



The influence of habitat preferences on shell morphology in ecophenotypes of *Trochulus hispidus* complex

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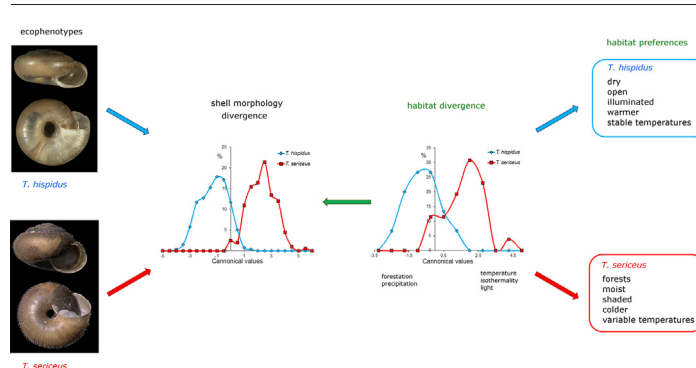
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HIGHLIGHTS

- Why *T. hispidus* and *T. sericeus* are not biological species but show different morphology.
- Illumination, forestation, precipitation and temperature discriminate their habitats.
- Significant relationships between the morphology and environmental parameters
- Phenotypic plasticity and selection associated with habitat preferences affect shell morphology.
- Morphological variability has no genetic basis, thus the snails are ecophenotypes.

GRAPHICAL ABSTRACT



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ABSTRACT

Trochulus hispidus and *T. sericeus* are hairy snails widely distributed in Europe. They differ in shell morphology and are usually found in various land habitats. However, their morphology does not match genetic distance as they do not form distinct clades. Therefore, it is interesting to determine to what extent environmental factors can control their phenotypes. We analysed the morphological traits and many environmental features of their habitats to find relationships between these parameters and explain ecological reasons for this plasticity. We found many statistically significant correlations between morphological traits and environmental variables. Illumination, forestation, precipitation and temperature occurred the most important features discriminating habitats of these snails. It turned out that *T. sericeus* prefers forests and moist shaded places, while *T. hispidus* chooses more dry habitats and open areas exposed to the sun. *T. sericeus* is also probably more tolerant to low and variable temperatures. The hair durability is also correlated with their habitats: the shell of *T. hispidus* is mostly hairless but hairs almost always cover the shell of *T. sericeus*. These results support the hypothesis that the lack of hairs is associated with the loss of a potential adaptive function due to the change from wet to dry habitats. The hairs facilitate the adherence of snails to herbaceous plants during feeding when the humidity levels are high. The morphological divergence of *T. hispidus* and *T. sericeus* is the result of phenotypic plasticity and selection associated with the habitat, which affect both the shell shape and the hair durability. Since *T. hispidus* and *T. sericeus* do not represent separate biological species and their variability has no genetic basis, they should be considered as ecophenotypes. This and our previous studies suggest that phenotypic plasticity in widely distributed *Trochulus* species is quite common and may have been of ancestral origin.

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1. Introduction

Morphological variation among populations of a species may be attributed either to genetic differences or to environmental stresses, as it was observed in snails (Kemp and Bertness, 1984). This variation is thought to be associated with adaption of organisms to specific environmental conditions (Price et al., 2003) and such changes may be crucial to survival in heterogeneous and variable conditions (Zunzunegui et al., 2011). Organisms that live in a broad range of diverse habitats and are highly mobile seem to demonstrate many adaptive traits (Piersma and van Gils, 2011) and small variation across the habitats. On the other hand, organisms with poor dispersal ability and a flexible phenotype are predestined to develop local forms, which finally can lead to speciation in the isolated areas. In heterogeneous environments, the immobile species with populations restricted to local habitats are expected to evolve into: (i) locally developed ecophenotypes, i.e. non-genetic modifications of the phenotype induced by the environment and showing plasticity across generations in response to their currently inhabited conditions or (ii) ecotypes, if this variation is supported by a genetic basis, i.e. inheritable traits (Charrier et al., 2013; Hollander et al., 2006).

Phenotypic plasticity is strongly associated with its ecological effects and can speed up evolutionary changes and speciation. Ecophenotypic modifications are most often observed in morphological characters, e.g. body size, and occur very fast compared with other evolutionary processes. Such changes can already occur in one or a few generations and are also easily reversible (Huntley et al., 1997). The most obvious examples of ecophenotypic variation are displayed in plants (Rowe and Speck, 2005), in which major groups of vegetative and floral characters were found to co-vary with different combinations of ecological parameters (Tetsana et al., 2014). The ecophenotypic variation affects often entire populations, and thus can be detected in statistical population analyses. The morphology of some plants, orchids, ultimately reflects the interaction of their genomes with its particular environment, both operating within the constraint of the size/maturity of the plant (Bateman et al., 2017).

