germany, Baden-Württemberg, Konstanz-Egg, ditch Hockgraben, N 47°40'57.3" E 9°11'34.2"	FR797894	FR797834	VINARSKI *et al.* 2011
Lymnaea stagnalis 4	Moll 53109	Germany, Baden-Württemberg, Konstanz-Egg, ditch Hockgraben, N 47°40'57.3" E 9°11'34.2"	FR797895	FR797835	VINARSKI *et al.* 2011
Radix auricularia (LINNAEUS 1758)					
Radix auricularia 1	Moll 51980	Bulgaria: river Kamchia 2,5km north of Staro Oryahovo, N 43,0195° E 27,8245°	LS974261	LS974218	This paper
Radix auricularia 2	Moll 52857	Russia, Novosibirsk Region, Novosibirsk, Reservoir near Kirza River, N 54°14.224' E 81°39.63114'	HE557667	HE557647	SCHNIEBS *et al.* 2011
Radix auricularia 3	Moll 52859	Russia, Novosibirsk Region, Novosibirsk, Reservoir near Kirza River, N 54°14.224' E 81°39.63114'	HE557668	HE557646	SCHNIEBS *et al.* 2011
Radix auricularia 4	Moll 53070	Germany, Bavaria, Weichering, pond in riverside forest, N 48°43'34.1" E 11°19'23.6"	FR797902	FR797842	VINARSKI *et al.* 2011
Radix auricularia 5	Moll 53086	Switzerland, Lake Constance near Güttingen	LT623597	LT623582	Schniebs *et al.* 2018
Radix auricularia 6	Moll S6815	Russia, Republic of Buryatia, Lake Baikal, Kotovo Bay near Monakhovo, N 53°39.011' E 108°58.587'	LS974264	LS974219	This paper

Code	Collection No. SNSD	Locality	ENA/GenBank No.		References
			cyt-b	ITS-2	
Radix auricularia 7	Moll S6840	Tunisia, artificial water reservoir near Khammamet, N 36°26.658' E 10°40.598'	LS974265	LS974220	This paper
Radix auricularia 8	Moll S7384	Denmark: Soro, lake Degnemosen, N 55.699417 E 12.504774	LS974263	LS974221	This paper
Radix auricularia 9	Moll S8736	Russia: Volga River delta, Katyushkin Erik channel, N 45.779410 E 47.872876	LS974262	LS974222	This paper
Radix natalensis (KRAUSS 1848)					
Radix natalensis 1	Tissue coll. 3252	Egypt: Cairo, Lotus basin in front of the Egyptian Museum, N 30.047038 E 31.233659	LS974266	LS974223	This paper
Radix natalensis 2	Tissue coll. 3253	Egypt: Cairo, Lotus basin in front of the Egyptian Museum, N 30.047038 E 31.233659	LS974267	LS974224	This paper
Radix dolgini GUNDRIZER AND STAROBOGATOV 1979)					
Radix dolgini 1	Moll 52861	Russia: Tomsk Region, District Teguldet, lake near Novoshumilovo village, N 57°25'30” E 88°31'13”	LS974268	KT030064	This paper VINARSKI *et al.* 2016
Radix dolgini 2	Moll 52862	Russia: Tomsk Region, District Teguldet, lake near Novoshumilovo village, N 57°25'30” E 88°31'13”	LS974269	KT030065	This paper VINARSKI *et al.* 2016
Radix dolgini 3	Moll 52863	Russia: Tomsk Region, District Teguldet, lake near Novoshumilovo village, N 57°25'30” E 88°31'13”	LS974270	KT030066	This paper VINARSKI *et al.* 2016
Radix dolgini 4	Moll S5217	Russia: Altay Territory, a swamp in the floodplain of Kulunda River, N 50°59'50” E 80°00'07”	LS974271	KT030061	This paper VINARSKI *et al.* 2016
Radix dolgini 5	Moll S5218	Russia: Altay Territory, a swamp in the floodplain of Kulunda River, N 50°59'50” E 80°00'07”	LS974272	KT030062	This paper VINARSKI *et al.* 2016
Radix labiata (ROSSMÄSSLER 1835)					
Radix labiata 1	Moll 51863	Montenegro, Žabljak, Black Lake, sand pools, 43°08'50''N 19°05'42''E	HE798507	HE798455	SCHNIEBS *et al.* 2013
Radix labiata 2	Moll 52415	Austria, Carinthia, Hermagor, N 46°37' E 13°22'	HE798484	HE798457	SCHNIEBS *et al.* 2013

Code	Collection No. SNSD	Locality	ENA/GenBank No.		References
			cyt-b	ITS-2	
Radix labiata 3	Tissue 3956	Spain: Santa Marina del Valdeon, Picos de Europa, Vega de Liordes, marshy headwater region, 1940m, 43°09'11''N 4°50'09''W	HE798491	HE798464	SCHNIEBS *et al*. 2013
Radix labiata 4	Moll S172	France, Bourgogne, Département Cote-d'Or	HE798492	HE798465	SCHNIEBS *et al*. 2013
Radix labiata 5	Moll S284	Switzerland, Basel City, Riehen, well Nollenbrunnen, 47°34'41''N 7°40'17''E	HE798493	HE798466	SCHNIEBS *et al*. 2013
Radix labiata 6	Moll S2904	Germany, Saxony, small brook north of Tharandt, N 51°00'08'' E 13°34'19''	HE798496	HE798469	SCHNIEBS *et al*. 2013
Radix balthica (LINNAEUS 1758)					
Radix balthica 1	Moll 51292	Switzerland, canton Basel City, Riehen, Wiesengriener, 47°35'21''N 07°38'32''E	HE573134	HE573083	SCHNIEBS *et al*. 2011
Radix balthica 2	Moll 51860	Sweden, Øland, east shore near Lille Seby, 56.345°N 16.565°E	HE573141	HE573090	SCHNIEBS *et al*. 2011
Radix balthica 3	Moll 52412	Croatia, lake Milanovac near Plitvica, 44°53'45''N 15°36'34''E	HE573139	HE573089	SCHNIEBS *et al*. 2011
Radix balthica 4	Moll 52663	Germany, Baden-Württemberg, river Danube near Sigmaringendorf, 48°03'45.54'' N 09°15'49.36''E	HE573120	HE573080	SCHNIEBS *et al*. 2011
Radix balthica 5	Moll 52907	Germany, Saxony: pond Rummelteich southwest of Trebsen, R 4552057 H 5683105	LS974275	LS974227	This paper
Radix balthica 6	Moll 53106	Germany, Bavaria, river Amper near lake Ammersee, 48°04'43''N 11°07'43''E	LS974276	HE573150	This paper SCHNIEBS *et al*. 2011
Radix balthica 7	Moll 53111	Germany, Baden-Württemberg, Konstanz-Egg, pond near University, N 47°41'09'' E 09°11'29''	HE573116	HE573078	SCHNIEBS *et al*. 2011
Radix balthica 8	Moll S2151	Germany, Mecklenburg-Western Pomerania, lake Torgelower See, N 53°34.252' E 12°46.622'	HE573159	HE573096	SCHNIEBS *et al*. 2011
Radix balthica 9	Moll S2174	Germany, Mecklenburg-Western Pomerania, lake Tiefwarensee, N 53°32.332' E 12°41.258'	HE573160	HE573097	SCHNIEBS *et al*. 2011

Code	Collection No. SNSD	Locality	ENA/GenBank No.		References
			cyt-b	ITS-2	
Radix lagotis (Schrank 1803)					
Radix lagotis 1	Moll 49868	Germany, Saxony, pond Vierteich near Freiteilsdorf, 13°41'57''E 51°15'43''	HE573114	HE573076	Schniebs *et al.* 2011
Radix lagotis 2	Moll 51858	Germany, Brandenburg, Strodehne, N 51.74555° E 12.22396°	LN874262	LN874255	Schniebs *et al.* 2015
Radix lagotis 3	Moll 52563	Saxony, pond Goldgrubenteich near Linz, 13°43'09''E 51°19'46''N	HE573115	HE573077	Schniebs *et al.* 2011
Radix lagotis 4	Moll 53236	Saxony, dam Doellnitzsee near Mutzschen, 12°55'18''E 51°15'45''N	LS974273	LS974225	This paper
Radix lagotis 5	Moll S1777	Ukraine: Donetsk Region, Staromarjevka, Kalmius River	LS974274	LS974226	This paper
Radix lagotis 6	Moll S3765	Bulgaria. Dragoman marshland 40km west of Sofia, N 42°56'11.7'' E 22°57'9.3''	LN874264	LN874257	Schniebs *et al.* 2015
Radix lagotis 7	Moll S3766	Bulgaria. Dragoman marshland 40km west of Sofia, N 42°56'11.7'' E 22°57'9.3''	LN874265	LN874258	Schniebs *et al.* 2015
Radix lagotis 8	Moll S3770	Bulgaria. Dragoman marshland 40km west of Sofia, N 42°56'11.7'' E 22°57'9.3''	LN874266	LN874259	Schniebs *et al.* 2015
Radix ampla (Hartmann 1821)					
Radix ampla 1	Moll 51112	Russia, Chelyabinsk Region, river Miass near Dynamo village, N 45°57' E 60°02'	HE798470	HE798448	Schniebs *et al.* 2013
Radix ampla 2	Moll 51113	Russia, Chelyabinsk Region, river Miass near Dynamo village, N 45°57' E 60°02'	HE798471	HE798449	Schniebs *et al.* 2013
Radix ampla 3	Moll 51409	Germany, Brandenburg, Neuzittau near Erkner, river Spree, N 52°23'26'' E 13°44'47''	LS974277	LS974228	This paper
Radix ampla 4	Moll 53082	Germany, Baden-Württemberg: Lake Constance, subbasin Überlinger See	LS974278	LS974229	This paper
Radix ampla 5	Moll 53083	Germany, Baden-Württemberg: Lake Constance, subbasin Überlinger See	LS974279	LS974230	This paper
Radix ampla 6	Moll 53098	Germany, Bavaria, Lake Ammer, Stegen, 11°08'07''E 48°04'32''N	HE573110	HE573072	Schniebs *et al.* 2011

