

The frictional and adhesive properties of the shells of terrestrial hairy snails

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Introduction

Hair-like structures on the surface of the shell occur in different even distantly related snail families (Pfenninger *et al.*, 2005; Wade *et al.*, 2007; Allgaier, 2011), suggesting that these features evolved several times independently. The hairs have various lengths, densities, and inclinations. These characters are used as taxonomic features (Allgaier, 2011; Duda *et al.*, 2011; Duda, 2012). Among all mollusks, the hair-like structures are most common and developed in the helicoid land snails (Helicoideas. I., Stylommatophora): different distantly related genera of Hygromiidae, Helicidae, Bradybaenidae, and some other families (Gural-Sverlova & Gural, 2012). One of the best-known examples of the group containing many species with the hairy shells is genus *Trochulus* Chemnitz, 1786 (Hygromiidae) (Proćków, 2009). In all *Trochulus* species, juvenile shells are covered with hairs, in adult snails they can get lost. These structures might be evolutionary expensive and should provide a selective advantage (Emlen, 2001). It was suggested by some previous researchers that shell structures of snails provide camouflage and defense against predators and parasites (Wilson *et al.*, 2004), mechanical stability (Britton,

Abstract

Hair-like structures of the shell in some terrestrial snails have been suggested to represent an adaptive advantage. One of the recently proposed explanations was the hypothesis that the possession of hairs facilitates the snails' adherence to the leaves of the plants during foraging, when humidity levels are high. In order to obtain some reliable data to test this contact mechanics hypothesis, the present paper deals with the frictional and adhesive properties of the smooth and hairy shells of two species of terrestrial mollusks, *Trochulus hispidus* and *T. villosulus*. Additionally, actively generated traction force of the snails was measured on different substrates. The conducted experiments revealed that in wet conditions the hairiness indeed increases friction and that adhesion through the water film is high enough to retain the weight of the snail. However, possession of long hairs decreases the adhesion, in comparison with the animals with short hairs. Our data suggest that hair-like structures require more energy expenditure during locomotion and only provide the advantage of higher adhesion on wet substrates. Obtained maximal traction forces were about 30 times higher than friction forces of the shell surface dragged over the substrate. Neither wetting condition of the substrate nor surface roughness had considerable effect on the traction forces of snails. Therefore, the function of the hair-like structures seems not to be related with snail locomotion and adhesion.

1995), or involved in sexual selection (Schilthuizen, 2003). However, these statements for hairy snails have not been tested experimentally yet. Thus, the role of mollusks' hair-like structures still remains unknown.

Species with the haired shells are usually observed in snails living in moist habitats (Proćków, 2009), that is why one of the suggested functions of the hairs was to facilitate movement in wet environments (Pfenninger *et al.*, 2005). An attempt to find a correlation between habitat humidity and shell hairiness was made, and it was hypothesized that the haired shells facilitates locomotion. The hairs were also speculated to relieve surface tension and decrease the minimum force necessary to move a snail shell over a wet surface (Suvorov, 2002). However, the experimental results have shown a weak opposite effect: The presence of hairs increased this force (Pfenninger *et al.*, 2005). Therefore, the same authors proposed an alternative hypothesis, according to which haired shells bear a selective advantage by increasing the ability of the snail to adhere to the leaves during foraging. Observation showed that snails in moist habitats usually forage on herbaceous plants, while in dry habitats they avoid plants and feed on the material lying on the ground (Pfenninger *et al.*, 2005). This hypothesis also

is supported by the fact that the other families of hairy snails are found in the same habitats and have similar niches. However, this hypothesis still has to be tested.

In this study, we aimed at testing the frictional and adhesive properties of the shells of terrestrial hairy snails on different substrate roughnesses and various substrate conditions (dry vs. flooded). Additionally, we measured traction force of intact snails on these substrates. Three questions were asked: (1) Do the hairs influence shell friction on dry substrates with different roughness? (2) Do the hairs influence the shell friction on wet substrates compared to dry ones? (3) Does the traction force of the intact snail during active locomotion on various substrates depend on the presence of hairs? For this study, we have chosen two species from the genus *Trochulus*: *T. hispidus* (Linnaeus, 1758) and *T. villosulus* (Rossmässler, 1838).

Materials and methods

Animals and shells used in experiments

Trochulus hispidus is a widely distributed European species, inhabiting various damp and dry habitats from urban areas to rocky alpine sites (Proćków, 2009; Duda *et al.*, 2011). In adult stage, the shell is 5.5–10.0 mm in width and 3.0–6.7 mm in height. The snails usually possess short hairs (0.20–0.31 mm) that often are lost in adult specimens (Proćków *et al.*, 2018). *Trochulus villosulus*—a rare West-Carpathian species, that prefers humid habitats in mountains up to 1,600 m a.s.l. and in foothills. Shell width reaches 7.0–9.0 (10) mm and height—4.5–6.0 mm. Shells covered with sparse long (0.6–1.0 mm) hairs during the snail's lifetime (Proćków, 2009; Gural-Sverlova & Gural, 2012).

The shells of *T. hispidus* and *T. villosulus* were collected in western Ukraine in summertime 2016–2017 (Fig. 1a,b). Living specimens of *T. hispidus* were collected in the Ternopil' region of Ukraine in July 2017. The snails were kept in cages with food (cucumber slices and grass collected around the laboratory) under a natural temperature regime. The cages were humidified by spraying water twice per day. The animals were individually weighed on the AG 204 Delta Range scale (Mettler Toledo GmbH, Greifensee, Switzerland, $d = 0.1$ mg). Images of the shells were taken with the Keyence VR 3100 camera. Smooth shells were used as the control in all experiments: shells of *T. hispidus* without hairs (lost naturally) or shells of both species with mechanically removed hairs (it was accentuated in experiment description if hairs were removed artificially). All experiments were conducted in the laboratory conditions ($T = 21$ – 25°C , $\text{RH} = 24.7$ – 34.1%)

Friction on the leaf surface

For the experiment, we chose a generic plant substrate with a rather smooth surface at the macroscale and rather hydrophobic surface that is typical for the vast majority of plants. We decided for *Acer platanoides* and *Quercus laevis* because the upper surface of the leaves best fulfilled our requirements.

