Forest snail faunas from Georgian Transcaucasia: patterns of diversity in a Pleistocene refugium

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Thirty forest sites in Georgia were sampled to obtain inventories of their land snail faunas. The sites, in nine sampling areas, ranged from Batumi near the Turkish border to Lagodekhi near the Azerbaijan border, and included parts of both the Lesser and Greater Caucasus mountains and the Colchic lowlands. Abundance was generally low, and most sites held less than 20 species. Ninety species were found overall. Although sites within some sampling areas had very similar faunas, the overall levels of similarity among sites and sampling areas was low, especially when compared with similar forests from northern central Europe. Both climate and geographical position affected similarity: although these were correlated, independent effects of each were detected, and the effect of position alone was strongest. Despite this effect, most species have ranges extending into Turkey or Ciscaucasia, a few have very limited ranges, and there is no evidence of patterns of allopatric replacement among congeners. These patterns resemble those seen in faunas from forests in lower latitudes, and suggest a slow build-up of regional diversity by differentiation *in situ*, without the repeated restrictions to a multiplicity of small refugia that are characteristic of areas where fluctuations in aridity are important. The refugium has remained isolated, and has contributed little to the Holocene recolonization of areas further north.   © 2011 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2011, **102**, 239–250.

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INTRODUCTION

Land snail faunas in Europe show a characteristic latitudinal gradient in regional richness that can be related to Pleistocene climatic fluctuations, and in particular to the glaciated or periglacial conditions prevailing north of the main mountain ranges at glacial maxima (Hausdorf & Hennig, 2003; Cameron, 2004; Cameron, Pokryszko & Horsák, 2010). Forest faunas in the north are essentially immigrant assemblages, with subsets and mixtures of faunas surviving in refuges further south. In Mediterranean and Balkan Europe, high diversity is associated with a multiplicity of small refuges; vicariance and

short-distance dispersal across barriers promotes differentiation among congeners. Such differentiation is, however, most evident in open but calcareous habitats, and the structure of regional diversity seems to relate mainly to fluctuations in aridity, sea level, and the periodic isolation of montane and alpine habitats (Cameron *et al.*, 2003). Within forest faunas, species richness at the level of small sites, termed alpha diversity by many authors (Magurran, 2004), does not vary greatly with latitude (Pokryszko & Cameron, 2005).

These patterns can be contrasted with some of those at lower or similar latitudes elsewhere, where although area and connectivity of forests varied, substantial areas of forest habitats remained within the region. Here, although there is evidence for speciation

as a result of the isolation of fragments of suitable habitats, regional diversity seems to have built up from isolation by distance alone (Solem, 1984; Barker, 2005): very few species have tightly restricted geographical ranges, and congeneric ranges overlap at the regional level. Local site diversities vary, but most are not much greater than those recorded for the immigrant faunas of northern European forests (Seddon *et al*., 2005; Stanisic *et al*., 2007). This runs counter to the general trend for site diversities of many organisms to be much greater at lower latitudes, other than in deserts (Ricklefs, 2004). Clearly, the balance and structure of local and regional diversity varies with the history of the regions concerned, and this raises questions about the assembly rules for local faunas.

For snails, these global comparisons are made less telling by the different spectra of taxonomic composition, size, and shape distribution, and known trophic status of snails between biogeographic realms (Cameron *et al*., 2005). Although the snail fauna of western Transcaucasia, including the extreme northeast of Turkey, contains many endemic species and genera (Schütt, 2005; Sysoev & Schileyko, 2009), the taxonomic composition of the forest snail fauna at family level and above is characteristically Western Palaearctic. The region is known to be one of the few places in the Western Palaearctic to have retained mixed broadleaf forests in large quantities throughout the Pleistocene, in either a single large refugium spreading along the south-eastern Black Sea Coast (van Andel & Tzedakis, 1996) or in multiple large and small refuges (Velichko & Kurenkova, 1990). Its flora is regarded as belonging to the broad Euro-Siberian assemblage, rather than to those of the Mediterranean or Irano-Turanian regions (Davis, 1971; Tumajanov, 1971). Although there were certainly shifts in climate sufficient to alter the altitudinal limits of some forest types by up to 1000 m (Tarasov *et al*., 2000), the combination of high mountains and exposure to warm and humid winds across the Black Sea evidently moderated the extremes of cold or aridity that characterized most of the region at glacial maxima (Denk, Frotzler & Davitashvili, 2001, and references therein). Areas to the north (Ciscaucasia), east, and south were, as now, dominated by arid conditions, with steppe vegetation predominating.

We report here on a survey of forest snail faunas in sites within refugial parts of the Caucasus, where forests persisted for millions rather than a few tens of thousands of years, along with others where most of the forests were likely to have been replaced by steppe at glacial maxima. We compare the patterns of richness and diversity found with those recorded elsewhere, and in particular with those from Poland and Transcarpathian Ukraine, typical of faunas established by northwards migration in the Holocene (Cameron, Pokryszko & Horsák, 2010). This gives us a deeper insight into the processes of the formation of the contemporary forest snail faunas and, more generally, to the dynamics of the refugial forests of the Caucasus ecoregion.