Since animals are far less plastic than plants their ecophenotypic variation is noteworthy. Phenotypic plasticity is observed in insects, in which it is often a highly adaptive trait (Whitman and Ananthakrishnan, 2009). The alternative phenotypes manifest themselves in polyphenisms, a process in which different phenotypes can arise from a single genotype under the influence of various environmental conditions (Nijhout, 2003). The most spectacular cases of polyphenic traits are found in social castes of Hymenoptera, termites and aphids (Lüscher, 1960; Wheeler and Nijhout, 1983; Stern and Foster, 1996), seasonal forms in butterflies (Shapiro, 1976), dispersal phenotypes in wing-dimorphic insects (Zera and Denno, 1997), asexual and sexual reproductive phases in aphids (Moran, 1991) and alternative male morphologies in thrips (Crespi, 1988) and beetles (Emlen, 1994). It was also reported that certain rocky shore gastropods develop thicker-walled shells at sites sheltered from wave action, where crabs are often abundant and attack the snails in contrast to exposed sites where crabs are scarce (Crothers, 1985; Pascoal et al., 2012; Trussell and Etter, 2001). In unionid freshwater bivalves shell morphologies are also highly variable due to environmental factors and sometimes result in extreme ecophenotypic plasticity (Inoue et al., 2013; Watters, 1994).

Terrestrial snails with their restricted mobility and extraordinary diversity of shell morphology are ideal organisms to study habitat-related evolution and ecophenotypic plasticity. The morphological variation of snail species has been among others connected with soil (Gould, 1969; Gould, 1971), predators (Cook and Pettitt, 1979) and climate (Goodfriend, 1986; Lazaridou-Dimitriadou et al., 1994; Proćków et al., 2017a). However, the shell variations between populations in different habitats have been poorly examined. *Cepaea nemoralis* (L., 1758) is a classic example of the divergence in shell colour and banding among

open vs. wood habitat types (e.g. Cook, 2008; Schilthuizen, 2013). The large quantitative variability in shell shape is also well known in the hygroimid *Trochulus hispidus* (L., 1758) (Duda et al., 2014; Naggs, 1985; Proćków, 1997). This species is widely distributed in Europe. It occurs from southern Scandinavia to the northern areas of the Mediterranean peninsulas and reaches the Ural and St. Petersburg on the eastern border of its geographical range (Proćków, 2009). This euryoecious species inhabits a wide variety of habitats from anthropogenic to rocky alpine sites (Duda et al., 2011; Proćków, 2009). It also appears phenotypically plastic because is represented by two morphological forms described as *T. hispidus* and *T. sericeus*, which can freely interbreed giving fertile offspring in the laboratory conditions (Proćków et al., 2017b). *T. hispidus* and *T. sericeus* were also regarded as a species complex with a high genetic similarity (Dépraz et al., 2009; Duda et al., 2014; Kruckenhauser et al., 2014). Nevertheless, they differ in their shell morphology, mainly in absolute and relative umbilicus diameter (Proćków et al., 2017c), which are considerably smaller in *T. sericeus* (Proćków et al., 2013). Other characteristic traits of *T. sericeus* are long curved hairs, a weak lip and convex whorls with coarse growth lines (Falkner, 1990). According to Falkner (1990) it inhabits the herb layer in damp forests and shrubs. Although *T. hispidus* and *T. sericeus* are conchologically distinct, their morphology does not match genetic distance as they do not form distinct phylogenetic clades (Proćków et al., 2013; Proćków et al., 2017c).

The enormous intraspecific morphological variation of the *Trochulus hispidus* complex is particularly interesting due to possible strong interactions between these organisms and their environment. These snails can be a good model to study interactions between environment and phenotypic plasticity. Therefore, the aim of this study was to determine to what extent environmental factors control the shell phenotypes in a wide range of their habitats. We also tried to find the main predictors that can explain ecological reasons for this variation.

2. Materials and methods

2.1. Morphology, sampling and ecological recording

The shell morphology was characterized by eight measurements, which were taken from the adult shells, using a calibrated eyepiece in a stereomicroscope with accuracy 0.1 mm: shell height (H), shell width (W), body whorl height (bwH), aperture height (h), aperture width (w), umbilicus major diameter (U) (i.e. the longest diameter parallel to the shell diameter, D), umbilicus minor diameter (u) (i.e. perpendicular to umbilicus major diameter) and shell diameter (D) taken from the bottom part of the shell. The whorls (whl) were counted according to Ehrmann's method (Ehrmann, 1933). 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). Moreover, 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 study included 275 *T. hispidus* and 201 *T. sericeus* individuals. The distinction between *T. hispidus* and *T. sericeus* populations was based on the average value of umbilicus diameter/shell diameter ratio, i.e. relative umbilicus diameter (U/D), because it was proved that it is a good discriminating parameter (Proćków et al., 2013).

Hairs were inspected in live-collected adults ($n = 397$) in terms of their durability, which was recorded as 0 for no hairs or 1 for their presence. Different stages of their development from only a few hairs to more hairs regularly covering the whole shell were regarded as present.