| Code | Collection No. SNSD | Locality | ENA/GenBank No. | | References |
			cyt-b	ITS-2	
Radix ampla 7	Moll 53099	Germany, Bavaria, Lake Ammer, Stegen, 11°08'07''E 48°04'32''N	HE573111	HE573073	SCHNIEBS *et al.* 2011
Radix ampla 8	Moll 53285	Germany, Mecklenburg-Western Pomerania, lake Tollensesee near Klein Nemerow, N 53.4909° E 13.2146°	LS974280	LS974231	This paper
Radix ampla 9	Moll S2193	Germany, Mecklenburg-Western Pomerania, lake Luebkowsee 2km east of Schwichtenberg, 13°44.567'E 53°40.967'N	HE573112	HE573074	SCHNIEBS *et al.* 2011
Radix ampla 10	Moll S2924	Switzerland: Lake Biel, near bridge to St. Peter's Island	LS974281	LS974232	This paper
Radix ampla 11	Moll S2925	Germany, Brandenburg, lake Oberuckersee near Warnitz, N 53°10'52.3" E 13°52'15.7"	LS974282	LS974233	This paper
Radix ampla 12	Moll S5186	Czech Republic, Františkov nad Ploučnicí, Ploučnice River near the bridge, N 50°43'26" E 14°19'27.5"	LS974283	LS974234	This paper
Radix ampla 13	Moll S5340	Czech Republic, Samechov, Sázava River, N 49°53'02" E 14°51'28"	LS974284	LS974235	This paper
Radix ampla 14	Moll S5344	Czech Republic, Žizníkov, Ploučnice River, N 50°40'48" E 14°34'02"	LS974285	LS974236	This paper
Radix ampla 15	Moll S5347	Czech Republic, Vsetín, Vsetínská Bečva, N 49°19'29" E 17°59'48"	LS974286	LS974237	This paper
Radix ampla 16	Moll S5348	Czech Republic, Vsetín, Vsetínská Bečva N 49°19'29" E 17°59'48"	LS974287	LS974238	This paper
Radix ampla 17	Moll S5587	Czech Republic, river Ohře in Jindřichov, N 50°06'25" E 12°23'48"	LS974288	LS974239	This paper
Radix ampla 18	Moll S5588	Czech Republic, Chrudimka in Hostovice, N 50°00'22" E 15°51'16"	LS974289	LS974240	This paper
Radix ampla 19	Moll S5589	Czech Republic, Chrudimka in Hostovice, N 50°00'22" E 15°51'16"	LS974290	LS974241	This paper
Radix ampla 20	Moll S6511	Croatia, Zrmanja River near Bilišane, N 44°11'38" E 15°47'35''	LS974291	LS974242	This paper
Radix ampla 21	Moll S6512	Croatia, Zrmanja River near Bilišane, N 44°11'38" E 15°47'35''	LS974292	LS974243	This paper

Code	Collection No. SNSD	Locality	ENA/GenBank No.		
			cyt-b	ITS-2	References
Radix ampla 22	Moll S7456	Mecklenburg Western-Pomerania, Müritz-Elde-Channel neari Güritz, R 4466187 H 5902966	LS974293	LS974244	This paper
Radix ampla 23	Moll S7478	Germany, Mecklenburg-Western Pomerania, lake Tollensesee, N 53.470790 E 13.174872	LS974294	LS974245	This paper
Radix ampla 24	Moll S7479	Germany, Mecklenburg-Western Pomerania, lake Tollensesee, N 53.470790 E 13.174872	LS974295	LS974246	This paper
Radix ampla 25	Moll S7480	Germany, Mecklenburg-Western Pomerania, lake Tollensesee, N 53.470790 E 13.174872	LS974296	LS974247	This paper
Radix ampla 26	Moll S7497	Germany, Mecklenburg-Western Pomerania, lake Tollensesee near Klein Nemerow, N 53.499711 E 13.220142	LS974297	LS974248	This paper
Radix ampla 27	Moll S7498	Germany, Mecklenburg-Western Pomerania, lake Tollensesee near Klein Nemerow, N 53.499711 E 13.220142	LS974298	LS974249	This paper
Radix ampla 28	Moll S7499	Germany, Mecklenburg-Western Pomerania, lake Tollensesee in Wustrow, N 53.479150 E 13.158684	LS974299	LS974250	This paper
Radix ampla 29	Moll S7500	Germany, Mecklenburg-Western Pomerania, lake Tollensesee in Wustrow, N 53.479150 E 13.158684	LS974300	LS974251	This paper
Radix ampla 30	Moll S7501	Germany, Mecklenburg-Western Pomerania, lake Tollensesee in Wustrow, N 53.479150 E 13.158684	LS974301	LS974252	This paper
Radix monnardi 1	Tissue coll. 9762	Russia, Pskov Region, Velikaya River near Kholoye village, N 56°22'28'' E 28°58'05''	LS974302	LS974253	This paper
Radix monnardi 2	Tissue coll. 9764	Russia, Pskov Region, Velikaya River near Kholoye village, N 56°22'28'' E 28°58'05''	LS974303	LS974254	This paper
Radix monnardi 3	Moll S2986	Russia, Chelyabinsk Region, pond Ust-Katavskij at Katav River, N 54.916538 E 58.159363	LS974304	LS974255	This paper

Code	Collection No. SNSD	Locality	ENA/GenBank No.		References
			cyt-b	ITS-2	
Radix monnardi 4	Moll S2987	Russia, Chelyabinsk Region, pond Ust-Katavskij at Katav River, N 54.916538 E 58.159363	LS974305	LS974256	This paper
Radix monnardi 5	Moll S8730	Russia, Moscow region, river Oka near Turovo, 54°50.836'N, 37°52.799'E	LS974306	LS974257	This paper
Radix monnardi 6	Moll S8731	Russia, Moscow region, river Oka near Turovo, 54°50.836'N, 37°52.799'E	LS974307	LS974258	This paper
Radix monnardi 7	Moll S8733	Russia, Lipetzk Region, river Baigora near Plastinka, 52°14.855'N, 40°02.373'E	LS974308	LS974259	This paper
Radix monnardi 8	Moll S8734	Russia, Lipetzk Region, river Baigora near Plastinka, 52°14.855'N, 40°02.373'E	LS974309	LS974260	This paper

in the tissue collection of the SNSD by assigning a tissue voucher number and a corresponding collection number in the mollusc collection of SNSD, and are stored at -80°C.

To analyse the variability of the most important distinguishing morphological characters of *R. ampla* 30 individuals were examined from several European countries as well as six specimens of *R. monnardi* from Eastern Europea and Western Siberia. Characters examined were: 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. Sequence data of the nuclear ITS-2 spacer and the mitochondrial cyt–b gene (329bp fragment) were obtained from both species. An additional 52 individuals of *R. ampla* were dissected in order to measure the parts of the copulatory apparatuses – the praeputium and the penis sheath – the parts that are reputed to have a high taxonomic and diagnostic importance (Kruglov, 2005). Most of the dissected snails originated from Russia, excluding two specimens collected from Vallersee, Austria. An additional two specimens of *R. monnardi* were added to the molecular genetic analyses, but without morphological analyses because we only had foot tissue.

We made an effort to find and examine the type series of *R. ampla* and the two closely related and conchologically similar species, *R. hartmanni* and *R. monnardi*. The lectotype of *R. ampla* was designated and illustrated by Vinarski & Glöer (2007), while the type series of *R. monnardi* was taken on loan from the Naturmuseum Saint-Gallen, Switzerland (NSG). The lectotype of *R. hartmanni* was unavailable for us: but it is depicted and illustrated in Forcart (1957).

For the taxonomy of the freshwater molluscs used in the molecular genetic analyses, we followed the current European checklists (Falkner *et al.*, 2001; Bank, 2011a).

MOLECULAR TECHNIQUES AND SEQUENCE ANALYSES

For the molecular analyses, we obtained sequence data of the nuclear ITS-2 spacer (partial, with 28S ribosomal RNA gene, partial sequence), which is 280 bp long in *Aplexa hypnorum* and up to 495 bp in *Lymnaea stagnalis* (the length of the ITS-2 spacer varies within genera and families) and a 329 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 Schniebs *et al*. (2011). All DNA-sequences have been placed in the European Nucleotide Archive (ENA, see http://www.ebi.ac.uk/ena/), available also from GenBank (Table 1).

We used new sequences and published sequences from our earlier publications (Schniebs *et al*. 2011, 2013, Vinarski *et al*. 2011, 2012). For outgroup comparison, we used the Palaearctic species *Aplexa hypnorum* (Linnaeus, 1758) from the freshwater gastropod family Physidae Fitzinger, 1833 as well as *Lymnaea stagnalis* (Linnaeus, 1758) from the freshwater gastropod family Lymnaeidae Rafinesque, 1815, since they have proven useful for this purpose (e.g. e.g. Schniebs, Glöer, Vinarski, Quiñonero-Salgado, Lopez-Soriano & Hundsdoerfer 2017; Schniebs, Glöer, Quiñonero-Salgado, Lopez-Soriano & Hundsdoerfer 2018). We included sequences of *Radix auricularia*, *R. natalensis* (Krauss, 1848), *R. balthica*, *R. lagotis*, *R. dolgini* (Gundrizer & Starobogatov, 1979), and *R. labiata* in the ingroup for inter- and intraspecific comparisons.

