



Microgeographic Morphophysiological Divergence in an Amazonian Soil Mite

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Abstract

Geographic isolation plays a major role in biological diversification. Yet, adaptive divergence also can occur with ongoing gene flow, but the minimal spatial scale required for this is unclear. Here, we hypothesized that local gradients in soil clay and water contents respectively select for anti-adherent and hypoxia-tolerant phenotypes in soil invertebrates, thereby driving intraspecific phenotypic divergence despite unlimited, passive dispersal. We tested this idea using the parthenogenetic oribatid mite *Rostrozetes ovulum*, an abundant species in tropical forest soils. We obtained 40 individuals from valleys and uplands within 4 km² of rainforest in central Amazonia, and estimated soil clay and water contents for each site. Then, we experimentally assessed submersion tolerance of each individual, measured its body size, shape and structural traits, and inferred anti-adherence from the extent of debris attached to its body. We found that morphological distance was greater between than within habitats while being independent of geographic distance, which itself was unrelated to habitat. Further, using structural equation modelling, we found that clayish soils harboured mites with fewer, larger dorsal pits that were less likely to have attached debris, consistent with an anti-adherent morphology. To a lower degree, individuals from moister soils tended to survive submersion longer, likely through anaerobiosis. These patterns could reflect phenotypic plasticity, local adaptation or some combination thereof. Altogether, they suggest that environmental gradients may trigger local-scale animal diversification in soils, contributing to the exceptional biodiversity of this substrate.

Keywords Adaptation · Functional analysis · Gradient model · Passive dispersal · Phenotypic plasticity · Sympatry

Introduction

The relative importance of geography vs. ecology in biological diversification has been highly debated (Sexton et al. 2014; Shafer and Wolf 2013). On the one hand, geographic distance can foster diversification by limiting gene flow while increasing the likelihood of contrasting selective

regimes (Fitzpatrick et al. 2009; Gillespie et al. 2012; Kisel and Barraclough 2010). In this view, environmental variation within an organism's dispersal range is generally expected to favour plasticity (Hollander 2008). On the other hand, there is increasing evidence for local adaptation at relatively small spatial scales (Richardson et al. 2014), as well as for fixation of plastic phenotypes through quantitative genetic or epigenetic mechanisms (Schlichting and Wund 2014; Vogt 2017). These findings suggest that specialization and plasticity might be complementary rather than alternative evolutionary responses to selection, in which case local environmental gradients might play a key role as selective agents (Caruso et al. 2017; Kaspari et al. 2010; MacColl 2011).

Most described species are invertebrates, most of which dwell in soils during at least some life stage (Decaëns et al. 2006). Despite this, the mechanisms underlying the evolutionary origins of these animals remain poorly understood, and their remarkable species diversity has long been considered enigmatic (Anderson 1975). The small body size of soil fauna correlates with high population density, high passive

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dispersal (e.g. through wind, water or attachment to larger animals) and short generation time, which may foster local adaptation along subtle environmental gradients (Kaspari et al. 2010). For instance, soil invertebrates are commonly challenged by natural variation in soil texture and moisture (Villani et al. 1999). Finer, clayish soils have smaller interstices (Grimaldi et al. 2003) and exert stronger adhesion than coarser, sandy soils (Khan et al. 2010), both of which could hinder microarthropod foraging (Hohberg and Traunspurger 2005). Therefore, clayish soils may select for smaller or more slender bodies capable of moving through smaller interstices, as well as for body surface depressions that decrease the contact area with the soil matrix, thus increasing anti-adherence and mobility (Rasputnig and Matischel 2010; Ren et al. 2001).

Likewise, soil waterlogging and flooding expose soil invertebrates to hypoxia (Villani et al. 1999). Thus, moister, flood-prone soils may select for higher tolerance to hypoxia or submergence. This could involve a physiological response, such as shifting to anaerobic metabolism (Adis and Junk 2002), or morphological traits. For instance, a smaller, more slender body may increase hypoxia tolerance by reducing internal oxygen diffusion time (Losi et al. 2013; Tufová and Tuf 2005). Likewise, cuticular rugosity facilitates the formation of plastron structures, air films that support underwater aerobic respiration (Marx and Messner 2012). Moreover, natural soil texture and moisture gradients can be correlated, as soil texture affects water holding capacity and often depends on topography, which in turn affects drainage (Luizão et al. 2004). Such a correlation is important as it may select for trait correlations (MacColl 2011), thus shaping further response to selection (Laughlin and Messier 2015). For instance, if soil clay and water contents correlate negatively or positively, then trait values conferring anti-adhesion and hypoxia tolerance may function as alternative or synergic adaptations, respectively.

Tropical forests have offered important insights on biological diversification (Rull 2020; Leite and Rogers 2013; Moritz et al. 2000). Yet, this knowledge has been highly biased towards vertebrates, whose diversification is likely to depend more on processes occurring at larger spatial extents. Here, we used the soil mite *Rostrozetes ovulum* (Berlese, 1908) (Oribatida: Haplozetidae) to test for morphophysiological divergence along soil gradients within 4 km² of Amazonian rainforest. This species is numerically dominant in tropical forest soils worldwide, feeds mainly on dead plant matter, and has an average life cycle of 4–5 months (Beck 1969; Pequeno et al. 2017). Also, *R. ovulum* is parthenogenetic: all individuals are female clones (Heethoff et al. 2013; Norton and Palmer 1991). In central Amazonia, there is much variation in body size (280 to 450 µm long) and shape (stouter or more slender body) among individuals, as well as in body surface structure (e.g. size and density of

pits, occurrence of grooves connecting pits) (Beck 1965). The reason is unknown, but the irregular topography of this region (40–110 m a.s.l.) creates an alternation between plateaus with clayish, well-drained soils and valleys with sandy, poorly-drained soils over a few hundreds of meters (Chauvel et al. 1987; Luizão et al. 2004). Such variation could select for morphophysiological divergence in soil-dwelling animals.

We first predicted that morphology should diverge between contrasting habitats (plateaus and valleys), but be unrelated to geographic distance between individuals due to passive dispersal. Likewise, there should be no relationship between environmental and geographic distances at this spatial extent. Next, we used structural equation modelling to investigate relationships among environment, form and function. We predicted that clayish soils should favour smaller and/or slenderer bodies with more surface depressions, which should be less likely to have attached debris (anti-adherence). Likewise, moister soils should favour smaller and/or slenderer bodies with a higher capacity to survive experimental submersion (hypoxia tolerance). Hypoxia tolerance should also increase with soil moisture, as a physiological response. Lastly, we tested whether sandier, valley soils were moister than clayish, plateau soils, which should drive a trade-off between anti-adhesion and hypoxia tolerance.