The samples were collected in 56 localities in various regions of Europe, including 30 inhabited by *T. hispidus* and 26 by *T. sericeus*. To characterise the microhabitat conditions, 25 environmental variables

were recorded at each sampling site. Based on three to five most abundant herbaceous plant species recorded at each sampling site, the mean indicator values (Ellenberg et al., 1991) were determined: light, temperature, moisture, acidity and nitrogen. Moreover, the exposure of each locality to sun and wind was assessed by the degree of afforestation, which could take values 0 for the site not located in a wood, 1 in the case of the site with single trees or located at the forest edge, and 2 for the site situated in wood. Furthermore, we considered 19 bioclimatic variables (Table 1), which were extracted from the climate layers with a spatial resolution of 30 arc-s (Hijmans et al., 2005). Exact values of the studied parameters for each locality are given in Appendix A.1.

2.2. Statistical analyses

Appropriate statistical tests were performed to assess statistical significance of differences in shell measurements and environmental parameters between two snail forms and their habitats, respectively. To check if the analysed variables fulfil the normal distribution, we applied the Shapiro-Wilk test. When the assumption about the normality of distribution was fulfilled, the unpaired t-Student test was applied; otherwise, its non-parametric counterpart, i.e. the unpaired Wilcoxon-Mann-Whitney test was used. Using this approach, we also tested the hypothesis about a relationship between the presence/absence of hairs on snail shells and moisture or precipitation level. Moreover, we carried out Discriminant Function Analysis (DFA) with Canonical Analysis (CA). In the comparison of shell morphology, we included all variables except for umbilicus relative diameter (U/D) because this parameter was used to differentiate the populations of *T. hispidus* and *T. sericeus* at the beginning. In the case of environmental variables, we successively added variables ranked according to *p*-values obtained in the statistical tests and we omitted the redundant variables that were correlated with already added and caused a numerical problem with matrix. The final set in this type of analysis included 15 variables: acidity, afforestation, light, moisture, nitrogen and temperature as well as BIO1-BIO3, BIO7, BIO9, BIO13-BIO15 and BIO18.

To find a set of linearly uncorrelated environmental variables, we also applied Principal Component Analysis (PCA) assuming a correlation matrix. For further analyses, we selected the first four components explaining in total almost 79% of variance. We also calculated pair-wise Spearman's correlation coefficients ρ between shell measurements and the principal components as well as selected poorly intercorrelated environmental variables. The obtained *p*-values were corrected using the conserved Bonferroni method. Differences were considered significant when *p*-value was smaller than 0.05.

Table 1
Bioclimatic variables used in the study.

Acronym	Description
BIO1	Annual mean temperature
BIO2	Mean diurnal range [mean of monthly (max temp. – min temp.)]
BIO3	Isothermality (BIO2/BIO7) * 100
BIO4	Temperature seasonality (standard deviation * 100)
BIO5	Max temperature of warmest month
BIO6	Min temperature of coldest month
BIO7	Temperature annual range (BIO5-BIO6)
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

To model relationships of shell morphometric features with many environmental conditions, we applied a generalized estimating equation approach (GEE), which is a nonparametric way to estimate the parameters of a generalized linear model for clustered data with a possible unknown correlation between outcomes (Liang and Zeger, 1986). To avoid correlation between the environmental variables, we used their principal components and selected environmental variables without significant correlation. Sites (localities) of collected samples were assumed as a random factor. We also considered models with and without interactions between selected uncorrelated environmental parameters. The models were compared using the Quasi-AIC (QIC) criterion (Pan, 2001) and the model with the smaller value was selected as better fitted.

The statistical tests, GEE analysis using function `geeglm` {geepack} and the model selection using function `model.sel` {MuMIn} were carried out in R package 3.3.3. (R Development Core Team, 2017), while CA and PCA in Statistica 13 (Dell, Inc. 2016, software.dell.com).

3. Results

3.1. Variation in shell morphology between two snail forms

The average and the range of all shell measurements are presented in Appendix A.2 and the box-plots of the most discriminative variables are shown in Fig. 1. *T. hispidus* and *T. sericeus* are characterized by different shell morphologies. *T. hispidus* showed significantly statistically smaller shell height (H), body whorl height (bwH), aperture height (h), aperture width (w), and shell height/width ratio (H/W) but larger umbilicus major and minor diameters (U and u), number of whorls (whl) and umbilicus relative diameter (U/D) than *T. sericeus*. The greatest difference refers to U, u and U/D. This denotes that *T. hispidus* has usually more flattened shells with larger umbilicus diameter as well as umbilicus relative diameter. Representative 'typical' shells of both phenotypes are shown in Fig. 2.

The analysis of hair durability revealed that hairs were present in 85% individuals of *T. sericeus*, while specimens with hairs constituted no >27.5% in *T. hispidus*. This feature occurs to be associated with the precipitation level because forms with hairs were most often found in sites characterized by significantly ($p < 0.001$, Wilcoxon-Mann-Whitney test) higher values of six out of eight precipitation or moisture parameters (BIO13, BIO14 and BIO16 to BIO19).