Alignments were performed using the sequence alignment editor BioEdit (Hall,1999). The ITS-2 alignment was obtained using the Clustal algorithm of MEGA4 (Tamura, Dudley, Nei & Kumar, 2007) and improved by eye. For post-alignment editing, see Schniebs *et al*. (2017). MEGA 4 was also used to check the mitochondrial sequences for stop codons. Consequently, we chose an analytical approach under the maximum parsimony (MP) criterion to be able to include the gap code information. Losing this information by analysing under distance or maximum likelihood criteria would mean losing the greatest part of the phylogenetic signal. The phylogenetic analyses for the ITS-2 spacer and cyt–b fragment were carried out using PAUP (version 4.0b10; Swofford, 2002; settings: gapmode=NewState, addseq=closest, maxtree=10000; number of bootstrap replicates=10000). For presentation of the MP results for ITS-2 and cyt–b, one of the 4360 and 592 best trees respectively was chosen to be able to illustrate branch lengths (one showing the same overall topology as the majority rule consensus tree was chosen).

For maximum-likelihood analyses including bootstrap support, we used RAxML (raxml-GUI 0.9 beta 2, Silvestro & Michalak, 2010; Stamatakis, Ludwig & Meier, 2005). Although often misleading, the best-suited nucleotide models were determined using jModelTest v. 0.1.1 (Posada, 2008), since the parameters of the GTR model are estimated in RAxML and this also includes all the simpler models. The best fit model for cyt–b as well as for ITS-2 selected by Akaike information criteria (AIC) was GTR+G. In accordance, GTRGAMMA was selected for the maximum-likelihood analyses. The settings were "ML+thorough bootstrap" with 100 (replicate) runs and 1000 (bootstrap) repetitions.

A haplotype network (median joining; Bandelt, Forster & Röhl, 1999; strictly a non dichotomous tree, called a network due to its representation, although it has no loops) was computed with the program "Network" (www.fluxus-engeneering.com).

RESULTS

Morphology

Shell   The shells of the examined specimens vary from nearly spherical and ear-shaped to egg-shaped and conical egg-shaped (e.g. Fig. 1). The height of the shells ranges from 9.33 to 20.07mm, their width from 9.33 to 20.10mm and the number of whorls between 2.5 and 3.5. Six of the 27 examined shells were spherical-oval in outline, approximately as high as wide (e.g. Fig. 1A–D). The other 21 shells varied from ear-shaped to egg-shaped and conical egg-shaped (e.g. Fig. 1E–H). In only two of all examined specimens the upper margin of the aperture is higher than the apex (Fig. 1A–B).

Mantle pigmentation   Mantle pigmentation of the examined 32 specimens shows a broad polymorphism (Fig. 2). The primary colour of the mantle varies from black (24 specimens, e.g. Fig. 2A, C, D, G) and brownish black (three specimens, e.g. Fig. 2E, F) to greyish brown (three specimens), grey (one specimen, Fig. 2H), and light-brown (one specimen, Fig. 2B). On top of this primary colour the mantle shows spots and/or dots of different size and colour. In nine of the examined specimens, the mantle shows only a few large white or yellow spots, of which some are fused (Fig. 2A–B). In two specimens, a relatively large number of large and small white spots were found and many of these spots were fused (Fig. 2D). In most of the specimens (14), the white, grey or yellow spots were smaller and only two or three of them fused (e.g. Fig. 2C, E). In seven specimens, all spots and dots were well separated (e.g. Fig. 2D).

The mantle collar is from whitish to grey or yellowish grey in colour and has numerous irregular patches of black or brownish black. The
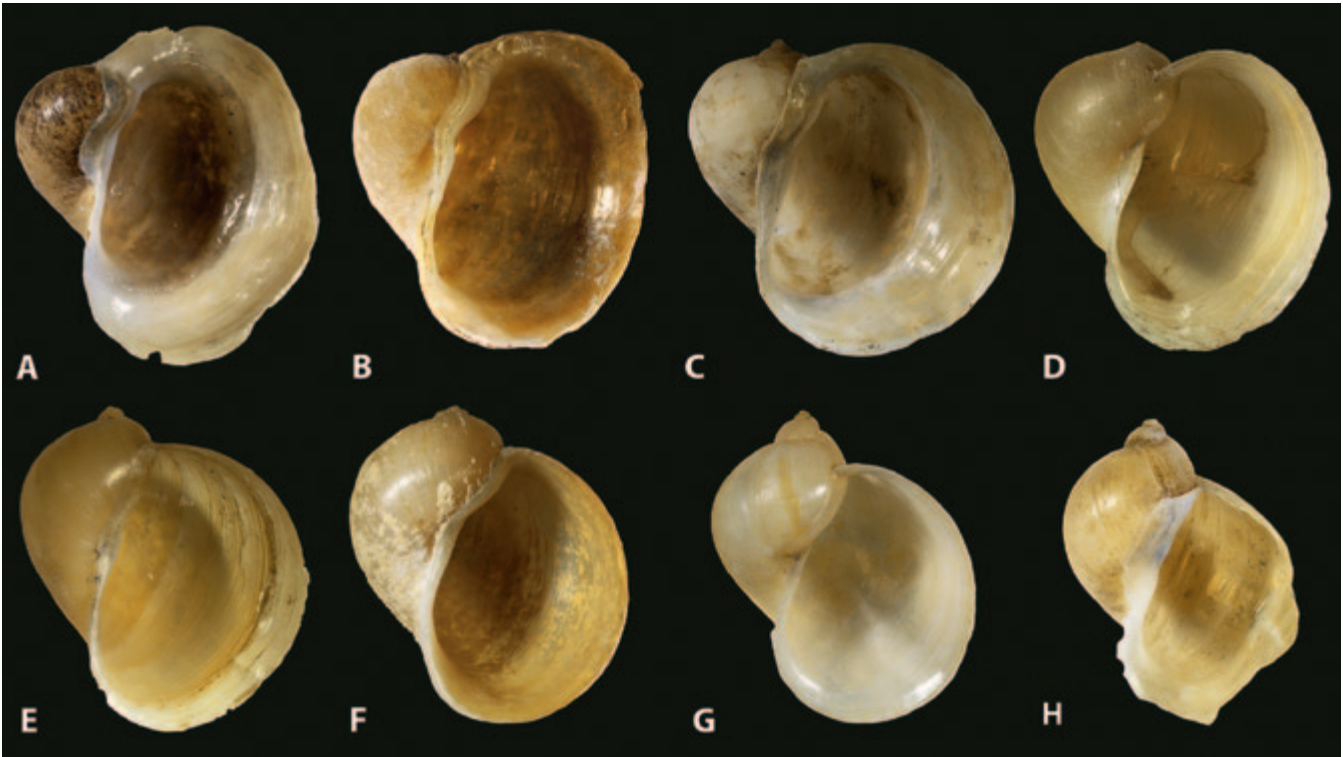
Figure 1 Variability in *Radix ampla* shells: A) Germany, Mecklenburg-Western Pomerania, Lake Tollensesee (*Radix ampla* 8, shell height 20.1mm); B) Germany, Mecklenburg-Western Pomerania, Lake Tollensesee (*Radix ampla* 24, shell height 13.4mm); C) Germany, Baden-Württemberg: Lake Constance (*Radix ampla* 4, shell height 14.8mm); D) Russia, Chelyabinsk Region, Miass River (*Radix ampla* 1, shell height 13.5mm); E) Germany, Mecklenburg-Western Pomerania, Lake Tollensesee (*Radix ampla* 29, shell height 10.0mm); F) Croatia, Zrmanja River near Bilisane (*Radix ampla* 21, shell height 13.4mm); G) Germany, Baden-Württemberg: Lake Constance (*Radix ampla* 5, shell height 15.4mm); H) Czech Republic, Frantiskov nad Ploucnicí, Ploucnice River (*Radix ampla* 12, height of the shell fragment 15.1mm).

colour of the mantle edge varies from white or grey to yellow and bluish green.

Male genitalia The measurements of praeputium and penial sheath of 22 of the specimens examined by molecular genetics from Western and Central Europe as well as from Western Siberia, are reported in Table 2 (see also Fig. 3). The ratio of the length of the praeputium to that of the penial sheath varies from 0.60–1.42 (n=22). A similar range of variation in this character is observed in a sample (n=52) of *R. ampla* collected in Russia (Fig. 4), with limits of variation being 0.57–1.21 (mean 0.88±0.13).

The praeputium is white, light yellow (e. g. Fig. 3B–D) or yellowish grey (e.g. Fig. 3A) in most specimens. One specimen showed a grey of the praeputium (Moll S6512, Fig. 3F), and two others a yellow colour of the praeputium (Moll S7499, Fig. 3E). In four specimens, the yellowish praeputium had an additional grey pigmentation.

The phallotheca was white or light yellow in 24 specimens studied. Only two specimens showed an additional grey pigmentation.

Bursa copulatrix The length of the bursa duct varied in 17 specimens between nearly not visible (n=7; Fig. 5F), up to half of the length of the bursa between 0.30 and 0.59mm (n=5; Fig. 5E), and nearly as long as the bursa between 1.31 and 1.73mm (n=5; Fig. 5A–D). The shape of the bursa was very variable, from nearly spherical (e.g. Fig. 5A) to elongate (e.g. Fig. 5C). In most specimens examined, the bursa duct entered on the ventral side into the provagina above the female vent. The bursa duct entered laterally in only two specimens.