THE REGION AND SAMPLING SITES

Figure 1 shows the region, and the location of clusters of sampling sites (hereafter SA, sampling area) within it. Samples were made from just south of Batumi near the Turkish border in the west to Lagodekhi near the Azerbaijan border in the east. Denk *et al*. (2001) gave a detailed account of the region and of the types of forest flora to be found within it. Our SAs are mostly near named localities of their account. The region has sharp west-to-east gradients in rainfall, and in the contrast between summer and winter temperatures. Areas near Batumi have a very oceanic climate: January mean temperatures are above 0 °C, and rainfall in the mountains nearby can exceed 4000 mm year^{-1}. Further east the climate is more continental, with rainfall generally being in the range 760–2000 mm year^{-1}, and winters are colder. Natural forest cover is mainly of broadleaved deciduous trees, principally beech, *Fagus orientalis*, and chestnut, *Castanea sativa*, with conifers, *Abies nordmanianna* and *Picea orientalis*, at higher altitudes, and with locally abundant alders, *Alnus barbata*, ash, *Fraxinus excelsior*, hornbeams, *Carpinus caucasica* and *Carpinus orientalis*, maples, *Acer* species, and elm, *Ulmus glabra*. Within each SA, sites were chosen to maximize the probability of obtaining a representative fauna, moderated by accessibility in difficult terrain. Details of location and descriptions are given in Appendix S1. Most accessible forests are subject to management resulting in near monocultures, and the combination of management, very steep slopes, and heavy rainfall over mainly acidic rocks (typically andesite volcanics) results in large areas in which land snail faunas are impoverished both in density and species richness. Typically, our sites were on less steep slopes with streams or run-off channels, with a greater than normal diversity in tree species. Although such sites have undoubtedly been subject to human disturbance (the presence of walnut *Juglans regia* being an indicator; Tumajanov, 1971), the presence of alders *Alnus barbata*, maples *Acer* species, and ash *Fraxinus excelsior* indicate soils with higher moisture and base status than those on the very steep and frequently unstable slopes around. Sites in the south-west (Batumi, Kintrisi, and Mtirala) frequently had *Rhododendron ponticum* as a dominant species in the understory: this species is missing in the easternmost parts of our survey area
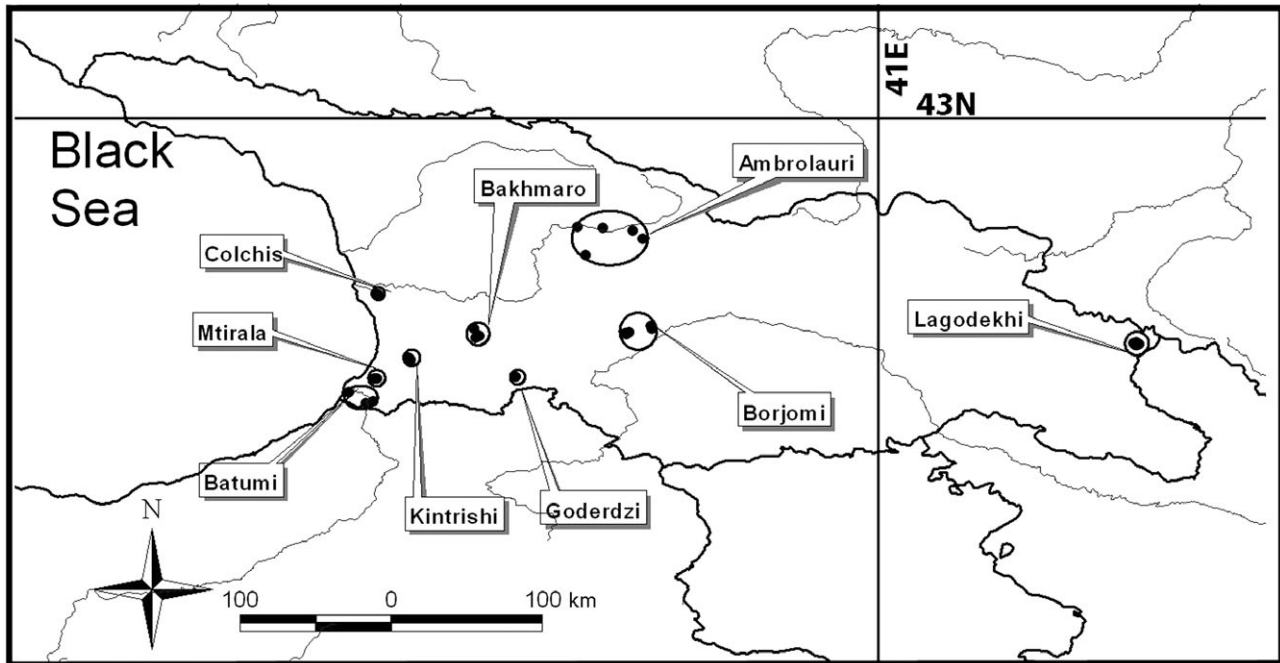
Figure 1. Map of the western Caucasus with sampling sites: black dots, sampling sites (as listed in Appendix S1); ellipses unify the sites into nine larger study areas (SAs).

(Tumajanov, 1971). One site at Ambrolauri (A1) was subject to much heavier and more destructive human disturbance than the remainder, a product of grazing by cattle. Only the sites at Ambrolauri (Fig. 1) had limestone as the underlying rock. Here, and at the Goderdzi Pass and at Bakhmaro, a few sites were sampled at elevations above 1400 m; the remainder were all below 1100 m. The Colchis site is unique in lying in swampy ground in the Colchic lowlands between the Lesser and Greater Caucasus.

