

THE INFLUENCE OF CLIMATE ON SHELL VARIATION IN *TROCHULUS STRIOLATUS* (C. PFEIFFER, 1828) (GASTROPODA: HYGROMIIDAE) AND ITS IMPLICATIONS FOR SUBSPECIES TAXONOMY

MAŁGORZATA PROCKÓW^{1,*}, ELŻBIETA KUŹNIK-KOWALSKA²
and PAWEŁ MACKIEWICZ³

¹*Museum of Natural History, University of Wrocław, Sienkiewicza 21,
50-335 Wrocław, Poland; e-mail: malgorzata.prockow@uwr.edu.pl*

²*Department of Invertebrate Systematics and Ecology, Institute of Biology,
Wrocław University of Environmental and Life Sciences, Koźuchowska 5b,
51-631 Wrocław, Poland*

³*Department of Genomics, Faculty of Biotechnology, University of Wrocław,
Joliot-Curie 14a, 50-383 Wrocław, Poland*

**Corresponding author*

Abstract.— *Trochulus striolatus* is a land snail showing great morphometric variation in its shell, which is the basis for recognition of its subspecies. However, this variability can result from an influence of environment. To verify the possible effect of bioclimatic and spatial variables on the shell size and shape, we studied many samples collected from four biotic zones (lowland, submontane, montane and subalpine). Many of its shell features appeared significantly negatively correlated with a precipitation parameter and altitude, whereas positively correlated with temperature parameters. The shells were smaller at higher altitudes and in colder environment with greater precipitation. The reduced growth period can be an adaptive response to the shorter growing season in mountainous regions compared to lowland areas, where the longer season permits a longer growth resulting in larger mean adult body size. This conforms to the converse Bergmann's cline. The synergetic interactions between seasonality, temperature and moisture best explain the size variation in *T. striolatus* resulting from the influence of local environmental and/or climate factors. Therefore, there is no sufficient justification for subspecies recognition and the subspecific epithets for *T. striolatus* should be discarded.



Key words.— altitude, converse Bergmann's rule, phenotypic plasticity, shell morphology, *T. s. abluensis*, *T. s. danubialis*, *T. s. juvavensis*.

INTRODUCTION

Land snails with their restricted mobility and particular habitat requirements (Cowie 1984, Baur 1993, Pfenninger *et al.* 1996) appear to be ideal organisms to study phenotypic evolution (Pfenninger and Magnin 2001) and their differentiation over space and time.

They often display a wide variation of shell shape, colour, banding pattern and other visible traits. The phenotypic appearance is influenced by a complex of factors such as phylogenetic history, gene-flow, environment and developmental processes (Cook and Cain 1980, Baur 1984, 1988, Goodfriend 1986), and may reflect adaptation to local conditions (Chiba 2009).

Many surveys of qualitative shell characters, such as shell colour and banding, have been carried out on different species and correlations with environment were examined (e.g. Lewis 1977, Heller 1981, Heller and Gadot 1987, Hazel and Johnson 1990, Honěk 1993). However, quantitative variation, which causes much uncertainty about the evolutionary and taxonomical relevance, has not been sufficiently explored. Intraspecific variation in body size is particularly intriguing because it suggests strong associations between an organism's size and its environment. For example, several studies have found that shell size exhibited substantial variation depending on altitude, and thus also on thermal differences among habitats (Burla and Stahel 1983, Baur and Raboud 1988, Sulikowska-Drozd 2001, 2011), while others revealed shell differences according to other environmental variables (Lazaridou-Dimitriadou *et al.* 1994, Mulvey *et al.* 1996, Pfenniger and Magnin 2001).

Trochulus striolatus (C. Pfeiffer, 1828) is a euryoecious species which inhabits a wide range of habitats: from lowlands, most often adjoining rivers, to rocky alpine sites (Duda *et al.* 2014). It is found in human-affected forests and nettle patches as well as in damp and shaded natural forests or on tall grasses in open areas (Proćków *et al.* 2014). *T. striolatus* is common in the British Isles, north of France and Switzerland, southern Germany, southern part of the Netherlands, Austria (except its southern part), reaching south-western Slovakia and north-western Hungary (Proćków 2009). Its wide shell size and shape variation (Naggs 1985, Proćków 2009, Proćków *et al.* 2014) has most likely led to recognition of several separate species or subspecies in the past (Locard 1894, Erhmann 1933), and subsequent difficulties in their identification (Perrin *et al.* 1984, Naggs 1985, Forcart 1965, Welter-Schultes 2012).

A large flat form regarded as typical was found in Germany, Switzerland (Falkner *et al.* 2001), as well as eastern and south-eastern England (Ellis 1969). A smaller form, often with a higher spire, described as *T. s. abludens* (Locard, 1888), is regarded to be restricted to the north-west of Europe including Ireland and France (Ellis 1969, Moorkens and Speight 2001, Falkner *et al.* 2002, Cucherat and Demuyneck 2006). However, it is unclear whether the invasive and highly variable populations found now across the British Isles can be unambiguously distinguished from forms found elsewhere in Europe (Anderson 2005). The other subspecies are: *T. s. danubialis* (Clessin, 1874) found in the Danube valley, *T. s. juvavensis* (Geyer, 1914) known from Höllengebirge in Austria and *T. s. austriacus* (Mahler, 1952) from mount Schlenken. Generally, their shells are variable in size but with a higher spire and narrower umbilicus than the typical form. Aside from the differences in the shell shape, some taxa are

anatomically very similar in terms of proportions of genital morphology (Proćków 2009), though, a slight differentiation in the cross-section of the penis between the subspecies has been recently detected (Duda *et al.* 2014). Nevertheless, it seems that the genital morphology among the *Trochulus* species is less discriminatory (Proćków *et al.* 2013b, 2014) than in other pulmonates. Additionally, genetic investigations reveal no clear separation of *T. striolatus* subspecies (Kruckenhauser *et al.* 2014), regardless of the monophyly of the species (Kruckenhauser *et al.* 2014, Proćków *et al.* 2014, 2017). It suggests that the variation of these morphologically variable forms can result from an influence of environmental factors.

Therefore, we assessed the potential effect of climate factors and altitude on shell morphology of *T. striolatus*. The obtained results were discussed in context of the taxonomic status of *T. striolatus* subspecies.

MATERIALS AND METHODS

Sampling and morphological characterization of populations

The material, encompassing the entire distribution range of *T. striolatus*, consisted of 1251 individuals from 56 localities (Fig. 1), and included field-collected snails as well as those coming from the following museum collections:

MNHN – Muséum National d'Histoire Naturelle, Paris, France;

NBCN – Naturalis Biodiversity Center, Leiden, the Netherlands;

SNM – Slovak National Museum, Bratislava, Slovakia.

We assigned specimens to subspecies based on the geographic distribution of the intraspecific taxa (Geyer 1914, Ellis 1969, Moorkens and Speight 2001, Falkner *et al.* 2002, Cucherat and Demuyneck 2006). Two syntypes of the Locard's collection (coll. Locard G. p. 243, MNHN), labelled as '*Helix abludens*, Boulogne', and five paratypes of Rusnov's collection (R/105/5, NHMW), labelled as '*T. striolata juvavensis*, Schafberges im Salzkammergut, Ob. Oest.', were examined. None of the type specimens of other taxa is extant. Thus, topotypical material was used in these cases. We did not investigate *austriacus* subspecies because its material was not available. The detailed information about samplings is shown in Appendix 1.

To analyse body size variation eight measurements were taken from the adult shells (reflected lip) using a calibrated eyepiece in a stereomicroscope with accuracy 0.1 mm:

H – shell height,
 W – shell width,
 bwH – body whorl height,
 h – aperture height,
 w – aperture width,
 U – umbilicus major diameter (i.e. the longest diameter parallel to the shell diameter, D),
 u – umbilicus minor diameter (i.e. perpendicular to umbilicus major diameter),
 D – shell diameter.

The whorls (whl) were counted according to Ehrmann's (1933) method. Since the systematic measurement error (with 1% error probability) does not compromise results (Duda *et al.* 2011), the specimens were measured once by the same person (MP) in standardised views (Proćków 2009). Besides, the following coefficients of shell proportions were calculated: height/width ratio (H/W), relative height of body whorl = body whorl height/shell height ratio (bwH/H), umbilicus relative diameter = umbilicus major diameter/shell diameter ratio (U/D), ratio of umbilicus minor to its major diameter (u/U), aperture height/width ratio (h/w). The measurement data and shell volume, calculated with the formula: shell volume = $0.312 \times [(\text{shell breadth})^2 \times \text{shell height}] - 0.038$ (Baur 1994), were subjected to a Principal Component Analysis (PCA).

Data analysis

To determine significance of the differences in the shell size between the populations, Kruskal-Wallis non-parametric analysis of variance (ANOVA) was performed using both shell volume and the first principal component (PC1) as the dependent variables, and the population as the independent variable. However, because results obtained in both analyses did not differ, we only present those based on shell volume. The intra-population variation was calculated with the coefficient of variation (CV%) of all the morphometric characters.