The surface roughness of the upper leaf surface was measured using white light interferometer (WLI) New View 5000 (Zygo Corporation, Middlefield, CT, USA). We cut 2×2 cm

squares out of the fresh leaf using a razor blade and fixed them on a glass slide. The roughness of the samples was measured at magnifications $\times 5$ and $\times 50$ (examined areas were 1.40×1.05 mm and 0.14×0.11 mm, respectively).

The surface of the *Q. laevis* leaf has rather big swells of irregular shape (Figs S5 and S6) with mean effective roughness (R_a) of 9.53 ± 1.17 μm and maximum height (R_z) of 132.97 ± 17.20 μm (WLI lens $\times 5$, $n = 9$). The swells are covered with the smaller oval-shaped bumps (Figs S7 and S8), which exhibit a mean roughness (R_a) of 1.42 ± 0.15 μm and a maximum height (R_z) of 14.57 ± 5.56 μm (WLI lens $\times 50$, $n = 9$). The leaves of *A. platanoides* have the veins that slightly tower over the leaf lamina (Figs S1 and S2). The mean effective roughness (R_a) is 7.12 ± 2.66 μm , and the maximal height (R_z) is 104.53 ± 31.24 μm (WLI lens $\times 5$, $n = 13$). The lamina has microasperities of irregular shape (Figs S3 and S4) with the roughness of 1.53 ± 0.74 μm and a maximum height (R_z) of 12.24 ± 3.58 μm (WLI lens $\times 50$, $n = 12$).

The force necessary to move *Trochulus* shells with upwards (naturally) oriented apex, with or without hairs, over dry and wet (flooded), and horizontal leaf surfaces was measured. For this experiment, both species *T. hispidus* and *T. villosulus* were used. Ten shells of each species were glued to one end of a 15–20 cm long human hair with a droplet of molten wax. The hair was fixed near the shell aperture (Fig. 2), so the shells were pulled in a similar orientation to that used during snail movement. The other end of the hair was fixed to a FORT-25 force transducer (25 g capacity; World Precision Instruments Inc., Sarasota, FL, USA). A leaf was fixed on a platform, which could move in a direction perpendicular to force sensor. A shell was placed on the leaf surface, and the platform was moved for 20 s with a constant speed of 6 mm/s. The experiments were repeated five times for each shell on both wet and dry surfaces. The 'wet' conditions were made by placing a water drop (0.5 mL) on the leaf surface with the shell placed on the water. Then, the hairs were mechanically removed to obtain smooth shells and the procedure was repeated. A new leaf was used with every new shell (five repeats of dragging per leaf, approximately 2 min). Force–time data were recorded using AcqKnowledge 3.7.0 software (Biopac Systems Ltd, Goleta, CA, USA). For each curve, the average and maximal values of the force were determined. For each condition, the differences in force needed to move the shells with or without hairs were tested for significance using ANOVA (software Sigma Stat 3.11, San Jose, CA, USA).

Adhesion measurements

The force necessary to detach *Trochulus* shells with or without hairs from wet vertical glass surface was measured. For this purpose, smooth and hairy shells of *T. hispidus* and hairy shells of *T. villosulus* were used. Five shells were used for each group. The force transducer and the software were used as described above (section Friction on the leaf surface). The human hair was fixed on the shell close to its apex. The shell was placed in the water drop of 5 μL on the vertical glass surface. The sensor was moved with the constant speed (200 $\mu\text{m/s}$)

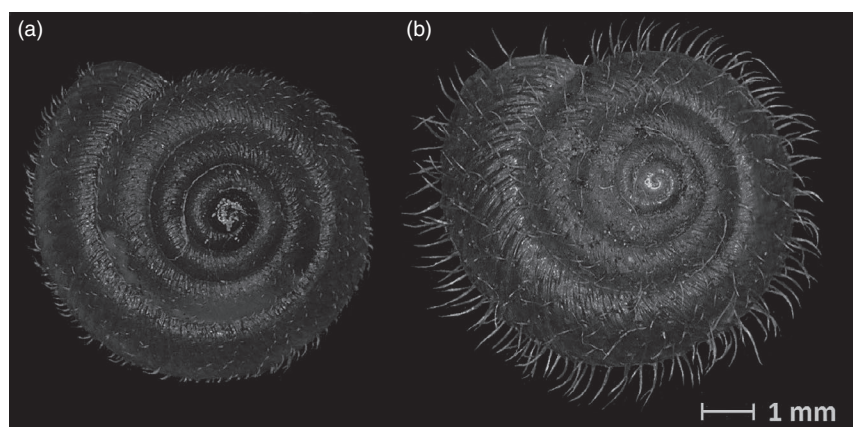


Figure 1 Microscopy images of the shells of *Trochulus hispidus* (a) and *T. villosulus* (b).

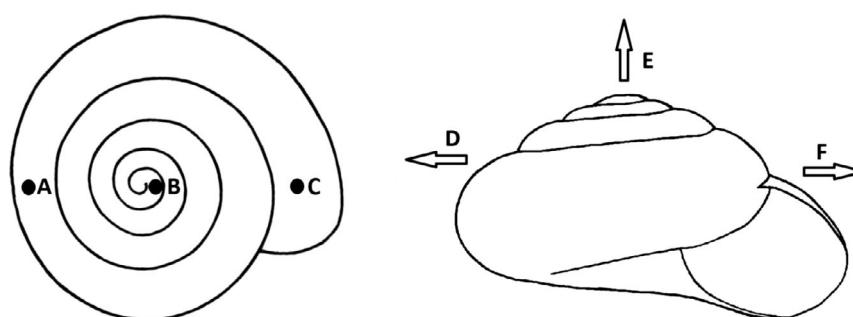


Figure 2 The spots of the shell connection to the force transducer (using the human hair) and the directions in which the force was measured during traction (A, D), adhesion (B, E), and friction (C, F) experiments.

in the direction perpendicular to the glass surface using motorized micromanipulator and the force–time curve was recorded. Five measurements per each shell were made. The force needed to separate the shell from the substrate was recorded as the highest peak of the curve.