MATERIAL AND METHODS

SAMPLING AND IDENTIFICATION

At each site, snails from an area of *c*. 400 m^2 were collected by hand in 5 person hours of searching; in addition, about 20 litres of litter was collected and sieved through 8-mm mesh sieves (Cameron & Pokryszko, 2005). Larger specimens were removed from the sieve, and material passing through the sieve was bagged, dried, and then sorted in the laboratory. Slugs (collected by hand in the field) were preserved separately, but, as in other studies (Cameron & Pokryszko, 2005), this method is not appropriate for obtaining a full inventory. Slug species found (identified by A. Wiktor) are listed by site in Appendix S2, but are excluded from our analyses. At each site location and altitude were determined by GPS (with the exception of sites at Batumi; see Appendix S1), and the dominance and occurrence of tree and understory shrubs were noted, as was the slope. Details are given in Appendix S1.

All living and empty but fresh shells were identified to species level as far as possible. Very eroded shells and unidentifiable juveniles are excluded from Appendix S2. Likharev (1962), Riedel (1966), Schileyko (1975, 1978, 1984), and Schütt (2005) were the principal sources for identification, supplemented by A. Riedel's collections of Zonitidae *s.l.* and others in the Museum and Institute of Zoology, Polish Academy of Sciences, Warsaw. Nomenclature generally follows that of Sysoev & Schileyko (2009); authorities and deviations from their list are given in Appendix S2. In some cases our records mark a significant extension of geographical range, and a few species are not mentioned in their checklist. Voucher specimens are deposited in the Museum of Natural History, Wrocław, and in the Institute of Ecology, Ilia State University, Tbilisi.

ANALYSES AND COMPARATIVE DATA

Sampling efficiency (the completeness of inventories at each site) was assessed by using the Chao 1 estimator of missing species (Southwood & Henderson, 2000), as this appears to be one of the most reliable estimators (Walther & Moore, 2005). As standard errors of this estimator are very large when the number of singletons and doubletons are small, we have used the median value as an overall estimator (Cameron, Pokryszko & Long, 2006).

Several statistical approaches were used for estimating the overall site-specific species richness, the level of differentiation between the sites, and for associating these variables with geography and climate. The overall pattern in faunas was first exposed by detrended correspondence analysis (DCA), using log-transformed abundances, and without down-weighting rare species (Leps & Smilauer, 2003). In order to describe the climates of the individual sites, bioclimatic data from WorldClim v1.4 (http://www.worldclim.org/) was used. This is a set of 19 global climate layers (bioclimate grids, including means and yearly dynamics of temperature and precipitation) with a spatial resolution of 1 km^2 (Hijmans *et al*., 2005). For all locations studied, the values of the bioclimatic variables were scored using ARCVIEW v.3.3 GIS software, and standardized in a way making the mean of each variable equal to zero and the standard deviation equal to unity. Two runs of canonical correspondence analysis (CCA; Manel *et al*., 2003) were applied in order to detect association of faunas/species with: (1) climate and (2) geography. For individual species, the tolerance indexes inferred from CCA analyses were scored to estimate the species-specific breadth of climatic niche and extent of occurrence of a species. This was to identify the species for which distribution is directly limited by geography rather than by climate (i.e. the species positioned above the regression line connecting climatic and geographic tolerance), and those in which distribution is limited by climate rather than by geography (the species positioned below the regression line). The software used for DCA and CCA was CANOCO v4.5 (ter Braak & Smilauer, 2002).

'Climatic distances' between the sites were calculated as Euclidean distances based on the 19 standardized bioclimatic variables and geographic distances: i.e. as Euclidean distances based on the exact latitude and longitude of a site. Partial Mantel tests (Manly, 1997) were applied to test for the significance of association of faunal differences (estimated as the inverse value of Simpson's similarity indexes, 100-SI) with geographic distances and climatic differences between the sampling sites. The Simpson index of similarity is the proportion of species in common with those in the least rich of any pair, diminishing the effect of mere impoverishment or inadequate sampling. The significance of the relationship was estimated for the Mantel tests with 10 000 permutations, using IBD software (Bohonak, 2002).

Some direct comparisons were made with data from Polish and Transcarpathian Ukrainian forest sites sampled in the same way (Cameron, Pokryszko & Horsák, 2010). Many of the latter samples contained more individuals than found in any of those reported here. To reduce the effect of differing median sample sizes, comparisons are limited to samples from Poland and Transcarpathian Ukraine containing no more than the maximum number recorded here (530). There are 100 of these, spread unevenly over the whole region involved.

RESULTS

SITE SPECIES RICHNESS, SAMPLING ERROR, AND FREQUENCY OF OCCURRENCE

Ninety species of snail represented by 6993 individuals were found in 30 samples. Appendix S3 lists the numbers of each species found at each site. The basic statistics for the faunas are shown by SA in Table 1. In general, the numbers of individuals were smaller than those found (by comparable means) in Central European or British forests, and in nine cases they fall below the recommended minimum requirement of

Table 1. Basic data

SA	Sites	Mean numbers	Range numbers	Mean species	Range species	Total species	Unique species
Colchis	1	251	–	20	–	20	3
Batumi	3	117	85–140	14	12–16	25	4
Mtirala	3	309	67–449	16.2	13–19	24	2
Kintrisi	3	186	148–206	16.3	14–19	24	2
Bakhmaro	3	150	60–247	15.3	12–20	28	0
Goderdzi	1	151	–	17	–	17	2
Borjomi	5	251	78–385	16.5	14–21	29	2
Ambrolauri	5	283	170–530	18.2	13–21	52 (42)	20 (10)
Lagodekhi	6	272	167–417	12.7	8–16	25 (24)	6 (5)
TOTAL	30	233	60–530	15.9	8–21	90 (80)	

