*Invertebrate Zoology, 2022, 19(4):* 404–424 

# Phylogeography of the closely related *Littorina* (*Neritrema*) species in the North-East Atlantic

**A.L. Maltseva[1*], M.A.Z. Panova[2], M.A. Varfolomeeva[1], D.V. Vikhreva[1], D.V. Smutin[3], P.A. Pavlova[1], G.P. Maslakov[1], L.G. Danilov[3], N.A. Mikhailova[4], A.I. Granovitch[1]**

*[1] Department of Invertebrate Zoology, St Petersburg State University, St Petersburg, Russia.*
*[2] Department of Marine Sciences - Tjärnö, University of Gothenburg, Sweden.*
*[3] Department of Genetics and Biotechnology, St. Petersburg State University, St. Petersburg, Russia.*
*[4] Centre of Cell Technologies, Institute of Cytology Russian Academy of Sciences, St Petersburg, Russia.*
** Corresponding author: arina.maltseva@spbu.ru*
*Arina Maltseva: ORCID 0000-0003-1973-4728*
*Marina Panova: ORCID 0000-0002-4147-6473*
*Marina Varfolomeeva: ORCID 0000-0003-0887-8486*
*Polina Pavlova: ORCID 0000-0002-3213-1967*
*Georgii Maslakov ORCID 0000-0003-0462-7284*
*Lavrentii Danilov ORCID 0000-0002-4479-3095*
*Natalia Mikhailova: ORCID 0000-0003-1650-9330*
*Andrei Granovitch: ORCID 0000-0002-5203-104X*

ABSTRACT: Phylogeographic studies of evolutionary young species co-existing over a vast geographic area can provide insights in the process of evolutionary divergence and its cohesiveness in different parts of the species ranges. The *Littorina* snails of the 'saxatilis' cryptic group diverged in near-glacial time, and tend to live in sympatry. *L. saxatilis* is widely distributed on both sides of the North Atlantic, while *L. arcana* and *L. compressa* are patchy distributed on the shores of Europe and Atlantic islands. The biogeographic history of the *Littorina* 'saxatilis' cryptic group is still obscure, with *L. saxatilis* studied much better than the other two species. We evaluated the population structure of the three 'saxatilis' species on the coasts of Wales, the Norwegian and the Barents seas using several genetic markers: the mitochondrial cytochrome b gene (cytb, partial, 26 haplotypes for 268 sequences), nuclear (5 microsatellite loci in 458 individuals) and whole-genome (2bRAD, 63 417 loci in 114 individuals) markers.

Analyses based on the cytb and microsatellite markers showed a deep divergence between the British and the North European populations of all three species with a high genetic similarity between their sympatric populations from Wales. *L. compressa* had the highest differentiation from both *L. arcana* and *L. saxatilis* and demonstrated the clear population structure due to allele frequency. The degree of the genetic differentiation between sympatric *L. arcana* and *L. saxatilis* in some regions was lower than between the regions within a species. Moreover, analyses of all three types of used markers indicate that the continental populations of *L. arcana* include individuals with contrasting genomic profiles. Our results suggest that *L. arcana* and *L. compressa* separated from their common ancestor *L. islandica* after *L. saxatilis*. The three sibling species survived glaciation in a refugium (or refugia) on the British coasts, separated from the mainland refugium (or refugia). After the glaciation, *L. compressa* colonised the mainland, most likely from a single European refugium. Post-glacial continental repopulation by *L. arcana* could have occurred from at least two sources, with two differentiated lineages still recognisable. Further inclusion of

Devoted to the 150 anniversary of Department of Invertebrate Zoology, Saint Petersburg State University.
Посвящается 150-летию кафедры зоологии беспозвоночных Санкт-Петербургского государственного университета.

*Littorina* populations from South Norway and France is needed to complete the reconstruction of biogeographic history in these three evolutionary young species of *Littorina* snails.
How to cite this article: Maltseva A.L., Panova M.A.Z., Varfolomeeva M.A., Vikhreva D.V., Smutin D.V., Pavlova P.A., Maslakov G.P., Danilov L.G., Mikhailova N.A., Granovitch A.I. 2022. Phylogeography of the closely related *Littorina* (*Neritrema*) species in the North-East Atlantic // Invert. Zool. Vol.19. No.4. P.404–424, Suppl. Materials. doi: 10.15298/invertzool.19.4.05

KEY WORDS: phylogeography, cryptic species, sympatric speciation, *Littorina*, cytochrome b, microsatellites, 2bRAD.

# Филогеография близкородственных видов *Littorina* (*Neritrema*) в северо-восточной Атлантике

**А.Л. Мальцева[1], М.А.З. Панова[2], М.А. Варфоломеева[1], Д.В. Вихрева[1], Д.В. Смутин[3], П.А. Павлова[1], Г.П. Маслаков[1], Л.Г. Данилов[3], Н.А. Михайлова[4], А.И. Гранович[1]**

*[1] Кафедра зоологии беспозвоночных, Санкт-Петербургский государственный университет, Санкт-Петербург, Россия.*
*[2] Отдел морских наук – Чарно, Гетеборгский университет, Швеция.*
*[3] Кафедра генетики и биотехнологии, Санкт-Петербургский государственный университет, Санкт-Петербург, Россия.*
*[4] Центр клеточных технологий, Институт цитологии РАН, Санкт-Петербург, Россия.*

РЕЗЮМЕ: Филогеографические исследования эволюционно молодых видов, сосуществующих на значительной части их ареалов, могут дать представление о процессе дивергенции в ходе видообразования в разных частях ареалов видов. Моллюски рода *Littorina* из группы криптических видов “saxatilis” дивергировали в предледниковый период и живут в условиях симпатрии на большей части современных ареалов. Вид *L. saxatilis* широко распространен на обеих сторонах северной Атлантики, тогда как *L. arcana* и *L. compressa* имеют более ограниченные ареалы на побережьях континентальной Европы и атлантических островов. Биогеографическая история группы криптических видов “saxatilis” до сих пор неясна; при этом для *L. saxatilis* она изучена значительно лучше, чем для двух других видов. Мы проанализировали популяционную структуру трех видов группы “saxatilis” на побережьях Уэльса, Норвежского и Баренцева морей, используя несколько генетических маркеров: митохондриальный ген цитохрома b (cytb, 26 гаплотипов среди 268 последовательностей), ядерные (пять микросателлитных локусов из 458 особей) и общегеномные (2bRAD, 63 417 локусов из 114 особей) маркеры.
Проведенный анализ cytb и микросателлитов показал глубокую дивергенцию между британскими и североевропейскими континентальными популяциями всех трех видов при высоком генетическом сходстве между их симпатрическими популяциями из Уэльса. Вид *L. compressa* демонстрировал значительные отличия от двух других видов (*L. arcana* и *L. saxatilis*) и сильно выраженную популяционную структуру по частотам аллелей. Уровень генетической дифференциации между симпатрическими популяциями *L. arcana* и *L. saxatilis* в некоторых точках сбора была ниже, чем внутривидовая дифференциация между удаленными популяциями. Более того, анализ всех трех типов используемых маркеров указывает на то, что континентальные популяции *L. arcana* включают особей с контрастно-различающимися геномными профилями.

Суммарно наши результаты предполагают, что *L. arcana* и *L. compressa* отделились от общего предка *L. islandica* после отделения *L. saxatilis*. Три близкородственных вида пережили период оледенения в британском рефугиуме (или рефугиумах) без контакта с популяциями, выжившими в материковом рефугиуме (или рефугиумах). Наиболее вероятно, что после ухода ледника *L. compressa* колонизировала континентальные побережья из одного европейского рефугиума. Напротив, послеледниковое восстановление материковых популяций *L. arcana* должно было произойти как минимум из двух источников; при этом две ясно дифференцированные линии этого вида все еще легко распознаваемы на генетическом уровне в современных популяциях. Дополнительное включение в анализ популяций из южной Норвегии и Франции поможет более полно понять биогеографическую истории трех эволюционно молодых видов *Littorina*.

Как цитировать эту статью: Maltseva A.L., Panova M.A.Z., Varfolomeeva M.A., Vikhreva D.V., Smutin D.V., Pavlova P.A., Maslakov G.P., Danilov L.G., Mikhailova N.A., Granovitch A.I. 2022. Phylogeography of the closely related *Littorina* (*Neritrema*) species in the North-East Atlantic // Invert. Zool. Vol.19. No.4. P.404–424, Suppl. Materials. doi: 10.15298/invertzool.19.4.05

КЛЮЧЕВЫЕ СЛОВА: филогеография, криптические виды, симпатрическое видообразование, *Littorina*, цитохром b, микросателлиты, 2bRAD.

## Introduction

Genetic patterns in young species provide valuable insights in the evolutionary process of divergence (see e.g. Michel *et al*., 2010; Brawand *et al*., 2014; McGee *et al*., 2016, etc.). In particular, phylogeographic studies of closely related sister-species, which coexist in a wide geographic range, can tell us about a geographic scene of the speciation process, the cohesiveness of divergence in the different part of the species ranges, and the role of the glacial range fluctuations in the recent speciation events (see e.g. Trewick, Wallis, 2001; Habel *et al*., 2005; Stewart *et al*., 2010; Tarnowska *et al*., 2012, etc.).

*Littorina* snails are common inhabitants of the intertidal zone worldwide and provide an excellent opportunity to study these questions. Species of the Atlantic branch of the subgenus *Neritrema* Récluz, 1869 diverged very recently, in the Plio-Pleistocene (Reid, 1996). This branch includes two groups of cryptic species: 'obtusata' (*L. fabalis* (Turton, 1825) and *L. obtusata* (Linnaeus, 1758)) and 'saxatilis' (*L. arcana* Hannaford Ellis, 1978, *L. compressa* Jeffreys, 1865 and *L. saxatilis* (Olivi, 1792)); the divergence time of species of the latter group is estimated as ~1 Mya (Reid, 1996; Panova *et al*., 2011; Reid *et al*., 2012). During the Plio-Pleistocene, at least two speciation events occurred in the Pacific *Neritrema* branch with daughter species having adjoining but not overlapping ranges: sibling pairs *L. sitkana* / *L. horikawai* and *L. natica* / *L. aleutica* (Reid, 1996). In contrast, the Atlantic *Neritrema* species tend to live in sympatry. *Littorina saxatilis* is the most widely distributed species on both sides of the North Atlantic, while two other species of the 'saxatilis' cryptic group demonstrate patchy ranges on the shores of Europe and Atlantic islands, having a more limited distribution, constantly accompanied by *L. saxatilis* (Reid, 1996; Granovitch *et al*., 2008). These three species have been recently in focus of several comparative studies, looking at the ecological and functional differences (Maltseva *et al*., 2016, 2020, 2021a,b; Lobov *et al*., 2021; Panova *et al*., 2022), reproductive barriers (Stankowski *et al*., 2020; Maltseva *et al*., 2021c) and whole-genome similarity (Panova *et al*., 2014). Further, the whole-genome analyses of *L. saxatilis* and *L. arcana* in the southern part of the distribution (Wales and France) suggested that the species are genetically distinct, although very closely related (Stankowski *et al*., 2020). Up to date there is no analysis including both northern and southern populations of these species.