Friction coefficient measurements on surfaces with different roughness

Three groups of shells were used for friction coefficient measurements: smooth and hairy shells of *T. hispidus* and hairy shells of *T. villosulus*. The epoxy resin (A. R. Spurr, J. Ultrastruct. Res., 1969, 26, 31–43) casts of a clean glass surface and polishing papers with different asperity sizes (polishing papers with grain sizes 3 μm and 12 μm , sandpapers of grit sizes P1200 and P320) were used as the substrates. The casts were made using a two-step molding method (Gorb, 2007). This eliminated the influence of differences in the chemical properties of different substrates, and only the effect of the surface roughness on friction was studied. Measurements were performed using an inclined plane friction tester (Berthé *et al.*, 2009). The surface of the epoxy resin casts was discharged using an ionizing blow-off gun (6115, Ion systems, Alameda, CA, USA). Shells with the apex oriented upwards were placed

on the epoxy resin cast fixed on a tilted plane, and the angle of tilt was continuously increased until the shell began to slide. The tangent of the final tilting angle is equal to the coefficient of friction μ ($\mu = \tan\theta = F_f/F_n$, F_f – friction force [N]; F_n – normal force, [N]). The measurements were performed for dry and wet shells. As the water film covered the epoxy resin casts irregularly and the capillary forces, caused by water, dramatically affected the measurements, for the measurements in the ‘wet’ conditions the shells were first placed in water for 2 min and then on filter paper for 1 min before the measurements were performed.

Traction force measurements

Prior to the experiment, one end of a 15–20 cm long human hair was fixed with a droplet of molten wax on the shell of a living snail of *T. hispidus*. The other end of the hair was fixed to a FORT-25 force transducer. Force–time data were recorded using AcqKnowledge 3.7.0 software. To investigate the effect of the surface roughness, five epoxy resin casts (smooth, and asperity sizes 3 μm , 12 μm , P1200, and P320) were used as substrates. Also, the effect of the substrate hydrophobicity was tested using two surfaces: glass (hydrophilic) and the epoxy resin replica of glass (hydrophobic) (Hosoda & Gorb, 2012).

Time–force curves were recorded when snail pulled on the hair. 'All individuals were tested on all surfaces, but as some did not move on some surfaces', and the experiments were conducted with various numbers of individuals (2–6) per surface. The generated traction force depended on both the strength of the animal (Rospars & Meyer-Vernet, 2016) and attachment to the substrate.

Results

Friction force generated on leaves

There were no significant differences between friction forces obtained on the leaves of different plant species (Kruskal–Wallis test, $P < 0.001$). Average values of friction force on the dry surfaces of leaves were significantly lower than on the flooded surfaces for all four groups of snails (smooth and hairy shells of *T. hispidus* and *T. villosulus*) (Holm–Sidak method, $P < 0.001$) (Fig. 3). Friction force on the dry surface varied from 0.05 mN to 0.75 mN, on the wet one—from 0.45 to 3.21 mN. For the dry surface, mean force values were more than 5 times lower than for the wet one (Table 1). In wet conditions, shells were wetted, and water film covered the bottom side of the shell. There was some volume of water between the shell and the leaf, and the thickness of the water film approximately depended on hairs' length.

Some differences were found between the friction forces of different snails' groups on the *Q. laevis* surface. The average force necessary to move smooth shells of *T. hispidus* was significantly lower than the forces needed to move shells of other experimental groups (*T. hispidus* intact and *T. villosulus* smooth and intact) (Holm–Sidak method, $P < 0.001$). However, a completely different situation was observed on the wet surface. Only hairy shells of *T. villosulus* required significantly higher forces to move them (Holm–Sidak method, $P < 0.001$), while other groups did not differ. The difference depended on

both factors—species and hairiness (for both effects $P < 0.001$). No differences were found between the groups on neither wet, nor dry *A. platanoides* leaf.

Adhesion measurements

Absolute values of adhesive forces were comparable to the absolute values of frictional forces obtained on the wet substrate. The values varied between 0.78 and 3.27 mN (1.28 ± 0.57 mN). The highest forces (1.67 ± 0.83 mN, $SDM = 0.039$) were achieved for the smooth shells of *T. hispidus*. Adhesion was significantly reduced for long-haired shells of *T. villosulus* (0.99 ± 0.11 mN, $SDM = 0.015$) (Kruskal–Wallis test, $P < 0.001$) (Fig. 4).

Friction coefficient (μ)

For the dry conditions, the slight increase of friction coefficient (μ) was observed with an increasing diameter of substrate asperities (Fig. 5a). The shells with long hairs (*T. villosulus*) had the lowest values (both mean and minimal) on almost all surfaces except the one with the grain size of $12 \mu\text{m}$, while friction coefficients of the hairless shells (*T. hispidus*, smooth) were the highest, except for the surface with the grit size P320. μ on the substrates with the highest roughness (P320 and P1200) differed significantly from those on the smooth substrate for all three groups of shells (Fig. 6a–c). The results obtained on the hairy shells of *T. hispidus* on the two roughest substrates were significantly higher than on the other surfaces (Fig. 6b), whereas for *T. villosulus* only values on the smooth surface differed from all the rough substrates (Fig. 6c).