The significant difference between these two snails in shell morphology is well confirmed by.

the discriminant analysis with canonical analysis, which produced one discriminant function (root) with p -value < 0.0001. Its distribution is presented in Fig. 3. These two snail forms show distinct almost non-overlapping groups. The greatest contribution to the discriminant function, according to the standardised function coefficients, is recorded for shell height (H) (–3.6), body whorl height (bwH) (2.4), umbilicus major diameter (U) (–1.7) and shell width (W) (1.3). The factor structure coefficients indicate that the discriminant function is negatively correlated with umbilicus major diameter (U) (–0.71) and umbilicus minor diameter (u) (–0.70), while positively with shell height/width ratio (H/W) (0.41), body whorl height (bwH) (0.26), and shell height (H) (0.21).

3.2. Differentiation of habitats occupied by two snail forms

The basic statistics of all environmental parameters that were used to characterise habitats occupied by *T. hispidus* and *T. sericeus* are included in Appendix A.3, while the most discriminative in Fig. 4. Generally, habitats of *T. sericeus* are characterized by greater forestation, and some precipitation parameters (BIO14 and BIO18), whereas habitats of *T. hispidus* show larger illumination and some other temperature parameters (BIO5, BIO9 and BIO11). These parameters show >5% difference between these habitats of the two forms.

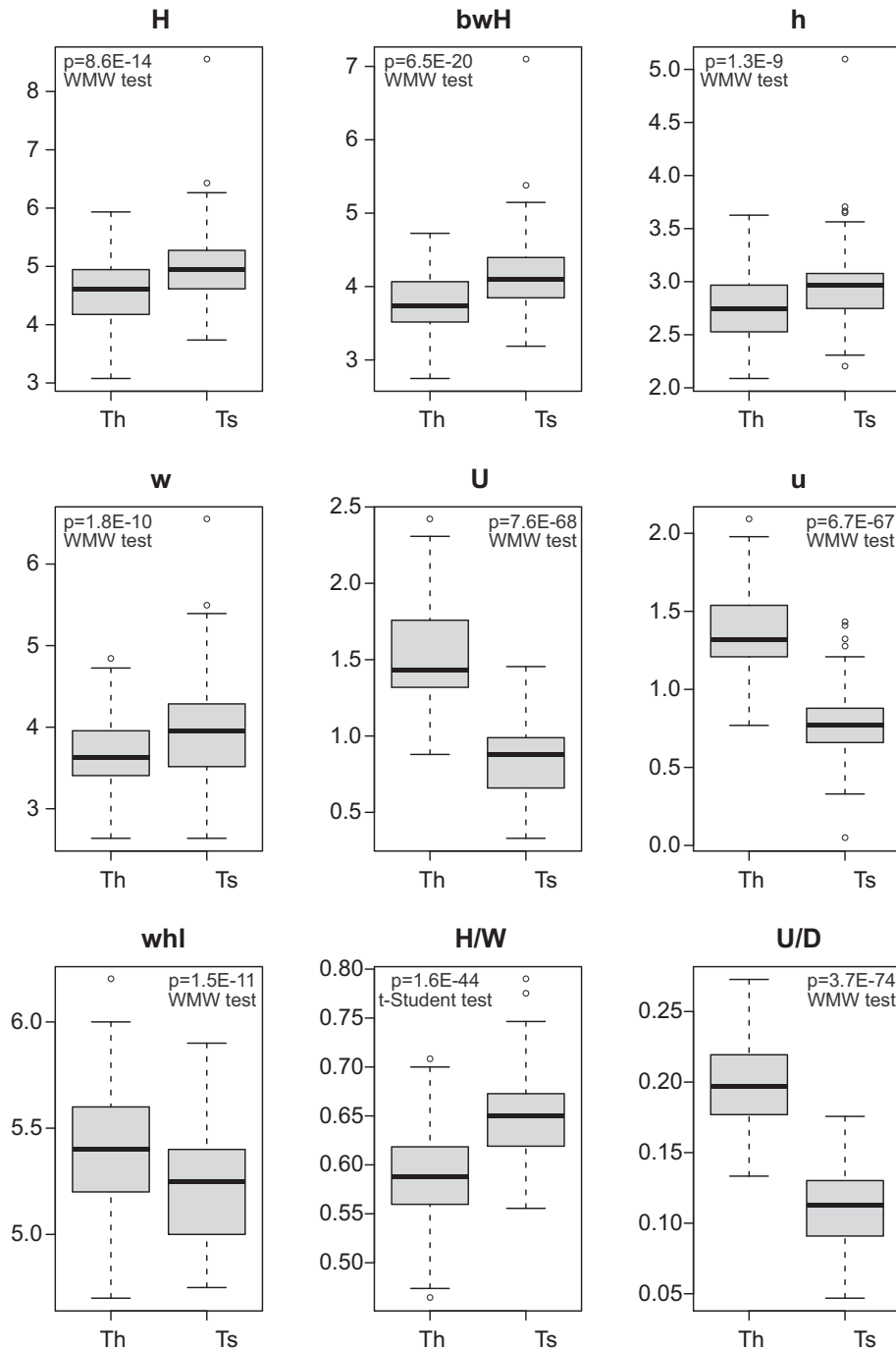


Fig. 1. Box-plots of selected discriminative shell measurements for *T. hispidus* (Th) and *T. sericeus* (Ts). The thick line indicates median, the grey box shows quartile range and the whiskers denote the range without outliers. *P*-values and test type are shown. WMW test means Wilcoxon-Mann-Whitney test.