The common ancestor of the Atlantic *Neritrema* snails was of the Pacific origin and colonised Atlantic shores during the Great Trans-Arctic Biotic Interchange (~ 3.5 Mya; Vermeij,

1991; Reid, 1996; Briggs, 2003). The most probable migration pathway was along the North American coast (Reid, 1996). Moreover, there are rather old populations of *L. saxatilis* on the Atlantic coast of North America deeply diverged from the European populations (Panova *et al*., 2011), but no records of neither *L. arcana* nor *L. compressa* were ever reported from the American coast, Greenland, and Iceland. The history of the modern range of *L. saxatilis* was reconstructed in several studies (Doellman *et al*., 2011; Panova *et al*., 2011; Blakeslee *et al*., 2021): its northern part was strongly affected by glacial cycles with source populations survived in several refugia during Last Glacial Maximum (LGM). The existence of such refugia was suggested on both sides of the Atlantic, being the source of independent recolonisation of the American coast, British Isles, and the European mainland (Reid, 1996, Doellman *et al*., 2011; Panova *et al*., 2011; Maltseva *et al*., 2020; Blakeslee *et al*., 2021). Nevertheless, the biogeographic history of *L. arcana* and *L. compressa* in the North East Atlantic (NEA) is still obscure. How populations from different parts of their ranges are related? Can their oldest populations be recognised? Was the dispersal route collinear and synchronous in all three species or did they colonise new shores independently? Did these species hybridize during active expansion and afterwards? When did the divergence of the three species happen compared to the separation of the American and the European clades of *L. saxatilis*? Can *L. arcana* and *L. compressa* have an American origin with a single or multiple events of European colonisation? Can the centers of divergence be established?

In this study, we analysed a number of populations of all three species of the 'saxatilis' group. We used several genetic markers to evaluate the population structure in *L. arcana*, *L. compressa* and *L. saxatilis:* the mitochondrial (cytochrome b gene), nuclear (microsatellites) and whole-genome (2bRAD). Based on these results, we discuss possible scenarios that have shaped contemporary genetic variation of these three evolutionary young species.

## Material and methods

MATERIAL COLLECTION. Snails of three species of the 'saxatilis' group were collected from wild populations, sample sizes are shown in Table 1. Only female individuals were in the samples of *L. arcana* and *L. saxatilis* from populations of Dalnye Zelentsy (Chevry, Oscar Bay) and Tromsø, Norway. In all other samples, there were individuals of both sexes; species identification was performed as described before (Maltseva *et al*., 2021a,c). Trematode-infected and immature individuals, as well as individuals with an intermediate state of definitive traits, were excluded from the analysis.

The E.Z.N.A.® *Mollusc DNA Kit* (Omega Bio Tec) was used for the genomic DNA isolation. The fragment of the cytb gene was amplified with 50 ng of the genomic matrix, 1 pmol of the forward TTCCCGCACCTTCAAATCTT and reverse GGACTAGGGCCGAAAGTATAAATA primers and the ready PCR mix ScreenMix-HS (Evrogen) in the Verity™ 96-Well Thermal Cycler (Applied Biosystems) based on protocol from Panova *et al*., 2011. The amplified region of 625 bp length was the same as in Panova *et al*., 2011; and only partially overlapped (367 bp) with the amplified fragment of mtDNA in the study of Doellmann *et al*. (2011). The amplicons were purified with the GeneJET PCR Purification Kit (Thermo Fisher Scientific) and sequenced with the ABI Prism 3500xl Genetic Analyzer (Thermo Fisher Scientific). Microsatellite loci were genotyped as described in Panova *et al*. (2008). Preparation of the 2bRAD libraries was done based on Wang *et al*. (2012) following the publicly available protocols https://github.com/z0on/2bRAD_denovo. The restrictase BcgI (BioLabs) was used fragmentise DNA; adapters were synthesised in the Genterra Company and T4 DNA-ligase was used to ligate them with DNA fragment; the concentration of amplicons was measured using DNA HS Assay Kit (Thermo Fisher Scientific) and the equimolar pool of all samples was obtained. The quality of the final library was evaluated using Agilent 2200 TapeStation System (Agilent Technologies). The sequencing was performed with Illumina NovaSeq6000 with addition 25% of PhiX-phage DNA by the Evrogen Company.

Cytb ANALYSIS**.** DNA sequences were aligned and substitutions were counted in the SeaView software (Gouy *et al*., 2010). Nucleotide diversity ð was calculated and a haplotype network was produced in the Popart software (Leigh, Bryant, 2015), using the TCS algorithm (Clement *et al*., 2000). Bayesian phylogeny was inferred in MrBayes v.3.2 software (Ronquist *et al*., 2012) based on aligned DNA sequences. Nucleotide substitution model was GTR + G + I (Lanave *et al*., 1984); optimal nucleotide substitution model was chosen using MrModeltest v.2.3 (Nylander, 2004) software. Analysis was performed as two independent runs, five chains in each (four heated and one cold; the first 25% samples

Table 1. Details on studied sites and samples taken for different analyses.
Таблица 1. Данные о точках сбора и образцах, полученных для различных анализов.

| Site name | Region | Coordinates, °N, °E | Microsatellites analysis | Analysis of cytb | 2bRAD |
|---|---|---|---|---|---|
| Levin Navolok, Russia | White Sea | 66.305<br>33.413 | – | 6S | – |
| Dalnye Zelentsy, Chevry, Russia | Barents Sea | 69.114<br>36.151 | 137(49A+42C+46S) | 72(24A+24C+24S) | – |
| Dalnye Zelentsy, Oscar Bay, Russia | Barents Sea | 69.117<br>36.070 | 142(50A+40C+52) | 2S* | – |
| Kola Bay, Russia | Barents Sea | 69.077<br>33.130 | – | 6S | – |
| Kiberg, Varangerfjord, Norway | Barents Sea | 70.283<br>30.977 | – | 3(2A+1S) | 23(11A+12S) |
| Tromsø, Telegraph Bay, Norway | Norwegian Sea | 69.631<br>18.910 | 95(31A+32C+32S) | 70(23A+23C+24S) | – |
| Bodø#1, Saltstraumen, Norway | Norwegian Sea | 66.947<br>13.704 | – | 30(17A+8S+5C) | 55(27A+9C+19S) |
| Bodø#2, Saltstraumen, Norway | Norwegian Sea | 66.951<br>13.607 | – | 8**(5C+3S) | 36(10A+10C+16S) |
| Black Rock, Wales | Irish Sea | 51.775<br>–5.119 | 84(24A+28C+32S) | 71(24A+24C+23S) | – |

A — *Littorina arcana*, C — *L. compressa*, S — *L. saxatilis*; * in the cytb-analysis these sequences were pooled with those from the Chevry site; ** in the cytb-analysis these sequences were pooled with those from the Bodø#1 site.

A — *Littorina arcana*, C — *L. compressa*, S — *L. saxatilis*; * в cytb-анализе эти последовательности были объединены с последовательностями с сайта Чевры; ** в cytb-анализе эти последовательности были объединены с последовательностями из сайта Буде#1.

Table 2. Descriptive statistics for the cytb polymorphisms in the populations of *L. arcana*, *L. compressa*, *L. saxatilis*.

Таблица 2. Описательная статистика полиморфизма по cytb в популяциях *L. arcana*, *L. compressa*, *L. saxatilis*.

| **Species** | **N seqs** | **π** | **N haps (by clades)** | **N clades** | **N SS-haps** |
|---|---|---|---|---|---|
| ***L. arcana*** | 90 | 0.01040 | 12 ($2A_1$;$1A_2$;1A;5C;1F;2G) | 4 | 7 |
| ***L. compressa*** | 81 | 0.00762 | 8 (3A;1C;4F) | 3 | 5 |
| ***L. saxatilis*** | 97 | 0.00645 | 13 ($4A_1$;$1A_2$;4A;3C;1E) | 3 | 9 |
| **TOTAL** | 268 | 0.00981 | 26 ($5A_1$;$1A_2$;6A;7C;1E;4F;2G) | 5 | 21 |
| | **N RS-haps** | **N PM sites** | **N SS subs** | **N NS subs** | |
| ***L. arcana*** | 5 | 25 | 7 | 1 | |
| ***L. compressa*** | 5 | 17 | 3 | 1 | |
| ***L. saxatilis*** | 9 | 19 | 6 | 0 | |
| **TOTAL** | 19 | 34 | – | 2 | |

SS — species-specific, RS — region-specific, PM — polymorphic, NS — non-synonymous, hap — haplotype, seq — sequence, subs — substitutions, clades — phylogenetic clades, the clade A was counted jointly with its subclades (see Fig. 2).

SS — видоспецифичные, RS — регионоспецифические, PM — полиморфные, NS — несинонимичные, hap — гаплотип, seq — последовательность, subs — замены, клады: филогенетические клады, гаплотипы клады A учитывали вместе с ее субкладами (см. рис. 2).

from the cold chain were discarded) for 15,000,000 generations with a sample frequency of 1,000, print frequency of 1,000 and diagnostics calculated every 1,000 generations; the results were visualised by the FigTree v 1.4.3 software (Rambaut, 2009). Population differentiation statistics were calculated in the Arlequin v. 3.5.2.2 (Excoffier, Lischer, 2010).

MICROSATELLITE ANALYSIS. Genetic differentiation between the species, regions and populations within the regions was tested using Nei's Gst estimator calculated in the R package adegenet (Jombart and Collins, 2008) and with the analysis of molecular variance (AMOVA) in Arlequin v. 3.5.2.2 (Excoffier, Lischer, 2010).

Discriminant analysis of principal components (DAPC; Jombart *et al*., 2010) was performed in R to assess and visualise population structure using the R package adegenet (Jombart, Collins, 2008). DAPC first uses principal component analysis to reduce the total genetic variation, and then identifies discriminant functions that maximise between-group differences while minimising within-group variation. The optimal number of PCs was chosen according to the a.score (a statistic that measures the quality of discrimination, based on group reassignment probabilities). Clusters were visualised using scatterplots and density plots of the discriminant functions using ggplot2 package (Wickham, 2016).

An admixture proportion model was run using the program Structure v. 2.3.4 (Pritchard *et al*., 2000). The analysis was performed using K = 2 to K = 7 with the burn-in period of 10,000 permutations followed by 100,000 MCMC replications. The results were clustered and visualised using CLUMPAK (Cluster Markov Packager Across K) web server (Kopelman *et al*., 2015), which runs clumpp (Jakobsson, Rosenberg, 2007) and distruct (Rosenberg, 2004) programs.

Spatial analysis of molecular variance (SAMOVA; Dupanloup *et al*., 2002) in SAMOVA 2.0 was performed to choose the grouping of populations in K groups which best explains the genetic variation. We tested K from 2 to 7 at 100 initial conditions. The best grouping of populations was chosen based on the maximum *Fct* statistics among different K.