On the wet surfaces, friction coefficients dramatically increased (Fig. 5b) in comparison with those obtained on dry surfaces. Friction coefficients of both hairy and smooth shells of *T. hispidus* were significantly higher on wet surfaces (Fig. 6a–b), except results for smooth shells on $12 \mu\text{m}$ and P1200. However, for *T. villosulus*, the friction coefficients appeared to be similar, except those for the smooth surface (Fig. 6c). Unlike in dry conditions, shells of *T. villosulus* in wet conditions showed the highest value on the smooth substrate. The surface with the medium roughness (grain size $12 \mu\text{m}$) represented the lowest friction coefficients regardless of the shells' hairiness. For two groups (smooth shells of *T. hispidus* and hairy ones of *T. villosulus*), the results on this substrate differed significantly from the substrate with the asperity size P320 (Fig. 6a,c). However, any other significant differences were not observed.

The shells of *T. villosulus* represented the lowest μ , which differed significantly from the results for smooth and hairy *T. hispidus* on the majority of substrates in dry conditions (except for the surface with the grain size of 3 and $12 \mu\text{m}$) (Fig. 7). For wet conditions, differences were observed on surfaces with high roughness (P1200 and P320) and a grain size of $3 \mu\text{m}$ (Fig. 7b,d,e). Friction properties of both shell types of *T. hispidus* were usually similar. Only in dry conditions, μ of hairy shells were lower on relatively smooth substrates (smooth and $12 \mu\text{m}$) (Fig. 7a,c), while in wet conditions, smooth shells had higher μ on the substrate P1200 (Fig. 7d).

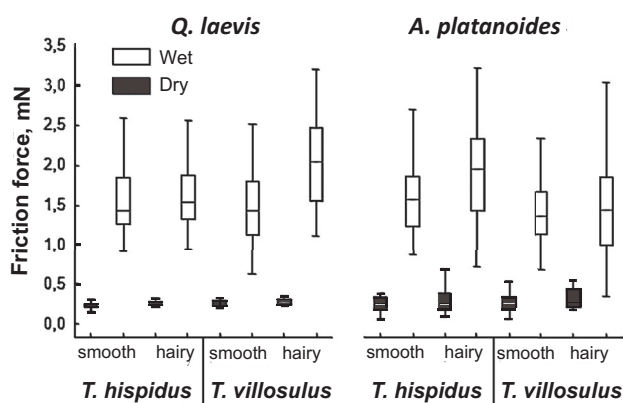
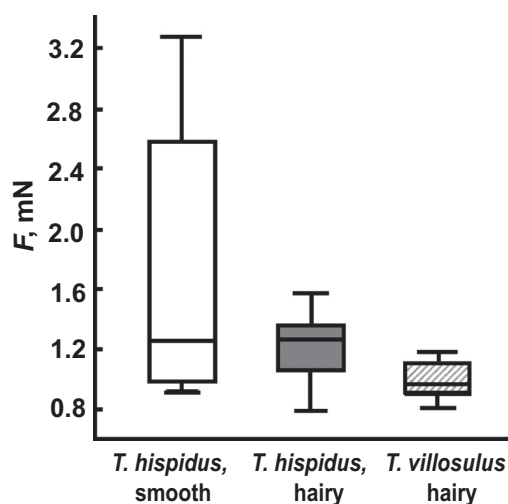


Figure 3 Average friction forces of the smooth and hairy shells of *Trochulus hispidus* and *T. villosulus* on wet and dry horizontally placed upper leaf surfaces of *Quercus laevis* and *Acer platanoides*. The ends of the boxes define the 25th and 75th percentiles, with a line at the median and error bars defining the 10th and 90th percentiles. ($N = 50$ for each group).

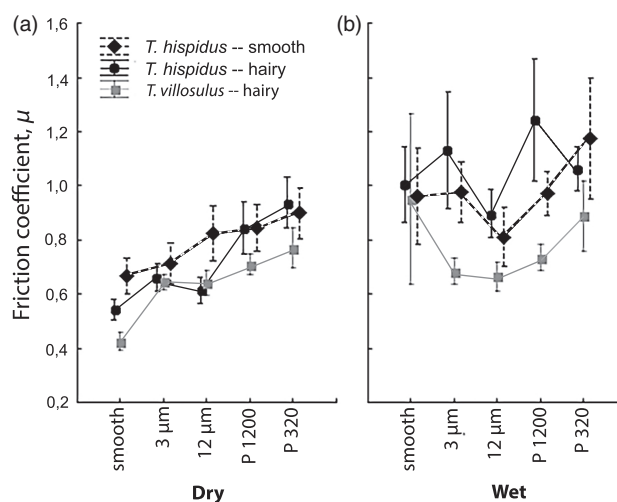
Table 1 Friction forces (mN) for smooth and hairy shells of *Trochulus hispidus* and *T. villosulus* on dry and wet leaf surfaces ($N = 50$ for each group)

Snail species	Hairiness	Wetness	<i>Acer platanoides</i>		<i>Quercus laevis</i>	
			Mean	SD	Mean	SD
<i>T. hispidus</i>	Smooth	Dry	0.254901	0.088533	0.238440	0.034113
		Wet	1.565129	0.394764	1.550476	0.395348
	Hairy	Dry	0.300533	0.154820	0.268712	0.026581
		Wet	1.900436	0.599099	1.639819	0.439938
<i>T. villosulus</i>	Smooth	Dry	0.279639	0.127527	0.268334	0.044842
		Wet	1.432855	0.391334	1.452355	0.432293
	Hairy	Dry	0.321003	0.122838	0.280402	0.041013
		Wet	1.443871	0.680340	2.074331	0.581267

**Figure 4** Adhesive force of the smooth and hairy shells of *Trochulus hispidus* and *T. villosulus* on the wet vertical glass surface. The ends of the boxes define the 25th and 75th percentiles, with a line at the median and error bars defining the 10th and 90th percentiles.