Among the studied parameters six variables appeared to differentiate statistically at the 0.05 level the habitats typical of *T. hispidus* and *T. sericeus* (Fig. 4). The most important factors are related with the absolute temperature, isothermality, precipitation of driest and warmest periods as well as the level of forestation associated with light availability. Isothermality may be interpreted as stability of environment in terms of the daily vs. annual temperature range.

The discriminant analysis with canonical analysis revealed a significant difference between the two habitats and proposed one discriminant function (root) with $p = 0.0007$. The two sets are clearly separated, although they partially overlap, with regard of distribution of this canonical root (Fig. 5). Interestingly, the distributions are multi-

modal, which implies that some snails of one ecophenotype were found in other habitats, including those typical of the other snail. According to the standardised function coefficients, BIO12 (−8.2), BIO2 (−4.0), BIO3 (2.1), BIO18 (4.0), BIO7 (4.1) and BIO14 (5.6) had the greatest contribution to the discriminant function. The most negative correlation coefficients with the discriminant function were revealed for temperature (−0.32), BIO3 (−0.29) and light (−0.26) while the highest positive coefficients showed forestation level (0.26), BIO18 (0.23) and BIO14 (0.21). These results imply that *T. sericeus* lives in forested and shady habitats with larger precipitation, while *T. hispidus* inhabits more open and dry environments with greater illumination. Considering temperature parameters, *T. sericeus* tolerates colder

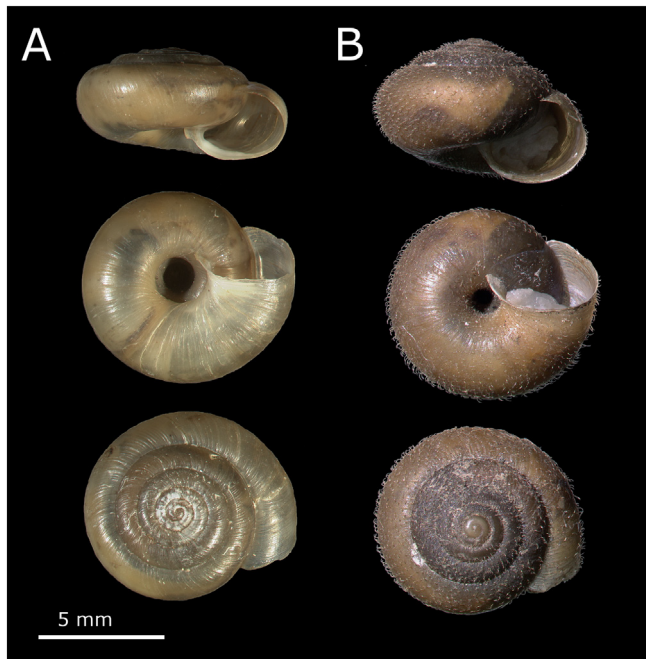


Fig. 2. Shells of 'typical' *T. hispida*, a specimen from Grande Chartreuse, France (A) and 'typical' *T. sericeus*, a specimen from Reischenau, Germany (B). It is clearly visible that the *T. hispida* shell is more flattened and has much larger umbilicus than the shell of *T. sericeus*.

conditions with larger oscillations of the day-night temperatures relative to the annual oscillations, while *T. hispida* prefers warmer habitats with more stable temperatures.

3.3. Selection of uncorrelated environmental variables

Since environmental variables were mostly intercorrelated, for further study we selected only such variables that significantly differentiated the snail populations and did not show significant correlations between themselves, i.e. absolute temperature, isothermality, precipitation of the driest (BIO14) and the warmest (BIO18) periods, forestation level and light availability. Among these traits, Spearman's correlation coefficient turned out significant only between BIO14 and BIO18 (0.58) as well as between forestation and light (−0.49). Therefore, we chose from these pairs such parameter that more significantly differentiated the studied populations, i.e. precipitation of the warmest quarter

and light availability, and added them to the final set including also absolute temperature and isothermality.

Moreover, to extract uncorrelated sets of parameters from environmental variables, we applied Principal Component Analysis. The first four principal components explained 33.5%, 26.7%, 10.3% and 8.4% of variance, respectively, which gives in total almost 79% of the explained variance. The components have sensible interpretation and relationships with the original variables. In the interpretation, we included only these variables whose absolute value of correlation coefficient with components was larger than 0.7 and the absolute value of component coefficients was >0.25.