2bRAD DATA ANALYSIS. The algorithms of the analysis were performed in accordance to https://github.com/z0on/2bRAD_denovo protocols. The quality of the reads obtained was assessed using the MultiQC program (Ewels *et al*., 2016). The reads were trimmed to a length of 50 nucleotides using the Trimmomatic program (Bolger *et al*., 2014). The resulting sequences were aligned to the reference *Littorina saxatilis* genome (Westram *et al*., 2018; the unmasked version from Marina Panova). Alignment was performed using Bowtie2 algorithms (Langmead, Salzberg, 2012; Langmead *et al.*, 2018). Annotation, indexing, and conversion of aligned reads were performed using SAMtools 1.9 (Li, 2011; Danecek, 2021). The ANGSD algorithms were used

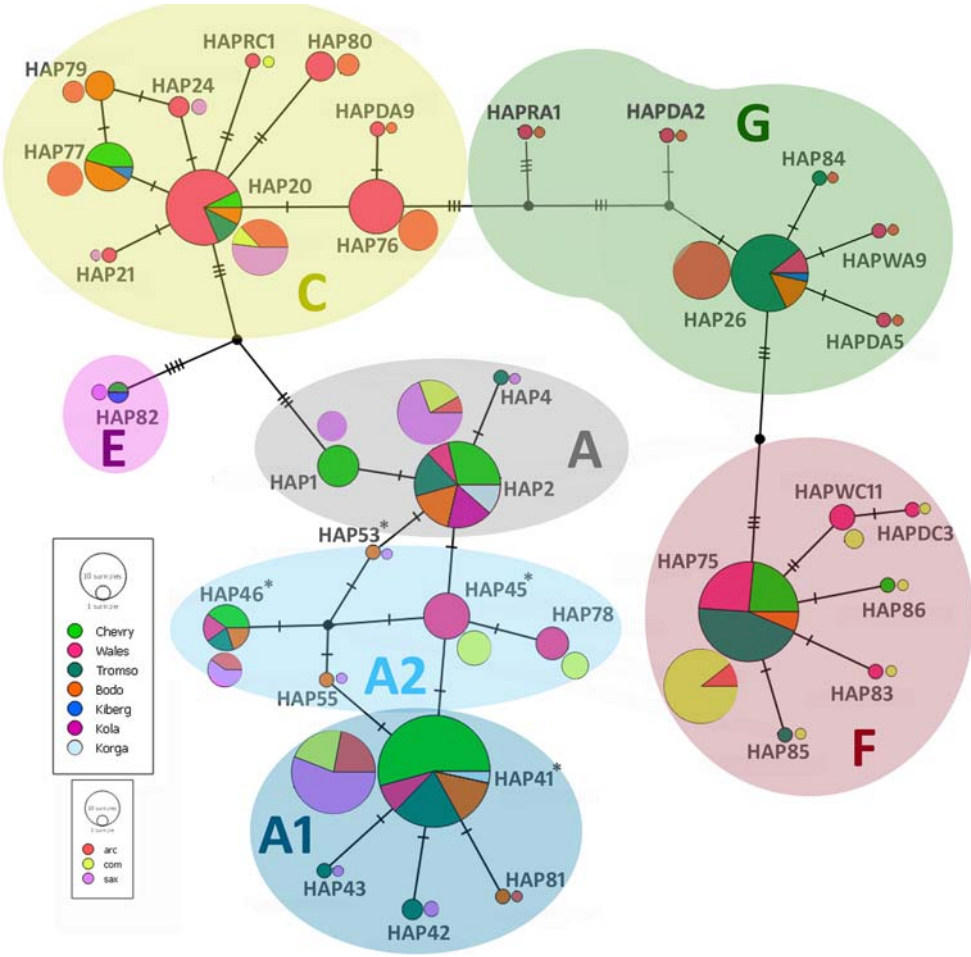


Fig. 1. TSC network of the cytb haplotypes registered in populations of *L. arcana*, *L. compressa*, *L. saxatilis*. Main circles in the network (connected by lines) correspond to individual haplotypes coloured by regions; additional circles (near main circles) represent distribution of corresponding haplotypes among species. The background colour indicates phylogenetic clades to which the haplotypes belong, see Fig_2 and text. In the network, 26 haplotypes registered in this study were supplemented with available cytb sequences on *L. arcana* and *L. compressa* (HAPDA02 ID JF501848.1; HAPDA05 ID JF501849.1; HAPDA09 ID JF501850.1; HAPDC01 ID JF501852.1; HAPDC03 ID JF501856.1; HAPRA01 ID U46791.1; HAPRC01 ID U46811.1; HAPWA08 ID AJ237716.1; HAPWA09 ID AJ237717.1; HAPWA10 ID AJ237718.1; HAPWC11 ID AJ237719.1). The network with sequences of *L. saxatilis* from Doellman *et al*., 2011 and Panova *et al*., 2011 can be found in Supplement SM1.

Рис. 1. TSC-сеть гаплотипов cytb, обнаруженных в популяциях *L. arcana*, *L. compressa*, *L. saxatilis*. Основные круги в сети (соединены линиями) соответствуют отдельным гаплотипам, раскрашенным согласно регионам; дополнительные кружки (рядом с основными кружками) представляют распределение соответствующих гаплотипов между тремя видами видов. Цвет фона указывает на филогенетические клады, к которым принадлежат гаплотипы, см. рис. 2 и текст. В сети 26 гаплотипов, зарегистрированных в нашем исследовании, были дополнены доступными последовательностями cytb из *L. arcana* и *L. compressa* (HAPDA02 ID JF501848.1; HAPDA05 ID JF501849.1; HAPDA09 ID JF501850.1; HAPDC01 ID JF501852.1; HAPDC03). ID JF501856.1; HAPRA01 ID U46791.1; HAPRC01 ID U46811.1; HAPWA08 ID AJ237716.1; HAPWA09 ID AJ237717.1; HAPWA10 ID AJ237718.1; HAPWC11 ID AJ237719.1). Сеть с включенными последовательностями *L. saxatilis* из Doellman *et al*., 2011 и Panova *et al*., 2011 можно найти в Приложении SM1.

to obtain population genetic information (Korneliussen *et al*., 2014). The results were calculated using ADMIXTURE 1.3 (Alexander *et al*., 2009). The visualisation of the obtained results was carried out in the R 4.2.0 program (R Core Team, 2022) using the package ggplot2 (Wickham, 2016).

## Results

MITOCHONDRIAL MARKER: Cytb. In the analysis, there were 268 individual cytb sequences from snails of three species belonging to the "saxatilis" cryptic group collected from sympatric populations in five locations (Table 1). The genetic diversity was quite high ($\pi$=0.0098), which is similar to values previously reported for the cytb of *L. saxatilis* ($\pi$=0.0099, Panova *et al*., 2011). The number of polymorphic sites was 34, the vast majority of which were in the third codon position, causing no amino acid substitutions (Table 2).

These 268 sequences grouped in 26 cytb haplotypes (Table 2, Fig. 1), of which 12 were newly identified, mainly those specific for *L. arcana* and *L. compressa*. The remaining 14 haplotypes were previously found in *L. saxatilis* (Panova *et al*., 2011). Four haplogroups of closely related sequences can be distinguished in the haplotype network. These groups are separated by 6-8 substitutions (Fig. 1), and they correspond to the earlier described clades of the *L. saxatilis* haplotypes (Panova *et al*., 2011). Across 26 analysed cytb haplotypes, five were identified in more than one species; one haplotype (HAP45) was detected only in *L. compressa* in our study, while in the previous study it was also registered in the North America *L. saxatilis* population (Panova *et al*., 2011). Interestingly, these multispecies haplotypes took a node-position of star-like patterns in three different groups, and were registered in four different locations (while derived haplotypes were usually region-specific; Fig. 1). Besides the five multispecies haplotypes found in multiple regions, there were two *L. arcana* haplotypes detected in more than one region. The rest 20 (see remark on HAP45 above) haplotypes were species-specific (nine to *L. saxatilis*, five to *L. compressa*, seven to *L. arcana*) and region-specific (Table 2, Fig. 1).

The Bayesian phylogenetic analysis of the cytb confirmed the existence of five clades, corresponding to those described earlier (Fig. 2; clades B and D were not registered in our samples, their sequences HAPs 27–40 and HAPs 13–19, respectively, were taken from Panova *et al*. [2011] and included to reconstruct an overall tree topology), as well as the clade F, comprising haplotypes of *L. arcana* and *L. compressa*. The group G did not form a clade; haplotypes of this group individually took a basal position within the 'saxatilis' clade (Fig. 2); nevertheless, the group G will be mentioned as the 'clade G' later on due to appearance as a monophyletic clade on the tree with a reduced haplotype number in the analysis (Supplement SM2). The cytb sequences of *L. arcana* were represented in the four clades, *L. saxatilis* — in the three clades (plus two clades absent from our samples); *L. compressa* — in the three clades. Further, *L. arcana* and *L. saxatilis* had species-specific clades (G and B,D,E, respectively, Fig. 2); two basal clades (F and G) did not contain *L. saxatilis*. In the three multi-species clades, the multi-species and multi-region sequences generally took a basal position, while monospecific haplotypes with a limited geographic distribution formed derived branches. Four (HAPs 2,41,20 and 75 of clades A, A1, C and F, respectively) of the five multi-species haplotypes were shared by species in sympatric populations. No species-specific haplotypes were found to be descended from heterospecific haplotypes, only from multispecific or conspecific ones.

The presence of different cytb clades in particular populations varied significantly between species and regions (Fig. 3). For example, the clade C was abundantly represented in the Wales populations of all three species, but was found in only one species (mainly *L. arcana,* once *L. saxatilis*, and never *L. compressa*) in the mainland populations. Similarly, haplotypes of the clade F were registered in both *L. arcana* and *L. compressa* in Wales, but exclusively in *L. compressa* along the North European coast. The opposite tendency was observed for the A clade and its subclade A1: these were far more often detected in the mainland populations of all three species than in Wales. Haplotypes of the F clade were registered in all four tested populations of *L. compressa*; the highest diversity of this clade was detected in this species. Besides *L. compressa*, one haplotype of the clade F was registered in the Wales population of *L. arcana*. Similarly, the clade A with the subclades A1 and