Materials and Methods

Mite and Environmental Sampling

The study was performed in ca. 4 km² of Amazonian non-flooded forest or “terra firme” area in Manaus, Northern Brazil (03° 04' 34" S; 59° 57' 30" W). Annual rainfall is 2200 mm and mean monthly air temperature usually ranges between 24 and 27 °C, with a drier, warmer season (monthly rainfall below 100 mm) from July to September (Castilho et al. 2006).

In April 2016, we sampled 17 sites over the study area. Seven sites were located in valleys (drained either by temporary or permanent streams), while the remaining transects were located in uplands at least 150 m away from any stream (Online Resource 1, Fig. S1). The distance among transects varied from 156 to 2989 m, averaging 1204 ± 653 m (mean ± SD). At each site, we recorded geographic coordinates and altitude with a handheld GPS, and randomly harvested the litter-soil interface along a 20 m-long transect down to 5 cm deep, until filling a 30 L plastic bag. This material was taken to the laboratory, where soil animals were extracted into neutral water using Berlese-Tullgren apparatus (Franklin and Morais 2006). Extracted animals

were surveyed under a stereomicroscope for living and undamaged *R. ovulum* adults, yielding 85 individuals.

In central Amazonia, soil clay content (%) increases very tightly with altitude (m) ($r = 0.94$) (Castilho et al. 2006; Chauvel et al. 1987; Luizão et al. 2004). Accordingly, we used published granulometric and topographic data from a well-studied forest reserve 3.7 km north of the study area, Reserva Ducke (Luizão 2001), to calibrate a model to predict clay content from altitude for each sampled transect ($r^2 = 0.91$, $n = 72$; Online Resource 1, Fig. S2). Soil water content (%) was measured directly for each transect using the gravimetric method during a preceding drier season (April 2014), to assess the propensity of soils to retain hypoxic conditions. Soil sampling followed the same design described above, and water content was averaged over soil cores for each transect.

Submersion experiment

Submersion tolerance was determined by subjecting collected specimens to experimental submersion in water. Each experimental unit consisted of a plastic pot (4 cm tall, 2.5 cm wide) with a 2.5 cm-thick layer of plaster of Paris as substrate. One oat flake was added to feed the mite *ad libitum* throughout the experiment. In each pot, a single mite was introduced and completely submerged with sterilized, neutral water to a depth of 0.5 cm. Experimental units were kept in a BOD chamber (12 h with light at 27 °C, and 12 h without light at 21 °C), and were monitored on alternate days to determine submersion survival time (days). Mites were suspected dead if unresponsive to tactile stimuli; in such cases, they were further monitored for 2 weeks to confirm death, which could be unambiguously determined by the simultaneous spread of all legs. Throughout the experiment, experimental units were cleaned from growing algae, and water was replaced whenever it became turbid. Some individuals were lost, probably by hiding in unforeseen fractures in the experimental substrate. Thus, submersion survival time (days) was determined for 59 individuals, which were preserved in 95% ethanol.

Morphological measurements and anti-adhesion scoring

Specimens recovered from the submersion experiment had their dorsal surfaces micrographed under an environmental scanning electron microscope (Quanta 250, FEI Company, USA), and these micrographs were used to measure several traits (Online Resource 1, Fig. S3). Body length (L) and width (W) were measured (Fig. 1) and used to estimate body mass with an allometric equation having high predictive power: $-17.17 + 3.0 \log(L + W)$ (Caruso and Migliorini 2009). Body shape was expressed as length:width ratio, so

that larger values indicated a more slender body. Surface rugosity was examined in a $50 \times 50 \mu\text{m}$ quadrat that was delimited over the top of each mite's dorsal surface, at the intersection between the longitudinal and transversal axes of the body. Within this area, pit density (i.e. number of pits) and mean pit diameter (i.e. average of four random pits) were determined. Further, we estimated the proportion of the dorsum covered by grooves connecting pits; when present, grooves always spread from the posterior to the anterior part of the dorsum, forming a diffuse network. We scored "groove cover" as an ordinal variable: (1) grooves absent; (2) grooves covering 0–33% of the dorsum; (3) grooves covering 33–66% of the dorsum; and (4) grooves covering 66–100% of the dorsum.

Anti-adhesion was inferred from the occurrence of debris attached to the body, as observed in micrographs. In general, oribatid mites look "shiny" and "clean" under the microscope, but they can carry debris (Raspotnig and Matischel 2010). Accordingly, individuals were classified as "dirty" if they had debris attached to their body, or "clean" otherwise (Online Resource 1, Fig. S4), and anti-adhesion was coded as 1 if clean or 0 if dirty. Although we could not determine the precise nature of debris—it could derive from the parent soil or from the experimental setup, e.g. plaster of Paris and/or organic matter—we judged this feature to be informative because plaster resembles the granulometry of clayish soils, and the latter also have more organic matter than sandy soils (Castilho et al. 2006).

Because of body damage and the lower quality of some micrographs, morphological traits could be reliably measured for only 40 individuals from 12 sites, with 23 individuals from seven plateaus and 17 individuals from five valleys. These individuals composed the sample analyzed in this study.

Data analysis

We assessed relationships between geographic, environmental and morphological distances among individuals using partial Mantel tests based on 999 permutations. All distances were computed as pair-wise Euclidian distances between individuals. Geographic distance was computed from geographic coordinates of collection sites in UTM. Environmental distance was computed from habitat coded as 0 (valley) or 1 (plateau), so that the resulting distance matrix represented individuals occurring either in the same or in different habitats. Morphological distance was based on the five measured morphometric traits (body mass, length:width ratio, pit diameter, pit density and groove cover), all scaled to zero mean and unit variance to have similar weight on Euclidian distance. We tested whether geographic and environmental distances were related, as well as if they affected morphological distance independently of each other. To