Traction force

While moving on a surface, the snail contacts it with its foot and one side of the shell (Video S1). Snails did not pull continuously when tethered, but started to pull, then relaxed and started to pull again with increasing strength. The animal usually repeated this action a couple of times, making a series of pull attempts. A typical force curve obtained in the experiments is shown in Fig. 8. After maximal effort, the snail usually changed its position: It moved in a sideward direction and then started pulling again (Video S2), producing the next group of peaks on the force curve. In such a way, a single snail generated 3–15 groups of peaks per treatment. Only the highest peak in each group of the time–force curves was used for further data processing. The traction forces achieved on the same substrates strongly varied among individuals and did not correlate linearly with body mass (m). The physiological state of the animals was considered to be the same, so the force (F) must be proportional to the physiological cross-sectional area (A) of the muscle (Hettinger, 1961). The

**Figure 5** Friction coefficient (μ) depending on substrate roughness and shell hairiness on dry (a) and on wet (b) surfaces. Vertical bars denote confidence intervals ($N = 30$ for each group).

density (ρ) of the snail's body was expected to be the same, so as $V = m/\rho$ and $A = V^{2/3}$ (Hettinger, 1961), then F should be proportional to animal mass $m^{2/3}$. Therefore, for all snails, the measured forces F were normalized on $m^{2/3}$. On the average, the obtained forces (17.79 ± 8.72 mN) were about 30 times higher than the average snail weight (0.57 ± 0.21 mN) and more than 10 times higher than the friction forces generated. The highest traction forces on the dry surface were achieved on the substrate P1200 (mean value— 102.14 ± 49.88 mN/g $^{2/3}$ and maximal— 190.6 mN/g $^{2/3}$). On the flooded surface, the highest values were achieved on the smooth substrate (mean value— 110.03 ± 47.56 and maximal— 186.56 mN/g $^{2/3}$) (Fig. 9). Lower traction was found on the flooded substrate P320 (58.7 ± 15.92 mN/g $^{2/3}$). The traction forces hardly ever varied depending on the substrate condition (dry or wet): Only on the smooth substrate were the differences significant ($P = 0.013$). The surface roughness did not affect the traction forces on dry substrates (Kruskal–Wallis test, $P = 0.064$). On the wet surface, the forces measured on the substrate P320 differed significantly from the forces on both the smooth substrate and P1200 (Kruskal–Wallis test, $P = 0.037$ and $P < 0.01$, respectively).

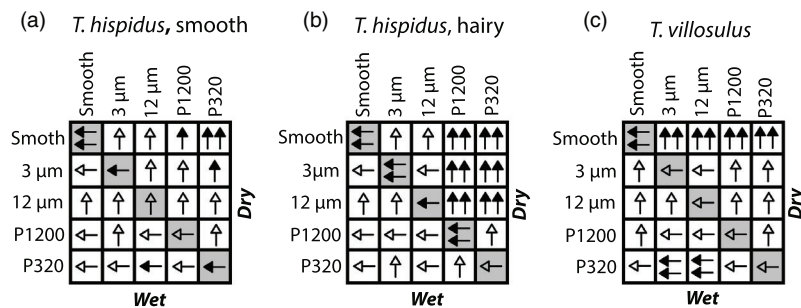


Figure 6 Comparison of the friction coefficients (μ) depending on the surface roughness in wet and dry conditions for smooth (a) and hairy (b) shells of *Trochulus hispidus* and hairy shells of *T. villosulus* (c) (Tukey test). Arrows direction indicates where the higher μ was found. Filled arrows are used, when significant differences between friction coefficients were observed (one arrow $0.01 \leq P < 0.05$; two arrows $P < 0.01$). Arrows in gray boxes show the differences between μ on the dry and wet substrate with the same roughness. Boxes above gray diagonal show differences between μ obtained on different surfaces in dry conditions. Boxes below gray diagonal, same in wet conditions. (Example. The first line of table A. Comparison of smooth wet surface with dry surfaces. **Box 1:** there were significant differences between μ on dry and wet smooth surfaces, and the values on the wet substrate were higher, $P < 0.01$; **Boxes 2-3:** No significant differences between wet smooth and dry surfaces with asperities 3 and 12 μm were observed; **Box 4:** μ on the dry surface with asperity P1200 was significantly higher than on wet smooth surface, $0.01 \leq P < 0.05$; **Box 5:** The values were higher on dry P320 surface, $P < 0.01$. First column—comparison of dry smooth surface with wet surfaces, no significant differences).

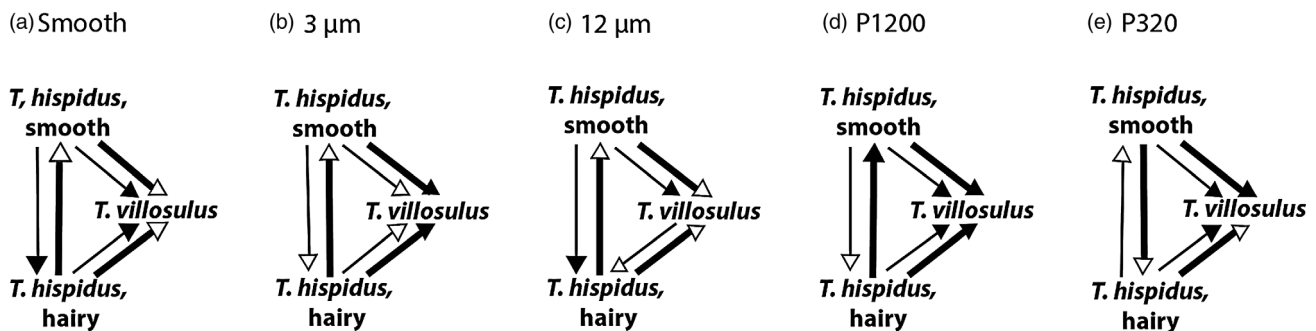


Figure 7 Comparison of the friction coefficients (μ) depending on the shell hairiness on the substrates with different roughnesses (a-e) (Holm–Sidak method). Three levels of shell hairiness are presented: no hairs (*Trochulus hispidus*, smooth), short hairs (*T. hispidus*, hairy), and long hairs (*T. villosulus*). Arrow direction indicates which species had the lowest μ . Filled arrow tips indicate significant differences. Thin arrows are used for dry conditions, thick arrows—for wet conditions. (Example. 7a, smooth surface: μ differed significantly for all three groups in dry conditions; values were lower for hairy shells (*T. hispidus* hairy and *T. villosulus*), the minimal values were observed for *T. villosulus*; Fig. 7b, $R_a = 3 \mu\text{m}$: The values for *T. villosulus* in wet conditions were significantly lower than for both other groups, and there were no significant differences between smooth and hairy *T. hispidus*).