To study a potential influence of climate and geographic conditions on shell morphology, we gathered information about long-term climate conditions of particular sampling sites from WorldClim (Global Climate Data, <http://www.worldclim.org>, Hijmans *et al.* 2005) and used with a resolution of 30 arc-s. Altitude, longitude and latitude were either recorded on site using a handheld GPS unit or determined via Falling Rain Genomics, Inc. (1996–2010). Altogether 22 variables were included in the study: all 19 bioclimatic variables (Table 1) as well as spatial location of specimens, i.e. latitude, longitude and altitude. To reduce the number of these parameters, we performed Principal

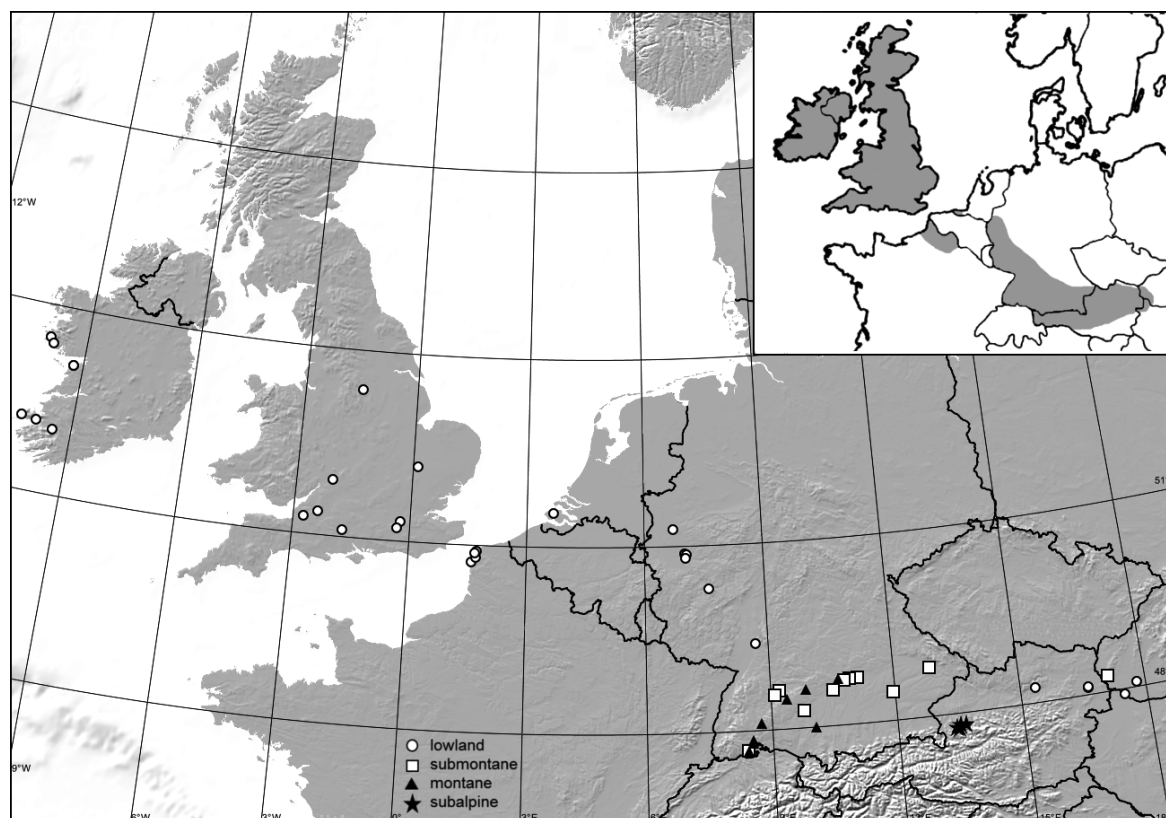


Figure 1. Distribution of *T. striolatus* (modified from Kerney *et al.* 1983) and sites of specimens' collection according to biotic zones.

Table 1. Description of bioclimatic variables used in the study. The parameters left after data reduction based on pair-wise correlations are indicated in bold.

Acronym	Description
BIO1	Annual mean temperature
BIO2	Mean diurnal range [mean of monthly (max–min temp.)]
BIO3	Isothermality (BIO2/BIO7)*100
BIO4	Temperature seasonality (standard deviation*100)
BIO5	Max temperature of warmest month
BIO5	Max temperature of warmest month
BIO6	Min temperature of coldest month
BIO8	Mean temperature of wettest quarter
BIO9	Mean temperature of driest quarter
BIO10	Mean temperature of warmest quarter
BIO11	Mean temperature of coldest quarter
BIO12	Annual precipitation
BIO13	Precipitation of wettest month
BIO14	Precipitation of driest month
BIO15	Precipitation seasonality (coefficient of variation)
BIO16	Precipitation of wettest quarter
BIO17	Precipitation of driest quarter
BIO18	Precipitation of warmest quarter
BIO19	Precipitation of coldest quarter

Component Analysis (PCA) using a correlation matrix and used the first two principal components in further studies. Moreover, we selected the variables that showed the largest contribution to the principal components, i.e. the absolute value of correlation coefficient > 0.9 . We also computed pair-wise Spearman's correlation coefficients (ρ) involving these variables to further reduce the number of correlated parameters. We assumed the pairwise absolute correlation cut-off 0.9 and applied re-evaluation the average correlations at each step of the elimination. Based on this result, we selected three bioclimatic parameters (BIO1, BIO6 and BIO18) and altitude as the least correlated (Table 1). Spearman's correlation coefficients were also calculated to assess a relationship between the principal components for environmental features, individual environmental/geographic factors and shell variables including two first principal components for morphometric features. Moreover, using the Mantel test, we estimated the significance of correlation between two matrices, one describing pairwise absolute differences between shell measurements of particular specimens and the second one, including geographical distance

between the sites, where the specimens were found. The geographical distances were calculated as orthodromic distances based on geographical latitudes and longitudes of the sites assuming the mean earth radius 6371 km. The Benjamini-Hochberg method for correction of the obtained p -values was applied to control the false discovery rate considering tests for all variables.

To further estimate and model relationships of shell morphometric features with environmental conditions (described by three selected bioclimatic variables) and altitude, and their combinations expressed by principal components, we carried out various linear regression analyses. At first, each of the shell characters was regressed onto each of environmental and altitude variables using linear and non-linear (two-order polynomial) functions,

$$f(x) = b_0 + b_1x \text{ and } f(x) = b_0 + b_1x + b_2x^2,$$

respectively. The models were compared using Akaike's Information Criterion (AIC) and the one with the smaller AIC value was selected as better fitted. Moreover, we applied the automated stepwise model simplification procedure based on AIC on the polynomial function to check, if the more complex model can be simplified to the linear one. According to Cabanne *et al.* (2014), the comparison of these models can show, if observed variation follows a continuous or step cline. In the first case, we should expect that the linear function will be better fitted to the data, whereas in the second case, the polynomial function should show a better goodness of fit.

Similarly, as other authors (Legendre and Legendre 1998, Ruggiero and Kitzberger 2004, Botes *et al.* 2006, Cardini *et al.* 2007), we also performed a trend surface analysis to determine the best-fitted combination of two spatial variables (geographic coordinates) that contribute significantly to explaining the variation in each of shell morphometric features. These relationships were modelled by a two-order (cubic) polynomial function:

$$f(x,y) = b_0 + b_1x + b_2y + b_3x^2 + b_4xy + b_5y^2 + b_6x^3 + b_7x^2y + b_8xy^2 + b_9y^3,$$

where x and y are longitude and latitude, respectively. The model was subjected to a stepwise simplification based on AIC to remove non-significant terms and then was compared with simple linear models:

$$f(x,y) = b_0 + b_1x + b_2y.$$

Similar approach we performed for two principal components derived from bioclimatic/spatial parameters.

Furthermore, to evaluate the significance of combined effects influenced by bioclimatic and spatial predictors on shell characteristics and estimate the proportion of variance explained by the models with these

components, we used multivariate regression analysis. We considered three sets of independent variable components: only spatial (i.e. altitude, latitude and longitude), only environmental (i.e. three selected bioclimatic variables) and spatially structured environmental (three selected bioclimatic variables and altitude). Each of these sets was modelled by three functions: a simple linear model, a linear model including pairwise interactions (and also three-way interaction in the case of spatial variables), and a model considering in addition to the previous one, quadratic terms for all variables. The models were simplified by AIC-based stepwise procedure and finally the best-fitted model was selected according to AIC value. To easily compare AIC values, we computed AIC weight, which is expressed by $AIC_w = \exp((AIC_{\min} - AIC_i)/2)$, where AIC_{\min} is the minimum of compared AIC values and AIC_i is AIC of a given model. The parameter can be interpreted as conditional probability for a given model (Burnham and Anderson 2002). Obtained p -values of determination coefficient R^2 for all models were corrected by the Benjamini-Hochberg method. In the regression analyses, we also considered the adjusted R^2 , which gives the percentage of variation explained by only those independent variables that in fact affect the dependent variable.

The statistical analyses were carried out in R package 3.1.1. (R Core Team, 2014, R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, <http://www.R-project.org>) and Statistica 10 (Stat Soft, Inc. 1984–2011). The elimination of correlated bioclimatic/spatial parameters was made by find Correlation from caret package in R. The Mantel test was calculated by mantel.rtest function from ade4 package also in R assuming 100 permutations. The automated stepwise model simplification procedure based on AIC was carried out using step function implemented in R package.

RESULTS

Shell size variation within and among populations

The inter-population shell variation of *T. striolatus* is conspicuous. The mean shell width (W) for the 56 populations ranged from 9.0 mm (Scharflingerpass) to 13.0 mm (Heidelberg) and the mean shell height (H) from 5.5 mm (Schafberg) to 7.6 mm (Cambridge). The mean shell volume ranged from 134 mm³ (Scharflingerpass) to 395 mm³ (Heidelberg), which indicated that the mean volume in the smallest-sized population was almost three times smaller than the mean size of the largest-sized population. When the populations from the highest altitudes (i.e. those with the extremely

small shells) were excluded from the comparison, the snails from Gosheim had the smallest mean shell volume (220 mm³), which was still over one-third smaller than the largest value. At the same time, the shell volume appeared to be the most variable character within the populations and within the biotic zones, as indicated by the coefficient of variation (CV%), which ranged from 9.2% (Schafberg) to 28.6% (Box Hill) and from 22.9% (submontane zone) to 30.2% (subalpine zone). Two more characters and one ratio, i.e. umbilicus major and minor diameters (U, u) as well as umbilicus relative diameter (U/D), had CV values spanning from 8.2% to 22.2% for the populations and from 13.1% to 20.9% for the biotic groups. The variability coefficients of the remaining characters were smaller and ranged from 1% to 17%. For the biotic groups CV values of these characters varied from 3% to 11%. For details, see Appendix 2.

