



# Ecological gradients and environmental impact in the forest dwelling *Haplothrips subtilissimus* (Thysanoptera: Phlaeothripidae) phenotypic variability



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## ABSTRACT

Classification of phenotypic variability in forest dwelling arthropods, especially in applied bioindication, remains difficult due to the complex and synergic ecological interactions. Ecologically, it corresponds with the concept of alternative ontogenies in order to maintain high population fitness. The research on the geobiont *Haplothrips subtilissimus* (Thysanoptera: Phlaeothripidae) morphometric plasticity alongside selected ecological gradients (temperature, humidity, food availability) and the environmental impact in the xerothermous hilly oak wood (Martinský les, SW Slovakia), have revealed that (MAN(C)OVA and Kruskal–Wallis test) the head width ( $p < 0.001$ ) and anterior pronotum width ( $p \leq 0.002$ ) as the only statistically significant variables. The Kruskal–Wallis test (non parametric ANOVA) revealed significant variation (more noticeable in males) in ecologically more diverse ecotone stands. A significant correlation ( $p < 0.001$ ) could be shown between the morphometric parameter pairs “head width” and “anterior pronotum width” for both sexes (0.729 for females and 0.822 for males) and between anterior and posterior pronotum width only for males (0.859). Pillai's statistics revealed an interaction of soil humidity and soil temperature which confirms female body parameters depend on habitat conditions ( $p = 0.011$ ). Females responded mainly to soil temperature ( $p = 0.018$ ) and food availability ( $p = 0.030$ ). Soil humidity interacts with the male's morphology (soil humidity  $p = 0.040$ ), while the relationship to food availability (Acarina) was not statistically significant ( $p = 0.350$ ). No body parameter has been significantly affected by environmental stress.

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## 1. Introduction

Bioindication has become an essential component of conservation biology, using suitable species (or their communities) to analyse the disturbance of their environment and environmental stress. Arthropods have been used as indicators of a wide range of environmental conditions since the turn of the century (Brown, 1997; Cairns and Pratt, 1993; da Rocha et al., 2010; de Moed et al.,

1997; Nelson, 2007; Wallner et al., 2013, etc.), and forest-dwelling species are no exception. Insects are thought to make good indicators because they respond quickly to environmental stress, have a short generation time, and are usually easily sampled and identified (Peck et al., 1998). Amongst many bioindicator categories detectors may provide a valuable response based on their body morphology (e.g. Spellerberg, 2005).

Morphology (body size and shape) has always been considered as an important factor which affects the patterns of inter- and intra-specific competition in ecological communities (Fedor et al., 2009, 2014; Hernández et al., 2011; Romero et al., 2014). Virtually all organisms, as well as biological processes, exhibit some degree of plasticity (e.g. Glukhov and Prokhorova, 2011; Nacua et al., 2010; Savič et al., 2008; Sukhodolskaya and Saveliev, 2014, etc.). It is defined as “phenotypic plasticity” (Ananthkrishnan, 2005),

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**Table 1**  
Detailed characteristics of the study sites (with ranking scores in brackets).

Study site	P	PV	MK	E	S	L
Average tree age (years)	80	100	15	95	95	95
Tree species diversity	High (0)	High (0)	Low (1)	High (0)	High (0)	High (0)
Tree age evenness	Low (0)	Low (0)	High (1)	Low (0)	Low (0)	Low (0)
Rotten wood in undergrowth	Absent (1)	Present (0)	Absent (1)	Absent (1)	Present (0)	Present (0)
Allochthonous tree species	Absent (0)	Absent (0)	Present (1)	Absent (0)	Absent (0)	Absent (0)
Total environmental impact	Low (1)	No (0)	Intensive (4)	Low (1)	No (0)	No (0)
Average soil/leaf litter temperature (°C)	16.4	16.5	17.1	17.5	16.4	16.4
Average soil/leaf litter humidity (%)	9	7	15	2.6	15	14
Food availability (average acarine specimens per site)	20,380	23,650	21,005	13,775	12,450	15,620

which can serve as a buffering mechanism against environmental changes (Schlichting and Pigliucci, 1998). It is affected by external conditions (West-Eberhard, 2003), where a single genome displays a tremendous ability to accommodate to a wide range of environmental conditions by often highly disparate phenotypes (Moczek, 2010), and genetically where a genome is changed (Ananthakrishnan, 2005). This occurs mainly within a generation, when variation is frequently adaptive because it allows individuals to adjust to the environment in real time (Whitman and Agrawal, 2009). Plasticity represents a complex evolved response in dealing with environmental changes, allowing organisms to maintain high fitness in the face of environmental variability (Schlichting and Pigliucci, 1995). Therefore it enables a species to enlarge its ecological niche (Ananthakrishnan, 2005). Thus, phenotypic plasticity has to be regarded not merely as an intrinsic variation, but as a fundamental characteristic of a species, subject to evolutionary pressures, and of practical concern for taxonomy or ecology (Ananthakrishnan, 2005).