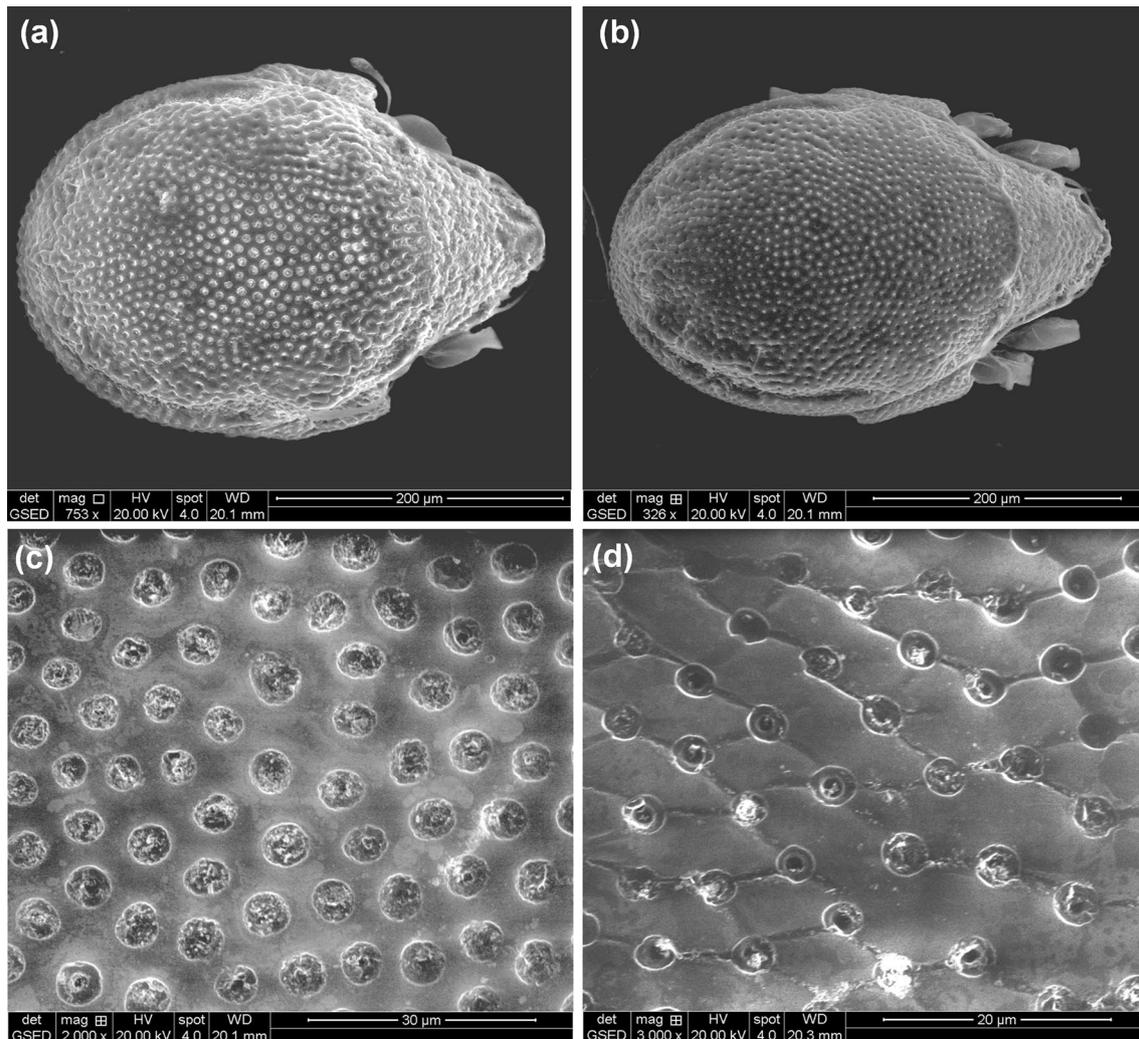


Fig. 1 Variation in body surface structure of the Amazonian soil mite *Rostrozetes ovulum*. Individuals spanned a gradient from relatively large pits at a lower density, without grooves connecting them (a, c),

to relatively small pits at a higher density, connected by grooves (b, d). Images were obtained with environmental scanning electron microscopy

visualize possible between-habitat variation in morphology, scaled traits were summarized with Principal Component Analysis (PCA). The first two principal components accounted for 80% of the variance in the original traits, and were used in further analyses.

To test for the expected relationships among environment, form and function, we used piecewise Structural Equation Modelling (Lefcheck 2016). Our path diagram included direct effects of soil clay and water contents on PC1 and PC2, representing selection on morphology, as well as correlated errors between soil clay and water contents, given their expected relationship with topography and drainage in the region. Further, we considered direct effects of morphology (PC1 and PC2) on function (anti-adhesion and submersion survival time); a direct effect of soil water content on submersion survival time, to test for a physiological

response; and correlated errors between survival time and anti-adhesion to test for a potential trade-off. Survival time was log-transformed to account for heteroscedasticity. Gaussian errors were assumed for all continuous response variables, whereas binomial errors were assumed for anti-adherence, whose path coefficient was computed following Menard (2011). Overall model fit was assessed using Fisher's *C* statistic, which tests the null hypothesis that the data were generated by the model; if so, $P > 0.05$ (Lefcheck 2016). Because individuals were clustered by transect, this could induce some autocorrelation in the data. However, the standard model assumption is of independent residuals, which may still hold if within-cluster residual variance is relatively large after accounting for fixed effects. This is important because overfitting the residual correlation structure reduces statistical power and precision and, thus, should

be avoided (Barnett et al. 2010; Guerin and Stroup 2000). Therefore, we fit one structural model with transect as random factor and another one without it, and compared them using Akaike's Information Criterion (AIC) (Barnett et al. 2010; Guerin and Stroup 2000; Lefcheck 2016). The simpler model was clearly favoured ($\Delta\text{AIC} = 6.21$), and thus we used it for interpretation.

Prior to analysis, we observed that submersion survival time had a markedly bimodal distribution: individuals either survived less or more than three weeks, with a gap between them (Online Resource 1, Fig. S5). This was supported by a direct test of the null hypothesis of unimodality (Silverman's test, $P = 0.034$). Further, bimodality was not related to habitat, as upland and valley mites both survived 20 days, on average (t test on log-transformed survival times; $t = 0.009$, $P = 0.82$). Thus, bimodality was likely caused by some other, uncontrolled factor in our experiment. Therefore, we assigned individuals to "experimental groups" surviving either less than or more than three weeks, and included this variable as another predictor of survival time to control for any confounding effect this contrast could have. Significant effects ($P < 0.05$) were visualized by plotting partial residuals against predictors whenever there was more than one predictor (Breheny and Burchett 2017). All analyses were performed in R 3.6.3 (Core Team 2020), with aid of packages "vegan" (Oksanen et al. 2019), "diptest" (Maechler 2016), "lme4" (Bates et al. 2015), "piecewiseSEM" (Lefcheck 2016) and "visreg" (Breheny and Burchett 2017).