Figures in brackets represent totals after removal of subalpine and anthropochorous species (see text).
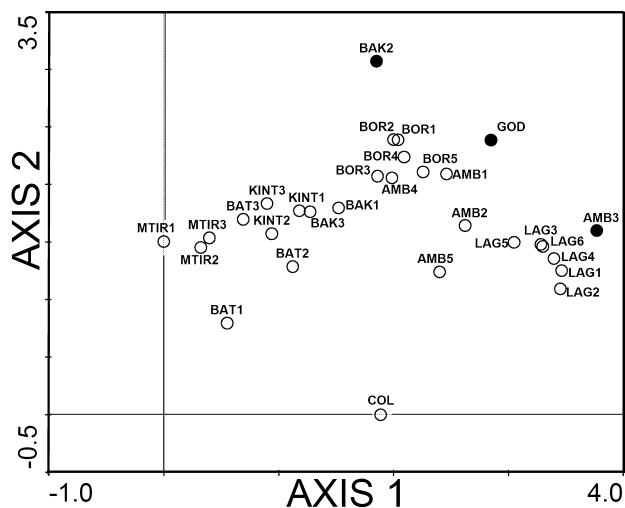
Figure 2. Positions of sites on a detrended correspondence analysis biplot: BAT, Batumi; MTIR, Mtirala; KINT, Kintrishi; COL, Colchis; BAK, Bakhmaro; GOD, Goderdzi; BOR, Borjomi; AMB, Ambrolauri; LAG, Lagodekhi; ●, BAK 2, GOD, and AMB 3, sites above 1400 m a.s.l.; AMB 1, disturbed site.

ten times as many individuals as species (Cameron & Pokryszko, 2005). Eight species are represented in the whole array by single individuals, 29 species (about one-third of the total species number) were recorded from a single SA, and 22 of those from a single site. There is a positive correlation between numbers of individuals and numbers of species ($R = 0.425$, $P < 0.05$), suggesting that species have been missed in some samples. The Chao 1 estimator of missing species gives widely varying estimates among samples (data not shown), but these do not correlate with the number of individuals per sample (Spearman's R on ranks, 0.165). The estimates are not normally distributed, with a few high values, but the median estimate is 1.1 missing species per site.

Ecological differentiation and casual species

The favourable combination of mixed dominants among trees and gentle slopes has a significant effect on the number of individuals found (Spearman's R on ranks, 0.41, $P < 0.05$) (Appendix S1). Species richness, however, shows no significant association with any single environmental factor recorded at the site, nor with any combination of two or more factors regarded as favourable or the reverse on evidence from other studies. DCA analysis (Fig. 2) indicates the separation of faunas representing different longitudes (axis 1) and elevations (axis 2). Sites within the same study area tend to cluster together along both axes.

Table 2. Species found at Ambrolauri excluded from analyses of faunal similarity and distance decay

Species	Distribution	Habitat
Truncatellina cylindrica	Widespread	Open
*Poiretia mingrelica**	Transcaucasian	Generalist
*Xeropicta derbentina**	Widespread	Open
Phenacolimax annularis	Widespread	Alpine
Vitrina pellucida	Widespread	Here Alpine
*Paralaoma servilis**	Very widespread	Generalist
Sphyradium doliolum	Widespread	Here Alpine
*Zebrina detrita**	Widespread	Open
Pupilla triplicata	Widespread	Alpine
Vallonia pulchella	Widespread	Open

*Species associated with human activity.

It is evident (Table 1) that the aggregate fauna of Ambrolauri sites is far greater than those of other clusters containing more than one sample. This cluster also contains far more unique species than any other. Sites there include the highest and the most disturbed in the whole array. Table 2 lists species recorded uniquely in Ambrolauri that, on external evidence, are either characteristic of the subalpine zone (here recorded only in Ambrolauri 3) or are more typical of open habitats, and may be present as a result of grazing disturbance. These species have been eliminated from the analyses of faunal differentiation and distance decay, as has *Truncatellina strobeli* from Lagodekhi, represented by a single empty shell, which was possibly a product of flood transport. None are restricted endemics. Even with these eliminations, the aggregate fauna of Ambrolauri samples is richer than those from elsewhere. However, the span of distances within the Ambrolauri SA is similar to that for all Batumi, Kintrisi, and Mtirala samples combined. These have 41 species recorded compared with 42 within Ambrolauri.

Faunal similarities and their dependence on geography and climate

All pairwise values of the Simpson index of similarity among sites are shown in Appendix S4. The faunas are strongly differentiated, and in four cases there are no species in common between pairs. Table 3 shows the mean values of the index in site-by-site comparisons within and among SAs. Although the numbers used in comparisons are limited, it is evident that the SAs at Mtirala, Kintrisi, Borjomi, and Lagodekhi are coherent, with much higher mean within-SA values in comparison with others. The single isolated sites at

Table 3. Mean values (%) of the Simpson index of similarity in site-by-site comparisons

|	Col	Bat	Mti	Kin	Bak	God	Bor	Amb	Lag
Colchis	–	52.1	35.0	42.6	28.2	29.4	34.9	36.2	34.5
Batumi		**58.3**	56.1	53.7	41.1	16.6	39.0	30.2	27.8
Mtirala			**81.9**	50.5	40.1	16.9	34.0	32.5	16.0
Kintrisi				**81.8**	54.4	23.6	50.4	41.0	35.7
Bakhmaro					**59.8**	39.9	53.9	37.7	32.6
Goderdzi						–	44.0	36.2	20.9
Borjomi							**72.8**	49.6	39.4
Ambrolauri								**47.7**	38.4
Lagodekhi									**79.7**

Within-cluster values are given in bold; these values are not available for Colchis and Goderdzi, each represented by only one sample.