Some of the most spectacular cases of polyphenic alternatives are found among insects (Moczek, 2010): Whitman and Agrawal (2009) claim that any trait can show phenotypic plasticity. In terms of the traits which can be influenced by the environment, phenotypic plasticity acts on a wide range of biological characteristics from morphology to physiology and behaviour (Ananthakrishnan, 2005; Bryan and Smith, 1956; Emlen and Nijhout, 2000; Stige et al., 2007; West-Eberhard, 2003). Body size and parameters are key traits in life history evolution (Atkinson, 1994). Populations of many thrips, for example, exhibit considerable diversity (Ananthakrishnan, 1961, 1970, 2005), where extreme forms or individuals are almost unrecognisable. Blanckenhorn (2009) states that the plasticity in body size can be caused by a variety of ecological variables, most notably food availability, predators, temperature, season length, photoperiod and humidity. Primarily, temperature has pervasive effects on all biological systems and strongly influences phenotypic plasticity e.g. in insects, which are known to differ in the sensitivity to temperature (de Jong and van der Have, 2009). Despite many theories on factors affecting intraspecific variability in thrips, none of the hypotheses has been statistically evaluated in situ to interconnect phenotypic plasticity with applied forest ecology and bioindication in the category of morphologically sensitive detectors (e.g. Spellerberg, 2005; Fränzle, 2006; Glukhov and Prokhorova, 2011).

A significant intraspecific variability of *Haplothrips subtilissimus*, a common forest dwelling thrips, has inspired us to analyse a morphometric response of individuals to selected ecological (soil temperature and humidity, food availability) and environmental (anthropogenic impact and stress) factors. Our research hints at the questions of intraspecific variability of *H. subtilissimus* morphological (morphometric) characters in the forest ecosystem, their distribution within both sexes, plasticity and mutual correlation. Before making any conclusion, we suggest that studying the phenotypic variability of soil-dwelling insects may help us to understand some of the consequences for bioindication. Moreover, the

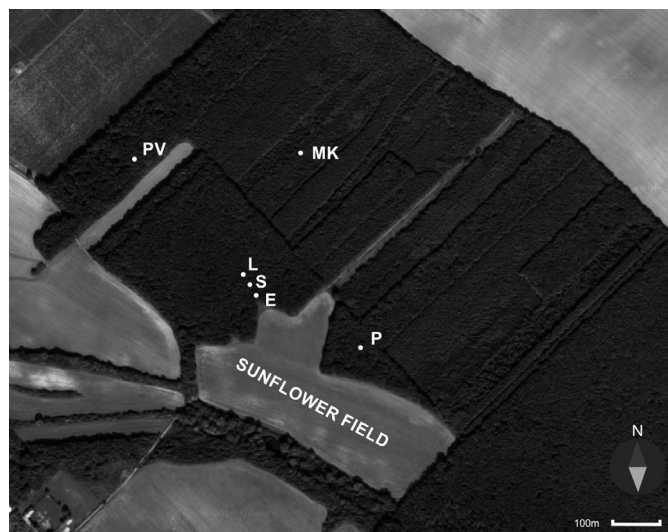


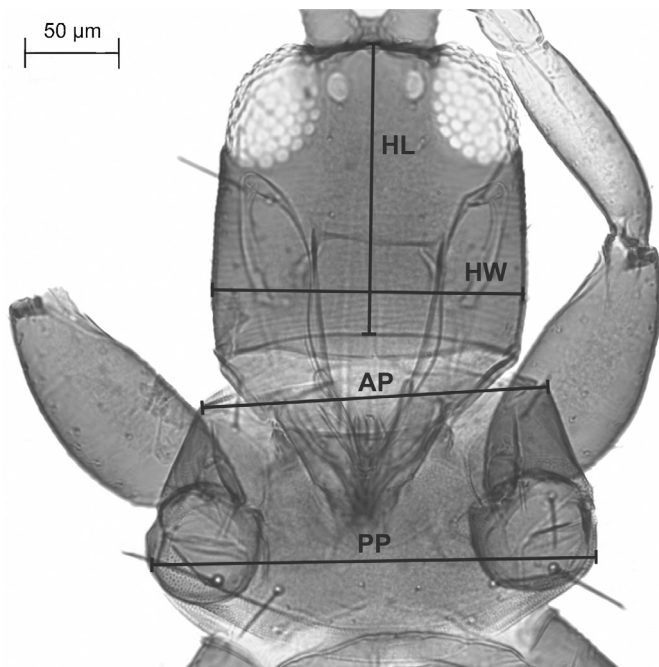
Fig. 1. Study area with the analysed sites (Google Maps, 2015).

statistical evaluation based on real, in situ (not within a laboratory) conditions, with no effect of geographic variation, offers a rather realistic view on the complex ecological interactions in forest ecosystems.