Analysis of shell volume as the dependent variable and population as the independent variable suggested that the populations distinctly differed in this parameter (Fig. 2). The groups of populations from different elevations, i.e. lowland, submontane, montane and subalpine, differed significantly from each other (Kruskal-Wallis test: $\chi^2 = 155.95$, $P < 0.001$). *Post hoc* Kruskal-Wallis test on shell volume significantly separated subspecies: *juvavensis* from *striolatus* and both from *abludens* and *danubialis* (Fig. 3). Similar results were obtained for h, bwH and PC1, whereas W, w, D and bwH/H separated all subspecies ($P < 0.05$, Fig. 3). All features except U and u separated *danubialis* and *juvavensis* ($P < 0.05$). Only U/D and h/w were not significant in the differentiation of *abludens* from *juvavensis* ($P > 0.05$). In recognition between *striolatus* and *abludens* the following traits were not significant ($P > 0.05$): U, u, u/U and h/w. The variables such as H, u/U and h/w could not significantly separate *striolatus* and *danubialis*, whereas whl, H/W, h/w and PC2 - *striolatus* and *juvavensis* ($P > 0.05$, Fig. 3). The shell volume constituted also the highest loading on PC1 of all studied shell variables. The PCA of the shell measurements showed that the specimens from different regions could not be distinguished and, thus, classified as separate subspecies (Appendix 3). Examples of differences in the shell size and shape between populations from various localities and biotic zones are shown in Fig. 4 and topotype specimens of *Trochulus* subspecies in Fig. 5.

We also found a significant ($P < 0.001$) negative relationship between shell shape (expressed as PC2 and H/W) and umbilicus major diameter ($\rho = -0.769$ and $\rho = -0.502$, respectively), which means that more globular shells had narrower umbilicus. Similar relationship ($\rho = -0.487$, $P < 0.001$) was between shell height/width ratio (H/W) and relative umbilicus diameter (U/D).

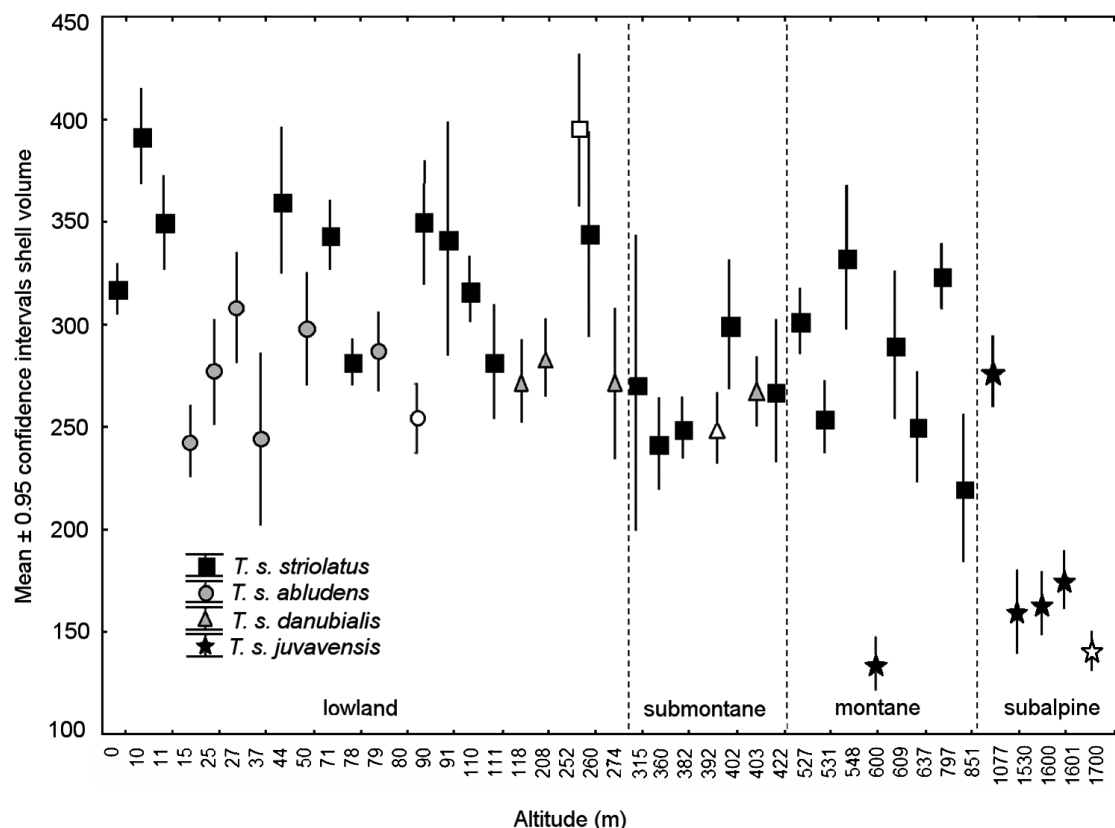


Figure 2. Means and variation in shell volume for populations of *T. striolatus* divided according to altitude and subspecies. Open symbols indicate respective topotype specimens.

Correlations of shell morphology with bioclimatic parameters and spatial distribution

All shell parameters were significantly correlated ($P < 0.05$) with at least several bioclimatic and spatial variables although values of correlation coefficients were not extremely large. We presented results only for three bioclimatic variables and altitude, which appeared the least correlated and showed the largest contribution in PCA analysis (Table 2). Considering cases with larger values of Spearman's correlation coefficient, e.g. $\rho > 0.25$ or $\rho < -0.25$, interesting types of relationships can be identified. The precipitation parameter, i.e. BIO18 was negatively correlated with shell width (W), volume and diameter (D), body whorl height (bwH), aperture height (h) and width (w) as well as PC1. On the other hand, the minimum temperature of coldest month (BIO6) and annual mean temperature (BIO1) were positively correlated with h and w, whereas whl showed negative correlation. The BIO6 was additionally positively correlated with W, D, bwH, bwH/H, volume and PC1, with the assumed threshold $\rho > 0.25$. Relatively small coefficients (and many

insignificant) were also present for relationships involved umbilicus measures (U, u), PC2 as well as shell ratios H/W, U/D, u/U and h/w.

Moreover, Spearman's correlation coefficients < -0.25 were found for relationships of altitude with W, H, bwH, D, h, w, volume and PC1 (Table 2). The largest coefficients were for aperture height (h) and width (w) with altitude ($\rho = -0.428$). Accordingly, the largest shells were found in populations from lower altitudes, and the smallest from subalpine sites (Fig. 6A–C).

To further avoid the interdependence between the bioclimatic/spatial parameters, we also considered their first two principal components explaining 50.6% (PC1_B) and 37.2% (PC2_B) of variance, respectively. With the first component, the most positively correlated were temperature measures BIO11 (0.936), BIO1 (0.935) and BIO6 (0.934), whereas negatively, precipitation parameters BIO18 (-0.967), BIO16 (-0.845) and BIO13 (-0.839) as well as altitude (-0.946). On the other hand, the temperature measures BIO5 (0.899), BIO8 (0.839) and BIO4 (0.837) were positively correlated with the second component. The precipitation of

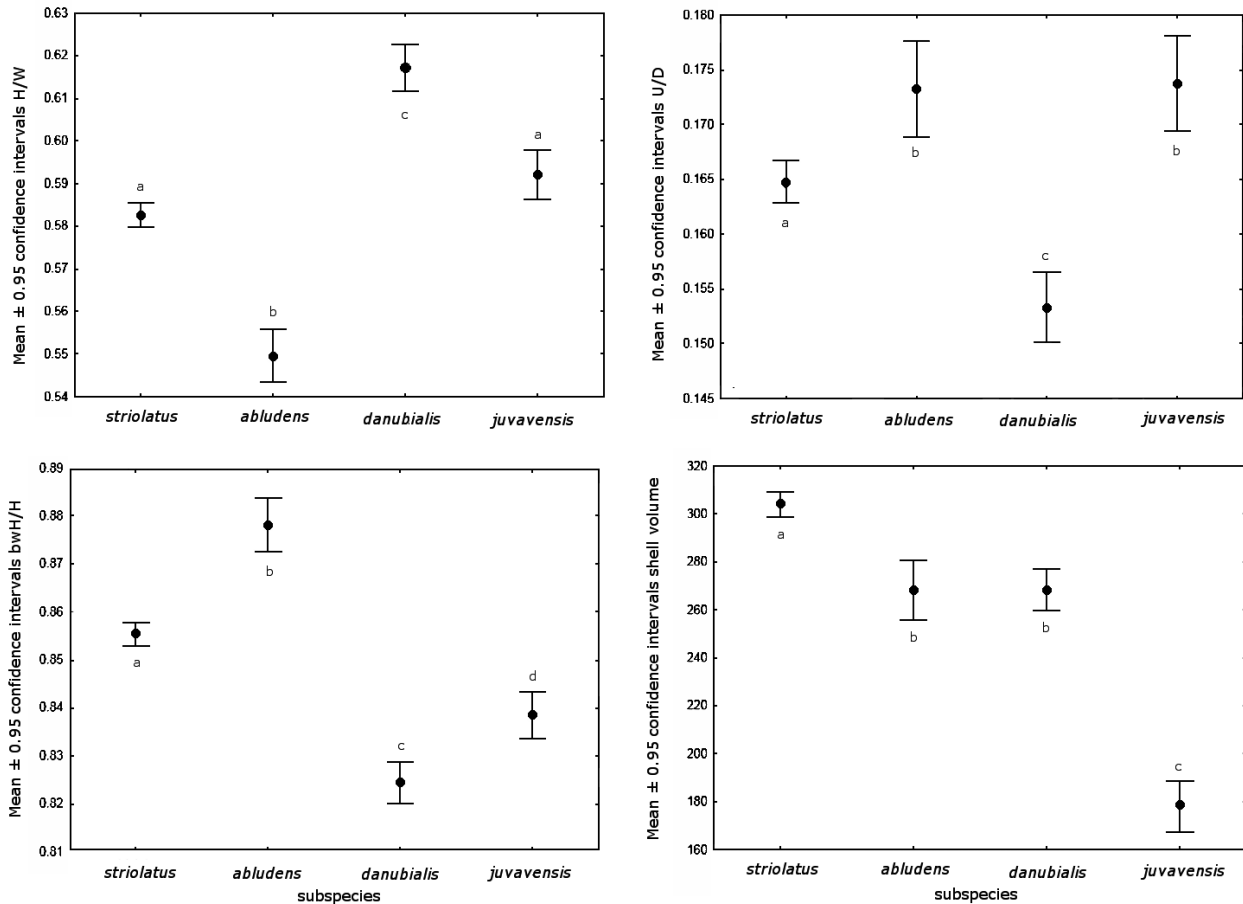


Figure 3. Shell shape (expressed as H/W, U/D and bwH/H) and volume in subspecies of *T. striolatus*. Letters 'a-d' indicate significant differences by Kruskal-Wallis test with $P < 0.05$.

coldest quarter, BIO19 had a negative coefficient (-0.833) with PC2_B. Morphometric features, aperture height (h) and width (w) showed the largest positive correlation with the PC1_B ($\rho = 0.446$ and $\rho = 0.418$, respectively) (Table 2). The positive correlation coefficients (from 0.325 to 0.363) were also shown in bwH, D, W and shell volume with the first principal component for morphometric parameters PC1. The negative correlation between PC1_B and whl was revealed ($\rho = -0.266$), whereas it was positively correlated ($\rho = 0.259$) with PC2_B. The largest negative correlation ($\rho = -0.266$) with PC2_B was shown by bwH/H.