Molecular phylogeny & phylogeography
The maximum-parsimony (MP) tree of the mitochondrial cyt–b marker (tree length=325, CI=0.6743, RI=0.9343) is illustrated in Fig. 6.
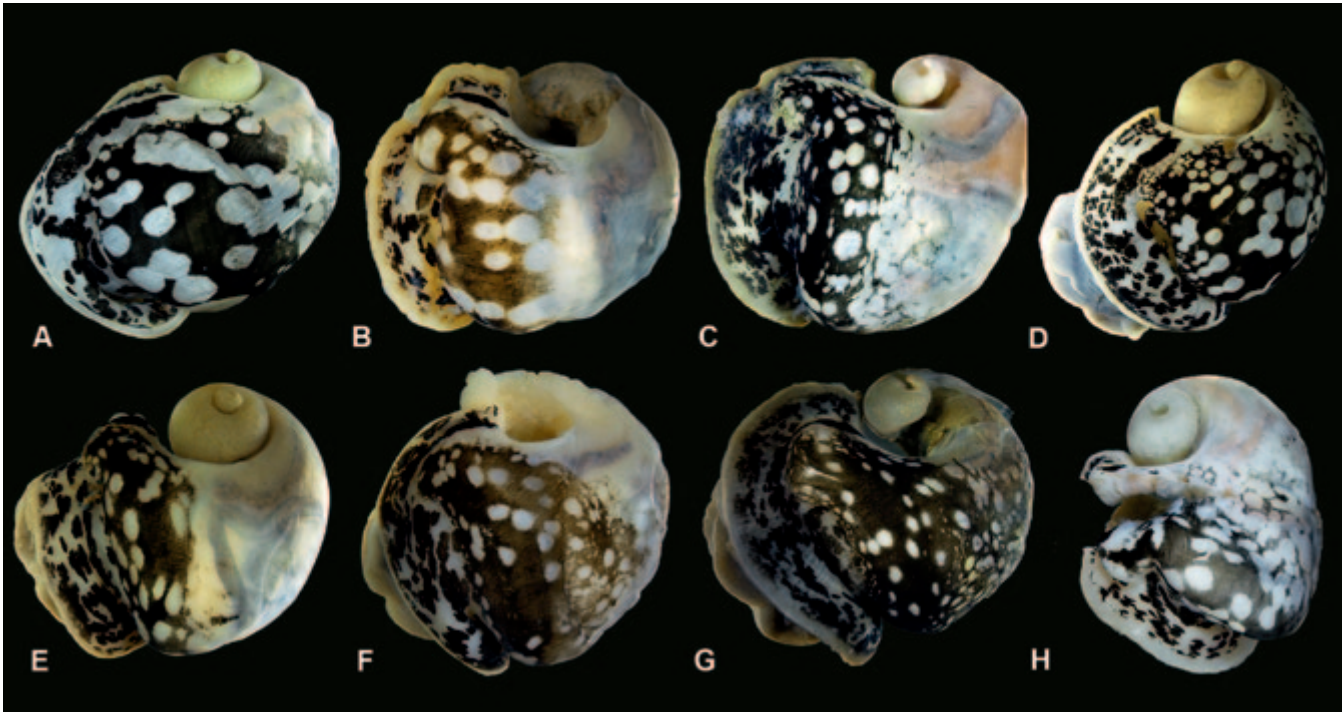
Figure 2   Variability in *Radix ampla* mantle pigmentation: A) Switzerland, Lake Biel (*Radix ampla* 10, shell height 13.4mm); B) Germany, Baden-Württemberg: Lake Constance (*Radix ampla* 4, shell height 14.8mm); C) Germany, Mecklenburg-Western Pomerania, Lake Tollensesee (*Radix ampla* 28, shell height 16.3mm); D) Czech Republic, Frantiskov nad Ploucnicí, Ploucnice River (*Radix ampla* 12, height of the shell fragment 15.1mm); E) Germany, Baden-Württemberg: Lake Constance (*Radix ampla* 5, shell height 15.4mm); F) Russia, Chelyabinsk Region, pond Ust-Katavskij (*Radix monnardi* 3, height of the shell fragment 12.0mm); G) Germany, Mecklenburg-Western Pomerania, Lake Tollensesee (*Radix ampla* 8, shell height 20.1mm); G) Croatia, Zrmanja River near Bilisane (*Radix ampla* 21, shell height 13.4mm).

Although the most basal branches have less than 81% bootstrap support, the clades of the species themselves have full support, except for *R. ampla* and *R. monnardi*.

The specimens of *R. ampla* plus *R. monnardi* form a distinct clade that is separated from *R. balthica* and *R. lagotis* with a bootstrap support of 77%. Within this clade, the specimens determined as *R. ampla* or *R. monnardi* from the European part of Russia and Western Siberia constitute a sister group (with 100% bootstrap support) to the *R. ampla* specimens analysed from Central Europe and Croatia.

In the RAxML tree of the cyt–b fragment (not shown) most of the basal branches show very low support (between 22 and 70%). The clades of the species themselves received full or nearly full support in most cases, except for *R. auricularia* (87%) and *R. ampla* (16%). The specimens of *R. ampla* form two subclades. One of these subclades, including six specimens of *R. ampla* and four of *R. monnardi*, groups sister to a branch

formed by the *L. stagnalis* specimens with 26% bootstrap support.

In the hypothesis of the phylogenetic relationships of *R. ampla* based on one of the 10000 best MP trees of the nuclear ITS-2 spacer (Fig. 7, tree length=1096, CI=0.7929, RI=0.9559), only a few of the basal branches are fully or very highly supported. The very low support of the others is expressed by a polytomy of *R. labiata*, *R. dolgini* and a polytomic cluster (83% support) of *R. lagotis*, *R. balthica*, and *R. ampla*. With the exception of the last three species, the clades of the species themselves have high (95 and 96%) or full support (*R. natalensis*, *R. dolgini*). All specimens identified as *R. ampla* and *R. monnardi* analysed fall into one cluster with 53% bootstrap support. Within the polytomic cluster of *R. lagotis*, *R. balthica* and *R. ampla*, one specimen identified by morphology and the mitochondrial marker cyt–b as *R. balthica* (specimen *Radix balthica* 6) groups sister to a cluster of all other specimens of *R. balthica* and *R. ampla* analysed.

Table 2 Measurements of praeputium and penial sheath of 22 specimens from Western and Central Europe as well as from Western Siberia examined by molecular genetics.

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 51112	3.50	5.80	1:1.66
Moll 51113	3.43	5.50	1:1.60
Moll 53082	4.02	3.45	1.16:1
Moll 53083	3.90	2.86	1.36:1
Moll 53098	3.25	2.90	1.12:1
Moll 53099	3.79	2.80	1.35:1
Moll S2924	3.77	5.18	1:1.37
Moll S2925	3.10	3.10	1:1
Moll S2986	2.97	2.44	1.21:1
Moll S5186	3.82	5.32	1:1.39
Moll S5348	2.80	3.45	1:1.23
Moll S5588	2.45	3.00	1:1.22
Moll S6511	3,08	2,16	1.42:1
Moll S6512	2.79	2.10	1.33:1
Moll S7456	3.30	4.30	1:1.30
Moll S7478	3.30	2.80	1.18:1
Moll S7479	1.25	1.43	1:1.14
Moll S7497	3.10	2.60	1.19:1
Moll S7498	2.70	2.10	1.28:1
Moll S7499	3.80	2.80	1.36:1
Moll S7500	1.70	1.50	1.13:1
Moll S7501	2.40	3.50	1:1.46
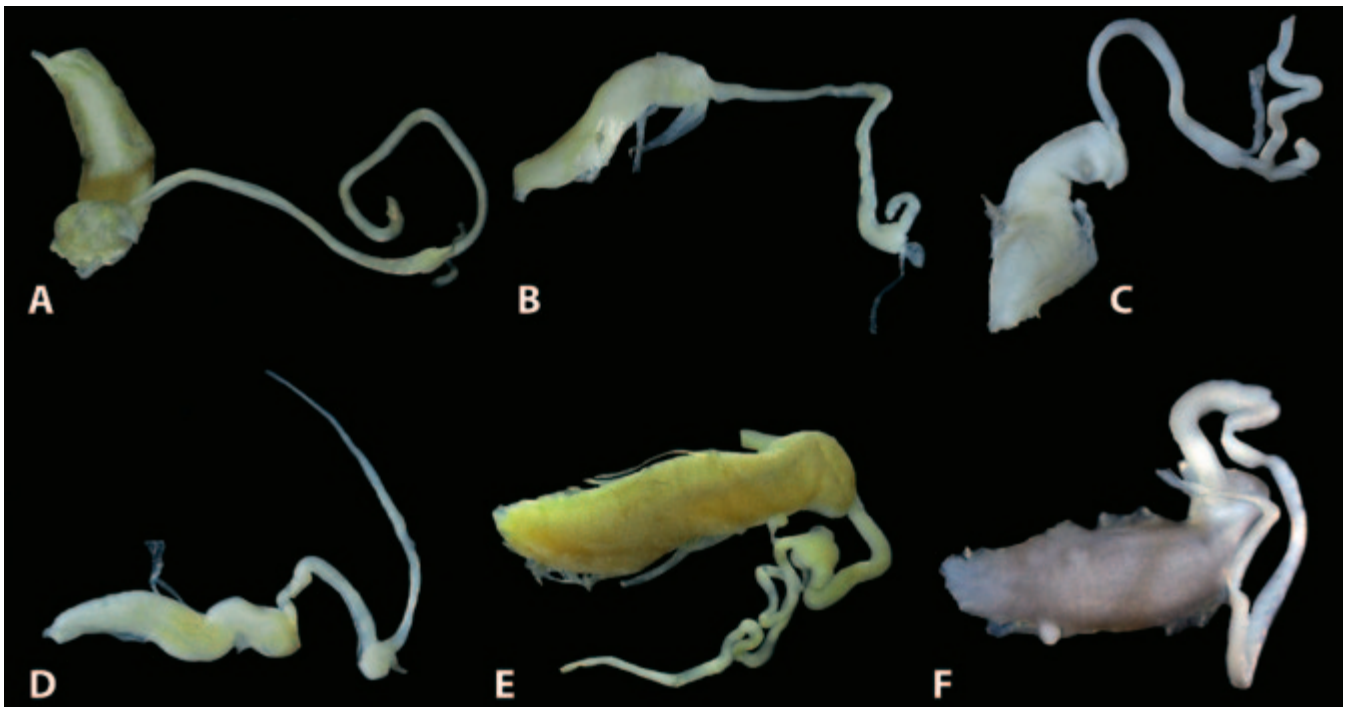
Figure 3 Variability in *Radix ampla* male genitalia: A) Russia, Chelyabinsk Region, Miass River (*Radix ampla* 2); B) Germany, Mecklenburg-Western Pomerania, Lake Tollensesee (*Radix ampla* 30); C) Czech Republic, Vsetin, Vsetínská Bečva (*Radix ampla* 16); D) Germany, Mecklenburg-Western Pomerania, Lake Tollensesee (*Radix ampla* 29); E) Germany, Mecklenburg-Western Pomerania, Lake Tollensesee (*Radix ampla* 28); F) Croatia, Zrmanja River near Bilisane (*Radix ampla* 21). Scale bar=1mm.
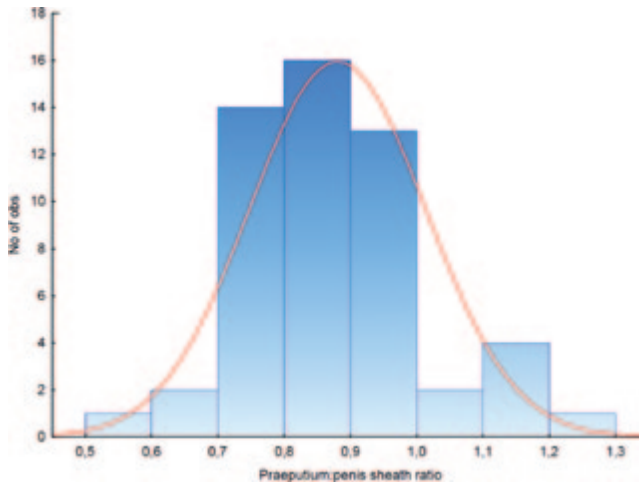
Figure 4 Distribution of the praeputium:penis sheath lengths ratio in a sample (n=52) of *R. ampla* from various localities of Central Europe (Vallersee, Austria), Eastern Europe, the Urals, and Western Siberia. The red line corresponds to the normal distribution curve.