Results

Morphological distance among *R. ovulum* mites was greater between than within habitats while controlling for geographic distance ($r = 0.25$, $P = 0.001$), but was unrelated to geographic distance while controlling for habitat ($r = -0.13$, $P = 0.98$). Also, there was no relationship between geographic and environmental distance ($r = 0.02$, $P = 0.16$). PCA revealed a major morphological gradient (PC1) comprising body surface structure (pit diameter, pit density and groove cover) and a secondary gradient of body size and shape (PC2) (Table 1). In general, individuals had either a lower density of larger pits without grooves between them, or the opposite suite of traits (Table 1; Fig. 1). In parallel, larger individuals tended to be stouter (i.e. lower length:width ratio) (Table 1). Individuals from different habitats clearly tended to diverge in morphospace, albeit with some overlap (Fig. 2).

Structural Equation Modelling revealed that the hypothesized relationships between environment, morphology and function of *R. ovulum* individuals provided a reasonable description of the data ($C_{16} = 14.36$, $P = 0.57$). Yet, the proportion of variance explained by submodels was generally

Table 1 Principal component analysis (PCA) applied to measured morphological traits of the soil mite *Rostrozetes ovulum* ($n = 40$)

Trait	Mean (range)	PC1 (62%)	PC2 (18%)
Pit diameter (μm)	4.05 (2.75–4.91)	0.47	– 0.37
Pit density (μm)	35.52 (31–49)	– 0.46	0.29
Groove cover (ordinal)	1.52 (1–4)	– 0.48	0.30
Body mass (μg)	6.06 (3.57–10.28)	0.39	0.60
Length:width ratio	1.64 (1.48–1.82)	– 0.41	– 0.55

Numbers in brackets indicate the proportion of explained variance

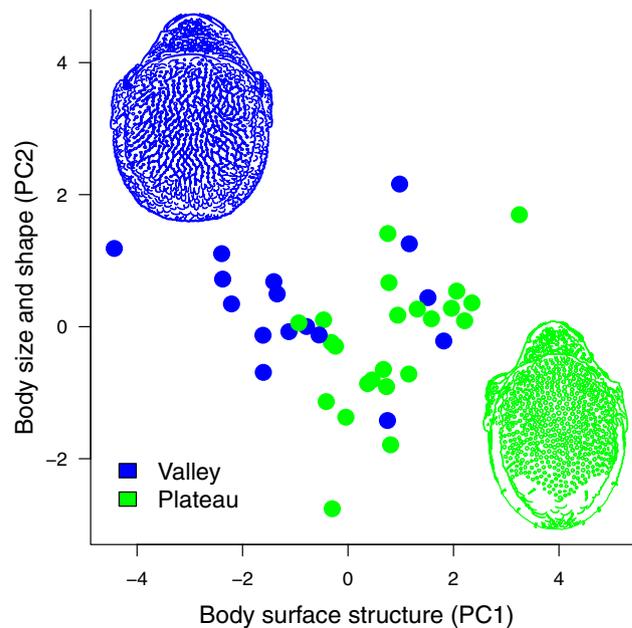


Fig. 2 Morphospace of the Amazonian soil mite *Rostrozetes ovulum*, as represented by Principal Component Analysis (PCA). Each point represents one individual ($n = 23$ for plateaus and 17 for valleys); colours represent habitats. Drawings represent the typical morphology of the mite at that particular position of the morphospace, based on the measured traits: larger, fewer pits without grooves between them in plateaus, and smaller, more numerous pits connected by grooves in valleys (see Table 1 for details)

low, and only some of the hypothesized effects were supported, all of which were positive (Fig. 3). First, soil clay content had a moderate effect on body surface structure, increasing PC1 scores (Fig. 3). Accordingly, individuals from clayish soils had larger but fewer, unconnected pits (Fig. 4a). Second, individuals with higher PC1 scores were more anti-adherent (Fig. 3), i.e. had a higher chance of having no debris attached (Fig. 4d). Third, soil water content had a weak effect on submersion survival time, with individuals from moister sites surviving somewhat longer to submersion (Figs. 3 and 4c), whereas soil water content explained a negligible part of the variance in body size and shape (PC2) (Figs. 3 and 4b).

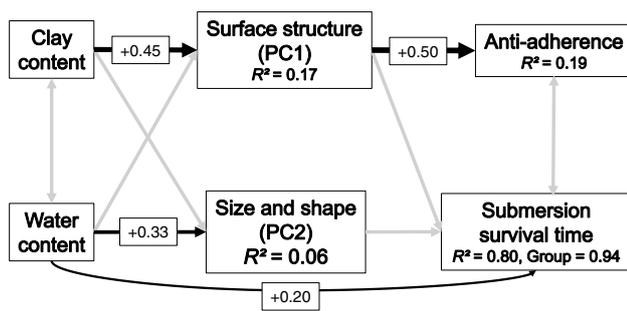


Fig. 3 Path diagram of structural equation model relating environment, phenotype and function of individuals of the Amazonian soil mite *Rostrozetes ovulum* ($n=40$). Arrows indicate hypothesized causal relationships; numbers in boxes indicate path coefficients. Unidirectional arrows indicate asymmetric causal effects; bidirectional arrows indicate correlated errors. Black arrows indicate significant effects ($P < 0.05$); grey arrows indicate otherwise. Arrow thickness is proportional to the path coefficient. The variable “experimental group” was omitted for clarity and is instead shown within the corresponding box of the response, along with its path coefficient

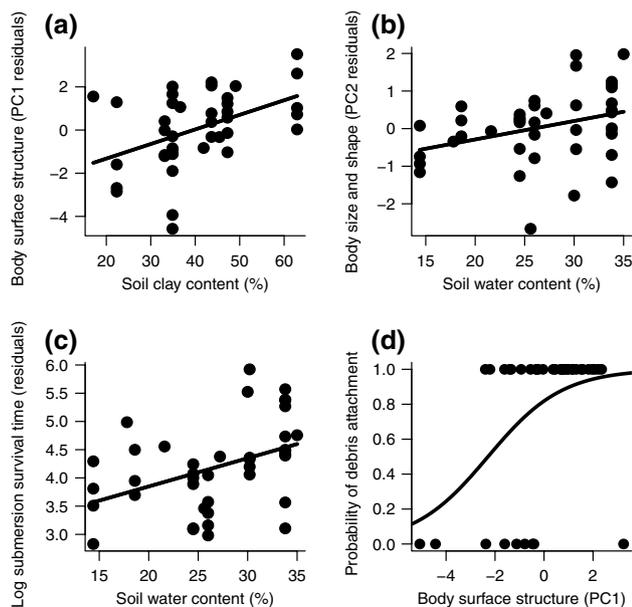


Fig. 4 Relationships between environment, phenotype and function of individuals of the Amazonian soil mite *Rostrozetes ovulum* ($n=40$). Partial residuals represent variation in the response variable after accounting for other predictors. Continuous response variables were modelled using standard multiple regression, whereas anti-adhesion (probability of no debris attachment) was modelled using logistic regression. PC: principal component