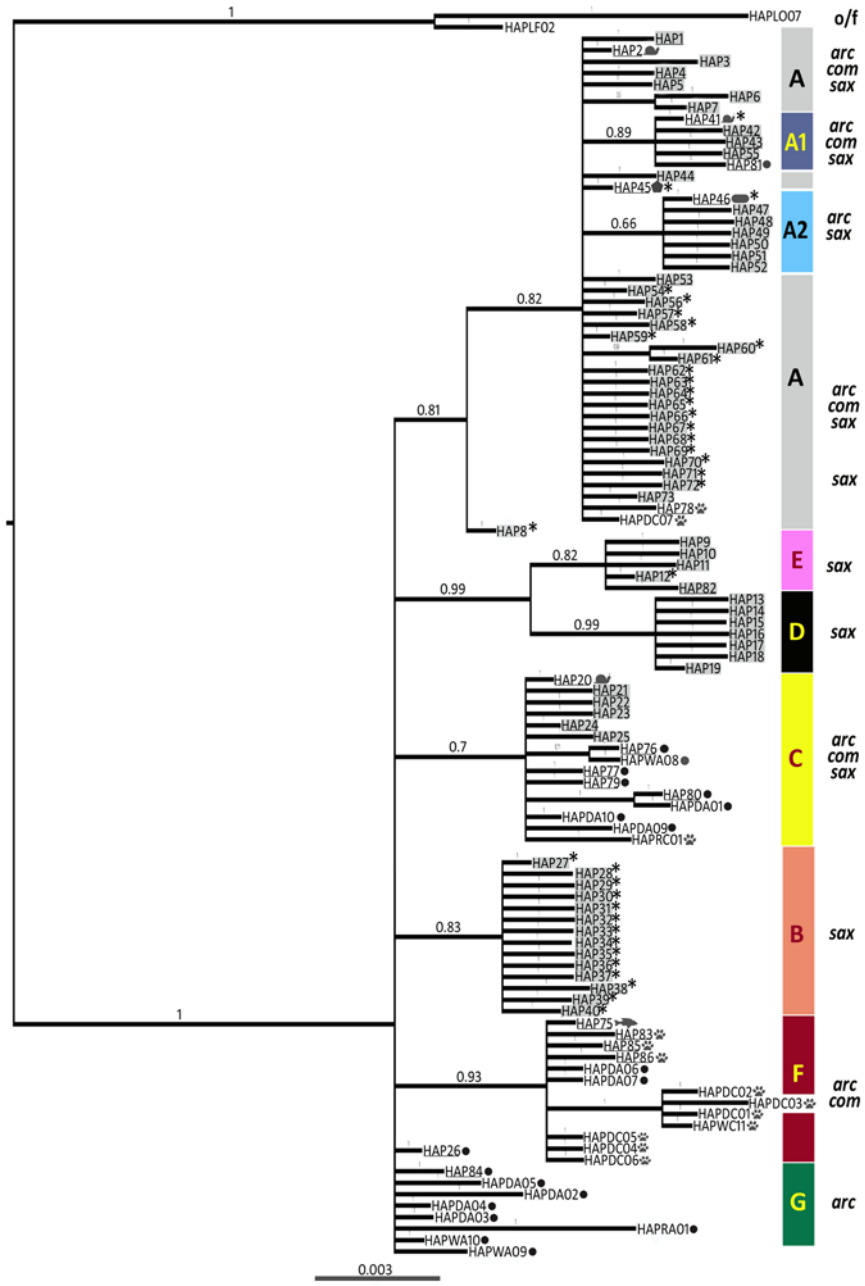


Fig. 2. Bayesian phylogenetic tree of the cytb haplotypes. Letter-names of the clades correspond to those in Panova *et al*., 2011; 73 unique haplotypes of *L. saxatilis* from Panova *et al*. 2011, 22 haplotypes of *L. arcana* and *L. compressa*, and sequences of *L. obtusata* and *L. fabalis* available from NCBI (HAPLO07 ID MN045776.1; HAPLF02 ID U46808; HAPDA02 ID JF501848.1; HAPDA05 ID JF501849.1; HAPDA09 ID JF501850.1; HAPDC01 ID JF501852.1; HAPDC03 ID JF501856.1; HAPRA01 ID U46791.1; HAPRC01 ID U46811.1; HAPWA08 ID AJ237716.1; HAPWA09 ID AJ237717.1; HAPWA10 ID AJ237718.1; HAPWC11 ID AJ237719.1; HAP75 ID OP133395 (*L. arcana*) and OP133396 (*L. compressa*); HAP76 ID OP133397; HAP77 ID OP133398; HAP78 ID OP133399; HAP79 ID OP133400; HAP80 ID OP133401; HAP81 ID OP133402; HAP82 ID OP133403; HAP83 ID OP133404; HAP84 ID OP133405; HAP85 ID

A2 were represented in all sampled *L. saxatilis* populations with the highest diversity in this species. Almost the same was true for the species-specific clade G in *L. arcana* (except the easternmost sample of *L. arcana*, Chevry of the Barents Sea; although the clade G was not detected in our Wales samples, it was reported by the other studies Reid *et al*., 1999; Doellman *et al*., 2011). In none of the analysed sites, all seven cytb clades/subclades were detected (considering all species). In two sites (Bodø and Tromsø) the differentiation estimates between sympatric populations of three species were higher than in Wales and Dalnye Zelentsy (mean *Fst* values were 0.35 and 0.60 vs 0.21 and 0.20, respectively). In the most eastern site Dalnye Zelentsy, all three species had a high representation of the clade A with its subclades, being clearly different from populations of Wales, the westernmost site. The contrast between the Wales and Barents populations was most prominent in the *L. arcana*: *L. arcana* had C, F, G clades in Wales vs A,A1,A2,C clades in Dalnye Zelentsy; while *L. saxatilis* had the clades C+A with subclades in Wales vs the clade A with subclades with an admixture of the clade E (also registered in Kiberg, another Barents site). This contrast was less evident in *L. compressa*.

Generally, the population differentiation by haplotype frequencies in *L. arcana* and *L. compressa* tended to be higher than in *L. saxatilis*: mean 'by frequency' *Fst* values were 0.35 and 0.41 vs 0.16, respectively. Differentiation estimates based on pairwise haplotype distances showed another tendency: In *L. arcana* and *L. saxatilis* 'by distance' *Fst* were higher than 'by frequency' *Fst* (0.49 vs 0.35 in *L. arcana*, 0.30 vs 0.16 in *L. saxatilis*), but lower in *L. compressa* (0.32 vs 0.41). Moreover, in the pair *L. arcana* / *L. saxatilis* the degree of genetic differentiation between the species within a region in some cases was lower than the differentiation between the regions within a species: e.g., 'by distance' *Fst* between *L. arcana* and *L. saxatilis* within the Barents Sea (Chevry) and Wales regions were 0.18 and 0.22, respectively, while *Fst* between the Barents Sea (Chevry) and Wales within *L. arcana* or *L. saxatilis* were 0.50 and 0.42, respectively (a complete set of *Fst* values with intervals can be found in Supplement SM4).

OP133406; HAP86 ID OP133407) were included. Asterisks mark sequences registered in the Northwest Atlantic populations; 26 haplotypes registered in this study are underlined; Among species-specific haplotypes, those registered exclusively in *L. saxatilis* are shown in the grey blocks, those found only in *L. arcana* are marked with a black dot, those detected only in *L. compressa* are marked with a paw. Among the multispecies haplotypes, those registered in both *L. arcana* and *L. compressa* are marked with a fish, the ones found in both *L. arcana* and *L. saxatilis* are marked with an ellipse; those found in both *L. compressa* and *L. saxatilis* are marked with a pentagon; finally, the haplotypes detected in all three species are marked with a snail. The Bayesian phylogenetic tree with included cytb sequences from Doellman *et al*., 2011 can be found in Supplement SM3.

Рис. 2. Байесовское филогенетическое дерево по последовательностям cytb гаплотипов. Буквенные названия клад соответствуют таковым в Panova et al., 2011; 73 уникальных гаплотипа *L. saxatilis* из Panova *et al*. (2011), 22 гаплотипа *L. arcana* и *L. compressa*, а также последовательности *L. obtusata* и *L. fabalis* доступны в NCBI (HAPLO07 ID MN045776.1; HAPLF02 ID U46808; HAPDA02 ID JF501848.1; HAPDA05 ID JF501849.1; HAPDA09; ID JF501850.1; HAPDC01 ID JF501852.1; HAPDC03 ID JF501856.1; HAPRA01 ID U46791.1; HAPRC01 ID U46811.1; HAPWA08 ID AJ237716.1; HAPWA09 ID AJ237717.1; HAPWA10 ID AJ2392AP7; HAPWA10 ID AJ2392JWC18.1; HAP75 ID OP133395 (*L. arcana*) и OP133396 (*L. compressa*); HAP76 ID OP133397; HAP77 ID OP133398; HAP78 ID OP133399; HAP79 ID OP133400; HAP80 ID OP133401; HAP79 ID OP133400; HAP80 ID OP133401; HAP79 ID OP133400; HAP80 ID OP133401; HAP79 ID OP133400; HAP80 ID OP133401; HAP79 ID OP134400; HAP80 ID OP133401; HAP84 ID OP133405; HAP85 ID OP133406; HAP86 ID OP133407). Звездочками отмечены последовательности, зарегистрированные в популяциях северо-западной Атлантики; 26 гаплотипов, зарегистрированных в данном исследовании, подчеркнуты; среди видоспецифичных гаплотипов в серых блоках показаны зарегистрированные исключительно у *L. saxatilis*, черной точкой отмечены обнаруженные только у *L. arcana*, отмечены отпечатком лапы обнаруженные только у *L. compressa*; среди многовидовых гаплотипов те, которые зарегистрированы у *L. arcana* и *L. compressa*, отмечены рыбой, обнаруженные у *L. arcana* и *L. saxatilis* отмечены эллипсом; обнаруженуыт у *L. compressa* и *L. saxatilis* отмечены пятиугольником; гаплотипы, обнаруженные у всех трех видов, отмечены улиткой. Байесовское филогенетическое дерево с включенными последовательностями cytb из Doellman *et al*., 2011 можно найти в Приложении SM3.

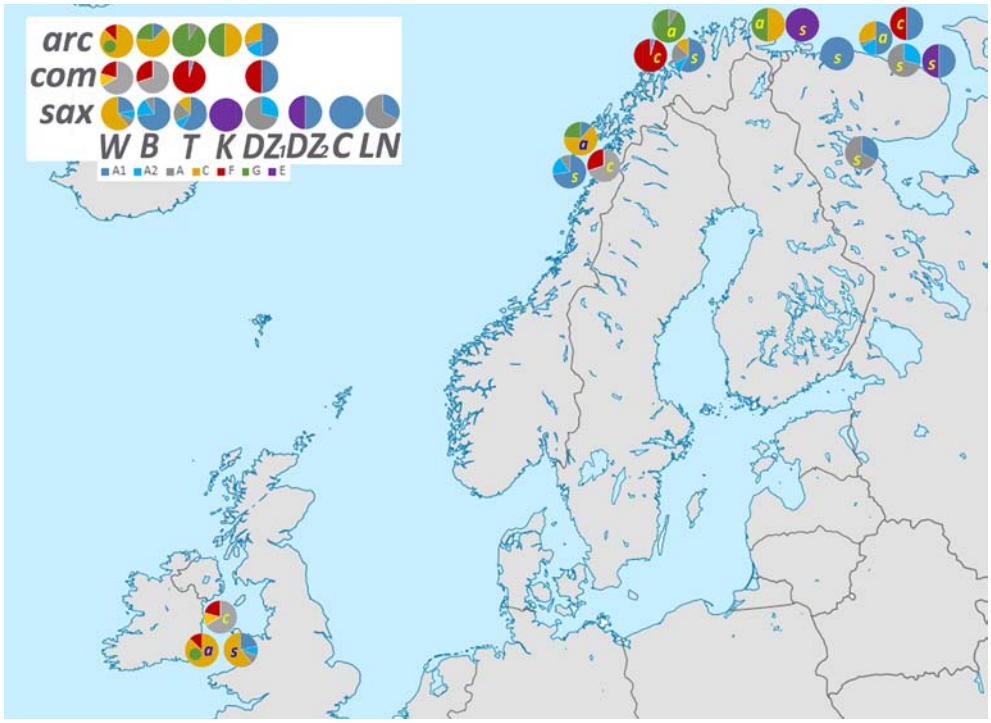


Fig. 3. Contribution of phylogenetic clades to the cytb diversity in local populations of *L. arcana* (a, arc), *L. compressa* (c, com), *L. saxatilis* (s, sax). Sectors within the pie-charts represent the percentage of haplotypes of a certain clade in the total diversity registered in a local population. Colours of clades are the same as in Fig. 2. There was just one sequence of *L. saxatilis* from the sites of Kiberg and two from Oscar Bay (DZ2, Barents Sea); these are displayed because they represent the rare clade E. Green dot in the Wales population of *L. arcana* indicates that the clade G was reported from the Wales populations of this species earlier (Doellman *et al*., 2011).

W — Wales, Irish Sea; B — Bodш, Norwegian Sea; T — Tromsø, Norwegian Sea; K — Kiberg, Barents Sea; DZ1 — Chevry, Dalnye Zelentsy, Barents Sea; DZ2 — Oscar Bay, Dalnye Zelentsy, Barents Sea; C — Kola Bay, Barents Sea; LN — Levin Navolok, White Sea.