Traction forces obtained on the smooth hydrophobic surfaces were smaller than on the hydrophilic for both flooded and dry substrates (Fig. 10). The forces on the dry epoxy resin (hydrophobic) showed the lowest values ($73.93 \pm 20.17 \text{ mN/g}^{2/3}$) and differed significantly from the forces obtained on both (dry and wet) glass surfaces (hydrophilic) ($P < 0.01$ for both cases).

Discussion

Snail locomotion

The snails have a unique mode of locomotion, which is termed *adhesive locomotion* (Denny, 1981, 1984). It is well established that snails can move and strongly adhere on a

wide variety of substrates almost regardless of the roughness of the latter, their physicochemical properties (e.g., hydrophobicity), and orientation. Snail locomotion was previously investigated in the context of physiology (Lissmann, 1945; Abbott & Lowy, 1958), biomechanics (Denny, 1981; Kobayashi et al., 2003; Chan et al., 2005; Lai et al., 2010), and tribology (Kim et al., 2010; Shirtcliffe et al., 2012). The chemical composition and the role of mucus were also widely discussed in this context (Burton, 1965; Denny & Gosline, 1980; Shirtcliffe, McHale, & Newton, 2012). Adhesion of the snail foot was reported to decrease on substrates with high roughness, and the significant effect of surface conditions (dry, wet, or oily) has previously been reported (Kim et al., 2010). The majority of abovementioned experiments was

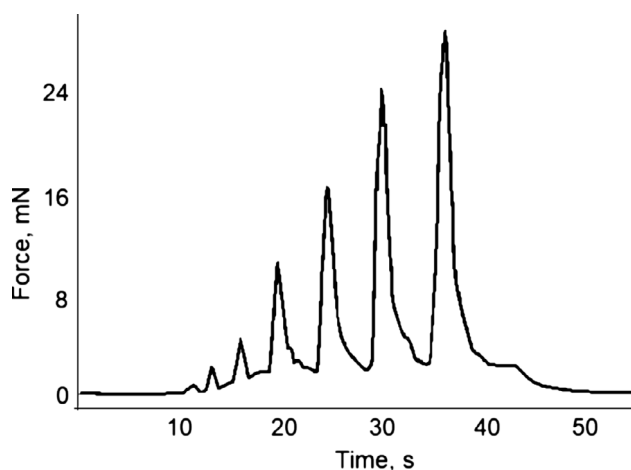


Figure 8 *Trochulus hispidus*, typical recording of traction force. The snail generated a number of peaks with increasing amplitude before reaching the maximum value. After each peak, the force has fallen down; presumably, due to the regular contraction and release of the foot muscle and sliding of the snail foot along the surface (see electronic supplementary materials, Videos S1–S3).

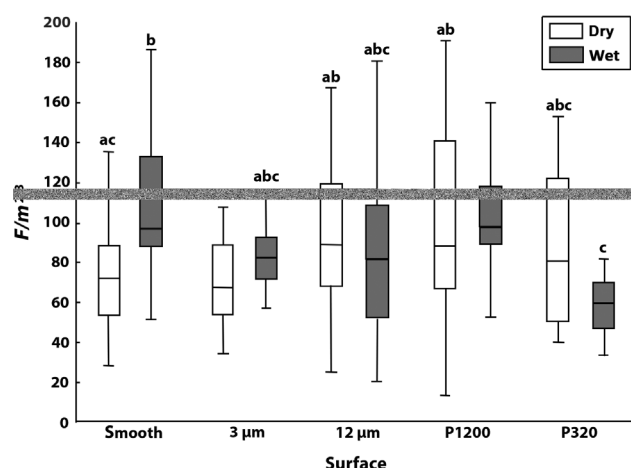


Figure 9 Normalized traction forces, generated by *Trochulus hispidus* on dry and wet substrates with different roughness. Groups indicated with different letters are significantly different. Groups having a couple of letters are not significantly different from groups indicated with at least one of those letters. The ends of the boxes define the 25th and 75th percentiles, with a line at the median and error bars defining the 10th and 90th percentiles.

performed on relatively big species whose body length can reach up to 25 cm (e.g., slugs *Deroceras reticulatum* and *Ariolimax columbianus* (Denny, 1980), snails *Helix aspersa* (Lai et al., 2010), and *H. pomatia* (Kim et al., 2010)). However, the body length of hairy snails hardly ever reaches more than 1.5 cm and is usually smaller. Small animals have relatively stronger adhesion compared with bigger animals due to the simple body-mass to body-surface relationship and have

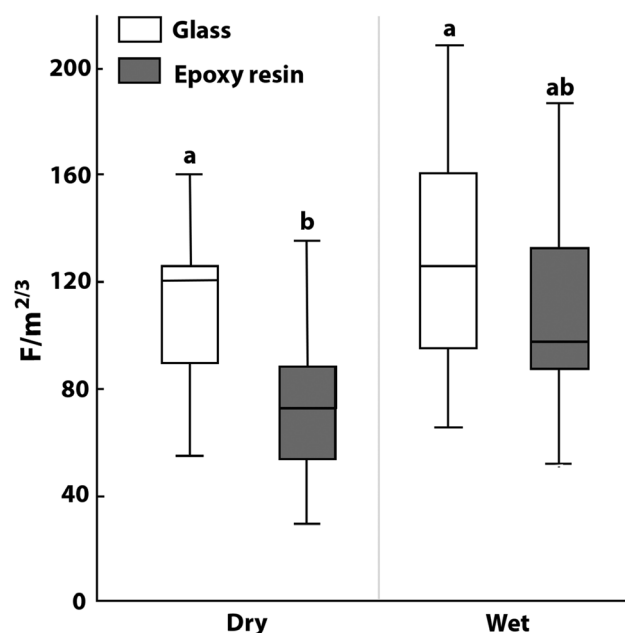


Figure 10 Normalized traction forces of *Trochulus hispidus* on dry and wet substrates with different hydrophobicity. Different letters indicate significant differences between corresponding groups. Groups marked with two letters do not differ significantly from groups with one of those letters. The ends of the boxes define the 25th and 75th percentiles, with a line at the median and error bars defining the 10th and 90th percentiles.