Table 4. Association of faunal dissimilarity between the sites (100 – Simpson's similarity index as a dependent variable) with geographic distance (Geodis) and distance based on the 19 normalized bioclimate variables (climdis) (independent variables): the output of partial Mantel tests

Independent variable	r	$P <$
Geodis	0.505694	0.0001
Climdis	0.350407	0.0001
Climdis controlled for Geodis	0.219106	0.0073
Geodis controlled for Climdis	0.438499	0.0001

Colchis and Goderdzi are similarly well differentiated from the others, but for Bakhmaro, Batumi, and Ambrolauri, with the greatest range of altitude, habitat, and distance between sites, the within-SA values are lower: in the case of Ambrolauri it is exceeded in the comparison with Borjomi; the effects of disturbance and altitudinal range, as well as limestone substrate may be significant here. There is an apparent geographical structure in differentiation among SAs, seen most clearly in the low levels of similarity between Lagodekhi, the most isolated by distance, and all others, and by the coherence of the three adjacent SAs in the south-west, Batumi, Mtirala, and Kintrisi. Partial Mantel tests (Table 4) show that this structure depends both on the climatic differences and geographic distances between the sites. Importantly, climatic differences between the sites influence the differences in species composition, even if controlled for the geographic distances and *vice versa*.

The CCA based on the (log-transformed) species abundances, on the one hand, and geographic coordinates, on the other, shows an ordination of the sites reflecting a geographic pattern that does not differ much from the DCA output (results not shown). CCA based on the species abundances versus values of
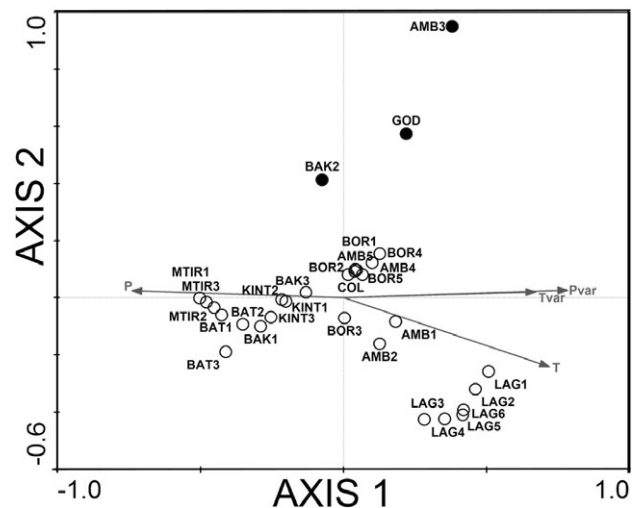
Figure 3. Ordination of the sites along the two canonical correspondence analysis axes determined by correlation between species abundances and standardized bioclimate variables. The four displayed bioclimatic variables are: T, mean annual temperature; T_{var}, temperature seasonality (standard deviation); P, annual precipitation; P_{var}, precipitation seasonality (coefficient of variation). The sites in the east are drier and more continental, but are warmer (in particular for Lagodekhi) than sites in south-west Georgia.

climatic variables showed a rather similar pattern (Fig. 3): although the first CCA axis reflected, to some extent, the eastern–western geographic gradient, the second axis separated the locations from different altitudes rather than those with different geography. There was significant ($P < 0.001$) correlation between species-specific geographic and climatic tolerances inferred from the CCA (Fig. 4). Twenty-two species showed climatic tolerances above the upper significance level ($P = 0.001$) of the regression line, indicating a more restricted geographical distribution than expected, and ten species had climatic tolerances
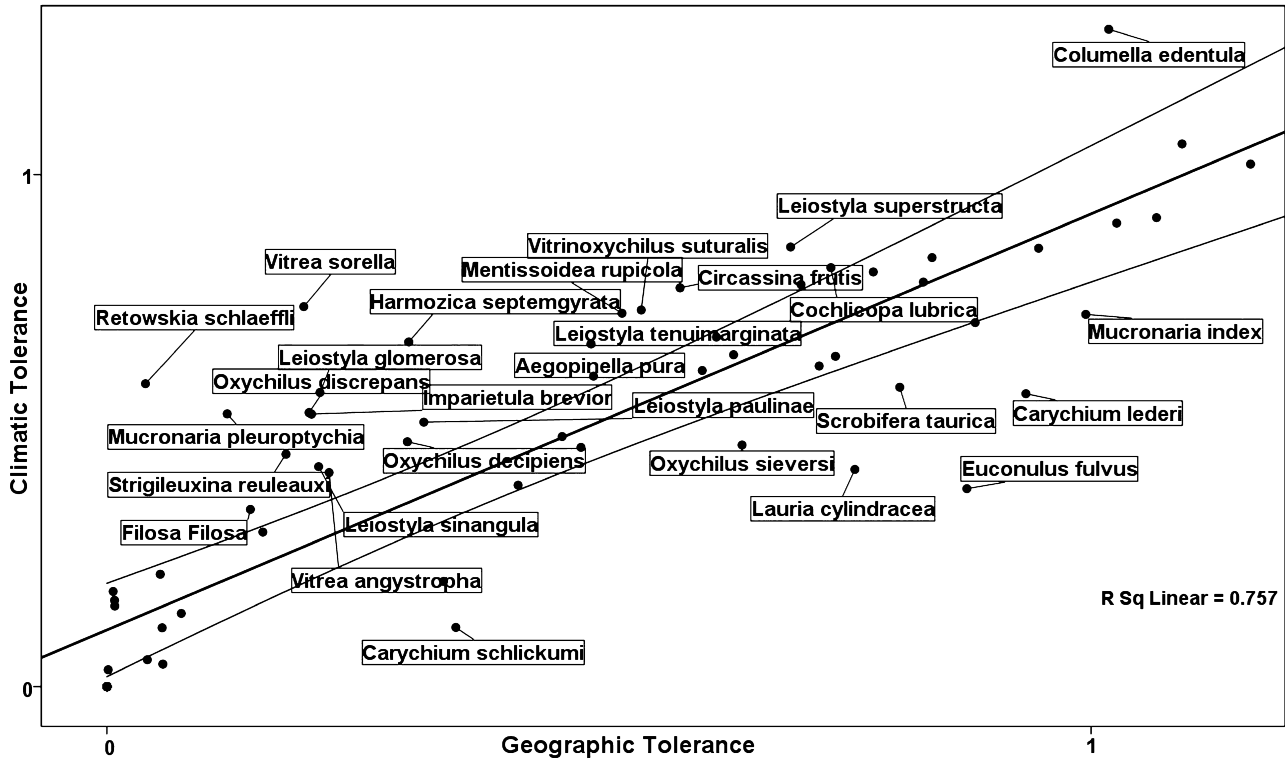
Figure 4. Regression line with $P = 0.001$ confidence limits, connecting species-specific 'geographic tolerance' (extent of occurrence of a species) and climatic tolerance. Species found at a single site (i.e. with zero tolerances) are not shown. Only names of the species that lie significantly above or below the regression line are shown.