In support of these results, the Mantel test showed significant correlations ($P < 0.04$) between matrices of differences between specimens for 10 shell measurements including PC1 and geographical distance between the sites, where the specimens were collected. The largest correlation coefficients referred to aperture height and width (0.16 and 0.12, $P = 0.019$).

Environmental characteristics of sites

PCA of the sites (Fig. 7) showed an environmental gradient (axis 1) from subalpine habitats at high altitudes in Austria and Germany characterized by a great amount of precipitation (e.g. BIO18) to marine-influenced lowland localities in the UK, the Netherlands and France with relatively high mean annual and winter temperature (BIO1, BIO11), as well as in Ireland with a highly isothermal environment (BIO3). The second important gradient (axis 2) separated the German, Austrian and Slovakian lowlands and submontane habitats mainly from the Irish ones. When the taxonomic description of sites were plotted on the PCA (Fig. 7), it became clear that the sites inhabited by the *T. s. abludens* and *T. s. danubialis* subspecies were situated among those occupied by *T. striolatus* s. str. On the contrary, *T. s. juvavensis* sites were well-separated from the others.

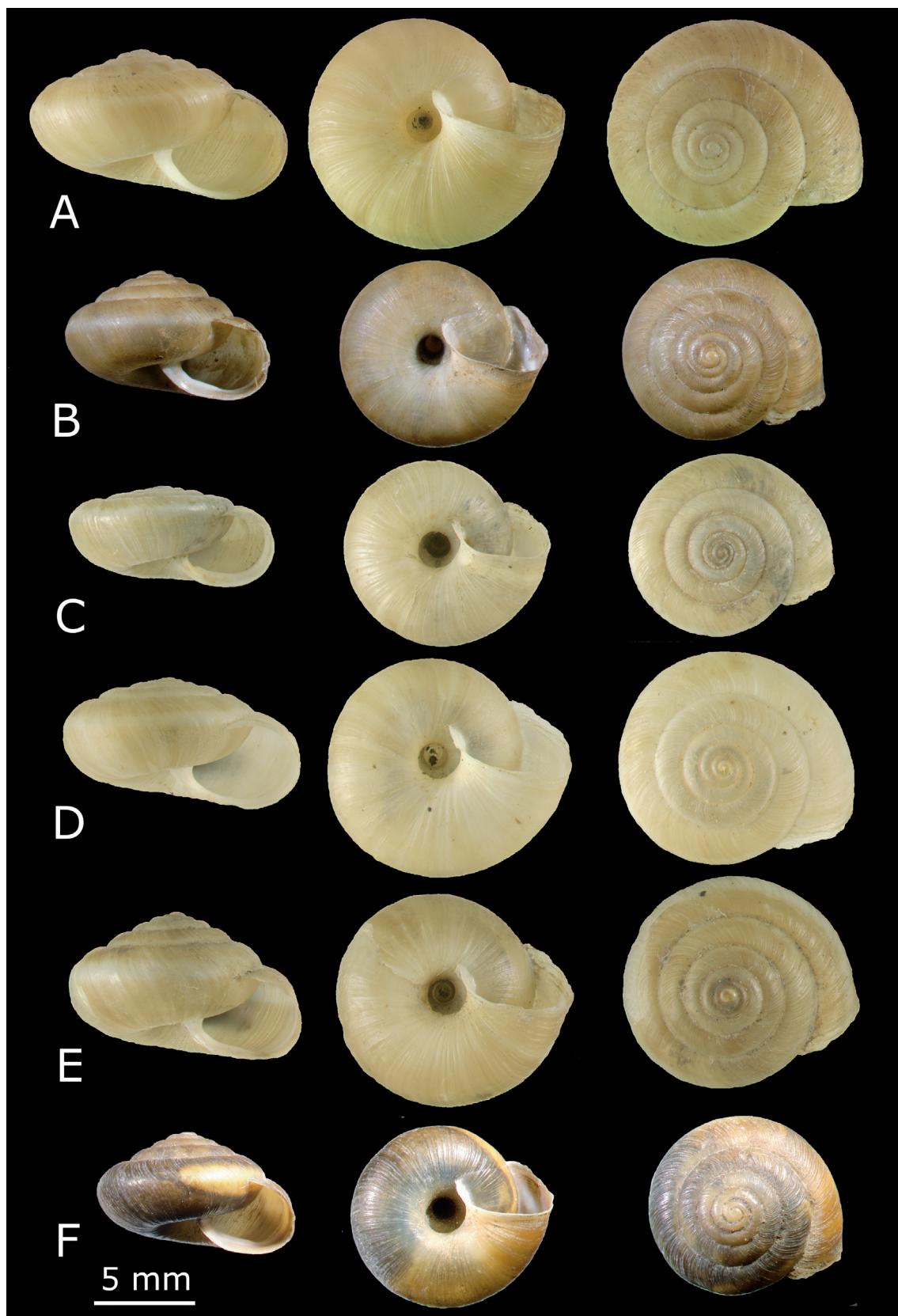


Figure 4. Specimens of *T. striolatus* from: A – Cambridge, UK (lowland); B – Gosheim, Germany (montane); C – Marquise, France (lowland); D – Cahir, Ireland (lowland); E – Donauwörth, Germany (submontane); F – Aichtal, Germany (submontane).

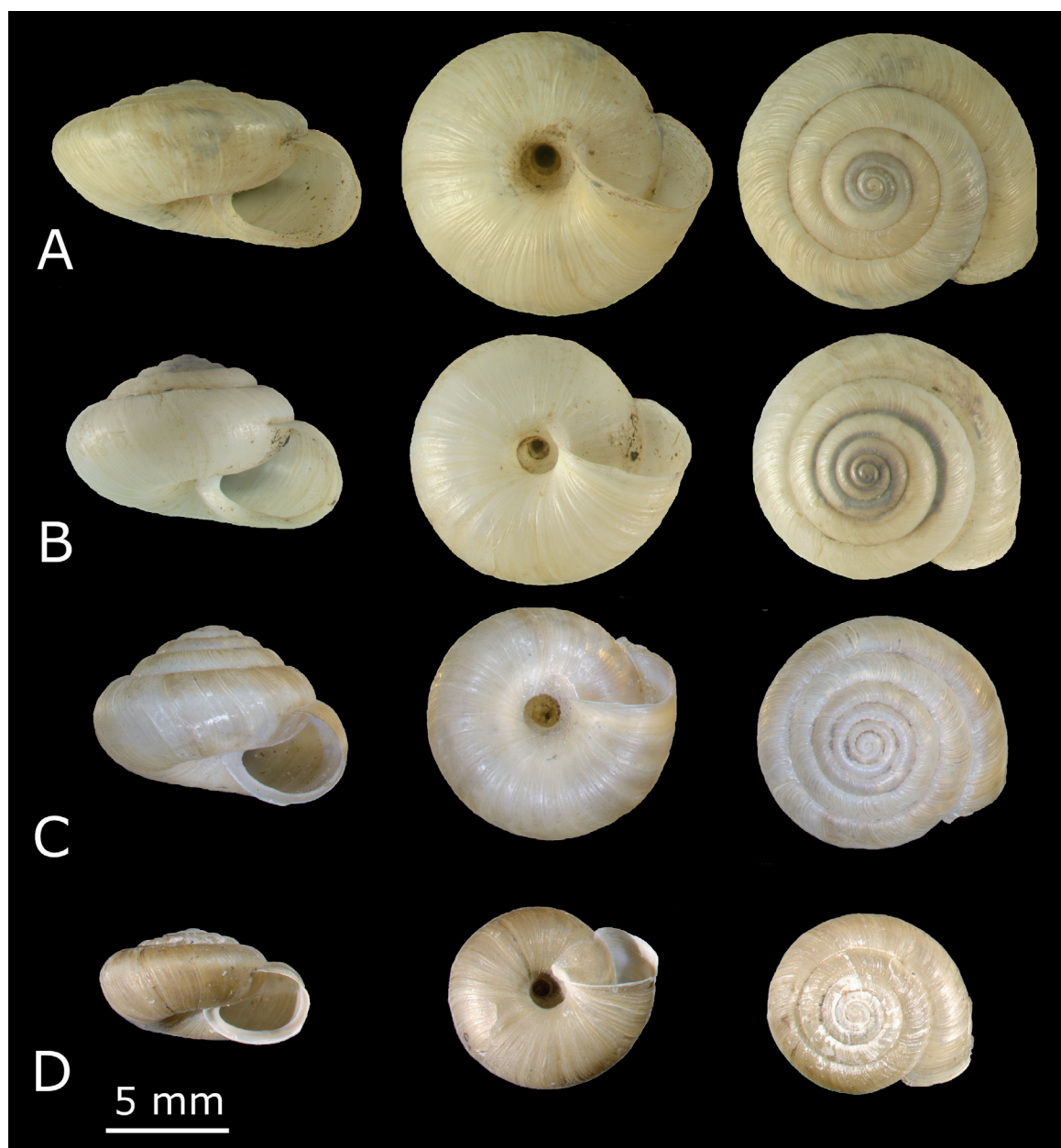


Figure 5. Topotype specimens of *T. striolatus* subspecies. A – *T. s. striolatus*, Heidelberg (Ref. no. 403377, NBCN); B – *T. s. danubialis*, Dillingen a. d. Donau (Ref. no. 403381, NBCN); C – *T. s. abludens*, Boulogne-sur-Mer (Ref. no. coll. Locard G. p. 243, MNHN); D – *T. s. juvavensis*, Schafberg.