Discussion

Although hundreds of animal species can be represented in a single square meter of soil (Anderson 1975), they have been largely neglected in research on biological

diversification. Here, we found evidence for local, habitat-driven morphological divergence in the Amazonian soil mite *Rostrozetes ovulum*, which is consistent with selection for anti-adhesive morphology in clayish soils. To a lower degree, soil moisture affected *R. ovulum*'s submersion tolerance. Yet, contrary to our prediction, there was no correlation between soil clay and water contents, or a trade-off between anti-adhesion and submersion tolerance. While shallow water tables in sandy soils increase soil moisture, the high porosity of clayish soils may counteract this effect, thus weakening a correlation between these variables and their potential to drive trade-offs. Nonetheless, these findings suggest that local soil gradients can drive intraspecific morphological divergence in soil animals, a hitherto neglected phenomenon in tropical forests.

The lack of relationship between morphological and geographic distance suggests no dispersal limitation, although this interpretation assumes that morphological distances reflect genetic distances to some degree. In parallel, the lack of relationship between environmental and geographic distance points to a decoupling between geographic isolation and divergent selection. As geographic isolation is generally expected to facilitate population divergence (Sexton et al. 2014; Shafer and Wolf 2013), these findings suggest that the observed morphological divergence is maintained by divergent selection with ongoing gene flow. Further evidence comes from an observed synchrony in *R. ovulum* density fluctuations across the study area (Pequeno et al. 2017), which is consistent with local populations connected by dispersal (Koelle and Vandermeer 2005). The small size and high population density of soil invertebrates renders them highly prone to passive dispersal (Karasawa et al. 2005; Schuppenhauer et al. 2019), which is supported by reports of no relationship between genetic and geographic distances up to many kilometres (Costa et al. 2013; Pfungstl et al. 2019).

Clayish soils favoured mites with fewer but larger pits without grooves connecting them, and these were less likely to have attached debris. Clayish soils exert stronger adhesion than sandier soils (Khan et al. 2010), and surface depressions provide anti-adherence by decreasing the available area for formation of a water film between body surfaces and the soil matrix (Ren et al. 2001). Thus, clayish soils may select for larger pits that decrease attrition between the mite's body and the surrounding substrate, thus facilitating movement. However, body surface structure might play other roles, e.g. anti-predator defence (Peschel et al. 2006).

Path coefficients and variance explained across the path connecting soil texture, morphology and anti-adhesion were clearly stronger than those related to soil water content, whose weak effect on body size and shape was actually contrary to our prediction (Fig. 3). Yet, as predicted, mites from moister sites survived submersion longer, albeit this effect was also weak. This is consistent with an adaptive

physiological response to hypoxia, possibly through anaerobic metabolism (Messner et al. 1992). It has been shown that *R. ovulum* individuals from floodplains have higher submersion tolerance compared to those from non-flooded forests (Franklin et al. 2001), but our results extend this phenomenon to a much finer scale. Overall, *R. ovulum*'s morphology appeared more related to soil physical constraints, and physiology to hypoxia risk.

Sympatric morphological divergence could reflect an environmental response of plastic genotypes, habitat-specialized genotypes, or some combination thereof. Preliminary attempts of performing common garden experiments and genetic studies with *R. ovulum* proved unexpectedly difficult, so clarifying this issue will require further study. Assuming that dispersal is not limiting, selection is generally expected to favour plastic genotypes (Hollander 2008), as long as plasticity is expressed after dispersal (Thibert-Plante and Hendry 2011). However, the adult cuticle of oribatid mites is fixed (Alberti and Coons 1999), so that morphological stability of dispersing adults could facilitate local adaptation (Thibert-Plante and Hendry 2011). Indeed, we have recently shown for the same study area that the thermal sensitivity of the mite's growth rate differs between habitats, suggesting locally adapted reaction norms (Pequeno et al. 2018). If local adaptation does occur, then it also may be geologically very recent, because the marked topo-edaphic gradient of central Amazonia is only ca. 45,000 years old (Pupim et al. 2019). Consistent with this idea, the also parthenogenetic oribatid mite *Oppiella nova* (Oudemans, 1902) has diverged morphologically and genetically between adjacent grasslands and temperate forests across central Europe, although selective agents are unknown (von Saltzwedel et al. 2014). More generally, phenotypic variance among ecotypes typically has both genetic and environmental components (Chevin and Lande 2011).

Models of tropical forest diversification have emphasized geographic isolation at large spatial extents, e.g. allopatric speciation driven by habitat isolation or large rivers (Rull 2020; Leite and Rogers 2013; Moritz et al. 2000). However, recent evidence on Amazonian insects indicates significant range overlap among congeneric species (Rosser et al. 2015) and dispersal over large Amazonian rivers (Santorelli et al. Jr. 2018; Rosser et al. 2020). Alternatively, the gradient model posits environmentally driven diversification despite gene flow (Endler 1977). Indeed, there are many examples of closely-related, parapatric species inhabiting forest-savannah ecotones (Moritz et al. 2000), and increasing examples of intraspecific adaptive divergence with little or no geographic isolation in Amazonia, e.g. from trees (Misiewicz and Fine 2014) and birds (de Abreu et al. 2018). For soil invertebrates, there is anecdotal evidence for intraspecific adaptive divergence between flooded and non-flooded forests (Adis and Junk

2002). Our results extend these observations by revealing morphophysiological divergence in *R. ovulum* at a much finer spatial and environmental scale, suggesting the gradient model may apply in sympatry for this and similar organisms. Over larger areas, large rivers might be corridors rather than barriers to dispersal (Schuppenhauer et al. 2019), but this requires testing.