below the lower significance level of the line, indicating a narrower climatic tolerance than expected from geographical range. It should be noted that some species were present in only two or three sites: it is the general trend rather than the precise position of each species that is meaningful. Of the 22 species apparently restricted by geography within our samples, 18 (86%) are also known from Turkey (see below), and three are widespread throughout the western Palaearctic.

DISTANCE DECAY AND BIOGEOGRAPHICAL RELATIONSHIPS

Analysis of the broader biogeographical pattern is hampered by the low intensity of previous sampling in the region. Many species are known only from scattered localities, and it would be premature to assume that all geographical ranges are adequately known. Several of our records (detailed in Appendix S2) represent significant range extensions. There are also some taxonomic uncertainties. Nevertheless, the extent to which differences among SAs are determined by limited geographical ranges can be examined by comparing the numbers and proportions of species known outside the area encompassed by our samples (Table 5). Nearly two-thirds (58) of all species recorded here are also known from Turkey. Thirty-one of these species recorded in Turkey are also known from Europe and/or Ciscaucasia: these include most of the anthropochoric and subalpine species. Only eight species not found in Turkey are previously recorded as confined to either the Lesser or Greater Caucasus, and two of these were found by us in a previously unrecorded range. The proportion of species also found in Turkey does not vary consistently or significantly with distance among groups of our samples, although it is slightly smaller in the Greater Caucasus than elsewhere (Table 5). Further evidence that actual ranges are not very restricted comes from considering the known distribution of the species found by us in only one cluster (Table 5). The proportion of such species also occurring in Turkey is lower, but is little different from that seen in the fauna as a whole.

Of the six species unallocated in Table 5, four raise taxonomic issues (Appendix S2). The remaining two are species of *Carychium*. The record of *Carychium lederi* from Colchis represents a major extension of known geographical range (southern Azerbaijan), and its presence also in Lagodekhi suggests a scattered but widespread distribution across Transcaucasia.

Table 5. Geographical distribution of species found in this study

A	Number of species	Europe	Ciscaucasia	Transcaucasia	Lesser + Black Sea	Greater	Unallocated
Also known in Turkey	58	19	12	6	17	3	1
Not known in Turkey	32	3	6	9 (11)	5 (4)	4 (3)	5
Total	90	22	18	15 (17)	22 (21)	7 (6)	6

B	Western Lesser Caucasus	Colchis	Borjomi	Greater Caucasus	Single SA
Known in Turkey	35 (73%)	14 (70%)	23 (79%)	38 (64%)	22 (54%)
Not known in Turkey	13 (27%)	6 (30%)	6 (21%)	21 (36%)	19 (46%)
Total	48	20	29	59	41

A, ranges of species: the column for Ciscaucasia (north of the Greater Caucasus watershed) indicates species found also in Transcaucasia; those for Transcaucasia, the Lesser Caucasus, and Black Sea coast, and for the Greater Caucasus indicate species known (outside Turkey) only in the regions named. Numbers in brackets take account of our findings (see text). For unallocated species see text. B, the representation of species known from Turkey in our samples, with clusters combined in groups, and ordered by distance from Pontic Turkey, and also for species recorded from only one sample area: Western Lesser Caucasus, Batumi, Mtirala, Kintrisi, Bakhmaro, and Goderdzi; Greater Caucasus, Ambrolauri and Lagodekhi. Distributional data from Schütt (2005), Likharev (1962), Sysoev & Schileyko (2009).

Table 6. The numbers of large and small species in different range categories, and the effect of *Leiostyla* species on the totals for small species

	Large	Small	*Leiostyla*	Small *ex Leiostyla*
Restricted	46 (85%)	21 (58%)	8 (100%)	13 (46%)
Widespread	8 (15%)	15 (42%)	0 (0%)	15 (54%)
Total	54	36	8	28

Widespread: ranging beyond the Caucasus and Pontic Turkey. The difference between large and small species is significant ($P < 0.01$) with or without *Leiostyla* (χ^2 tests).

Carychium schlickumi was previously known only as a Pliocene fossil, and, possibly, from beach debris on the Black Sea (Schütt, 2005): its occurrences in both Colchis and Borjomi also suggest a wide but discontinuous distribution within Transcaucasia.