## 2. Material and methods

The study material was sampled in 6 forest stands, all of them situated in an xerothermous hilly oak wood at Martinský les (SW Slovakia, Europe; N 48° 16', E 17° 22'), synecologically more or less derived from the original *Aceri tatarici*–*Quercetum*, Zolyómi 1957 climax (Fig. 1). All the sites were selected to cover a wide spectrum of ecological conditions and environmental impact (Table 1). The locality included 3 sites situated along a transect line (providing gradients of the studied ecological variables) from an ecotone community (E), between the forest and the nearby sunflower field towards the centre of a 95 year-old forest stand (S, L) without regular forestry management. 3 more sites with a different degree of ecological conditions and environmental impact were established in the surroundings of the transect: P – 90 year-old forest with the absence of rotten wood in the undergrowth; PV – 100 year-old forest with some management interventions in progress (cutting down and afforestation), and MK – 15 year-old oak stand with a 10% abundance of black locust (*Robinia pseudacacia*) (Doričová and Fedor, 2013).

We collected thrips during the vegetation seasons of 2008 and 2009 using the soil quadrat method and sieving the fallen leaves and wood material. In total, 1016 (261 males and 755 females) *H. subtilissimus* specimens were sampled at the six sites as follows: E (269, 77: 192), S (250, 64: 186), L (112, 9: 103), P (24, 15: 9), PV (171, 50: 121), MK (190, 46: 144).



**Fig. 2.** Photo of the analysed morphometric variables (HL – length of the head, HW – width of the head, AP – anterior pronotum width, PP – posterior pronotum width).

According to our previous experience with morphometrics and phenotypic plasticity (Fedor et al., 2008, 2009, 2014), we selected 4 morphometric variables, which are well-visible and easy to measure: width of the head (HW), length of the head (HL), anterior pronotum width (AP) and posterior pronotum width (PP) analysed separately for males and females (Fig. 2) under the digital image analysis system (microscope Leica DM1000 and image analyser software Leica LAS EZ, Version 2.0.0, © 2010).

As input data, the morphometric characters as well as the ecological variables: (1) soil temperature, (2) soil humidity, (3) the abundance of Acarids (sampled by sieving leaf-litter) as the preferred prey and (4) environmental impact were used. Each study site was given a total environmental impact ranking score between 0 and 4, with 0 as the lowest impact and 4 indicating the highest impact. Ranking categorical scores (0 or 1) stands for low tree species diversity (one tree species composition), high tree age evenness (same tree age stands), absence of old rotten wood in the undergrowth and presence of allochthonous tree species (*Robinia pseudacacia*). These parameters generally express the main human-induced impact affecting natural resistance and resilience mechanisms in Central European oak woods (Doričová and Fedor, 2013).

For the comparison of medians between populations from the various sites the Kruskal–Wallis test (Siegel and Castellan, 1998) was used. Subsequently, after determining at least one sample stochastically dominating another, sample contrasts between individual sample pairs with Bonferroni procedure was used (again by using Kruskal–Wallis test, which is in this case the Mann–Whitney *U* test). In order to investigate the main factors for the *H. subtilissimus* intraspecific variability, we used the multivariate analysis of (co)variance method (MAN(C)OVA, e.g. Mertler and Vannatta, 2004) and Kruskal–Wallis test (Siegel and Castellan, 1998).

MANOVA is a procedure for comparing multivariate sample means. Since the multivariate extension of the *F*-test is not completely direct (the actual distributions of these statistics are difficult to calculate), we rely on the approximation based on the *F*-distribution so-called Pillai's statistic (trace), which is considered to be the most powerful and most robust (to violations of

assumptions) and appropriate particularly when sample sizes are unequal (Mertler and Vannatta, 2004). Since we only needed the main effects of gender (not interactions), this variable can be considered as an explanatory covariate (control variable), which in fact yields MANCOVA. The assumption of normality was checked by (multivariate) Shapiro–Wilk normality test ( $p=0.255$ ) and the assumption of homogeneity of covariance matrices was checked by Box's *M*-test ( $p$ -values  $> 0.157$ ), which in all cases indicates that the null hypothesis cannot be rejected. Consequently, MANOVA was followed by univariate ANOVAs (with Bonferroni correction procedure) for each body part to understand why MANOVA yielded significance.