stronger force production in relation to their body mass. Our traction experiments revealed differences in generated forces only on very few rough substrates (section Traction force; Fig. 9), which probably has something to do with the challenge of proper contact formation between the foot and substrate. Additionally, locomotion on rough substrates might require production of a bigger volume of mucus (Kobayashi et al., 2003; Shirtcliffe et al., 2012), which can be more problematic for small animals.

In our experiments, we clearly demonstrated that maximum traction forces, generated by foot of small terrestrial snails during locomotion, are considerably higher than the highest values of friction of the shell passively dragged along the surface. The highest friction force values were obtained on wet substrates (sections Friction force generated on leaves and Traction force), and therefore, the differences between snail traction and shell friction were even stronger on dry substrates. Friction of the shell itself seems to minimally influence the actively generated traction force by the foot. However, friction caused by hairy shells may still result in some energy cost during locomotion, because snails must overcome it during their motion through terrain with obstacles.

As mentioned above, to overcome friction force, locomotion on flooded surfaces requires about twice as much energy than that on the dry substrate (section Friction force generated on leaves). Therefore, even if no significant differences were observed in measured traction forces of snails between flooded

and dry substrates, the hairy snail movement in wet conditions most likely would be costlier due to additional energetic expenditures for overcoming stronger shell friction. However, the contact area between the shell and the surface during mollusk movement is much smaller than it was during our friction experiments on shells, as a snail contacts the substrate only with one side of the shell, while in the experiment, the whole bottom side of the shell interacted with the surface. On the other hand, the friction forces may be much higher than those experimentally observed when the snail moves through litter or narrow spaces in the vegetation: In that case, lateral and upper surfaces of the shell interact with the substrate. Interestingly, snails partially solve this friction problem by lifting up and rotating the shell, in order to further minimize shell friction, when they feel that the shell is stuck to the substrate (Video S3).

Hairiness effect

The main goal of the experiments was not to measure friction and adhesive forces between shells and various substrates, but rather to estimate whether there are differences between the forces that depend on the hairiness of the shell. The presence of hair-like structures on the snail shell was previously considered to facilitate the adherence to plant surfaces when the humidity level is high (Pfenninger *et al.*, 2005). The experimental study in support of this hypothesis was previously performed on one species—*T. villosus*, so it remained unknown how differences in hair morphology of different species affect adhesion. The costlier structures are the more fitness advantage they must convey to their bearers, in order to be maintained over evolutionary time despite the significant costs associated with high hair densities (Arzt *et al.*, 2003; Labonte *et al.*, 2016). Therefore, if hair-like structures of snail shells provide some selective advantages by increasing adhesion on wet surfaces, an increase of the hair length should increase the adhesion strength. However, the results of our experiment have not shown such a correlation: The gradual increase in the friction coefficient in the series of samples *smooth*—*short hairs*—*long hairs* was not observed (section Friction force generated on leaves). Forces, needed to move shells *T. villosulus* having long hairs were significantly higher on the flooded surface, as well as in the previously published experiment (Pfenninger *et al.*, 2005). Nevertheless, this does not support the hypothesis about the biological role of the hairs' adhesive function. Shells with short hairs in *T. hispidus* did not generate significantly higher forces while being dragged in comparison with the shells of the same species without hairs. Thus, it seems that the short hair coverage does not provide any advantage in generating stronger adhesion on flooded substrates. Also, higher forces in *T. villosulus* may be also caused by an additional weight of trapped water, which was moved together with the shells attached to the sensor. *T. villosulus* has longer hairs than *T. hispidus*, and therefore, it can retain the higher amount of water on its surface. These results may lead us to two additional questions: (1) How often snails are in the wet conditions, where described effects can potentially take place and (2) whether living animals with hairy shells have the higher attachment to substrates.

Surface roughness and hair morphology

The forces obtained in the present study were about 10 times lower than in the previous experiment (Pfenninger *et al.*, 2005). A possible explanation is the effect of the substrate properties. As we did not know which plant was used, it was impossible to use the same plant species and reconstruct possible effects of the specific substrate. The type of the substrate 'smooth plant leaf' still may differ significantly from the plant we used. Various plants possess a wide range of surface textures (see review by Gorb & Gorb, 2017). Also, even leaves described as 'smooth' may be covered with waxes or moist secretions, thus having different degree of hydrophobicity. The difference in obtained results could be also due to different surface roughness, but difference was too large to be accounted for only by a difference in substrate texture (section Friction coefficient (μ); Fig. 5). It may be the case that usually hydrophobic features of the leaf surface might potentially have influenced the force (Opitz *et al.*, 2002), however, not to that extent.

The morphology of the hair impacts friction on substrates with different roughness. Short hairs of *T. hispidus* can interlock with the substrate irregularities and act like insect claws (Dai *et al.*, 2002), while long hairs of *T. villosulus*, due to their presumed lower bending stiffness, are strongly deformed under the same load, so their tips do not interlock with the surface. These shells are more likely to slide over the surface with lower friction resistance. Also, shell size and geometry may have an effect on the friction coefficient. Juvenile shells often have higher height/width ratio, so during the friction measurements they usually did not slide, but swung on the lateral side and then started to roll. Such situation often was observed for the shells of *T. villosulus*.