Рис. 3. Вклад филогенетических клад в генетическое разнообразие по cytb в локальных популяциях *L. arcana* (a, arc), *L. compressa* (в, com), *L. saxatilis* (s, sax). Сектора на круговых диаграммах представляют процент гаплотипов определенной клады в общем разнообразии, зарегистрированном в конкретной популяции. Цвета клад такие же, как на рис. 2. Всего одна последовательность *L. saxatilis* обнаружена точке Киберг и две — в Бухте Оскара (DZ2, Баренцево море); они отображены, т.к. представляют редкую кладу E. Зеленая точка в популяции *L. arcana* в Уэльсе указывает на то, что присутствие клады G ранее регистрировалось в популяциях этого вида в Уэльсе (Doellman *et al*., 2011).

W — Уэльс, Ирландское море; B — Будё, Норвежское море; T — Тромсё, Норвежское море; K — Киберг, Баренцево море; DZ1 — Чевры, Дальние Зеленцы, Баренцево море; DZ2 — Бухта Оскара, Дальние Зеленцы, Баренцево море; C — Кольский залив, Баренцево море; LN — Левин Наволок, Белое море.

Similar tendency was not registered in pairs with *L. compressa.* The latter species had the highest differentiation from both *L. arcana* and *L. saxatilis* (mean *Fst* = 0.37 and 0.38, respectively; mean *Fst* between *L. arcana* and *L. saxatilis* was 0.28).

NUCLEAR MARKER: microsatellites. Five loci were analysed in populations of the three 'saxatilis' species from three regions (four collection sites). Estimates confirmed a significant degree of genetic heterogeneity due to population structure (mean *Gst* = 0.15). Analysis of molecular variance (AMOVA) showed significant effects on genetic diversity of both factors: 'species' and 'region'. The analyses groping populations based on alleles frequencies and

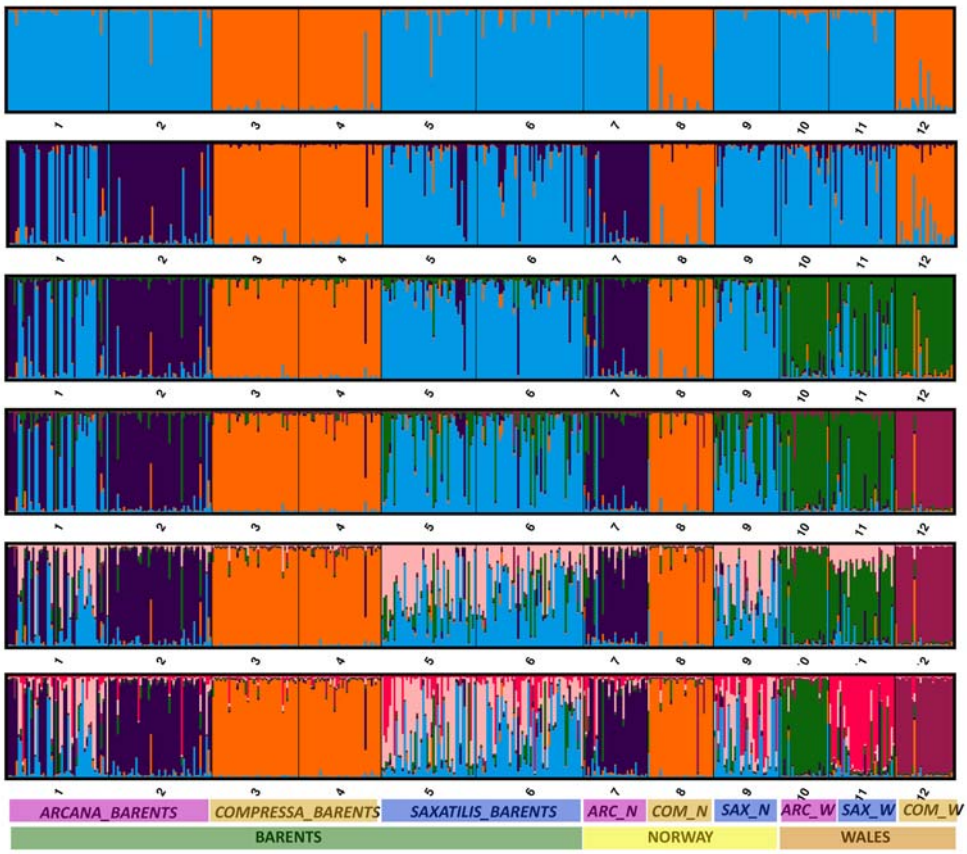


Fig. 4. Results of the admixture analysis of the populations of *L. arcana*, *L. compressa*, *L. saxatilis* based on microsatellite data; Barents: Dalnye Zelentsy (Oscar Bay 1,3,5 and Chevry 2,4,6); Norway: Tromsш (7,8,9); In the analysis the number of presumable ancestral populations K varied from two (upper panel) to seven (the lower panel).

Рис. 4. Результаты анализа ADMIXTURE популяций *L. arcana*, *L. compressa*, *L. saxatilis* по микросателлитным данным; Barents: Дальние Зеленцы (Бухта Оскара 1,3,5 и Чевры 2,4,6); Norway: Тромсё (7,8,9); при анализе количество предполагаемых предковых популяций K варьировало от двух (верхняя панель) до семи (нижняя панель).

free of any assumptions on Hardy-Weinberg equilibrium or linkage equilibrium (DAPC and SAMOVA) confirmed a higher similarity between *L. arcana* and *L. saxatilis,* and the differentiation of *L. compressa* (*Fst* values within the former species pair were lower than between any of them and *L. compressa*, SM5–6). The latter species demonstrated a clear between-region population structure agreeing well with results of the cytb analysis. Interestingly, the British population of *L. compressa* adjoined to sympatric populations of two other species, while its mainland populations were clearly separated from two other species as individual clusters. In contrast, the *L. arcana*/*L. saxatilis* pair formed in the DAPC analysis two very tight region clusters, both two-species: the British Isles versus the mainland (more information can be found in the SM5). The SAMOVA analysis corroborated the differentiation between the British vs continental populations in all three species (SM7).

The STRUCTURE analysis considers linkage disequilibrium during ancestry establishing (Kaeuffer *et al*., 2007); it verified the genetic differentiation of *L. compressa* from the *L. arcana* and *L. saxatilis* pair at the level of two groups (Fig. 4). Some mainland populations of

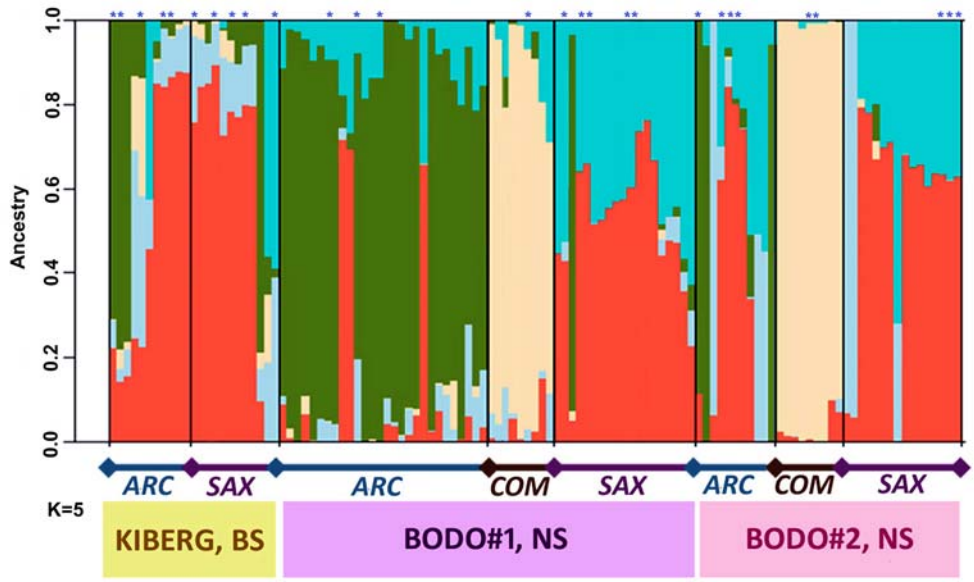


Fig. 5. Results of the ADMIXTURE analysis based on 2bRAD data at K=5. ARC — *L. arcana*, COM — *L. compressa*, SAX — *L. saxatilis*; BS — Barents Sea, NS — Norwegian Sea. Asterics mark genomic profiles of male individuals; unmarked individuals were females.
Рис. 5. Результаты анализа ADMIXTURE на основе данных 2bRAD при K=5. ARC — *L. arcana*, COM — *L. compressa*, SAX — *L. saxatilis*; BS — Баренцево море, NS: Норвежское море. Звездочками отмечены геномные профили самцов; все не отмеченные особи — самки.

*L. arcana* received a distinctive ancestry at the level of three groups but not in Wales. Instead, in Wales sympatric populations of all three species had a closer ancestry at K = 4, and *L. compressa* had an own ancestry at K=5 and higher, the mainland and the Wales populations separately. At K=5 and higher, individuals of *L. arcana* and *L. saxatilis* had more complex patterns of contribution from ancestral populations than *L. compressa*. This species pair had a similar set of ancestral populations in all locations tested, though the contributions of these populations into genomic profiles strongly varied between the species. Although each of the three species had generally similar ancestry patterns in the Norwegian and Barents seas, two closely located populations of *L. arcana* from the Barents Sea (Oscar Bay = 1 and Chevry = 2) demonstrated a different pattern. Some *L. arcana* individuals from the Oscar Bay demonstrated patterns similar to other mainland populations of *L. arcana*, while others to *L. saxatilis* (at K = 3 and higher). Some distinctiveness of *L. arcana* population from Oscar Bay was confirmed by DAPC as well (when Chevry and Oscar Bay sites were analysed separately; see Supplement SM5.2).

WHOLE-GENOME MARKERS: 2bRAD. After the filtering, 63,417 loci were successfully genotyped in total 114 individuals. Although a limited number of populations was included in this analysis (there was no 2bRAD data for the Wales populations), there were several interesting results (Fig. 5). First, samples of *L. compressa* demonstrated again the clear genetic differentiation from the *L. arcana*/*L. saxatilis* pair; both analysed populations had similar patterns with a predominant contribution of the same ancestral population. Second, in the case of the pair *L. arcana*/*L. saxatilis,* the contributions of several ancestral populations to genomic profiles differed in the Norwegian and in the Barents seas. Third, in two locations (Kiberg and Bodø#2) the species pair *L. arcana*/*L. saxatilis* had quite similar to each other genetic patterns while in Bodø#1 *L. arcana* had a distinctive ancestry compared to both sympatric *L. saxatilis* and *L. arcana* from the Bodø#2 and Kiberg populations. Rarely, *Littorina arcana* individuals with such distinctive genomic profiles were

registered in other populations as well. Also, several individuals of Bodø#1 *L. arcana* had a pattern resembling those of *L. arcana* from Bodø#2 and of sympatric *L. saxatilis*. The 'saxatilis'-like or -unlike pattern of genomic profiles in *L. arcana* was not related with sex of individuals and cannot be explained by misidentification of males (Fig. 5).