The RAxML tree of the ITS-2 spacer (not shown) shows lower bootstrap values for the basal branches as well as for the clades of the species themselves. Again the species *R. lagotis*, *R. balthica*, *R. ampla*, and *R. monnardi* show a polytomic cluster that groups sister with *R. dolgini* (74% bootstrap support). Within this polytomic cluster, two specimens of *R. monnardi* (*Radix monnardi* specimens 1 and 2) group sister to a polytomic cluster of all other specimens of *R. lagotis*, *R. balthica*, *R. ampla*, and *R. monnardi*. Only the specimens of all *R. lagotis* analysed, form a separate species clade with 53% bootstrap support. The *Radix balthica* specimen 6 is not part of the cluster of all other specimens of *R. balthica* analysed, but falls into a polytomic subcluster grouping sister to four specimens determined as *R. monnardi* from Moscow region and Lipetsk region in the European part of Russia (*Radix*
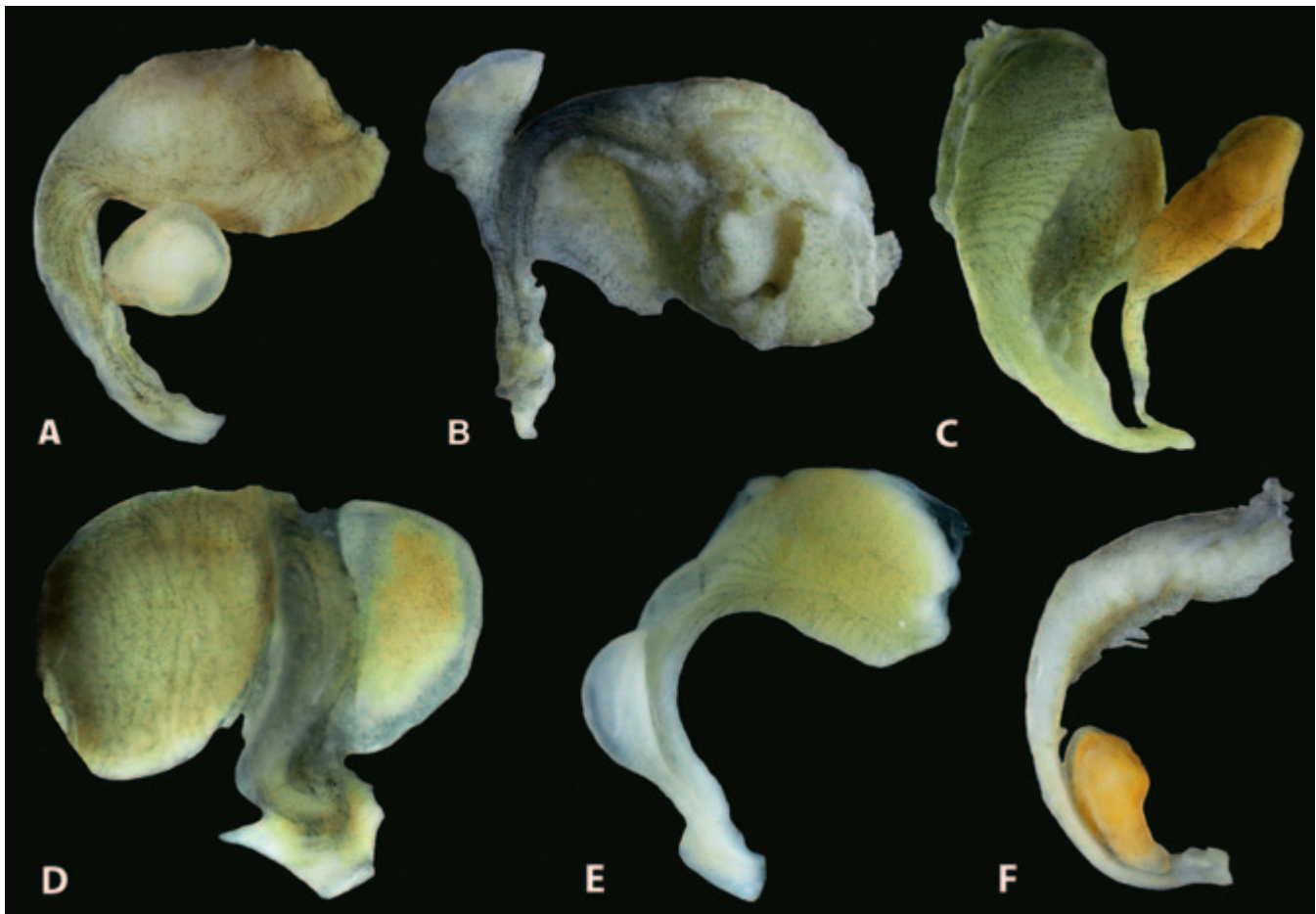