Humidity level effect

The thick water film or large water droplets taken from the wet substrates can cause dramatic shell weight increase, which may cause the observed differences in friction forces between dry and wet surfaces. It still remains unknown how often snails are exposed to such conditions, and which effects would be detected at lower humidity levels. When observed, Trochulin snails generally forage on leaves without visible water film, or in natural conditions, the water film is generally so thin that it contacts only hairs, but not the surface of the shell (and does not lead to an increase of the shell weight), as it was the case in our experiments. Also, the snails usually avoid heavy rain. A thin water film usually occurs on most natural surfaces after rain or morning dew and frequently forms in the moist habitats (Wolff & Gorb, 2012), where hairy snails usually occur (Freund *et al.*, 1999; Rabinovich *et al.*, 2002; Asay & Kim, 2006). Therefore, snails staying on plants may often come in contact with a thin water film.

In many cases, animal adhesion strongly differs at different environmental humidities, and in some cases capillary forces, generated by the presence of ambient water, is supposed to be the adhesion mechanism in attachment devices of different animals (Niewiarowski *et al.*, 2008; Wolff & Gorb, 2012). To generate capillary adhesion, the thickness of the water layer on the

surface has to be sufficient to build a liquid meniscus, but also cannot be too thick (Opitz *et al.*, 2002). Such a film can be easily formed in moist biotopes. Thus, it has been hypothesized that adhesion of hair-like structures of snails may be adapted for an increase of adhesion to the substrate by capillary interactions. However, the structure of snails' hairs is very simple (Proćków, 2009; Proćków, Mackiewicz, & Pienkowska, 2013): They do not possess any special thin terminal elements typical for the adhesive hairs of insects, spiders, and geckos (Gorb, 2001; Federle, 2006; Wolff & Gorb, 2012). Therefore, snails' hairs may not be able to generate adhesion in the same way as these animals do, and the humidity effects on their adhesion may be not similar. This was supported by our experiments, which clearly demonstrated an increase of adhesion on wet substrates in snails covered with hairs, when compared to the hairless snails.

The observed increase of the friction coefficient in wet conditions may be observed due to the increase of the hairs' flexibility and therefore their adaptability to the substrate, and in turn to the increase of the real contact area with the substrate. This might be the reason for the effect, which was noticed for long-haired *T. villosulus* on the smooth substrate (section Friction coefficient (μ); Fig. 6c). However, the friction coefficient of *T. villosulus* shells did not increase in wet conditions on the rough surfaces, while for smooth and short-haired shells, the friction coefficients were higher on higher roughness in wet conditions (Fig. 6a,b). Thus, the effect of the shearing capillary bridge might be an additional reason for the results obtained in our friction experiments. Also, the interaction between the shell and substrate will depend on the physicochemical properties of both surfaces, as discussed above.

Role of attachment to the substrate in snail biology

Proper attachment to the substrate might provide some selective advantages to the snails at rest, if adhesion force is sufficient for the shell retention on vertical or inverted surfaces. However, hairs decrease adhesion in the wet condition, when compared to smooth shells. When moving on vertical or inverted surfaces, the shell might come in contact with water film and hairs, which retain water more readily than the smooth shell and may contribute to increased drag during sliding locomotion. Thus, we can conclude that the selective advantages of hairy shells are presumably not related to the maintenance of adhesion and friction at rest or during locomotion. Moreover, in all *Trochulus* species, juvenile shells are covered with hairs, while adults may not be. Therefore, it may be the case that the adhesion to water film provides some advantages only for young/small snails.

Conclusions

The hair-like structure of the terrestrial snails appeared to be costly not only because they require material and energy to build them, but they also demand higher energy during locomotion in wet conditions. Traction forces of snails seem not to depend significantly on the wetness or the surface roughness. Friction does not influence the maximal traction forces actively generated by

the snails. Therefore, the hypothesis about the adhesive function of the mollusks' hairs (Pfenninger *et al.*, 2005) seems not to hold according to our data. Since hairy snails inhabit moist biotopes, shell ornamentation might potentially influence both the water exchange and thermoregulation. The hairs may condense water, as well as maintain evaporation. Additionally, the hairs still may perform such not related to humidity level function as defense against predators, maintenance of mechanical stability of the shell, and play a role in sexual selection.

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Competing interests

No competing interests declared.

Data availability statement

The averaged prey density and temperature data used as input in the models are available in Supporting Information provided with the online version of the manuscript. Raw data are not shared.

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Supporting Information

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

Figure S1. White light interferometric image of the surface of *Acer platanoides* leaf, magnification 5x.

Figure S2. White light interferometric image of the surface of *Acer platanoides* leaf, magnification 50x.

Figure S3. White light interferometric image of the surface of *Acer platanoides* leaf, magnification 50x.

Figure S4. White light interferometric image of the surface of *Acer platanoides* leaf, magnification 50x.

Figure S5. White light interferometric image of the surface of *Quercus laevis* leaf, magnification 5x.

Figure S6. White light interferometric image of the surface of *Quercus laevis* leaf, magnification 5x.

Figure S7. White light interferometric image of the surface of *Quercus laevis* leaf, magnification 50x.

Figure S8. White light interferometric image of the surface of *Quercus laevis* leaf, magnification 50x.

Video S1. The typical way of the snail's locomotion. While moving, the snail contacts the substrate with its foot and one side of the shell. The shell can be lifted a little bit.

Video S2. Sideward movements of the snail when the shell is stuck (on the video the shell is connected to the force sensor). The animal starts pulling, relaxes and moves aside, then starts to pull again with increasing strength. The snail repeats this action a couple of times, making a series of pull attempts. After the maximal effort, the snail changes its position: it moves in a sideward direction and then starts pulling again.

Video S3. The snail tries to minimize shell friction by lifting up and rotating the shell when it feels that the shell is stuck to the substrate.