Cline variation of shell morphology with bioclimatic and spatial parameters

In order to test, if the observed relationships of shell features with bioclimatic and spatial parameters show a continuous or step character, we compared linear and non-linear (two-order polynomial) models describing these relationships and used the procedure of automated stepwise model simplification on the polynomial function. Results of the two types of model selection were very similar. In only three cases from 68,

the first approach preferred the linear model to the nonlinear one, whereas the second approach proposed a simplified function $Y \sim \text{intercept} + X^2$. Since, the second approach is more general, we focused on the description only its results. Table 3 and Fig. 8 present the best-fitted models with adjusted R^2 larger than 0.25 that were selected according to the stepwise model simplification procedure on the polynomial function, whereas all models selected according to this procedure are included in Appendix 4.

Table 2. Spearman's rank correlation coefficients between 17 shell variables (including two first principal components, PC1 and PC2) vs 3 bioclimatic variables and altitude as well as their two first principal components (PC1_B and PC2_B). White cells indicate coefficients that are statistically significant with p -value < 0.05 after Benjamini-Hochberg correction, whereas shaded cells indicate coefficients that are not statistically significant.

Variable	W	H	bwH	h	w	D	U	u	whl	H/W	U/D	u/U	bwH/H	h/w	volume	PC1	PC2
BIO1	0.235	0.164	0.233	0.277	0.294	0.242	0.059	0.117	-0.290	-0.104	-0.091	0.152	0.182	0.000	0.217	0.223	0.002
BIO6	0.314	0.180	0.297	0.393	0.366	0.314	0.128	0.185	-0.351	-0.176	-0.053	0.149	0.293	0.077	0.277	0.296	-0.059
BIO18	-0.308	-0.225	-0.321	-0.399	-0.389	-0.318	-0.089	-0.135	0.247	0.106	0.103	-0.123	-0.234	-0.062	-0.291	-0.311	-0.017
altitude	-0.320	-0.287	-0.377	-0.428	-0.428	-0.316	0.009	-0.050	0.281	0.021	0.205	-0.151	-0.198	-0.052	-0.324	-0.322	-0.125
PC1_B	0.340	0.256	0.363	0.446	0.418	0.343	0.094	0.144	-0.266	-0.096	-0.109	0.135	0.251	0.097	0.325	0.344	0.027
PC2_B	-0.062	0.081	-0.045	-0.117	-0.107	-0.059	-0.078	-0.071	0.259	0.150	-0.098	0.008	-0.266	-0.055	-0.017	-0.051	0.158

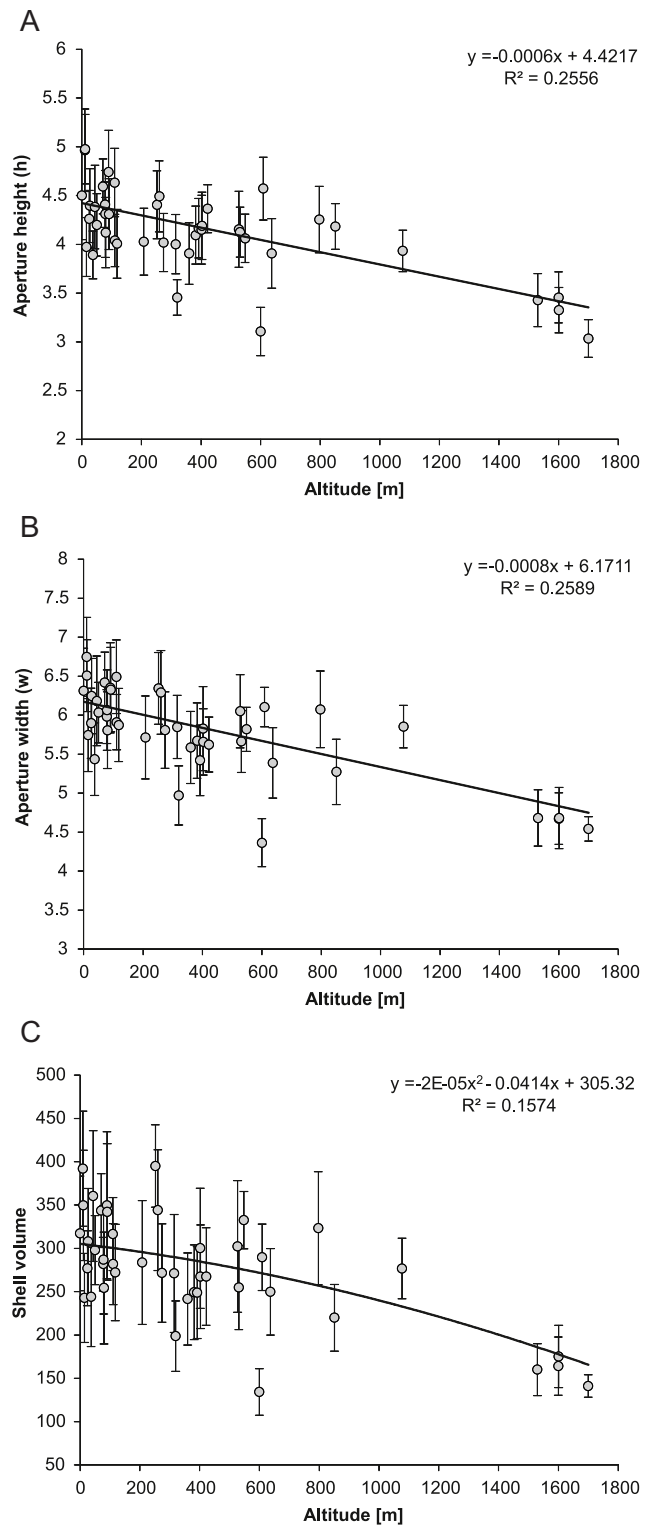


Figure 6. Relationship of altitude with (A) aperture height, (B) aperture width and (C) shell volume for *T. striolatus*. Circles and whiskers indicate an average and standard deviation of the given shell measure for the particular altitude. The best-fitted function found by an automated stepwise model simplification based on AIC on the polynomial function was plotted.

Among the studied relationships, only 4 were not statistically significant ($P > 0.05$). In 8 significant cases, the linear relationship was favoured and a non-linear function in 56. Interestingly, the top models with the largest adjusted R^2 (> 0.25) included aperture height and width in relation to precipitation of warmest quarter (BIO18), minimum temperature of coldest month (BIO6) and altitude (Table 3, Fig. 8). Moreover, among the models, there were relationships of shell diameter and width as well as PC1 with BIO6. The linear relationships were found for the two aperture parameters related with altitude indicating a continuous cline. The other relationships were best described by a non-linear function, which suggests a step character of these relationships.

When the bioclimatic and spatial parameters were reduced to two principal components, their relationship with shell morphometric was also in most cases

better described by non-linear models than linear ones (Fig. 8). Only three of 34 dependencies involving U/D, H and h/w with one of the principal components preferred the linear model in both model selection approaches (see Appendix 5 for the results of the stepwise model simplification procedure). Similarly to the results based on the individual bioclimatic/spatial parameters, the largest adjusted R^2 (about 0.3) was shown by aperture height (h) and width (w) in dependence on the first component PC1_B (Table 3).

Contribution of bioclimatic and spatial variables in explanation of shell morphology

In order to estimate the influence of combined effects by bioclimatic and spatial parameters on shell morphological characteristics, we carried out multivariate regression analysis. We considered five sets of

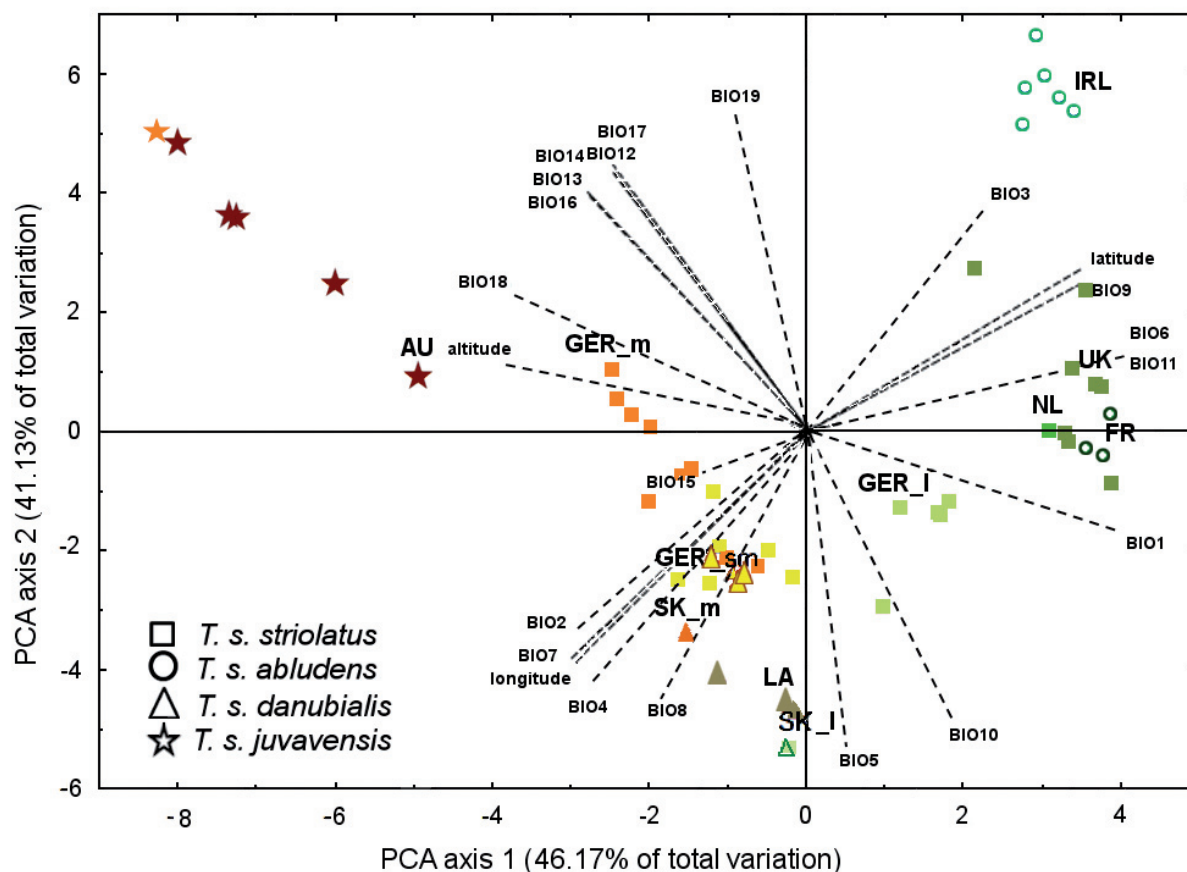


Figure 7. Principal component analysis (PCA) of environmental variables for sites of *T. striolatus*. Correlation of variables with PCA axes is indicated by dashed vectors. Abbreviations: AU – Austria subalpine, FR – France; GER_l – Germany lowland, GER_m – Germany montane, GER_sm – Germany submontane, IRL – Ireland, LA – Lower Austria lowland, NL – the Netherlands, SK_l – Slovakia lowland, SK_sm – Slovakia submontane, UK – the United Kingdom. Colours indicate type of habitat: green – lowlands, yellow – submontane, orange – montane, brown – subalpine. Symbols denote subspecies of *T. striolatus* found in the sites.

independent bioclimatic/spatial variables and tested several types of functions (see Material and methods for details). Table 4 summarizes the best-fitted models selected according to the stepwise model simplification procedure. Coefficients of the models are presented in Appendices 6–10. The adjusted R^2 coefficients were significant for all the models with $P < 0.0003$.