Figure 5 Variability of the form of the bursa copulatrix and the length of the bursa duct in *Radix ampla*: A) Russia, Chelyabinsk Region, pond Ust-Katavskij (*Radix monnardi* 3); B) Russia, Chelyabinsk Region, Miass River (*Radix ampla* 1); C) Germany, Mecklenburg-Western Pomerania, Lake Tollensesee (*Radix ampla* 26); D) Russia, Chelyabinsk Region, Miass River (*Radix ampla* 2); E) Switzerland, Lake Biel (*Radix ampla* 10); F) Germany, Baden-Württemberg: Lake Constance (*Radix ampla* 5). Scale bar=1mm.
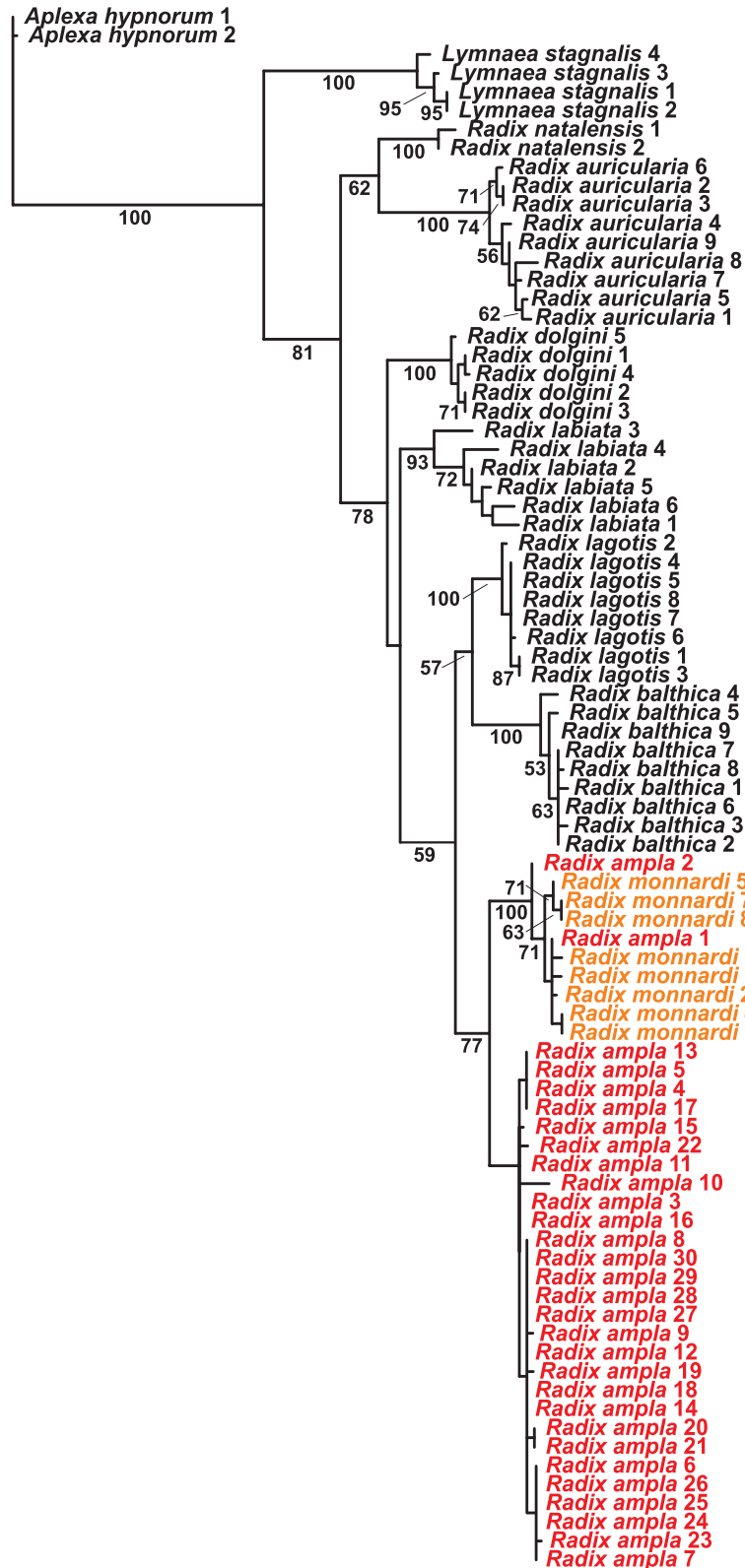
Figure 6 Hypothesis of the phylogenetic relationships of *R. ampla* and *R. monnardi* based on one of the 8421 best maximum-parsimony trees of the mitochondrial marker cyt–b (fragment of 329 bp; tree length=325, CI=0.6743, RI=0.9343). The overall topology corresponds to that of the strict consensus tree. Branch lengths are proportional to the number of substitutions. Bootstrap support values above 50% are reported below nodes. The *R. ampla* specimens are marked red and the *R. monnardi* specimens orange.
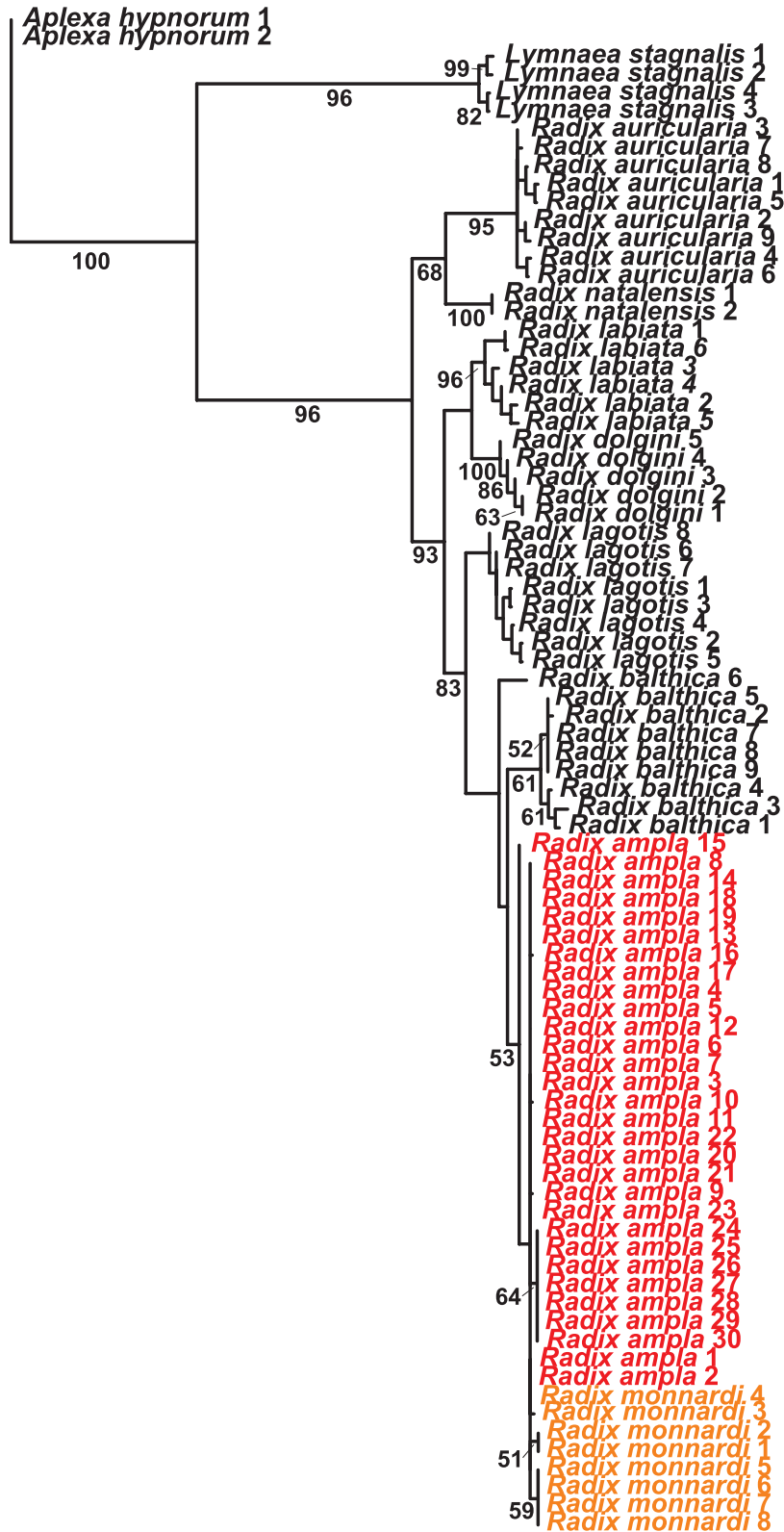
Figure 7 Hypothesis of the phylogenetic relationships of *R. ampla* and *R. monnardi* based on one of the best 10000 maximum-parsimony trees of the nuclear marker ITS-2 (tree length=1096, CI=0.7929, RI=0.9559). The overall topology corresponds to that of the strict consensus tree. Branch lengths are proportional to the number of substitutions. Bootstrap support values above 50% are reported below nodes. The *R. ampla* specimens are marked red and the *R. monnardi* specimens orange.
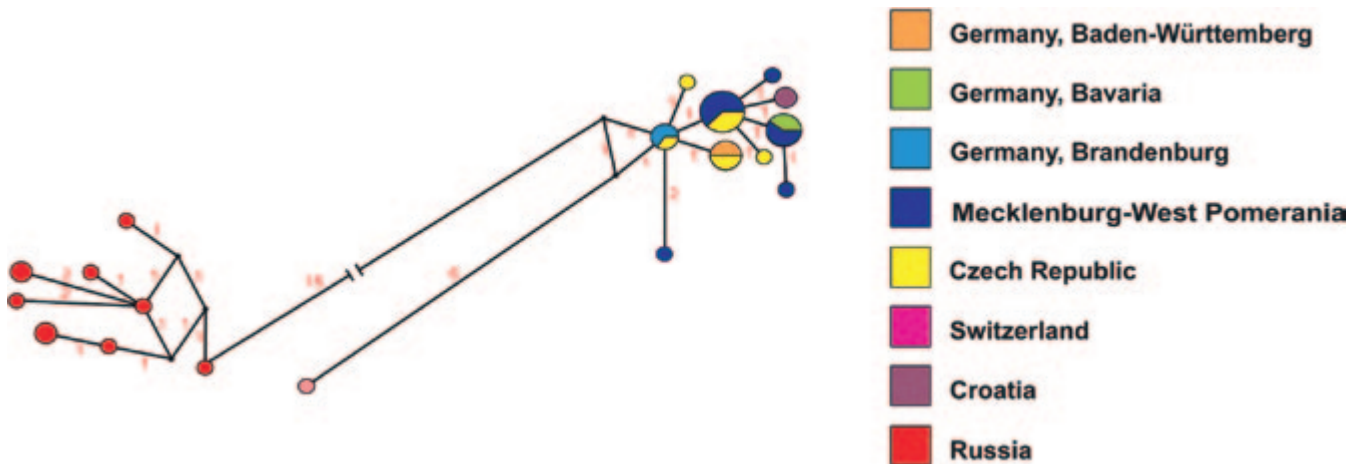
Figure 8 Haplotype network of cyt–b sequences (314 bp) of 38 *Radix ampla* specimens from different localities in Europe and from the Urals. The small black dots represent internal haplotypes not present in the dataset. The length of the connecting lines is (mostly) proportional to the number of substitutions between haplotypes (reported as red numbers on the lines).

monnardi specimens 5, 6, 7, 8), a clade of all *R. lagotis* specimens, as well as to a clade formed by the remaining specimens of *R. balthica* analysed. All specimens determined by morphology and the mitochondrial marker as *R. ampla* form one cluster with 58% bootstrap support that groups sister to two specimens determined as *R. ampla* and two specimens determined as *R. monnardi* from Western Siberia.