## Discussion

In the present study we performed a complex phylogeographic analysis of the three closely related *Littorina* species in the 'saxatilis' group in a first attempt to reconstruct the history of their divergence in the North Atlantic. The common ancestor of the Atlantic *Neritrema* clade is of Pacific origin; it colonised Atlantic shores at ~3.5 Mya and diverged to the 'obtusata' and 'saxatilis' clades at ~2–3 Mya. *Littorina saxatilis* speciated from *L. islandica*, the common ancestor of the "saxatilis" species group, at ~0.5 Mya (Reid, 1996; Panova *et al*., 2011; Reid *et al*., 2012). The timing and the order of two other species origin is obscure due to scarcity of molecular data and absence of paleontological records. Similarly, the preglacial fate of *L. islandica* is not well understood. It was predicted to be an egg-laying species (like all other *Neritrema*, except *L. saxatilis*) with preference to boreal conditions and a limited southward spread (due to benthic spawn) as it occurs in modern *L. arcana* and *L. compressa* (Reid, 1996; Símonarson *et al*., 2021). Fossils attributed to *L. islandica* were recorded in Bardarson's horizons of Iceland and dated ~2 Mya (Gladenkov *et al*., 1980; Reid, 1996; Símonarson *et al*., 2021). The native *Littorina* populations of Iceland and Greenland, including *L. islandica* and its possible oldest decedents, were extinct during glaciation (Doellman *et al*., 2011). The oldest records of *L. saxatilis* include shells from Boxgroove in Sussex (British Isles) and Sidi Abd er Rahmane near Casablanca (North Africa) dated at about 0.5 Mya (Reid, 1996), implying a wide distribution of the species during preglacial time. The exact place of origin of *L. saxatilis* is still unknown. The phylogeographic history of *L. saxatilis* was studied in details and described as waves of westward and eastward expansions in pre-, inter- and postglacial periods (without pointing the original source) (Doellman *et al*., 2011; Panova *et al*., 2011; Blakeslee *et al*., 2021). On the Northwest Atlantic (NWA) coasts, *L. saxatilis* survived in at least two refugia during glaciation cycles. The modern Northeast Atlantic (NEA) populations of *L. saxatilis* represent the result of recolonisation after LGM from at least three refugia: the southern (on the Iberian coast, the Galician populations are still strongly differentiated from all others, exclusively possessing clade D haplotypes), the British (which strongly contributed to recolonisation of the British Isles and enriched with the clade C haplotypes), and the northern mainland refugium hypothetically located on the North Sea coasts, which acted as a source for the recolonisation of the continental coasts (Doellman *et al*., 2011; Panova *et al*., 2011). Additional refugia were predicted to function in the NEA at the Faroe Islands and northern Norway (Maggs *et al*., 2008), where *L. saxatilis* could also survive.

In our study, we considered the European populations of all three descendants of *L. islandica*: *L. saxatilis*, *L. arcana* and *L. compressa.* The differentiation of individual clades in *L. saxatilis* most probably occurred in allopatry due to geographic and/or temporal factors. *Littorina arcana* and *L. compressa* have most likely specified in sympatry with *L. saxatilis* through a niche differentiation due to ecological factors (Reid, 1996; Maltseva *et al*., 2021a,b,c, 2022); that is, differentiation of their specific clades may have occurred in the presence of other clades with a possibility of early introgression and the inheritance of a shared ancestral polymorphism. It means, for example, that the history of *L. arcana* of the clade A and of the clade G could be challenging to trace separately, because these clades could have coexisted throughout the species lifetime. Generally, the presence of one species in several diverged mitochondrial clades on a phylogenetic tree, that is 'paraphyly' based on mitochondrial markers, is does not necessarily suggest a true paraphyly of this taxon, but appears due to incomplete lineage sorting or introgressive hybridisation during speciation event (see e.g. Ballard, Whitlock, 2004; Peters *et al*., 2007; Choleva *et al*., 2014). The mechanisms of premating isolation due to habitat choice and postmating prezygotic reproductive isolation have been suggested to play a crucial role in speciation events

between species of the 'saxatilis' group (Lobov *et al*., 2019; Maltseva *et al*., 2021c, 2022). The strength of these two types of isolating mechanisms may vary depending on local conditions (such as diversity of available microhabitats) and genetic characteristics of coexisting subpopulations, which may lead to different degrees of introgression between incipient species in different parts of their initial ranges. Altogether, this makes the overall patterns of genetic divergence of *L. arcana* and *L. compressa* quite difficult for the phylogeographic interpretations. The evolutionary reconstructions are further complicated by the absence in our analysis populations of *L. arcana* and *L. compressa* from France and South Norway and the fact that no fossil records are known for both species (Reid, 1996). Below we suggest a plausible fragmentary evolutionary scenario for this species group, most parsimoniously explaining the data at hand.

*LITTORINA ARCANA* AND *L. COMPRESSA* ARE LESS POTENT COLONISERS THAN *L. SAXATILIS*. Both *L. arcana* and *L. compressa* are egg-layers while *L. saxatilis* is the only brooder in the *Neritrema* clade. The benthic eggs-masses are vulnerable to diverse stressors of the intertidal zone such as temperature, desiccation, solar radiation, salinity variation, predators, etc. Further, the egg-layers probably have a more limited long-distance dispersal potential than the ovoviviparous *L. saxatilis*. Accordingly, neither *L. arcana* nor *L. compressa* are known from the NWA and the Northcentral Atlantic (NCA) coasts, the North East Atlantic (NEA) islands, warm-temperate climatic zones, estuarine areas (for details see Reid, 1996). No cryptogenic populations of these species have ever been reported, unlike Venice or San-Francisco populations of *L. saxatilis* (Panova *et al*., 2011; Blakeslee *et al*., 2021). Brooding strategy gives *L. saxatilis* an advantage for efficient breaking into estuaries, mudflats, islands, warm-climate zones (e.g., Spain, the North and South Africa) and extremely cold-climatic zones (e.g., Svalbard and Novaya Zemlya), predisposing this species to extremely wide distribution range (the broadest across *Littorina* species). Thus, a higher degree of connectivity can be expected in this species compared to more fragmented population ranges in *L. arcana* and *L. compressa,* which is illustrated by the lower values of between-region *Fst* in *L. saxatilis* than those in *L. arcana* and *L. compressa*. In this context it is meaningful that the level of differentiation between sympatric *L. arcana* and *L. saxatilis* was low in the westernmost and easternmost sites of the study with a high level of between-sites differentiation within the species. This makes plausible that the postglacial expansion of *L. arcana* occurred from at least two source populations, coexisted for a significant period with *L. saxatilis* with some degree of between-species genetic introgression in each source site.

IS *L. SAXATILIS* AN OLDER SPECIES THAN *L. ARCANA* AND *L. COMPRESSA*? Two new cytb clades F and G, grouping together on some reconstructions (Supplement SM2), were found in *L. arcana* (haplotypes of both clades) and *L. compressa* (clade F), but not *L. saxatilis*. Based on the positions on a phylogenetic tree, the speciation of these two 'without-saxatilis' clades occurred close in time to the separation of the main clades of *L. saxatilis*: A, B, C and DE (these have a common root). In turn, this implies that speciation of the *L. arcana* (G clade) and *L. compressa* (F clade) occurred when *L. saxatilis* was already widely dispersed on both sides of the North Atlantic. Based on Doellman *et al*. (2011) and Panova *et al*. (2011), only the clade A spread on both sides of the North Atlantic from north to south populations in each case, as well as at the coasts of Greenland and Iceland (NCA) demonstrating the widest distribution pattern. It takes a basal place to other clades in some reconstructions (Supplement SM3) and has the oldest mean TMRCA estimate to 220 Kya (Panova *et al*., 2011). The region of this pan-North-Atlantic clade emergence cannot be unambiguously established since the diversity levels are similar on both sides of the Atlantic, as if the NWA and the NEA populations existed in parallel throughout the species history. It could be hypothesised that the ancestral population of *L. saxatilis* have existed in the NCA, where the fossils of *L. islandica* were found. This population may have harboured the ancient clade A haplotypes and acted as an initial source for the first wave of parallel preglacial colonisation of both the NEA and the NWA coasts. Clades F and G are distributed in the NEA only: modern *L. arcana* and *L. compressa* are absent at the NCA and the NWA

coasts; both these species harbour haplotypes of the clade A and its subclades (with basal haplotypes shared with *L. saxatilis*). This latter fact may be interpreted as either an ancestral polymorphism inherited from *L. islandica* or an early introgression from ancient *L. saxatilis*. There are no reasons to surmise spreading of *L. islandica* beyond the NCA coasts (see above), and consequently, similar to *L. saxatilis*, the NCA region seems the most plausible location site of the ancestral populations of both *L. arcana* (clade G) and *L. compressa* (clade F). This surmise is compatible with the possibility of independent colonisation of the British and the European mainland coasts (see explanation on this below). The presumed ancestral populations together with the oldest *L. saxatilis* and *L. islandica* were lost during glaciation, when the NCA coasts were covered by the ice sheet, eliminating coastal fauna (Dunton, 1992; Ingolfsson, 1992; Thatje *et al*., 2005; Maggs *et al*., 2008; Doelmann *et al*., 2011). Nevertheless, *L. arcana* and *L. compressa* reached the North European and British coasts in preglacial time and survived in several refugia there together with *L. saxatilis* as explained below.

THERE IS A DEEP DIVERGENCE BETWEEN THE BRITISH AND THE CONTINENTAL POPULATIONS OF SPECIES OF THE 'SAXATILIS' GROUP. In our cytb analysis, the Wales populations of all three species had unique characteristics compared to the mainland: the unique presence of the clade C in *L. compressa* and its predominance in *L. saxatilis*, the unique presence of the clade F in *L. arcana*, the British *L. arcana* was not revealed to have clade A or its subclades, unlike its continental populations, and the diversity of the clade A in the British *L. saxatilis* was very limited. Considering the clade F, the diversity in the mainland vs British populations of *L. compressa* was similar (overlapping exclusively with the basal haplotype), indicating no shorter independent history of this clade on the continental coast than on the British Isles. Such a tendency was equally fair for the clade C in *L. arcana*. Taken together, these facts illustrate a deep divergence between the British and the mainland populations of all three species and low probability of postglacial repopulation of the continental coast from the British source. This conclusion is corroborated fully by the microsatellite analysis and agrees well with previous low estimates of the 'British Isles – mainland' migration in example of *L. saxatilis* (Panova *et al*., 2011).

The unique interchange of the cytb clades between three 'saxatilis' species together with the proximity of their microsatellite profiles in Wales implies that all three species reached the British coasts at the early stage of their divergence and continuously coexisted there during the glacial cycles. Moreover, some degree of gene flow should have occurred between them to provide the observed patterns. Modern *L. compressa* cannot hybridise with its sibling species due to efficient premating and postmating isolating mechanisms (Warwick *et al*., 1990; Maltseva *et al*., 2021c). However, it is well-known that pre- and postmating prezygotic barriers can evolve very quickly under sympatric conditions (e.g., Zigler *et al*., 2005; Matute, 2010; Turissini *et al*., 2018). The possibility of hybridisation between modern *L. arcana* and *L. saxatilis* was inferred from population data (Mikhailova *et al*., 2009; Granovitch *et al*., 2013). The possibility of productive hybridisation was demonstrated in laboratory experiments between modern British *L. saxatilis* males and *L. arcana* females (not vice versa) (Warwick *et al*., 1990), although this asymmetry may be evolutionarily recent and geographically variable as explained above. The strength of premating mechanisms based on habitat choice, playing a pivotal role in reproductive isolation between the Atlantic *Neritrema* snails (Maltseva *et al*., 2021c), obviously depends on local conditions. Hypothetically, both pre- and postmating may have been less efficient during glaciation time when *L. arcana*, *L. compressa* and *L. saxatilis* were locked together by ice in the British refugium (see Maggs *et al*., 2008) due to their evolutionary youth and to a possibly limited diversity of available microhabitats. On the mainland coast, the pattern of interrelation between three 'saxatilis' species turned out to be different.