Our study has some caveats. First, analyzable individuals came from 12 sites only, which nonetheless were well spread over the study area (Online Resource 1, Fig. S1). Second, soil clay content was predicted from altitude rather than directly measured, but this relationship is very strong in the region Chauvel et al. 1987; Castilho et al. 2006; Online Resource 1, Fig. S2) and is unlikely to be confounded by other factors. Third, our measure of anti-adhesion (i.e. with or without attached debris) was indirect and qualitative. Quantifying debris from micrographs is more uncertain than measuring morphological traits due to the amorphous nature of debris, which is why we used a parsimonious, binary scoring. Ideally, mite mobility would be experimentally measured in different soil textures. Yet, these caveats should introduce noise in the modelled relationships (e.g. relatively low R^2) rather than create spurious ones. Hence, our structural model should be viewed as conservative.

In summary, we have revealed sympatric morphological divergence in the Amazonian soil mite, *R. ovulum*. This divergence was related to soil texture, consistent with selection for anti-adherence. To a lower degree, soil moisture affected submersion tolerance, probably through anaerobic metabolism. These patterns could reflect phenotypic plasticity, local adaptation or some combination thereof. Most importantly, they suggest that local soil gradients may trigger diversification of soil animals, possibly contributing to the exceptional biodiversity of this substrate.

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Author Contributions All authors contributed to the study conception and design. Material preparation and data collection were performed by PACLP and EF. Analysis was performed by PACLP, who also wrote the first draft of the manuscript. All authors commented on previous versions of the manuscript, and read and approved the final manuscript.

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Data Availability All data generated and analysed during this study are included in this article as a supplementary information file (Online Resource 2).

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All experiments reported in this manuscript complied with the current laws of the country in which they were performed (Brazil).

References

- Adis, J., & Junk, W. J. (2002). Terrestrial invertebrates inhabiting lowland river floodplains of Central Amazonia and Central Europe: a review. *Freshwater Biology*, 47(4), 711–731. <https://doi.org/10.1046/j.1365-2427.2002.00892.x>.
- Anderson, J. M. (1975). The enigma of soil animal species diversity. In J. Vanek (Ed.), *Progress in Soil Zoology* (pp. 51–58). Prague: Academia.
- Barnett, A. G., Koper, N., Dobson, A. J., Schmiegelow, F., & Manseau, M. (2010). Using information criteria to select the correct variance-covariance structure for longitudinal data in ecology. *Methods in Ecology and Evolution*, 1(1), 15–24. <https://doi.org/10.1111/j.2041-210x.2009.00009.x>.
- Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1), 1–48. <https://doi.org/10.18637/jss.v067.i01>.
- Beck, L. (1965). Über Variabilität und Wertigkeit morphologischer Merkmale bei adulten Oribatiden (Arachnida, Acari). *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft*, 508, 1–64.
- Beck, L. (1969). Zum jahreszeitlichen Massenwechsel zweier Oribatidenarten (Acari) im neotropischen Überschwemmungswald. *Verhandlungen der Deutschen Zoologischen Gesellschaft*, 32, 535–540.
- Breheny, P., & Burchett, W. (2017). Visualization of regression models using visreg. *R package* (pp. 1–15). <http://myweb.uiowa.edu/pbreheny/publications/visreg.pdf>.
- Caruso, T., & Migliorini, M. (2009). Euclidean geometry explains why lengths allow precise body mass estimates in terrestrial invertebrates: the case of oribatid mites. *Journal of Theoretical Biology*, 256(3), 436–440. <https://doi.org/10.1016/j.jtbi.2008.09.033>.
- Caruso, C. M., Martin, R. A., Sletvold, N., Morrissey, M. B., Wade, M. J., Augustine, K. E., et al. (2017). What are the environmental determinants of phenotypic selection? A meta-analysis of experimental studies. *American Naturalist*, 190(3), 363–376. <https://doi.org/10.1086/692760>.
- Castilho, C. V., Magnusson, W. E., de Araújo, R. N. O., Luizão, R. C. C., Luizão, F. J., Lima, A. P., & Higuchi, N. (2006). Variation in aboveground tree live biomass in a central Amazonian Forest: Effects of soil and topography. *Forest Ecology and Management*, 234(1–3), 85–96. <https://doi.org/10.1016/j.foreco.2006.06.024>.
- Chauvel, A., Lucas, Y., & Boulet, R. (1987). On the genesis of the soil mantle of the region of Manaus, Central Amazonia, Brazil. *Experientia*, 43, 234–241.
- Chevin, L. M., & Lande, R. (2011). Adaptation to marginal habitats by evolution of increased phenotypic plasticity. *Journal of Evolutionary Biology*, 24(7), 1462–1476. <https://doi.org/10.1111/j.1420-9101.2011.02279.x>.
- Core Team, R. (2020). *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.
- Costa, D., Timmermans, M. J. T. N., Sousa, J. P., Ribeiro, R., Roelofs, D., & Van Straalen, N. M. (2013). Genetic structure of soil invertebrate populations: Collembolans, earthworms and isopods. *Applied Soil Ecology*, 68, 61–66. <https://doi.org/10.1016/j.apsoil.2013.03.003>.
- de Abreu, F. H. T., Schiatti, J., & Anciães, M. (2018). Spatial and environmental correlates of intraspecific morphological variation in three species of passerine birds from the Purus–Madeira interfluvium, Central Amazonia. *Evolutionary Ecology*, 32(2–3), 191–214. <https://doi.org/10.1007/s10682-018-9929-4>.
- Decaëns, T., Jiménez, J. J., Gioia, C., Measey, G. J., & Lavelle, P. (2006). The values of soil animals for conservation biology. *European Journal of Soil Biology*, 42, S23–S38. <https://doi.org/10.1016/j.ejsobi.2006.07.001>.
- Endler, J. A. (1977). *Geographic variation, speciation, and clines*. Princeton: Princeton University Press.
- Fitzpatrick, B. M., Fordyce, J. A., & Gavrillets, S. (2009). Pattern, process and geographic modes of speciation. *Journal of Evolutionary Biology*, 22(11), 2342–2347. <https://doi.org/10.1111/j.1420-9101.2009.01833.x>.
- Franklin, E., Morais, J. W., & Santos, E. M. R. (2001). Density and biomass of Acari and Collembola in primary forest, secondary regrowth and polycultures in central Amazonia. *Andrias*, 15, 141–153.
- Franklin, E., & de Morais, J. W. (2006). Soil mesofauna in central Amazon. In F. M. S. Moreira, J. O. Siqueira & L. Brussaard (Eds.), *Soil biodiversity in Amazonian and other Brazilian ecosystems* (pp. 142–162). Oxfordshire: CABI Publishing. <https://doi.org/10.1079/9781845930325.0142>.
- Gillespie, R. G., Baldwin, B. G., Waters, J. M., Fraser, C. I., Nikula, R., & Roderick, G. K. (2012). Long-distance dispersal: A framework for hypothesis testing. *Trends in Ecology and Evolution*, 27(1), 47–56. <https://doi.org/10.1016/j.tree.2011.08.009>.
- Grimaldi, M., Teixeira, W. G., & Huwe, B. (2003). Soil structure. In G. Schroth & F. L. Sinclair (Eds.), *Trees, crops and soil fertility: concepts and research methods* (pp. 191–208). Wallingford: CABI Publishing.
- Guerin, L., & Stroup, W. W. (2000). A simulation study to evaluate PROC MIXED analysis of repeated measures data. *Conference on Applied Statistics in Agriculture*. <https://doi.org/10.4148/2475-7772.1249>.
- Heethoff, M., Bergmann, P., Laumann, M., & Norton, R. A. (2013). The 20th anniversary of a model mite: A review of current knowledge about *Archegozetes longisetosus* (Acari, Oribatida). *Acarologia*, 53(4), 353–368. <https://doi.org/10.1051/acarologia/20132108>.
- Hohberg, K., & Traunspurger, W. (2005). Predator-prey interaction in soil food web: Functional response, size-dependent foraging efficiency, and the influence of soil texture. *Biology and Fertility of Soils*, 41, 419–427. <https://doi.org/10.1007/s00374-005-0852-9>.
- Hollander, J. (2008). Testing the grain-size model for the evolution of phenotypic plasticity. *Evolution*, 62(6), 1381–1389. <https://doi.org/10.1111/j.1558-5646.2008.00365.x>.
- Karasawa, S., Gotoh, K., Sasaki, T., & Hijii, N. (2005). Wind-based dispersal of oribatid mites (Acari: Oribatida) in a subtropical forest in Japan. *Journal of the Acarological Society of Japan*, 14(2), 117–122. <https://doi.org/10.2300/acari.14.117>.
- Kaspari, M., Stevenson, B. S., Shik, J., & Kerekes, J. F. (2010). Scaling community structure: How bacteria, fungi, and ant taxocenes differentiate along a tropical forest floor. *Ecology*, 91(8), 2221–2226.
- Khan, M. A. M. A., Qaisrani, R., & Li, J.-Q. (2010). The techniques of reducing adhesion and scouring soil by bionic: Review of literature. *The 3rd International Conference of Bionic Engineering*, 3(July), 41–50.