In terms of overall distribution there is a clear difference between large and small species (Table 6), with the latter defined as those with a maximum shell dimension of less than 5 mm. (Hausdorf & Hennig, 2003): of the few widespread large species, three are probably present in forest as a result of human intervention (*Fruticicola fruticum*, *Xeropicta derbentina*, and *Zebrina detrita*). Inspection of differences among higher taxa reveals one singularity. Unlike the remainder, *Leiostyla* (Pupillidae) species show a clear difference in richness and occurrence between the Lesser and Greater Caucasus (seven and two species, respectively, one in common), with a peak of diversity at Mtirala, the wettest cluster within the array, where five species were found in a single site. Five of the eight species are also found in Turkey, but none

are found outside the broad Caucasian region. The distribution of species in this genus runs counter to the general trend for small species.

COMPARISON WITH POLISH FOREST SNAIL FAUNAS

Table 7 shows comparisons of site species richness, overall similarity, and frequency of occurrence between the sites reported here and those from Poland, with no more than 530 individuals, a subset of those reported in Cameron, Pokryszko and Horsák (2010). Georgian forests are impoverished relative to those in Poland, with little more than half the median number of species. Median abundance is higher in Poland (even with a restriction to sites with less than 530 individuals) than in Georgia, but regressions of species on individuals over the same range of individuals per site (data not shown) confirm that the difference is not a product of differential sampling error: Polish sites are consistently richer for any given number of individuals. Species are also

Table 7. Comparative data on species richness, similarity, and frequency of occurrence between Georgian sites and those from Poland with less than 530 individuals per site

	Poland	Georgia
Samples	100	30
Species	101	90
Mean/median/site	25.2/26	15.9/15.5
Range	13–40	8–21
Median no. of individuals	333	233
Mean site-by-site Simpson index (±SE)	52.4 ± 0.28	41.4 ± 0.90
Overall Whittaker's index (S/α)	4.01	5.70
Species in less than 3.3% of sites	14 (14%)	30 (33.3%)

generally less frequent than in Poland: 33% of all species were recorded from only one site, compared with 14% found in the equivalent proportion of Polish sites (three or fewer). Faunal differentiation between sites and clusters is greater in Georgia than in Poland, despite the greater distances between clusters in the latter.

DISCUSSION

The snail faunas recorded in this study are characterized by relative poverty at the level of individual sites and by high levels of turnover among (and sometimes within) SAs, among which, however, there are only rather weak relationships between similarity and proximity. Although many species are endemic to the Caucasian region as a whole (including north-east Turkey), few have very restricted distributions, and although there are cases where congenerics have non-overlapping ranges, there is little sign of widespread allopatric or parapatric turnover of species within a genus. Detailed molecular studies would be needed to determine whether these parapatric or allopatric congeners were sister species diverging after confinement to separate refugia.

As in many studies of snail species richness and composition in tropical or subtropical forests (Emberton, Pearce & Randalana, 1996; de Winter & Gittenberger, 1998; Schilthuizen & Rutjes, 2001; Seddon *et al.*, 2005; Tattersfield *et al.*, 2006; Stanisic *et al.*, 2007), densities are low, and even with considerable sampling effort full inventories of site faunas are difficult to achieve (Cameron & Pokryszko, 2005). Failure to achieve a full inventory affects both estimates of species richness and estimates of difference or similarity between sites and SAs. Although several of our samples do not meet the minimum require-

ments set out by Cameron & Pokryszko (2005), we note that the Chao estimator does not indicate gross deficiencies, and in comparison with Polish forests, over the same range of sample sizes, these samples from Georgia are both poorer at site level, and differ more among themselves, despite the shorter distances involved. Although the refugial status of the area is reflected in the high regional diversity (Sysoev & Schileyko, 2009), it does not result in locally richer faunas. Among tropical or subtropical study areas, in which at least patches of forest are thought to have survived throughout the Pleistocene, site richness varies considerably, but many have faunas that are no richer than those reported here. Within the limits of our choice of sites, richness appears unaffected by the features of the habitat recorded by us, although the numbers found increased in favourable conditions.

Among our samples, both the variation in climate and in position affect composition, although the latter effect is stronger, as shown both by the partial Mantel tests and by partial CCA; twice as many species appear constrained by location as by climate. However, a broader consideration of geographical ranges, using published information for the whole region, shows rather few cases of very restricted distributions; only 18 out of 90 species are known just from the area encompassed by our samples. Only slightly more than a third of all species are missing from the neighbouring part of Turkey (Schütt, 2005). Given the relatively low intensity of sampling in the region, even these proportions may be too high. Several species appear to have disjunct ranges (e.g. Turkey and the Greater Caucasus, but not in between), and our own sampling has extended the known ranges of a few species. The case of *Leiostyla*, where five out of eight species recorded were found in a single site, emphasizes the lack of neat patterns of allopatric replacement. A rather similar pattern is seen in the same genus in the forests of Madeira, another forest refugium through the Plio–Pleistocene climatic changes (Cameron, Cunha & Martins, 2007). As elsewhere, small species tend to be more widely distributed than large ones (Pokryszko & Cameron, 2005). *Leiostyla* is a conspicuous exception to this general trend.