*LITTORINA ARCANA* AND *L. COMPRESSA* DIFFER IN THEIR HISTORIES ON THE EUROPEAN CONTINENTAL COAST. In earlier studies, the origin of the clade C was geographically related to the British Isles (Doellman *et al*., 2011; Panova *et al*., 2011), since its haplotypes were described as most diverse and

typical for the British populations of *L. saxatilis*. This study revealed that the clade C is present and equally diverse in *L. arcana*. Moreover, considering the wide spread of the clade C across populations of *L. arcana* far beyond the UK, its British origin in *L. saxatilis* seems doubtful, while its emergence in *L. arcana* sounds more reasonable.

Although the divergence of the British vs the continental populations seems to be similar in all species of the 'saxatilis' group, *L. arcana* demonstrates several peculiar phenomena. (1) Besides the clade C, most probably originated in *L. arcana*, this species also harboured the clade G haplotypes. While clade C was exchanged between three species at least in Wales, the clade G kept its strict specificity for *L. arcana* (even though its haplotypes were reported from Wales). Why did these two clades, evolved in *L. arcana* and coexisting in its modern populations, have such a different history? (2) *L. arcana* had a complex ancestry based on microsatellite analysis: all its populations had a common ancestry only at the level of two groups (when this species was completely indistinguishable from *L. saxatilis*). At K=3 and higher, some continental *L. arcana* populations received a unique ancestry. In the population of the Barents Sea there were individuals with contrasting genetic patterns. (3) Agreeingly, the 2bRAD genomic profiling of populations of the sites of Bodø and Kiberg also showed contrasting genetic patterns in populations of *L. arcana* with two fractions, originating from different sources: one was rather similar to sympatric *L. saxatilis*, and the second fraction had a unique origin. Even if these two fractions of *L. arcana* did coexist in the same populations, they still remained easily recognisable.

Thus, analyses of all three types of molecular markers (cytb, microsatellites, 2bRAD) clearly showed that continental populations of *L. arcana* are genetically heterogeneous and include individuals with strongly diverged genomic profiles. In a recent study (Maltseva *et al*., 2021c) describing the mating activity in the populations of the 'saxatilis' species in Kiberg (specimens were included in the 2bRAD analysis) and Dalnye Zelentsy (specimens were included in the microsatellite analysis), it has been hypothesised that *L. arcana* females, though morphologically indistinguishable, were represented by a mixture of two different lineages which significantly varied in their attractiveness for the males of both *L. arcana* and *L. saxatilis*. Our results on genotyping in these populations are in a good agreement with such a hypothesis. In turn, this implies that *L. arcana* appears in its northern populations as two partially reproductively isolated lineages (since these keep their genetic identity) and could be regarded as a 'semiparaphyletic' species. That is, lineages of *L. arcana* were separated at the very early stages of speciation (most probably in preglacial time), finalising its divergence in the presence of sympatric *L. saxatilis* with possibility of differential introgression from it in different parts of their ranges. Our data do not suggest that contrasting genetic profiles are present in the British populations of *L. arcana* and consequently do not contradict results of Stankowski *et al*. (2020) on populations of UK and South France.

Unlike *L. arcana*, continental populations of *L. compressa* generally had a more uniform clade composition: clades F + A (including A1 subclade) in all cases (and the same was in Wales plus admixture of the clade C). This uniformity is confirmed by the relatively low 'by distance' *Fst* compared to 'by frequency' *Fst* values on cytb data in *L. compressa*, while the reverse tendency was observed in *L. arcana*. Concordantly, in microsatellite analysis only allele-frequency-based methods (DAPC and SAMOVA) showed population differentiation in *L. compressa*, while the STRUCTURE analysis did not. These facts point to another source of the between-populations variation in *L. arcana* compared to *L. compressa*: predominance of variation between sequences or allele combinations over variation in their frequencies. *L. compressa* was clearly separated from the two other species in all the analyses performed based on all genetic markers used. Also, the distinctiveness of *L. compressa* from the *L. arcana* / *L. saxatilis* pair was previously described at the proteomic, metabolomic, morphological and ecological levels (Maltseva *et al*., 2020, 2021a) and in its mating patterns (Maltseva *et al*., 2021c). The separation of the British vs the mainland lineages is the only traceable historical event in this species. The modern continental populations of *L. compressa* may have originated from a single European source during postglacial recolonisation.

## Conclusion

In this study, we used a number of molecular markers to reconstruct the recent phylogeographic history of three *Littorina* species of the 'saxatilis' group in the Northeast Atlantic. Our results suggest that speciation of *L. arcana* and *L. compressa* from *L. islandica* occurred after separation of *L. saxatilis,* and most probably this happened on the NCA coasts. These species reached the British and the North European coasts during the eastward expansion in near glacial time. Similar to other coastal species, their ranges extremely shrank during glaciation. All three sibling species survived in the refugium (or refugia) on the British coasts, most probably on the south coasts of the Irish Sea, and significantly introgressed into genomes of each other there and then. On the continental coast, *L. compressa* persisted in the single refugium, the population of which acted as a source for the post-glacial mainland colonisation. *Littorina arcana* got through the glaciation in more than one refugium (besides the Irish Sea), having been locked by ice at the very early stages of speciation; of these two *L. arcana* lineages with contrasting genomic patterns, one had a genetic introgression from sympatric *L. saxatilis* and still demonstrates high similarity with this species. Thus, the post-glacial colonisation of the mainland *L. arcana* occurred from at least two sources. The inclusion of more southern populations (e.g., of the South Norway and France) is needed to make the picture more complete.

**Compliance with ethical standards**

CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest.

**Supplementary data.** The following materials are available online in one file.

SM1. TCS haplotype network based on the 367 bp cytb fragment.

SM2. Bayesian phylogenetic tree of the 625 bp cytb haplotype sequences.

SM3. Bayesian phylogenetic tree based on the 367 bp cytb fragment.

SM4. Fst values and corresponding p-values.

SM5. Discriminant analysis of principal components (DAPC) results.

SM6. Heatmap of pairwise Fst values bases on microsatellite data.

SM7. SAMOVA results of population of the three 'saxatilis' species.

**Acknowledgements**.This research was funded by the Russian Science Foundation, grant number 19-14-00321 PI Andrei I. Granovitch. The facilities for the molecular procedures were provided by the Resource Centre of St. Petersburg State University "Development of Molecular and Cell Technologies". The facilities for maintenance of experimental animals were provided by the Resource Centre Environmental Safety Observatory of St Petersburg State University. We thank Prof. John Grahame for providing us the snails from Wales many years ago. We also thank Emma Hermansson and Yulia Gracheva who performed part of the microsatellite genotyping within their degree projects.

## References

Alexander D.H., Novembre J., Lange K. 2009. Fast model-based estimation of ancestry in unrelated individuals // Genome Research. Vol.19. No.9. P.1655–1664. https://doi.org/10.1101/gr.094052.109

Ballard J.W.O., Whitlock M.C. 2004. The incomplete natural history of mitochondria // Molecular Ecology. Vol.13. No.4. P.729–744. https://doi.org/10.1046/j.1365-294X.2003.02063.x

Blakeslee A.M., Miller A.W., Ruiz G.M., Johannesson K., André C., Panova M. 2021. Population structure and phylogeography of two North Atlantic *Littorina* species with contrasting larval development // Marine Biology. Vol.168. No.7. P.1–16. https://doi.org/10.1007/s00227-021-03918-8

Bolger A.M., Lohse M., Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data // Bioinformatics. Vol.30. No.15. P.2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Brawand D., Wagner C.E., Li Y.I., Malinsky M. et al. 2014. The genomic substrate for adaptive radiation in African cichlid fish // Nature. No.513. P.375–381. https://doi.org/10.1038/nature13726

Choleva L., Musilova Z., Kohoutova-Sediva A., Paces J., Rab P., Janko K. 2014. Distinguishing between incomplete lineage sorting and genomic introgressions: complete fixation of allospecific mitochondrial DNA in a sexually reproducing fish (Cobitis; Teleostei), despite clonal reproduction of hybrids // PLoS One. Vol.9. No.6. Art.e80641. https://doi.org/10.1371/journal.pone.0080641

Clement M., Posada D.C.K.A., Crandall K.A. 2000. TCS: a computer program to estimate gene genealogies // Molecular Ecology. Vol.9. No.10. P.1657–1659.

Danecek P., Bonfield J. K., Liddle J. et al. 2021. Twelve years of SAMtools and BCFtools // GigaScience Vol.10. No.2, giab008. https://doi.org/10.1093/gigascience/giab008

Doellman M.M., Trussell G.C., Grahame J.W., Vollmer S.V. 2011. Phylogeographic analysis reveals a deep lineage split within North Atlantic *Littorina saxatilis* // Proceedings of the Royal Society B: Biological Sciences. Vol.278. No.1722. P.3175–3183. https://doi.org/10.1098/rspb.2011.0346

Dunton K. 1992. Arctic biogeography: the paradox of the marine benthic fauna and flora // Trends in Ecology and Evolution. Vol.7. No.6. P.183–189. https://doi.org/10.1016/0169-5347(92)90070-R

Dupanloup I., Schneider S., Excoffier L. 2002. A simulated annealing approach to define the genetic structure of populations // Molecular Ecology. Vol.11. No.12. P.2571–2581. https://doi.org/10.1046/j.1365-294X.2002.01650.x

Excoffier L., Lischer H.E.L. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows // Molecular Ecology Resources. Vol.10. P.564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Ewels P., Magnusson M., Lundin S., Käller M. 2016. MultiQC: summarize analysis results for multiple tools and samples in a single report // Bioinformatics. Vol. 32 No.19. P.3047–3048. https://doi.org/10.1093/bioinformatics/btw354

Gladenkov Y.B., Norton P., Spaink G. 1980. The Upper Cenozoic of Iceland. The Survey // Trudy Geologicheskii Instituta. Vol.345. P.1–116. https://doi.org/10.1093/molbev/msp259

Gouy M., Guindon S., Gascuel O. 2010. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building // Molecular Biology and Evolution. Vol.27. No.2. P.221–224. https://doi.org/10.1093/molbev/msp259

Granovitch A.I., Maximovich A.N., Avanesyan A.V., Starunova Z.I., Mikhailova N.A. 2013. Micro-spatial distribution of two sibling periwinkle species across the intertidal indicates hybrdization // Genetica. Vol.141. P.293–301. https://doi.org/10.1007/s10709-013-9728-3

Habel J.C., Schmitt T., Müller P. 2005. The fourth paradigm pattern of post-glacial range expansion of European terrestrial species: the phylogeography of the Marbled White butterfly (Satyrinae, Lepidoptera) // Journal of Biogeography. Vol.32. No.8. P.1489–1497. https://doi.org/10.1111/j.1365-2699.2005.01273.x

Ingolfsson A. 1992. The origin of the rocky shore fauna of Iceland and the Canadian Maritimes // Journal of Biogeography. Vol.19. No.6. P.705. https://doi.org/10.2307/2845711

Jakobsson M., Rosenberg N.A. 2007. CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure // Bioinformatics. Vol.23. No.14. P.1801–1806. https://doi.org/10.1093/bioinformatics/btm233

Jombart T., Devillard S., Balloux F. 2010. Discriminant analysis of principal components: A new method for the analysis of genetically structured populations // BMC Genetics. Vol.11. No.1. P.1–15. https://doi.org/10.1186/1471-2156-11-94