The main difference between the MP and the RAxML tree of the ITS-2 spacer is that only in the MP tree all specimens of *R. ampla* and *R. monnardi* form one cluster. In the RAxML tree, specimens of *R. ampla* and *R. monnardi* analysed group into four different clusters with very close phylogenetic relationships to specimens of *R. balthica* and *R. lagotis*.

The network representation of mitochondrial sequences (cyt–b, 314 bp; ingroup only, Fig. 8) shows differentiation into two main lineages, a Western European one (including the samples from Germany, Croatia, the Czech Republic and Switzerland) and a Russian one, including all *R. monnardi* and two *R. ampla* specimens.

DISCUSSION

Morphology

Shell The typical shell of *R. ampla* is described as auriculate or spherical-oval in outline, approximately as high as wide (Piechocki & Wawrzyniak-Wydrowska, 2016), with a strongly spherical, inflated last whorl (Andreyeva *et al.*, 2010; Glöer, 2002; Khokhutkin *et al.*, 2009; Piechocki & Wawrzyniak-Wydrowska, 2016). The aperture is very large (Piechocki & Wawrzyniak-Wydrowska, 2016) and square-shaped, rounded with palatal and parietal edges forming an obtuse angle or sometimes perpendicular to each other (Andreyeva *et al.*, 2010; Khokhutkin *et al.*, 2009), its upper margin is higher than the apex (Glöer, 2002; Piechocki & Wawrzyniak-Wydrowska, 2016) or at the level of the apex (Andreyeva *et al.*, 2010; Piechocki & Wawrzyniak-Wydrowska, 2016) or reaching only 0.85 of the shell height (Stadnichenko, 2004). The spire is very low (Andreyeva *et al.*, 2010; Glöer, 2002; Khokhutkin *et al.*, 2009; Kijashko *et al.*, 2016, Piechocki & Wawrzyniak-Wydrowska, 2016; Stadnichenko, 2004), up less than 0.2 of the shell height (Kijashko *et al.*, 2016), conical. The number of whorls is given with 3–3.5 (Piechocki & Wawrzyniak-Wydrowska, 2016), 4 (Georgiev, 2014), 3.50–4.25 (Andreyeva *et al.*, 2010), 4.0–4.25 (Khokhutkin *et al.*, 2009) up to 4.5 (Stadnichenko, 2004). These differences appear to be due to different schemes of whorls count used by various authors.

The columellar margin of aperture is straight and forms an almost straight, oblique line with the wall of the aperture (Glöer, 2002) without a bend (Georgiev, 2014; Khokhutkin *et al.*, 2009; Piechocki & Wawrzyniak-Wydrowska, 2016) or with a weak bend (Andreyeva *et al.*, 2010; Glöer, 2002; Georgiev, 2014; Khokhutkin *et al.*, 2009). The shell

is strong (Piechocki & Wawrzyniak-Wydrowska, 2016; Stadnichenko, 2004) or fragile (Andreyeva *et al.*, 2010; Stadnichenko, 2004), whitish or corneous (Piechocki & Wawrzyniak-Wydrowska, 2016), light yellowish or light brown (Andreyeva *et al.*, 2010; Khokhutkin *et al.*, 2009; Stadnichenko, 2004). Different shell heights are reported: 20mm (Glöer, 2002), up to 29.6mm (Stadnichenko, 2004), up to 30mm (Andreyeva *et al.*, 2010; Khokhutkin *et al.*, 2009), 20–40mm (Piechocki & Wawrzyniak-Wydrowska, 2016). The width of the shells is given as 19mm (Glöer 2002), up to 23.3mm (Stadnichenko, 2004), 19–40mm (Piechocki & Wawrzyniak-Wydrowska, 2016). The dimensions of the lectotype of *R. ampla* with 3.5 whorls are: shell height 21.5; shell width 21.0; spire height 1.2; spire width 1.5; body whorl height 20.3; body whorl width 21.0; aperture height 20.0; aperture width 17.0 (Vinarski & Glöer 2007).

Only six of 27 shells examined in our study show the typical nearly spherical or spherical-oval shape (e.g. Fig. 1A–D). The egg-shaped and conically egg-shaped shells of *R. ampla* specimens (e.g. Fig. 1E–H) identified in our study with molecular genetic methods could be confused with the shells of such forms in *R. balthica* or *R. auricularia*, especially because they show a relatively high spire (Fig. 1G–H).

The variability is also manifested by the different development of the ear-shaped aperture, which does not reach the apex in most specimens analysed (e.g. Fig. 1D–H). This is in contrast to descriptions in the literature (Glöer, 2002; Andreyeva *et al.*, 2010; Piechocki & Wawrzyniak-Wydrowska, 2016) and the original description (Hartmann, 1821: 251, pl. II). Moreover, in our study on intraspecific variability of *R. balthica* (Schniebs *et al.*, 2011) we have observed that the wandering pond snail could develop ear-shaped shells too, but their initial whorls are more convex (Piechocki & Wawrzyniak-Wydrowska, 2016). The Russian authors consider such specimens of *R. balthica* as belonging to a distinct species, *R. ampullacea* (Rossmässler, 1835) (Stadnichenko, 2004; Kruglov, 2005). Therefore, a clear distinction between *R. ampla* and *R. balthica* based only on the shells is difficult and not possible in some cases. Traditionally, the shell shape was one of the most important characters to determine snail species (Hubendick, 1951), but it has been rightly criticized to be too much determined by the environment and habitat (e.g. Schniebs *et al.*,

2011, 2013). However, in some cases it can still provide taxonomic information to experts, for example, *R. auricularia* in Central Europe has a specific shape to the first two whorls in most of the specimens (e.g. Glöer, 2002, Piechocki & Wawrzyniak-Wydrowska, 2016). An experienced person can therefore distinguish between shells of *R. auricularia* and other *Radix* species by the typical pointed and narrowly conical spire in most specimens of *R. auricularia*.

Mantle pigmentation   Our study found a higher variability in mantle pigmentation for *R. ampla* than previously published. Hitherto, the pigmentation of this species was described as dark with a few large white spots (Piechocki & Wawrzyniak-Wydrowska, 2016, Schniebs *et al.*, 2011) or as light grey, whitish grey or yellowish grey for *R. monnardi* (Stadnichenko, 2004).

One result of our study was the observation that just a small part (nine of 32 analysed specimens) of *R. ampla* show the typical mantle pigmentation (Piechocki & Wawrzyniak-Wydrowska, 2016; Schniebs *et al.*, 2011) (Fig. 2A–B). Most of the other (22 of 32 analysed specimens) show a mantle pigmentation typical for *R. balthica*: many medium-sized, light distinct spots on dark background (Glöer 2002, 2015; Piechocki & Wawrzyniak-Wydrowska, 2016; Schniebs *et al.*, 2011) (Fig. 2C, E–H).

That is the reason why mantle pigmentation could be used for differentiation from *R. balthica* only if the mantle shows only a few large white or yellow spots, of which some are fused (Fig. 2A–B).

Male genitalia   The ratio between lengths of praeputium and penis sheath for *R. ampla* is given as 0.7–1.0 (Kijashko *et al.*, 2016) and for *R. monnardi* as equal to 1.76 (Kruglov, 2005). In Siberian populations the ratio varies from 0.57 to 0.91 (Vinarski, 2009). Andreyeva *et al.* (2010) mentioned that the praeputium in *R. ampla* is significantly shorter than the penis sheath. Stadnichenko (2004) has also determined that the penis sheath in *R. monnardi* is significantly longer than the praeputium (1:0.76). The individuals studied in this analysis show ratios within the extremes reported and they do not differ much from the ratios found in *R. balthica* (0.7–1.3, Schniebs *et al.*, 2011). Thus, contrary to some claims (Kruglov, 2005), it is virtually impossible

to use this ratio as a reliable tool for species identification within *Radix*.

Most specimens analysed in our study confirm the observation of Georgiev (2014) concerning monochromatic pigmentation of the male genitalia. But a yellowish pigmented praeputium is more typical than a white praeputium (Schniebs *et al.,* 2011). In four specimens the yellowish praeputium had an additional grey pigmentation. That is why the pigmentation of the male genitalia is also not a very reliable distinguishing character.

Bursa copulatrix Hitherto, *R. ampla* bursa duct lengths were known only from nearly not visible (Schniebs *et al.*, 2011, Fig. 9.3) as very short (Stadnichenko, 2004) and short (Khokhutkin *et al.*, 2009), to shorter than the bursa (Georgiev, 2014) but not extending half of the length of the bursa (Piechocki & Wawrzyniak-Wydrowska, 2016; Schniebs *et al.*, 2011; Stadnichenko, 2004 as *Lymnaea patula, Lymnaea monnardi*; Vinarski & Glöer 2007, Fig. 2.D). But we found bursa ducts nearly as long as the bursa (e.g. Fig. 5A–B).

In 2011, Schniebs *et al*. mentioned the character "position of the bursa" and "bursa duct behind the vagina and provaginal duct" as characteristic for *R. labiata*, confirming the information given by Stadnichenko (2004) and Kruglov (2005). In the meantime, this position of the bursa and the bursa duct was found not only in specimens of *R. ampla* determined by molecular genetics, but also in newly analysed *R. balthica* and *R. lagotis*. That is why the position of the bursa and the bursa duct character is no longer useful as reliable character for the differentiation of these *Radix* species.