In all five approaches, the adjusted R^2 larger than 0.25 was reported for shell width (W), body whorl height (bwH), aperture height (h), aperture width (w), shell diameter (D) and the first principal component for morphological features (PC1). Such R^2 also showed shell volume for the models based on spatial variables, uncorrelated bioclimatic variables and altitude as well as two principal components for bioclimatic/spatial data. The largest proportion of variance was explained for h (the adjusted $R^2 = 0.42$) under the model including BIO1, BIO6, BIO18 and altitude. When all 19 bioclimatic and three spatial variables were implemented in the models, the adjusted R^2 exceeded 0.5 for W, h, w, D and PC1 (data not shown).

All the models with $R^2 > 0.25$ including both bioclimatic and spatial variables characterized by the largest AIC_w , i.e. were better fitted than models involving only spatial or bioclimatic variables. It means that the variation in shell features is at best explained when bioclimatic parameters together with spatial variables are considered. Almost all best models contained after parameter reduction still quadratic and cubic terms, which indicates non-linear relationships between shell morphometry and environmental conditions. In all cases but one, the models also included interaction terms between their variables suggesting complexity of these relationships.

DISCUSSION

Associations between the environment and morphological variability within *T. striolatus*

The study of morphometric parameters of the shells jointly shows that there is a considerable multivariate inter-population variation. The data indicate significant differences between the populations from various biotic zones across the entire distribution range of *T. striolatus* (Fig. 2). Given this finding and genetic evidence for a gene flow among populations (Proćków *et al.* 2014), it seems quite likely that the morphological variation results at least partially from environmental influences. Ten of seventeen features representing shell size (e.g. shell volume and PC1) and shape (bwH/H) exhibit moderate but significant relationship with bioclimatic factors considered in this study. After selection of bioclimatic variables explaining the most variation in studied localities and least correlated, we obtained annual mean temperature (BIO1), minimum temperature of coldest month (BIO6) and precipitation of warmest quarter (BIO18). This choice seems reasonable because BIO1 well describes the general condition during the whole year. BIO6 is likely a limiting factor for growth and survival of snails. Since snails are most active during warm periods with high precipitation, BIO18 is also an appropriate parameter. The strongest response to the environmental and spatial conditions is demonstrated for height and width of aperture. Given the strong correlation between these two measures and shell volume ($\rho = 0.789$ and $\rho = 0.855$ with $P < 0.001$, respectively), it may be assumed that the aperture size is also a good estimator of body size.

Table 3. Characteristics of the best-fitted models describing relationships between shell morphometric variables and selected environmental/spatial parameters as well as their two principal components (PC1_B and PC2_B). Only relationships with adjusted $R^2 > 0.25$ were presented. The models were selected according to the automated stepwise model simplification procedure based on AIC on the polynomial function and compared with the simple linear model. The data were sorted according to adjusted R^2 . The data for all relationships are presented in Appendices 4–5.

Relationship	Selected model	R^2	Adj. R^2	AIC_w
h ~ BIO6	$Y \sim \text{int} + X + X^2$	0.3147	0.3136	1.000
h ~ PC1_B	$Y \sim \text{int} + X + X^2$	0.3104	0.3092	0.808
w ~ BIO6	$Y \sim \text{int} + X + X^2$	0.3028	0.3017	1.000
w ~ PC1_B	$Y \sim \text{int} + X + X^2$	0.3025	0.3014	0.988
w ~ BIO18	$Y \sim \text{int} + X^2$	0.2927	0.2921	1.000
h ~ BIO18	$Y \sim \text{int} + X^2$	0.2909	0.2904	0.998
D ~ BIO6	$Y \sim \text{int} + X^2$	0.2601	0.2596	1.000
w ~ altitude	$Y \sim \text{int} + X$	0.2589	0.2583	1.000
W ~ BIO6	$Y \sim \text{int} + X^2$	0.2585	0.2579	1.000
h ~ altitude	$Y \sim \text{int} + X$	0.2556	0.2550	1.000
PC1 ~ BIO6	$Y \sim \text{int} + X^2$	0.2530	0.2524	1.000

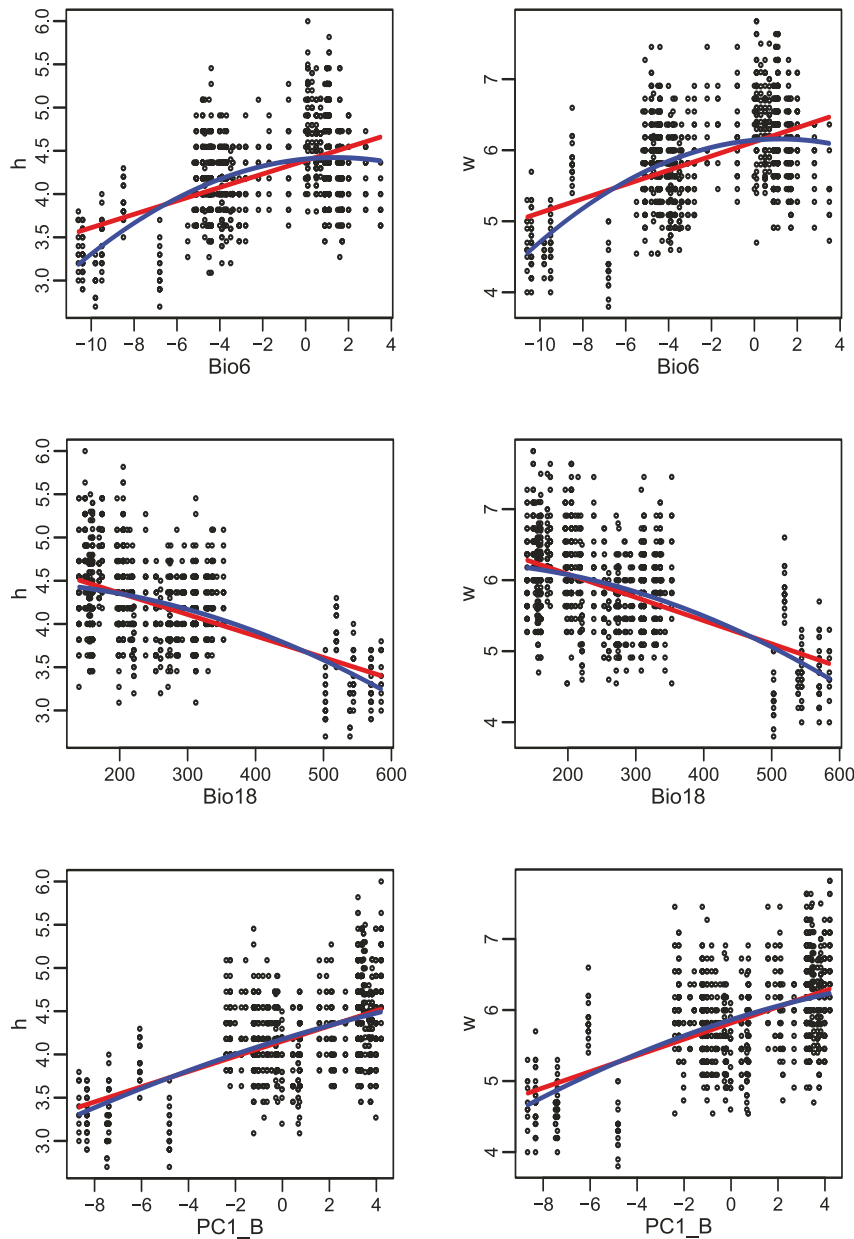


Figure 8. Selected relationships with the largest adjusted R² values between aperture height (h) or aperture width (w) and bioclimatic variables as well as two principal components based on the bioclimatic/spatial parameters. The red line indicates fitted linear function whereas the blue one means function found by an automated stepwise model simplification based on AIC on the polynomial function. Relationships for all parameter combinations are presented in Appendices 4 and 5.

Variation in shell size and shape in land snails was investigated many times and mechanisms underlying this variation were attributed to selection in different environments (Engelhard and Silk 1994, Welter-Schultes 2000b, Chiba 2009), whereas in other land-snail species the influence of population history was implicated (Gould and Woodruff 1990, Davison and Clarke 2000). In an extensive literature review, Goodfriend (1986) noted some individualistic responses in

body size of land snails along altitudinal, moisture, temperature/insolation, and calcium availability gradients. In ten of twelve taxa, he documented positive correlations between shell size and moisture. Similar results were obtained for *Cochlicopa lubrica* (Armbruster 2001). Our study, however, reveals an opposite pattern; snails from sites with higher precipitation and simultaneously with lower temperatures, tend to be smaller. The more intensive precipitation and lower

Table 4. Characteristics of the best-fitted models describing relationships between shell morphometric dependent variables and five combinations of bioclimatic/spatial independent variables. The models were selected according to the automated stepwise model simplification procedure based on AIC. The relationships involving only longitude and latitude and two principal components for bioclimatic/spatial data (PC1_B and PC2_B) were modelled by a two-order (cubic) polynomial function. For all shell variables, nonlinear model (nonlin) was favoured over the linear one. In the other three cases, the relationships were modelled by: a simple linear model (lin), a linear model including pairwise interactions and also three-way interaction in the case of spatial variables (inter), as well as a model considering in addition to the previous one, quadratic terms for all variables (full). AIC weights (AIC_w) were calculated for each of five best-fitted models for a given shell variable. The largest values of adjusted R² and AIC_w were bolded for the given shell variable.