The first principal component was positively correlated with parameters describing annual precipitation (BIO12) as well as precipitation in the coldest (BIO19) and the driest periods (BIO14, BIO17), while negatively correlated with seasonality of temperature (BIO4) and precipitation (BIO15) (Appendix A.4). Accordingly, corresponding coefficients of this component showed relatively large values (Appendix A.5). The second component was positively related with precipitation in the warmest (BIO18) and the wettest periods (BIO13, BIO16), and negatively with the annual mean temperature (BIO1) and temperature in the driest (BIO9) and the coldest periods (BIO6, BIO11). The third component can be well described by various temperature parameters: annual mean temperature (BIO1), mean diurnal range (BIO2) and temperatures of warmest periods (BIO5, BIO10). The fourth component is correlated with parameters describing illumination (negative correlation) and forestation (positive correlation) of the habitat. Coefficients of this component are also relatively large for absolute temperature and isothermality. These four components were used in the other study on relationships between morphological features and environmental characteristics.

3.4. Relationships between shell phenotypes and habitat

Among all possible 56 correlations measured by Spearman's rho coefficient (ρ) between four selected uncorrelated environmental variables and 14 shell measurements, 31 turned out statistically significant (Appendix A.6). Thirteen measurements were significantly correlated with at least two environmental variables. Only u/U showed no significant correlations. The top negative correlations (ρ from −0.50 to −0.54) were between BIO18 (precipitation in the warmest quarter) and: umbilicus major (U) and minor diameters (u) as well as umbilicus relative diameter (U/D). The largest positive correlations (ρ from 0.31 to 0.33) showed aperture height/width ratio (h/w) with light availability as well as umbilicus relative diameter (U/D) with BIO3 (isothermality) and absolute temperature.

In the case of correlations involving four principal components (PC) representing environmental parameters, 27 turned out statistically significant (Appendix A.7). Ten measurements were significantly correlated with at least two components, in that H/W parameter with all four components. PC4 was involved in 12 correlations. PC2 showed the strongest negative correlation (ρ from −0.37 to −0.41) with absolute and relative umbilicus diameters (U, u and U/D), while the strongest positive correlation (ρ from 0.4 to 0.49) was demonstrated by PC4 with body whorl height (bwH), shell height (H) and aperture width (w).

The analyses showed that the individual morphological traits are under the influence of many environmental variables. To study the impact of combined effects by these variables on the shell characters, we carried out a multivariate regression analysis using generalized estimating equation procedure. In the case of four selected uncorrelated environmental variables, we also considered models with interactions, which occurred better fitted. The coefficients of the fitted models and its significance are included in Appendix A.8. Eight out of 14 models, for W, H, bwH, h, w, D, H/W and bwH/H, had at least one coefficient significant. The significant relationship with the morphological traits were also shown by the interaction BIO18 - light in six cases, the interaction

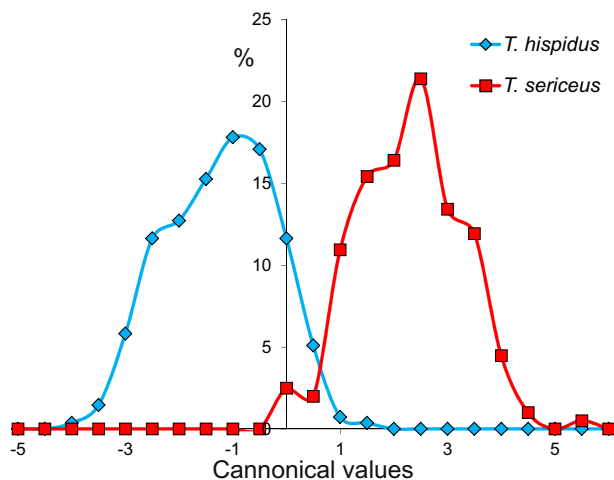


Fig. 3. Distribution of canonical values found in discriminant function analysis based on variables characterising *T. hispida* and *T. sericeus* shell morphology.