These patterns stand in sharp contrast to those found in regions where there has been either repeated restriction to isolated refugia created by fluctuations in aridity, or where the whole forest fauna is a product of the colonization of previously glaciated or periglacial landscapes. In the former, families and genera with stenotypic species typically show very characteristic patterns of allopatric replacement; some species may have tiny geographical ranges, the most extreme case being reported by Solem (1988). Even within Turkey as a whole the influence of aridity and multiple, tiny refugia shows in the fact that around 25% of more than

100 clausiliid species are known only from, or very near, their type localities (Schütt, 2005). In the latter case, faunas are characterized by species with large geographical ranges and by very low rates of distance decay in similarity (Nekola & White, 1999; Pokryszko & Cameron, 2005; Cameron *et al.*, 2010).

These various contrasts prompt consideration of the way in which regional diversity has developed in an area where forests have persisted, albeit with changes in character and connectivity, throughout the Pleistocene. In faunas from Africa, Australia, and New Zealand, it appears that several processes have been involved. Periodic isolation of optimal forest fragments have certainly played a part (Emberton *et al.*, 1996; Moritz *et al.*, 2001), and might account for some of the parapatric distributions noted above, as has the development of altitudinal differentiation (Stanisic, 1982; Tattersfield *et al.*, 2006). There is, however, a substantial proportion of species that show patchy, more-or-less discontinuous distributions (Solem, 1984; Barker, 2005; Stanisic *et al.*, 2007), for which isolation by distance alone, followed by back migration, with either niche separation or metapopulation dynamics allowing co-existence, seems the most robust explanation (Cameron *et al.*, 2003). A general analysis is made more problematic by the differences in taxonomic composition, trophic levels, and ranges of size and shape among faunas.

In the case of Georgian forests, with a typically western Palaearctic fauna, high-altitude faunas, at least within the limits of our sampling, hold a number of species with wide geographical ranges but with alpine habitat preferences. More generally, although there are some species restricted to parts of the region where a combination of climate and geographical isolation may be involved, there are many that are distributed widely but are not found in all, or even most, samples. In this context, the contrasting hypotheses of multiple glacial forest refuges in the Caucasus (Velichko & Kurenkova, 1990), or of a single larger refugium at the Black Sea coast (van Andel & Tzedakis, 1996), are relevant. Some recent molecular genetic data on other organisms (Tarkhnishvili, Thorpe & Arntzen, 2000; Murtskhvaladze, Gavashelishvili & Tarkhnishvili, 2010) lend support to the former, and it is clear that a part of the pattern shown here stems from a combination of isolation and the strong east-to-west climatic gradient. However, the presence of a number of species confined to, but not restricted within, the region of north-east Turkey, the Lesser Caucasus, and the Black Sea coast suggests a rather large and long-standing refuge in which differentiation occurred. Most of the species with a limited distribution irrespective of estimated climate tolerance are found in south-west Georgia, close to the major glacial refugium, from Batumi to Bakhmaro, irrespec-

tive to the altitude and climate differences. The range of some of them, including the largest species *Helix goderdziana* (Mumladze, Tarkhnishvili & Pokryszko, 2008) extends to north-east Turkey, within the extent of the major refugium, but not to the rest of the Caucasus ecoregion. Forests persisted not only in the mountains, but also into the Colchic lowlands well into the Holocene (Connor, Thomas & Kvavadze, 2007).

Although our results tend to confirm that the pattern of diversification in forested regions, where forest has persisted with some connectivity over millions rather than tens of thousands of years, show similar patterns regardless of the details of faunal composition in terms of systematics, trophic levels, or size and shape spectra, there are two cautionary notes. First, although much work has been carried out, the region has not been surveyed intensively, and adequate sampling is made difficult by both low densities and difficult terrain. Patchy distributions may become less so, as more data is gained. Furthermore, a greater number of known sites would make the distinction between historic factors and present climatic limits much easier to disentangle. Second, and perhaps of more significance, we note that some recent molecular studies show that populations showing very little morphological differentiation may differ in genetic material, to the extent that isolation may be dated to the Pliocene. High morphological conservatism was shown, for instance, in Caucasian salamanders (*Mertensiella caucasica*), in which geographic populations isolated from at least the early Pliocene are not recognizable morphologically (Tarkhnishvili *et al.*, 2000). Snail species are generally distinguished on morphological characters, especially of the shell, yet we know from studies elsewhere that relatively short periods of isolation can result in internal differentiation among externally similar populations (Martins, 2005).

The differential between large and small species in terms of geographical range reported here repeats a pattern found in other studies (Cameron *et al.*, 2005; Cameron *et al.*, 2010). It is even more pronounced when large species associated with human activity are excluded. Although this trend reflects the ease of passive dispersal in small species, we note that for small species with exacting requirements (*Leiostyla* species here, some charopids in Australia (Stanisic *et al.*, 2007), range sizes may be very small without showing patterns of strict allopatry.

Finally, we note that the character of this forest refugium, reputedly the largest in the western Palaearctic, is one of relatively permanent isolation for forest snails. There is indeed an endemic fauna, one that appears to have played little part in the recolonization of northern Eurasia in the Holocene, unlike refuges around the Alps, the Carpathians, the

Balkans, and Iberia (Hausdorf & Hennig, 2003). Even the forest fauna of the southern Urals, closer to Transcaucasia than any strictly European refugium, has the character of early Holocene forests in Central Europe: the only species in common with our Georgian samples are those widespread across the whole western Palaearctic (Horsák *et al*., in press). The very high barrier of the Greater Caucasus and the predominance of steppe on all sides of the region appear to have constrained movement.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Details of location and habitat of samples.
Appendix S2. List of species found with authorities, taxonomic notes, and details of known geographical ranges.
Appendix S3. Species/sites matrix for the samples used in this study.
Appendix S4. All pairwise Simpson indices of similarity (%) for samples used in this study.

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