Dependent variables	Independent variables													
	longitude and latitude				longitude, latitude, altitude				BIO1, BIO6, BIO18				BIO1, BIO6, BIO18, altitude	
	Model	Adj. R ²	AIC _w		Model	Adj. R ²	AIC _w		Model	Adj. R ²	AIC _w	Model	Adj. R ²	AIC _w
W	nonlin	0.30	5.5E-16		full	0.31	3.7E-14		full	0.32	2.8E-10	full	0.34	0.110
H	nonlin	0.19	6.8E-20		full	0.24	0.756		full	0.19	4.7E-19	full	0.24	0.244
bwH	nonlin	0.27	2.8E-20		full	0.30	2.2E-07		full	0.27	4.6E-19	full	0.32	1.000
h	nonlin	0.34	1.8E-32		inter	0.37	3.5E-23		full	0.37	7.2E-20	full	0.42	1.000
w	nonlin	0.35	4.0E-18		inter	0.37	6.0E-07		full	0.36	1.0E-14	full	0.39	1.000
D	nonlin	0.31	7.5E-18		full	0.31	3.1E-15		full	0.32	2.6E-11	full	0.34	1.8E-04
U	nonlin	0.16	1.000		full	0.12	3.4E-13		full	0.10	2.8E-17	full	0.13	2.9E-08
u	nonlin	0.18	1.000		full	0.15	7.6E-12		full	0.13	1.8E-18	full	0.16	5.8E-09
whl	nonlin	0.12	1.6E-18		full	0.16	1.5E-04		full	0.16	2.3E-04	full	0.18	1.000
H/W	nonlin	0.14	1.000		full	0.13	1.2E-04		full	0.07	1.2E-22	full	0.12	6.4E-06
U/D	nonlin	0.13	0.804		full	0.13	0.182		full	0.07	1.5E-20	full	0.13	0.014
u/U	nonlin	0.04	0.006		full	0.04	0.013		inter	0.04	0.261	full	0.05	0.708
bwH/H	nonlin	0.15	0.972		full	0.14	0.024		full	0.12	1.0E-08	full	0.14	0.004
h/w	nonlin	0.02	1.5E-07		full	0.03	8.0E-05		full	0.02	5.4E-08	full	0.02	1.5E-07
volume	nonlin	0.23	8.3E-15		full	0.26	4.8E-05		full	0.24	1.6E-10	full	0.27	1.000
PC1	nonlin	0.29	2.9E-17		full	0.31	2.0E-11		full	0.31	8.4E-09	full	0.33	1.000
PC2	nonlin	0.16	1.000		full	0.14	7.5E-07		full	0.06	2.0E-29	full	0.14	9.0E-07

temperature may restrict activity of snails and their growth. In agreement with that, we found that metric features of shells collected from locations with higher temperatures were larger. It is possible that *T. striolatus* reaches sexual maturity faster in wetter and colder places in order to reproduce earlier, like some other land-snail species (Berry 1963, Pfenninger and Magnin 2001, Proćków *et al.* 2012). It results in smaller size, because shell growth is limited after maturity. It was suggested that the early reproduction could constitute a selective advantage (Pfenninger and Magnin 2001) because winter mortality in juveniles was shown to be size-dependent in *Cornu aspersum* (Madec *et al.* 2000). As in the case of *Candidula unifasciata*, further experiments are needed to determine whether the size-precipitation correlation is due to phenotypic plasticity in response to the prevailing conditions or whether it has an adaptive significance (Pfenninger and Magnin 2001). Interestingly, opposite trends were shown by the number of whorls in this study. Shells from sites with the larger temperatures had smaller number of whorls. It may be related with some changes in development of shells.

Furthermore, we found that adult *T. striolatus* at high altitudes produced notably small shells in comparison to conspecific populations from lower elevations (Fig. 2, Fig. 5A, E, Fig. 6C). These results are consistent with those obtained for a helioid *Arianta arbus-torum* and clausiliids *Vestia turgida* and *V. gulo* (Burla and Stahel 1983, Baur and Raboud 1988, Sulikowska-Drozdz 2001, 2011), also occurring along elevation gradients. Since any data collected along altitudinal gradients reflect a combined effect of regional peculiarities and general altitude phenomena (Körner 2007), the interactions between temperature, precipitation, and duration of growing season make likely the presence of shell size correlations with elevation (see also further discussion). In fact, we showed significant and high correlations between altitude and almost all bioclimatic parameters (data not shown).

The so-called converse Bergmann's rule was demonstrated for many ectothermic species, including invertebrates (reviewed in Blanckenhorn and Demont 2004). This rule says that body size decreases as latitude/altitude increases. Contrary to the original Bergmann's rule, the converse effect is mediated by duration of growing season, as opposed to temperature *per se*. The shorter seasons at higher latitudes/altitudes progressively limit the time available for foraging, growth and development. Hence it results in a pattern of decreasing body size (Blanckenhorn and Demont 2004). Accordingly, we observed a negative correlation of temperature seasonality with shell metric features. Analogous seasonal time constraints, caused by altitude, were recorded in some insects (Blanckenhorn 1997, Fischer and Fiedler 2002, Chown

and Klok 2003). For particular species of terrestrial molluscs, however, such a pattern is not sufficiently documented. It was shown in only recently published studies on land-snail species (Kotsakiozi *et al.* 2013, Giokas *et al.* 2014) and other invertebrates (Puzin *et al.* 2014). Regional land snail faunas in north-west Europe demonstrated neither latitudinal nor altitudinal patterns in body size if phylogenetic effects are controlled (Hausdorf 2003). Observations of land snails across large extents (north-western Europe, eastern North America, and New Zealand) revealed only weak altitudinal variation in community body sizes which is probably obscured by other strong local environmental gradients such as precipitation (Nekola *et al.* 2013). It should be emphasised, that both Bergmann's rule and converse Bergmann's rule are not mutually exclusive because they are affected by different environmental factors (Blanckenhorn and Demont 2004). They are often taxon-specific and partially depend on the study design (Shelomi 2012). This pattern is also thought to be stronger at the intra-specific level than between closely related species (Meiri 2011). The crucial effect of body size, identified here, may be more important in short-lived taxa with a predominantly annual life cycle such as studied *Trochulus* species (Germain 1930, Proćków *et al.* 2013a). For organisms terminating their growth only over several months, the effects of end of season time constraints will get pronounced, and may hence strongly affect the overall growth strategy and final body size of the species.

Our results of PCA showed that differences between populations in some traits co-vary significantly with long-term climatic factors and the geographic location of their sampling sites. Snails found in climatically milder sampling sites in the British Isles tend to have larger shells (Fig. 4A, Fig. 7). This association allows formulating assumptions that conform to the above discussion about the causes for this pattern. An oceanic climate is generally characterized by warm (not hot) summers and cool (not cold) winters, and a relatively narrow annual temperature range. It lacks a dry season, as precipitation is more evenly dispersed throughout the year (Meteorological Office 2013). Additionally, relatively long growing season (e.g. approximately 252 days in UK, Meteorological Office 2013) is meaningful because snails remain active for a longer part of the year, than it is encountered in other places at a comparable latitude (e.g. 180–200 days in Germany, Rötzer and Chmielewski 2001), and may continue their growth with limited periods of aestivation or hibernation reaching larger shell sizes. This explanation is well confirmed by our global analyses of all samples, which revealed significant positive correlations between metric features and temperature parameters but negative correlations with temperature ranges. Some individuals of *T. striolatus* inhabiting the same area (i.e.

Bl – Blumberg and Sch – Schleithem) significantly differed in shell morphometry but showed no differences in COI sequences (Proćków *et al.* 2014: Fig. 5c, Fig. 14). It may indicate a great phenotypic plasticity in response to the prevailing local conditions. Our current studies showed that many shell size parameter is in some way related with a bioclimatic variable and altitude indicating that ultimate size of *T. striolatus* is influenced quite a lot by environment. In other land-snail species like vitrinids the larger size was a consequence of increased life span depending on local climate (Umiński 1975). Similarly, significant correlations between environmental factors and shell trait variables were found in *C. unifasciata*, showing that in areas with a more Mediterranean climate shells tended to be more depressed with smaller apertures and coarser and wider spaced ribs (Pfenninger and Magnin 2001). Finally, environmental variables such as the increasing insolation and temperature instability were suggested as possible important factors influencing the shell morphology in high mountain populations of *V. turgida* (Sulikowska-Drozd 2001).

The second axis in PCA plot (Fig. 7) separated the snails from low and very high altitudes (representing two extreme shell sizes, Fig. 5A, D) from snails of sub-montane and montane regions (intermediate shell-sized, Fig. 4B, E, F), where climate conditions show considerable variation (e.g. head vs. mouth of a valley, Viazzo 1989). Thus, climate harshness related to altitude does not influence species uniformly along the gradient but discrete changes could be distinguished, like in *V. turgida*, in which snails occurred above the timberline tended to be smaller than those living at the same altitude in forests (Sulikowska-Drozd 2001). Contrary to that, in our regression analyses including all specimens, we found that the strongest relationships of shell features (aperture width and height) with altitude showed a linear and continuous rather than non-linear and step-wise character. However, the most of other relationships of shell features with environmental variables were non-linear. The overall shell variability, especially at the population level, is affected by many different spatial and climatic features including topographic complexity of the area and local microclimate conditions. Most models including both bioclimatic and spatial variables explained the largest proportion of variance in the shell morphometrics and were better fitted than models considering the parameters separately.