We also used the function `heplot3d()` from the package {heplots} (Fox et al., 2009). This function visualises effects for pairs of variables in a hypothesis–error plot, showing the size and orientation of hypothesis variation in relation to error variation as ellipsoids (Friendly, 2006). The error ellipsoid is obtained by dividing the error sum of squares and products matrix of residuals by the error degrees of freedom, producing a data ellipsoid for the residuals. It shows main effects and interactions protruding outside the error ellipsoid (significance). The slope of the ellipses and lines (deformed ellipses) indicates a simple interpretation of the relationships depending on the specific significant effect. The orientation of the ellipsoid representing an independent variable (e.g. soil temperature) and the ordination space axis characterised by the dependent variable (e.g. head width) shows their mutual relationship. The range of independent variable values in descending order is represented by “+” and “–” (in our case only for significant variables).

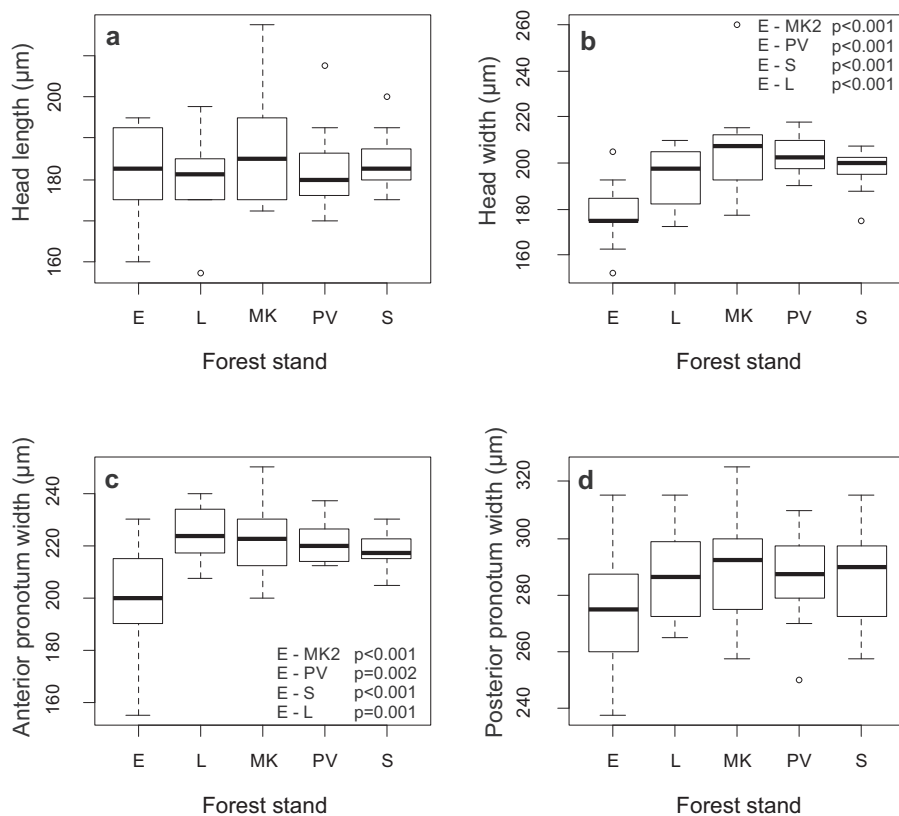
To correlate morphometric characteristics, we used Pearson's correlation coefficient as a measurement of linear dependence. Using all of the above mentioned statistical analyses, we tried to find out the statistical significance of the *H. subtilissimus* body parameters changing proportionally with the gradual changes of ecological conditions and environmental impact. For all statistical methods the R software 2.12.1. (2010) was used.