Kaeuffer R., Réale D., Coltman D.W., Pontier D. 2007. Detecting population structure using STRUCTURE software: Effect of background linkage disequilibrium // Heredity. Vol.99. P.374–380. https://doi.org/10.1038/sj.hdy.6801010

Kopelman N.M., Mayzel J., Jakobsson M., Rosenberg N.A., Mayrose I. 2015. Clumpak: A program for identifying clustering modes and packaging population structure inferences across K // Molecular Ecology Resources. Vol.15. No.5. P.1179–1191. https://doi.org/10.1111/1755-0998.12387

Korneliussen T.S., Albrechtsen A., Nielsen R. 2014. ANGSD: Analysis of Next Generation Sequencing Data // BMC bioinformatics. Vol.15. No.1. P.356. https://doi.org/10.1186/s12859-014-0356-4

Langmead B., Salzberg S.L. 2012. Fast gapped-read alignment with Bowtie 2 // Nature methods. Vol.9. No.4. P.357–359. https://doi.org/10.1038/nmeth.1923

Langmead B., Wilks C., Antonescu V., Charles R. 2019. Scaling read aligners to hundreds of threads on general-purpose processors // Bioinformatics. Vol.35. No.3. P.421–432. https://doi.org/10.1093/bioinformatics/bty648

Leigh J.W., Bryant D. 2015. POPART: full-feature software for haplotype network construction // Methods in Ecology and Evolution. Vol.6. No.9. P.1110–1116. https://doi.org/10.1111/2041-210X.12410

Li H. 2011. Improving SNP discovery by base alignment quality // Bioinformatics. Vol.27. No.8. P.1157–1158. https://doi.org/10.1093/bioinformatics/btr076

Lobov A.A., Maltseva A.L., Mikhailova N.A., Granovitch A.I. 2019. The molecular mechanisms of gametic incompatibility in invertebrates // Acta Naturae. Vol.11. No.3(42). P.4–15. https://doi.org/10.32607/20758251-2019-11-3-4-15

Lobov A.A., Babkina I.Y., Danilov L.G., Masharskiy A.E., Predeus A.V., Mikhailova N.A., Granovitch A.I., Maltseva A.L. 2021. Species-specific proteins in the oviducts of snail sibling species: Proteotranscriptomic study of *Littorina fabalis* and *L. obtusata* // Biology. Vol.10. No.11. P.1087. https://doi.org/10.3390/biology10111087

Maggs C.A., Castilho R., Foltz D. et al. 2008. Evaluating signatures of glacial refugia for North Atlantic benthic marine taxa // Ecology. Vol.89. No.11. P.S108–S122. https://doi.org/10.1890/08-0257.1

Maltseva A.L., Varfolomeeva M.A., Lobov A.A., Mikhailova N.A., Renaud P.E., Grishankov A.V., Volovik K.Y., Granovitch A.I. 2016. Measuring physiological similarity of closely related littorinid species: a proteomic insight // Marine Ecology Progress Series. Vol.552. P.177–193. https://doi.org/10.3354/meps11770

Maltseva A.L., Varfolomeeva M. A., Lobov A.A., Tikanova P., Panova M., Mikhailova N.A., Granovitch A.I. 2020. Proteomic similarity of the Littorinid snails in the evolutionary context // PeerJ. Vol.8. Art.e8546. https://doi.org/10.7717/peerj.8546

Maltseva A.L., Varfolomeeva M.A., Ayanka R.V., Gafarova E.R., Repkin E.A., Pavlova P.A., Shavarda A.L., Mikhailova N.A., Granovitch A.I. 2021a. Linking ecology, morphology and metabolism: niche differentiation in sympatric populations of closely related species of the genus *Littorina* (*Neritrema*) // Ecology and Evolution. Vol.11. No.16. P.11134–11154. https://doi.org/10.1002/ece3.7901

Maltseva A.L., Varfolomeeva M.A., Gafarova E.R., Panova M.A.Z., Mikhailova N.A., Granovitch A.I. 2021b. Divergence together with microbes: a comparative study of the associated microbiomes in the closely related *Littorina* species // PloS One. Vol.16. No.12. Art.e0260792. https://doi.org/10.1371/journal.pone.0260792

Maltseva A.L., Varfolomeeva M.A., Lobov A.A., Tikanova P.O., Repkin E.A., Babkina I.Y., Panova M., Mikhailova N.A., Granovitch A.I. 2021c. Premating barriers in young sympatric snail species // Scientific Reports. Vol.11. No.1. Art.5720. https://doi.org/10.1038/s41598-021-84407-2

Maltseva A.L., Lobov A.A., Pavlova P.A., Panova M., Gafarova E.R., Marques J.P., Danilov L.G., Granovitch A.I. 2022. Orphan gene in *Littorina*: an unexpected role of symbionts in the host evolution // Gene. Vol.824. Art.146389. https://doi.org/10.1016/j.gene.2022.146389

Matute D.R. 2010. Reinforcement can overcome gene flow during speciation in *Drosophila* // Current Biology. Vol.20. No.24. P.2229–2233. https://doi.org/10.1016/j.cub.2010.11.036

McGee M.D., Neches R.Y., Seehausen O. 2016. Evaluating genomic divergence and parallelism in replicate ecomorphs from young and old cichlid adaptive radiations // Molecular Ecology. Vol.25. No.1. P.260–268. https://doi.org/10.1111/mec.13463

Michel A.P., Sim S., Powell T.H.Q., Taylor M.S., Nosil P., Feder J.L. 2010. Widespread genomic divergence during sympatric speciation // Proceedings of the National Academy of Sciences. Vol.107. P.9724–9729. https://doi.org/10.1073/pnas.1000939107

Mikhailova N.A., Gracheva Y.A., Backeljau T., Granovitch A.I. 2009.A potential species-specific molecular marker suggests interspecific hybridization between sibling species *Littorina arcana* and *L. saxatilis* (Mollusca, Caenogastropoda) in natural populations // Genetica. Vol.137. No.3. P.333–340. https://doi.org/10.1007/s10709-009-9397-4

Panova M., Mäkinen T., Fokin M., André C., Johannesson K. 2008. Microsatellite cross-species amplification in the genus *Littorina* and detection of null alleles in *Littorina saxatilis* // Journal of Molluscan Studies. Vol.74. No.2. P.111–117. https://doi.org/10.1093/mollus/eym052

Panova M., Blakeslee A.M., Miller A.W., Mäkinen T., Ruiz G.M., Johannesson K., André C. 2011. Glacial history of the North Atlantic marine snail, *Littorina saxatilis*, inferred from distribution of mitochondrial DNA lineages // PloS One. Vol.6. No.3. P.e17511. https://doi.org/10.1371/journal.pone.0017511

Panova M., Johansson T., Canbäck B., Bentzer J., Rosenblad M. A., Johannesson K., Tunlid A., André C. 2014. Species and gene divergence in *Littorina* snails detected by array comparative genomic hybridization // BMC Genomics. Vol.15. No.1. P.1–21. https://doi.org/10.1186/1471-2164-15-687

Panova M.A., Varfolomeeva M.A., Gafarova E.R., Maltseva A.L., Mikhailova N.A., Granovitch A.I. 2022. First insights into the gut microbiomes and the diet of the *Littorina* snail ecotypes, a recently emerged marine evolutionary model // Evolutionary Applications. Art.e13447. https://doi.org/10.1111/eva.13447

Peters J.L., Zhuravlev Y., Fefelov I., Logie A., Omland K.E. 2007. Nuclear loci and coalescent methods support ancient hybridization as cause of mitochondrial paraphyly between gadwall and falcated duck (*Anas* spp.) // Evolution. Vol.61. No.8. P.1992–2006. https://doi.org/10.1111/j.1558-5646.2007.00149.x

Pritchard J.K., Stephens M., Donnelly P. 2000. Inference of population structure using multilocus genotype data // Genetics. Vol.155. No.2. P.945–959. https://doi.org/10.1534/genetics.116.195164

R Core Team. 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rambaut A. 2009. FigTree v1.3.1. http://tree.bio.ed.ac.uk/software/figtree.

Reid D.G. 1996. Systematics and evolution of *Littorina*. The Ray Society.

Reid D.G., Rumbak E., Thomas R.H. 1996. DNA, morphology and fossils: phylogeny and evolutionary rates of the gastropod genus *Littorina* // Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences. Vol.351. No.1342. P.877–895. https://doi.org/10.1098/rstb.1996.0082

Reid D.G., Dyal P., Williams S.T. 2012. A global molecular phylogeny of 147 periwinkle species (Gastropoda, Littorininae) // Zoologica Scripta. Vol.41. P.125–136. https://doi.org/10.1111/j.1463-6409.2011.00505.x

Rosenberg N.A. 2004. DISTRUCT: A program for the graphical display of population structure // Molecular Ecology Notes. Vol.4. No.1. P.137–138. https://doi.org/10.1046/j.1471-8286.2003.00566.x

Símonarson L.A., Eiríksson J., Knudsen K.L. 2021. The Marine Realm Around Iceland: A Review of Biological Research // Pacific-Atlantic Mollusc Migration. P.13–35. https://doi.org/10.1007/978-3-030-59663-7_2

Stankowski S., Westram A.M., Zagrodzka Z.B., Eyres I., Broquet T., Johannesson K., Butlin R.K. 2020. The evolution of strong reproductive isolation between sympatric intertidal snails // Philosophical Transactions of the Royal Society B. Vol.375. No.1806. Art.20190545. https://doi.org/10.1098/rstb.2019.0545

Stewart J.R., Lister A.M., Barnes I., Dalén L. 2010. Refugia revisited: individualistic responses of species in space and time // Proceedings of the Royal Society B: Biological Sciences. Vol.277. No.1682. P.661–671. https://doi.org/10.1098/rspb.2009.1272

Tarnowska K., Krakau M., Jacobsen S., Wołowicz M., Féral J.P., Chenuil A. 2012. Comparative phylogeography of two sister (congeneric) species of cardiid bivalve: strong influence of habitat, life history and post-glacial history // Estuarine, Coastal and Shelf Science. Vol.107. P.150–158. https://doi.org/10.1016/j.ecss.2012.05.007

Thatje S., Hillenbrand C.D., Larter R. 2005. On the origin of Antarctic marine benthic community structure // Trends in Ecology and Evolution. Vol.20. No.10. P.534–540. https://doi.org/10.1016/j.tree.2005.07.010

Trewick S.A., Wallis G.P. 2001. Bridging the "beech-gap": New Zealand invertebrate phylogeography implicates Pleistocene glaciation and Pliocene isolation // Evolution. Vol.55. No.11. P.2170–2180. https://doi.org/10.1111/j.0014-3820.2001.tb00733.x

Wang S., Meyer E., McKay J. K., Matz M.V. 2012. 2b-RAD: a simple and flexible method for genome-wide genotyping // Nature Methods. Vol.9. No.8. P.808–810. https://doi.org/10.1038/nmeth.2023

Warwick T., Knight A., Ward R. 1990. Hybridisation in the *Littorina saxatilis* species complex (Prosobranchia: Mollusca) // Hydrobiologia. Vol.193. P.109–116. https://doi.org/10.1007/BF00028070

Wickham H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York. https://doi.org/10.1007/978-0-387-98141-3

Zigler K.S., McCartney M.A., Levitan D. R., Lessios H.A. 2005. Sea urchin bindin divergence predicts gamete compatibility // Evolution. Vol.59. No.11. P.2399–2404. https://doi.org/10.1111/j.0014-3820.2005.tb00949.x

*Responsible editor E.N. Temereva*