Molecular phylogeny & phylogeography
Our molecular genetic analyses show that *R. ampla*, *R. balthica*, and *R. lagotis* are three closely related species. According to Russian authors (Kruglov, 2005; Andreeva *et al.*, 2010; Vinarski & Kantor, 2016) they should be classified within a separate subgenus *Peregriana* Servain, 1881, distinct from the nominative one (with *R. auricularia* as its type species). New taxonomic studies based on molecular genetics place *R. ampla* as well as *R. balthica* and *R. lagotis* into the genus *Ampullaceana* Servain, 1881 (Aksenova *et al.* 2018).

Whereas the seven *Radix* species used for this analysis differ very well in the mitochondrial marker cyt–b and the species clades themselves are fully or very highly supported (except for the clade of *R. ampla* and *R. monnardi*), both trees of the nuclear marker ITS-2 show a polytomy of *R. ampla*, *R. balthica* and *R. lagotis* with 85% bootstrap support in the MP tree (Fig. 7) and 74% in the RAxML tree (not shown). In our previous molecular genetic analyses of *Radix* species (Schniebs *et al.,* 2011, 2013, 2015) the species cluster of these three species *R. ampla*, *R. balthica*, and *R. lagotis* always showed full support. However, only a small number of *R. ampla* specimens (4–5) was used in these analyses.

In both the MP (Fig. 6) and the RAxML (not shown) tree of the mitochondrial marker cyt–b, the specimens of *R. ampla* and *R. monnardi* from Western Siberia and the European part of Russia form a subclade separated from the other European specimens of *R. ampla* analysed (with full bootstrap support in the MP tree and 79% support in the RAxML tree). Recently, a similar intraspecific dichotomy with two subclades, Western European and Russian, was found in *R. auricularia* (Aksenova *et al.,* 2017). In contrast, the sequences of the nuclear marker ITS do not form a highly supported cluster of the specimens from Western Siberia and the European part of Russia.

In addition, the species *Radix pinteri* Schütt, 1974 and *Radix relicta* Poliński, 1929 should be mentioned here as possible extreme morphological forms of *R. ampla*. *R. pinteri* is known only from Lake Prespa from three localities in Macedonia (Schütt, 1974; Albrecht *et al.*, 2008). *R. relicta* is recorded from Lake Ohrid between Macedonia and Albania (Poliński, 1929, Albrecht *et al.*, 2008). Unpublished phylogenetic analyses with COI sequences of several species of the genus *Radix* including *R. ampla* and the COI sequences of the *R. pinteri* and *R. relicta* specimens available from GenBank from Albrecht *et al*. (2008), also show (see Fig. 3 from Albrecht *et al.*, 2008) that based on this marker, *R. pinteri* and *R. relicta* fall into one cluster with *R. ampla* that could be interpreted as a species cluster. Unfortunately, no tissue material and/or nuclear ITS-2 markers of these two forms are available for us at this time. Therefore, we could not include them into our analyses.

The network relationships of the mitochondrial haplotypes coloured with respect to their geographic origin (Fig. 8) shows a distinct correlation of haplotype occurrence to geographic origin. All Russian samples, irrespective of whether they were determined as *R. ampla* or *R. monnardi*,
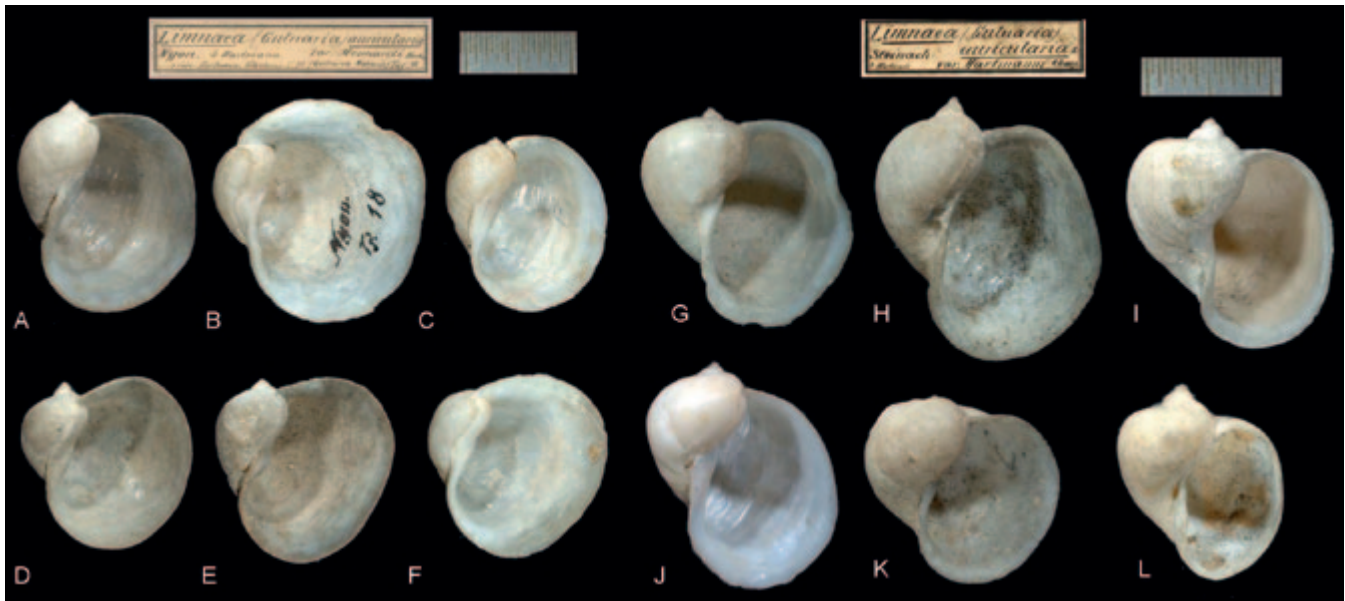
Figure 9 Shells (syntypes or topotypes) of *R. monnardi* and *R. hartmanni* (NSG). A–F) Switzerland, canton Vaud, Nyon (*R. monnardi*, syntypes); G–L) Switzerland: Lake Constance (*R. hartmanni*, topotypes; J. Hartmann's collection).

formed a cluster that is well separated from the European one by a minimum of 15 mutation steps.

Taxonomy
Kruglov (2005) delineated as many as three distinct species of snails that would correspond to *R. ampla sensu* Glöer (2002): *Lymnaea patula*, *L. hartmanni*, and *L. monnardi*. The first species name is, according to Vinarski & Glöer (2007), a synonym of *R. balthica* and will not be discussed further, whereas the two other species are still mentioned in Russian literature, although Vinarski & Kantor (2016) doubt their validity. According to Kruglov (2005), these two species may be distinguished from *R. ampla* (= *Lymnaea patula sensu* Kruglov) by an extremely enlarged aperture with an upper margin elevated above the shell apex. However, the examination of the type series of *R. monnardi* (Fig. 9) as well as a series of *R. hartmanni* topotypes (see Fig. 9) has shown that the position of the shell apex in relation to the aperture is quite variable. The lectotype of *R. hartmanni* (see Forcart, 1957, Taf. 5, fig. 28) has a high spire, and the apex of this shell lies much higher than the upper margin of the aperture. Among syntypes of *R. monnardi* (see Fig. 9), some specimens have a very inflated aperture, with its upper edge being higher than the apex, but other shells do not.

In our opinion, this character (upper edge of the aperture being higher than the apex) does not represent a species-specific trait and can neither be used for taxonomic nor for diagnostic purposes. This observation is in accordance with the above-mentioned absence of a reliable genetic distinction between *R. monnardi* and *R. ampla*. It thus appears reasonable to treat *R. monnardi sensu* Kruglov (2005) and *R. hartmanni sensu* Kruglov (2005) as full synonyms of *R. ampla*. The taxonomic revision thus formally reads:

  R. monnardi **syn. rev.** *R. ampla*
  R. hartmanni **syn. rev.** *R. ampla*
A nomenclatural note concerning the authorship of *R. hartmanni* must be added here. Hubendick (1951) and Kruglov (2005) referred this species to as *Lymnaea hartmanni* (Studer, 1820). Formally, the taxonomic name *Limneus hartmanni* Studer, 1820 has a priority before *Limneus auricularia* var. *ampla* Hartmann, 1821. However, Studer's name represents a nomen nudum (Forcart, 1957) since its description does not include a diagnosis. Thus, according to ICZN (art. 50.1), the authorship of this name should be ascribed to Hartmann (1821), whose description made it available. The two names, *Limneus auricularia* var. *ampla* and *Limneus auricularia* var. *hartmanni* were published simultaneuosly, and we, acting as First Revisers (ICZN, art. 24.2), choose the former one for the designation of this species.

CONCLUSIONS

With this new knowledge of the intraspecific variability of *R. ampla*, it is no longer possible in some cases to differentiate all specimens of *R. ampla*, *R. balthica*, and *R. lagotis* by morphological characters. This must be taken into account in new identification keys.

A possible reason for this high intraspecific variability could be the adaptation to very different conditions in the waterbodies. A wider shell aperture creates space for a larger foot, which can be beneficial in heavy water movement in larger lakes or to prevent sinking in muddy grounds. More egg-shaped and conically egg-shaped *R. ampla* shells, which are very similar to those of *R. balthica*, were found in specimens from rivers. This shape of the shell could have ecological advantages in flowing water.

The species status of *R. monnardi* is not justified and should be treated as a synonym of *R. ampla*. In addition, the species status of *R. pinteri* and *R. relicta* is questionable. Whether these forms can be granted a subspecies status requires further analysis.

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