- Kisel, Y., & Barraclough, T. G. (2010). Speciation has a spatial scale that depends on levels of gene flow. *American Naturalist*, 175(3), 316–334. <https://doi.org/10.1086/650369>.
- Koelle, K., & Vandermeer, J. (2005). Dispersal-induced desynchronization: From metapopulations to metacommunities. *Ecology Letters*, 8(2), 167–175. <https://doi.org/10.1111/j.1461-0248.2004.00703.x>.
- Laughlin, D. C., & Messier, J. (2015). Fitness of multidimensional phenotypes in dynamic adaptive landscapes. *Trends in Ecology and Evolution*, 30(8), 487–496. <https://doi.org/10.1016/j.tree.2015.06.003>.
- Lefcheck, J. S. (2016). piecewiseSEM: Piecewise structural equation modelling in R for ecology, evolution, and systematics. *Methods in Ecology and Evolution*, 7(5), 573–579. <https://doi.org/10.1111/2041-210X.12512>.
- Leite, R. N., & Rogers, D. S. (2013). Revisiting Amazonian phylogeography: Insights into diversification hypotheses and novel perspectives. *Organisms Diversity and Evolution*, 13(4), 639–664. <https://doi.org/10.1007/s13127-013-0140-8>.
- Losi, V., Moreno, M., Gaozza, L., Vezzulli, L., Fabiano, M., & Albertelli, G. (2013). Nematode biomass and allometric attributes as indicators of environmental quality in a Mediterranean harbour (Ligurian Sea, Italy). *Ecological Indicators*, 30, 80–89. <https://doi.org/10.1016/j.ecolind.2013.01.034>.
- Luizão, F. (2001). Granulometria do Solo de 72 Parcelas Permanentes na Reserva Ducke. Programa de Pesquisa em Biodiversidade (PPBio). *DataOne*. <https://search.dataone.org/view/fecosta.31.3>.
- Luizão, R. C. C., Luizão, F. J., Paiva, R. Q., Monteiro, T. F., Sousa, L. S., & Kruijt, B. (2004). Variation of carbon and nitrogen cycling processes along a topographic gradient in a central Amazonian forest. *Global Change Biology*, 10(5), 592–600. <https://doi.org/10.1111/j.1529-8817.2003.00757.x>.
- MacColl, A. D. C. (2011). The ecological causes of evolution. *Trends in Ecology and Evolution*, 26(10), 514–522. <https://doi.org/10.1016/j.tree.2011.06.009>.
- Maechler, M. (2016). diptest: Hartigan's dip test statistic for unimodality: corrected. *R package version 0.75-7*. <https://CRAN.R-project.org/package=dipstest>.
- Marx, M. T., & Messner, B. (2012). A general definition of the term “plastron” in terrestrial and aquatic arthropods. *Organisms Diversity and Evolution*, 12(4), 403–408. <https://doi.org/10.1007/s13127-012-0088-0>.
- Menard, S. (2011). Standards for standardized logistic regression coefficients. *Social Forces*, 89(4), 1409–1428. <https://doi.org/10.1093/sf/89.4.1409>.
- Messner, B., Adis, J., & Ribeiro, E. F. (1992). Eine vergleichende Untersuchung über die Plastronstrukturen bei Milben (Acari). *Deutsche Entomologische Zeitschrift*, 39(1–3), 159–176. <https://doi.org/10.1002/mmnd.19920390124>.
- Misiewicz, T. M., & Fine, P. V. A. (2014). Evidence for ecological divergence across a mosaic of soil types in an Amazonian tropical tree: *Protium subserratum* (Bursaceae). *Molecular Ecology*, 23(10), 2543–2558. <https://doi.org/10.1111/mec.12746>.
- Moritz, C., Patton, J. L., Schneider, C. J., & Smith, T. B. (2000). Diversification of rainforest faunas: An integrated molecular approach. *Annual Review of Ecology and Systematics*, 31, 533–563. <https://doi.org/10.1146/annurev.ecolsys.31.1.533>.
- Norton, R. A., & Palmer, S. C. (1991). The distribution, mechanisms and evolutionary significance of parthenogenesis in oribatid mites. In R. Schuster & P. W. Murphy (Eds.), *The acari: reproduction, development and life history strategies* (pp. 107–136). Dordrecht: Springer.
- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlenn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2019). vegan: Community ecology package. *R package version 2.5-6*. <https://CRAN.R-project.org/package=vegan>.
- Pequeno, P. A. C. L., Franklin, E., Norton, R. A., de Morais, J. W., & Guilherme, D. R. (2017). Spatial abundance pattern of a common soil arthropod changes suddenly with season in a tropical rainforest. *Pedobiologia*, 63, 46–51. <https://doi.org/10.1016/j.pedobi.2017.07.002>.
- Pequeno, P. A. C. L., Franklin, E., Norton, R. A., & de Morais, J. W. (2018). A tropical arthropod unravels local and global environmental dependence of seasonal temperature–size response. *Biology Letters*, 14, 1–4. <https://doi.org/10.1098/rsbl.2018.0125>.
- Peschel, K., Norton, R., Scheu, S., & Maraun, M. (2006). Do oribatid mites live in enemy-free space? Evidence from feeding experiments with the predatory mite *Pergamasus septentrionalis*. *Soil Biology and Biochemistry*, 38(9), 2985–2989. <https://doi.org/10.1016/j.soilbio.2006.04.035>.
- Pfingstl, T., Wagner, M., Hiruta, S. F., Koblmüller, S., Hagino, W., & Shimano, S. (2019). Phylogeographic patterns of intertidal arthropods (Acari, Oribatida) from southern Japanese islands reflect paleoclimatic events. *Scientific Reports*, 9(1), 1–20. <https://doi.org/10.1038/s41598-019-55270-z>.
- Pupim, F. N., Sawakuchi, A. O., Almeida, R. P., Ribas, C. C., Kern, A. K., Hartmann, G. A., et al. (2019). Chronology of Terra Firme formation in Amazonian lowlands reveals a dynamic quaternary landscape. *Quaternary Science Reviews*, 210, 154–163. <https://doi.org/10.1016/j.quascirev.2019.03.008>.
- Raspočnik, G., & Matischek, T. (2010). Anti-wetting strategies of soil-dwelling Oribatida (Acari). *Acta Societatis Zoologicae Bohemicae*, 74, 91–96.
- Ren, L. Q., Tong, J., Li, J. Q., & Chen, B. C. (2001). Soil adhesion and biomimetics of soil-engaging components: A review. *Journal of Agricultural and Engineering Research*, 79(3), 239–263. <https://doi.org/10.1006/jaer.2001.0722>.
- Richardson, J. L., Urban, M. C., Bolnick, D. I., & Skelly, D. K. (2014). Microgeographic adaptation and the spatial scale of evolution. *Trends in Ecology and Evolution*, 29(3), 165–176. <https://doi.org/10.1016/j.tree.2014.01.002>.
- Rosser, N., Kozak, K. M., Phillimore, A. B., & Mallet, J. (2015). Extensive range overlap between heliconiine sister species: Evidence for sympatric speciation in butterflies? *BMC Evolutionary Biology*, 15(125), 1–13. <https://doi.org/10.1186/s12862-015-0420-3>.
- Rosser, N., Shirai, L. T., Dasmahapatra, K. K., Mallet, J., & Freitas, A. V. L. (2020). The Amazon river is a suture zone for a polyphyletic group of co-mimetic heliconiine butterflies. *Ecography*, 43, 1–11. <https://doi.org/10.1111/ecog.05282>.
- Rull, V. (2020). Neotropical diversification: Historical overview and conceptual insights. In V. Rull & A. C. Carnaval (Eds.), *Neotropical diversification: Patterns and processes* (pp. 13–50). Cham: Springer Nature Switzerland.
- Santorelli, S. Jr., Magnusson, W. E., & Deus, C. P. (2018). Most species are not limited by an Amazonian river postulated to be a border between endemism areas. *Scientific Reports*, 8, 1–8. <https://doi.org/10.1038/s41598-018-20596-7>.
- Schlichting, C. D., & Wund, M. A. (2014). Phenotypic plasticity and epigenetic marking: An assessment of evidence for genetic accommodation. *Evolution*, 68(3), 656–672. <https://doi.org/10.1111/evo.12348>.
- Schuppenhauer, M. M., Lehmitz, R., & Xylander, W. E. R. (2019). Slow-moving soil organisms on a water highway: Aquatic dispersal and survival potential of Oribatida and Collembola in running water. *Movement Ecology*, 7(1), 1–14. <https://doi.org/10.1186/s40462-019-0165-5>.
- Sexton, J. P., Hangartner, S. B., & Hoffmann, A. A. (2014). Genetic isolation by environment or distance: Which pattern of gene flow