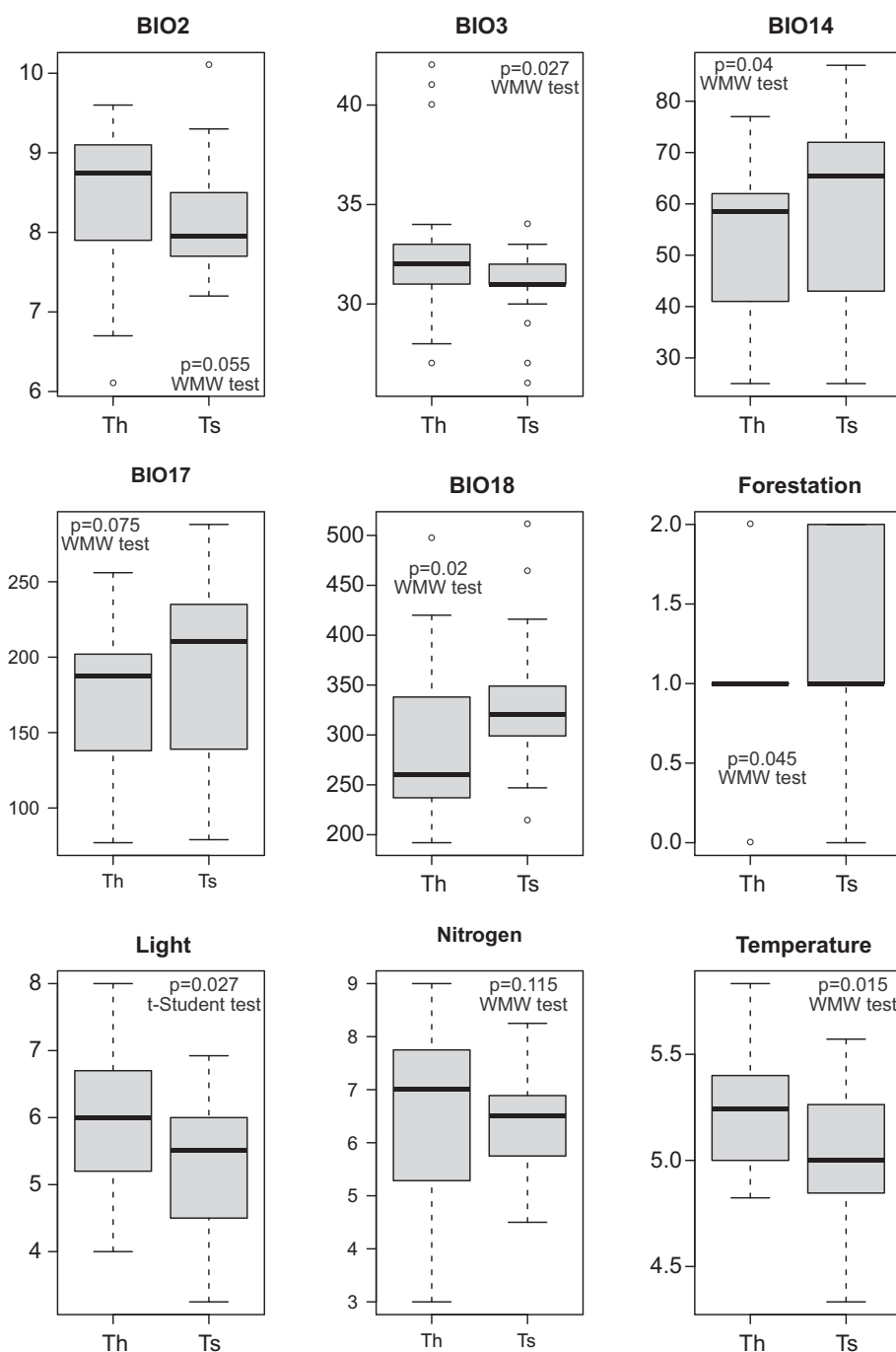


Fig. 4. Box-plots of selected discriminative environmental characterising habitats occupied by *T. hispidus* (Th) and *T. sericeus* (Ts). The thick line indicates median, the grey box shows quartile range and the whiskers denote the range without outliers. *P*-values and test type are shown. WMW test means Wilcoxon-Mann-Whitney test.

BIO3 - absolute temperature in three cases, the interaction BIO3 - temperature in two cases as well as light availability, the interaction BIO3 - temperature and the interaction temperature - light in one case.

When principal components were included as representatives of environment variables, at least one coefficient at the components was statistically significant in each model and in seven cases, at least two components were important to describe the relationships (Appendix A.9). The coefficients at the second component were significantly different from zero for 1 models including the following morphological traits: W, H, bwH, w, D, U, u, whl, H/W and U/D. PC4 showed significant coefficients in the models with W, H, bwH, h, w, D, whl and h/w, while PC1 only for the model with h/w and PC3 only for the model with bwH/H.

4. Discussion

Our analyses clearly indicate that two hairy snails *T. hispidus* and *T. sericeus* are characterized by different shell morphology. Nine out of fourteen morphological characters significantly distinguished these two forms, which are usually collected from different habitats. *T. sericeus* was mostly found in wet, shaded habitats, whereas *T. hispidus* preferred more open and dry environments with greater illumination, which often match a kind of 'rich' or eutrophic places such as nettle beds and waste places, where it can be commonly found (Anderson, 2016). *T. sericeus* is also probably more tolerant to low and variable temperatures. However, due to the habitat complexity it is

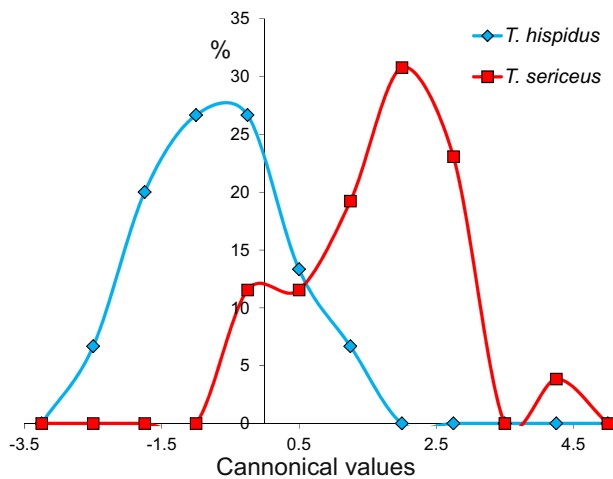


Fig. 5. Distribution of canonical values found in discriminant function analysis based on environmental variables characterising *T. hispidus* and *T. sericeus* habitats.

usually difficult to point out only single factors playing a crucial role in formation of shell in land snails. We found that many parameters, such as: light availability, forestation, isothermality as well as precipitation of driest month and warmest quarter seem to be important in the habitat preferences. The differences in morphology and habitats imply that the environmental conditions can influence the morphology. In fact, we observed statistically significant correlations between morphological traits and environmental parameters. We proved statistically for example that shells of snails living in more illuminated habitats have much lower shell height and more round aperture, while the reduced dimension of shell umbilici is associated with the increasing precipitation of warmest quarter. The significance of models and coefficients involving interactions implies a complex, e.g. synergistic or opposite influence of environmental features on morphological traits.