There are also many other unmeasured environmental or gene-by-environmental factors important for determining shell sizes as it was reported for some populations of *Oreohelix cooperi* (Anderson *et al.* 2007). In this study, we found shells from Gosheim to be smaller than those from the montane zone (Fig. 4). These dwarf forms were genetically confirmed

to represent *T. striolatus* (Proćków *et al.* 2017). *Trochulus* from “Heide von Gosheim bei Spaichingen” was mentioned by Geyer (1913: 101–106) but identified as *Helix suberecta*. The latter, however, most likely does not represent a separate species (Duda *et al.* 2014). Thus, our findings indicate that in this case an individualistic response to local environmental and/or climate variables can apply. It is worth to mention that the habitat in which we found the snails (humid forest) differs from that given by Geyer (1913) (heathland). Nevertheless, in both cases the snails remained small.

Taxonomic implications

Typically, subspecies are considered distinctive, geographically replacing forms of a species (O’Brien and Mayr 1991). The recognition of diversity below the species level enables deeper understanding intraspecific variation, gives insights into the adaptability of organisms and provides knowledge about the process of speciation (Braby *et al.* 2012).

As a result of the current study, showing the clear and significant influence of environment on morphometric variation of *T. striolatus* shell, the legitimacy of its division into subspecies is not confirmed, and formal recognition of these subspecies is not justified. Therefore, the following synonymy is proposed:

- Trochulus striolatus* (C. Pfeiffer 1828: 28), pl. VI, fig. 8. Locus typicus: Germany: near Heidelberg, in gardens.
 = *Helix danubialis* Clessin 1874: 184, pl. VIII, fig. 4. Locus typicus: Germany: Bavaria: near Dillingen, woods on the border of the Bavarian Danube.
 = *Helix abludens* Locard 1888: 334. Locus typicus: Jersey; Ireland: environs of Dublin; France: dep. Pas-de-Calais: Boulogne-sur-Mer. Syntypes: MNHN.
 = *Helix (Hygromia, Fruticicola) montana* var. *juvavensis* Geyer 1914: 276. Locus typicus: Austria: north slopes of Schafberg. Paratypes: NHMW.

The taxonomic status of *austriacus* subspecies could not be verified due to its material unavailability. Except for the original description (Mahler 1952), there is no other study that describes this taxon. However, taken into account our findings, we can suppose that its morphology might be also the product of phenotypic plasticity associated with the influence of environment.

When snail species are distinguished from each other by shell dimensions, the coefficient of variability (CV%) is helpful to solve the taxonomic doubts (Welter-Schultes 2000a, Örstan 2001). CV of about 3% to 8% was usually obtained for shell height and diameter in land snail samples consisting of a single species (Welter-Schultes 2000a, Örstan and Yildirim 2003). Subsequently, it was suggested that CV larger than 9% for an unbiased and sufficiently large sample would strongly indicate that the sample is a mixture of specimens of

two sympatric species with different mean dimensions (Örstan 2003). Shell volume as well as umbilicus major and minor diameters appeared to be the most variable traits within populations and biotic zones of *T. striolatus* (CV mostly exceeding 9%, Appendix 2). This finding may be important in considering infra-specific classification of *T. striolatus* suggested by some authors (Klemm 1974, Lisický 1991, Falkner *et al.* 2001). The most often recognized subspecies within *T. striolatus* is *T. s. danubialis*, whose distribution range has not been precisely defined. It was recorded not only along the Danube River but also in the southern part of the Little Carpathians and Žilinská kotlina basin (Hudec 1964, Čejka 2000, Čejka *et al.* 2008). According to Schileyko (1978), the taxon should be given a separate species status. Originally *T. s. danubialis* was described as having conical shell with a low spire [“Gewinde kurz, wenig erhoben”] and a very narrow umbilicus [“Nabel sehr eng”] (Clessin 1874: 184). However, inconsistent records of high-spined and narrow-umbilicated (Ehrmann 1933) versus low-spined and broad-umbilicated shells (Hudec 1964) of *T. s. danubialis* were reported from different regions. Both variation of shell in size and shape as well as umbilical structure have been attributed to environmental plasticity in some subspecies of *T. striolatus* (Mahler 1952, Forcart 1965). This statement is supported by our results showing that umbilicus diameter, umbilicus relative diameter (U/D) and shell volume are extremely variable characters within all populations of *T. striolatus* (Appendix 2). The relative height of spire (bwH/H) was also found to be significantly influenced by climate factors. Therefore, all these features considered as diagnostic so far, appear to be taxonomically useless. In addition, intra-population shell size of *T. s. danubialis* showed great variation and overlapped other populations (Fig. 2). For example, its shell shape is similar to those from Aichtal (Fig. 4E, F). Similarly, the shells of topotypic material overlapped the variation of many other populations (Fig. 2). Our recent investigations revealed that under constant laboratory conditions the average shell shape of *T. hispidus* changed significantly from flat with a wide umbilicus to more globular or even elevated with a narrower umbilicus (Pročków *et al.* in press), which indicates a great influence of environmental factors. In agreement with that, we found significant correlations of shell width and height as well as umbilicus major and minor diameter with bioclimatic features. Their strongest negative correlations were observed with the precipitation variable (Table 2). We also demonstrated the significant negative relationship between shell shape (PC2 and H/W) and umbilicus diameter or relative umbilicus diameter (U/D), which indicates that more globular shells have narrower umbilicus. On the other hand, among Helicinae, i.e. *Levantina*

spiriplana (Olivier, 1801) and *Iberus gualtierianus* (L., 1758), changes in the relative width of the umbilicus were considered genetically based and used to diagnose subspecies (Pfeiffer 1949, Falkner 1990). Generally, differences in shell shape and shell structure are usually heritable, genetically based, and phylogenetically meaningful, while intra-specific variation in size is more often plastic and under the influence of environment.

In the light of the results obtained in the current study, *T. s. danubialis* has failed to meet the criteria of subspecies status because it does not represent morphologically and geographically distinct populations. Admittedly, a slight morphological differentiation in the cross-section of the penis between *T. s. striolatus* and *T. s. danubialis* has been detected, however, it might be an artefact of a limited sampling dataset ($n = 8$, Duda *et al.* 2014). Moreover, as observed by those authors, in *danubialis* and *juvavensis*, the arrangement of the penial folds was the same as in *T. hispidus*, thus, very variable in *T. striolatus*.

Similarly, there is no evidence to support the validity of *T. s. abludens* as a diagnosable subspecies. We found no taxonomically useful features due to the immense variation of shell size and shape within and between populations of this taxon. It should be also noted that the diagnosis of this subspecies (based on larger shell, more depressed spire and wider umbilicus) established by Falkner *et al.* (2002) disagrees with Ellis's (1969) diagnosis (assuming smaller shell and often higher spire). Furthermore, none of these descriptions corresponds to the original designation by Locard (1888), i.e. medium sized, conical-subglobose shell, high spire and quite large umbilicus (see specimen from Locard's collection, Fig. 5C). Similar inconsistencies in sizes and shapes of shells were detected in this study among the topotype specimens from Boulogne-sur-Mer (Fig. 9), which display a very high variation in traits regarded as diagnostic so far, i.e. shell volume (CV = 25%), umbilicus diameter (15%) and relative umbilicus diameter (U/D) (13%). Subsequently, we were unable to establish restricted geographic occurrence of this taxon.

Conspicuously small shells were observed in highland populations of *T. striolatus* from the Salzkammergut mountain range of the Austrian Northern Limestone Alps, including a mountain Schafberg, the type locality of *T. s. juvavensis* (Fig. 1, Fig. 2, Fig. 3, Fig. 5D). Its restricted geographical range and the differentiation in its shell size from the other subspecies conform to historical definitions of subspecies that rely on geographically disparate phenotypes. However, we also found significant differences based on the environmental/spatial effects (e.g. altitude, precipitation seasonality), suggesting that some of the morphological variation may be associated with a cline or a particular



Figure 9. Apertural and umbilical views of topotype specimens of *T. s. abludens* from Boulogne-sur-Mer (Ref. no. 297640, NBCN).

habitat. More recent definitions regard subspecies as evolving allopatric and phenotypically distinct populations that represent partially isolated lineages of a species. The subspecies should have at least one fixed diagnosable character state. Moreover, according to some researchers differences in these characters should be correlated with evolutionary independence conforming to population genetic structure (Braby *et al.* 2012). This is incongruent with recent mitochond-

rial DNA data that did not show the clear separation of three *T. striolatus* subspecies (Kruckenhauser *et al.* 2014). The identical mitochondrial COI sequences in some individuals from the *T. striolatus* populations with the significant differences in size (Proćków *et al.* 2014) may suggest that at least part of the variation is environmentally dependent and has an effect on recognition of the traditional taxonomic categories such as subspecies. Quite opposite results were revealed for

two *Trochulus oreinos* subspecies, in which the distribution of mitochondrial haplotypes clearly corresponded to their distribution ranges in spite of difficulties in their distinction at the morphological level (Duda *et al.* 2011). This, however, can be well explained by their different evolutionary history (Duda *et al.* 2014). Nevertheless, the interpretation of a single gene data should be viewed with caution. Sometimes molecular results lead to different conclusions like in *A. arbustorum*. Arter (1990) using allozymes demonstrated a gene flow along Alpine drainage systems between lowland and high altitude populations of *A. arbustorum*. He also showed that recolonization accompanied by clinal variation led to the origin of the smaller form *alpicola*. It does not agree with phylogeographic studies of mtDNA (Haase *et al.* 2003, Gittenberger *et al.* 2004) that revealed a common lack of geographic pattern of variation: prominent differences among closely situated populations and no differences among regions. This may result from different scales of investigations: local versus global.

Subspecies are considered important taxonomic units for describing biodiversity and identifying early stages of population divergence, and thus are important for studies on both evolution and conservation (Braby *et al.* 2012). However, at the current stage of knowledge, it seems that *T. s. juvavensis* is not a valid taxonomic entity because at least some of its morphological variation is clinal and may be associated with a particular environment (e.g. subalpine zone). Nevertheless, *T. s. juvavensis* may reflect an incipient stage of differentiation from the whole population of *T. striolatus*. It may be in the process of allopatric speciation with still proceeding gene flow. These results also provide additional evidence for ongoing speciation and relatively recent diversification within *Trochulus* (Proćków *et al.* 2017) but recognition of many subspecies within *T. striolatus* is not justified.

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