- is most common? *Evolution*, 68(1), 1–15. <https://doi.org/10.1111/evo.12258>.
- Shafer, A. B. A., & Wolf, J. B. W. (2013). Widespread evidence for incipient ecological speciation: A meta-analysis of isolation-by-ecology. *Ecology Letters*, 16(7), 940–950. <https://doi.org/10.1111/ele.12120>.
- Thibert-Plante, X., & Hendry, A. P. (2011). The consequences of phenotypic plasticity for ecological speciation. *Journal of Evolutionary Biology*, 24(2), 326–342. <https://doi.org/10.1111/j.1420-9101.2010.02169.x>.
- Tufová, J., & Tuf, I. (2005). Survival under water - comparative study of millipedes (Diplopoda), centipedes (Chilopoda) and terrestrial isopods (Oniscidea). In K. Tajovský, J. Schlaghamerský & V. Pižl (Eds.), *Contributions to soil zoology in Central Europe I* (pp. 195–198). České Budějovice: Academy of Sciences of the Czech Republic.
- Villani, M. G., Allee, L. L., Diaz, A., & Robbins, P. S. (1999). Adaptive strategies of edaphic arthropods. *Annual Review of Entomology*, 44(32), 233–256. <https://doi.org/10.1146/annurev.ento.44.1.233>.
- von Saltzwedel, H., Maraun, M., Scheu, S., & Schaefer, I. (2014). Evidence for Frozen-Niche Variation in a cosmopolitan parthenogenetic soil mite species (*Acari*: Oribatida): PLoS ONE. <https://doi.org/10.1371/journal.pone.0113268>.
- Vogt, G. (2017). Facilitation of environmental adaptation and evolution by epigenetic phenotype variation: insights from clonal, invasive, polyploid, and domesticated animals. *Environmental Epigenetics*, 3(1), 1–17. <https://doi.org/10.1093/eep/dvx002>.

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