In agreement with these findings, *T. hispidus* easily changes its shell shape from flattened and wide-umbilicated to elevated and narrow-umbilicated in laboratory conditions, so thus it resembles *T. sericeus* merely within one generation. Most probably a constant high humidity and temperature, which change in a constant way, play an important role in determining shell shape in this case (Pročków et al., 2017b). This assumption may also refer to wild populations of *T. hispidus* and *T. sericeus*, which are associated with certain habitats: dry and sun exposed open areas versus moist and shady woodlands. Our study also shows that evaporation and humidity regimes seem to be important in the habitats occupied by these snails. In fact, precipitation variables were significantly negatively correlated with shell size and umbilicus diameter in the congener *T. striolatus*. In this species, a general pattern of shell morphology was also revealed: more globular shells have narrower umbilicus (Pročków et al., 2017a). A similar tendency is seen in *T. hispidus* complex, specifically in *T. sericeus*. This implies that phenotypic plasticity in *Trochulus* species is quite common and may have been of an ancestral origin. Molluscan shells are especially prone to environmental change, which is often the main determinant of morphological variability and has an unavoidable impact on development of morphological structures. For example in hygromiid land snail *Candidula unifasciata* shells are more depressed and have smaller apertures in areas characterized by more Mediterranean climate (Pfenninger and Magnin, 2001). The shell morphology of bradybaenid land snails *Mandarina* sp. also correlates with their microhabitat characteristics, so that taxa in wet and sheltered sites tend to have high-spired shells with small apertures, and those in dry and exposed sites have usually relatively low-spired shells with large apertures (Chiba, 2004). Sympatric species of freshwater snails *Biomphalaria* show homoplasies in their morphology in the same habitat (Plam et al., 2008) and several marine

cypraeid species demonstrate ecophenotypic variation and latitudinal clines in shell morphology (Irie, 2006).

Considering hair durability, *T. hispidus* can be described as mostly deprived of hairs and *T. sericeus* as hairy. This variation corresponds to differences in their habitats. Generally, haired forms are associated with moist environments. It was proposed that hairs of some *Trochulus* species facilitate their adherence to herbaceous food plants during foraging when humidity levels are high (Pfenninger et al., 2005). In support of this, an interesting correlation was observed in *T. hispidus* inhabiting the UK, where a nearly hairless, flattened form with very wide umbilicus was more frequent in dry calcareous pastures and coastal dunes (Anderson, 2016). Our study confirms that hair presence is associated with the precipitation level. The absence of hairs in the majority of *T. hispidus* specimens could be explained by the loss of the potential adaptive function of hairs linked to the shift from humid to dry habitats like in other *Trochulus* species (Pfenninger et al., 2005). Contrary to hair durability, hair length is very similar in *T. hispidus* and *T. sericeus* and their average length is 0.24 mm and 0.26 mm, respectively (Pročków et al., 2013).

T. hispidus is quite often found in the loess molluscan assemblages of Central Europe, which suggests that it was able to survive in the harsh climate of the glacial steppe (Ložek, 1964). This is probably the reason of highly variable ecophenotypic and genetic structure among its populations (Kruckenhauser et al., 2014), including also *T. graminicola* (Pročków et al., 2017c). Moreover, in *T. hispidus* the plasticity is also reflected in a bet-hedging strategy of life. Since it lives in the environment which may alter over short distances and even from one season to the next, its short-lived and fast-reproducing mode of reproduction may represent an optimum. Nominally this simple semelparous strategy is in fact a complex and flexible system that adjusts to very variable environmental conditions. (Pročków and Kuźnik-Kowalska, 2016).

The morphological divergence of *T. hispidus* and *T. sericeus* found in the wild populations can be explained by phenotypic plasticity and habitat-related selection, which influences the shell formation. Since *T. hispidus* and *T. sericeus* do not represent distinct biological species (Pročków et al., 2017b) and their variation is not supported by a genetic basis (Kruckenhauser et al., 2014; Pročków et al., 2017c), they should be regarded as ecophenotypes. They could evolve independently in separate phylogenetic lineages under the influence of local environmental conditions. Our results show that these snails can represent a good model to investigate interactions between the environment and phenotypic plasticity and constitute a step towards better understanding mechanisms responsible for morphological disparity. They also give insight into the relative importance of ecological factors in microevolutionary processes.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2018.02.311>.

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