### 3. Results

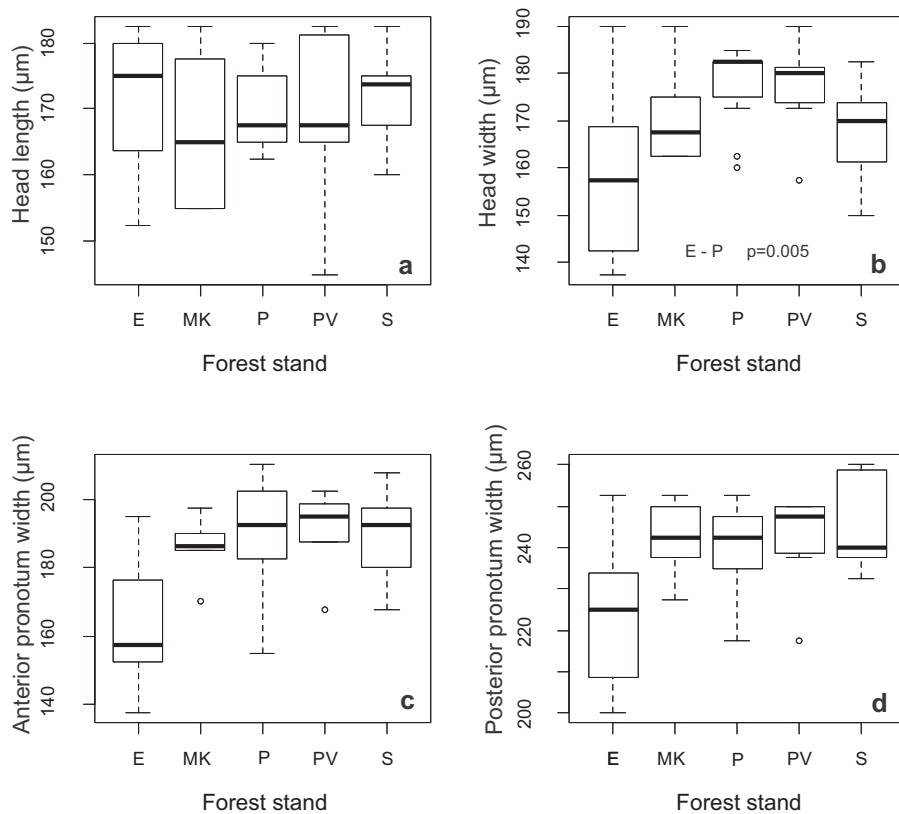
#### 3.1. Morphology and morphometrics in *H. subtilissimus* populations

Overall, the statistical evaluation of the thrips body morphometrics along the selected ecological factors indicated that the characters might differ in their response to the environment. Body size and shape varied with habitat conditions: smaller specimens occurred mostly in the ecotone zone (study site E), while their proportion rapidly decreased towards the forest interior (forest sites S, L, P, PV and also MK), being replaced by larger specimens. As shown by the Kruskal–Wallis tests, there were significant lower head width ( $p < 0.001$ ) and anterior pronotum width ( $p \leq 0.002$ ) in the ecotone (E) than in all other sample sites for females (Fig. 3), while in the case of males only head width was significant lower in the ecotone (E) compared to the old forest (site P;  $p < 0.001$ ) (Fig. 4). The other characters (head length and posterior pronotum width) remained relatively indifferent. To avoid any disputable conclusion, the sample with only 9 female specimens at the site P was excluded from the analysis.

Nonparametric ANOVA (Figs. 3 and 4) of the thrips body characters revealed high variation, more noticeable in males, especially in the population from the ecotone site (E) indicating a wider range of ecological conditions at the boundary between the forest and open sites compared to the forest interior (forest sites S, PV, P and MK). Indeed, regarding phenotypic plasticity, the ecotone offers more alternative ontogenies in order to provide high population fitness. Two of the variables measured, the head width and the anterior



**Fig. 3.** Box and whisker plots of the female morphometric characters measured in populations from different sites; *p* values from Kruskal–Wallis tests are given for significant differences between sites; a – length of the head, b – width of the head, c – anterior pronotum width, d – posterior pronotum width.



**Fig. 4.** Box and whisker plot of male morphometric characters measured in populations from different sites; *p* values from Kruskal–Wallis tests are given for significant differences between sites; a – length of the head, b – width of the head, c – anterior pronotum width, d – posterior pronotum width.

**Table 2**

Pearson's  $r$  correlation matrix of the analysed *Haplothrips subtilissimus* female morphometric variables (HL – length of the head, HW – width of the head, AP – anterior pronotum width, PP – posterior pronotum width).

Female	HL	HW	AP	PP
HL	1	0.277*	0.384***	0.419***
HW		1	0.729***	0.549***
AP			1	0.690***
PP				1

Significance levels: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , NS (not significant)  $p > 0.05$ .

**Table 3**

Pearson's  $r$  correlation matrix of the analysed *Haplothrips subtilissimus* male morphometric variables (HL – length of the head, HW – width of the head, AP – anterior pronotum width, PP – posterior pronotum width).

Male	HL	HW	AP	PP
HL	1	0.314 NS	0.271 NS	0.440*
HW		1	0.822***	0.742***
AP			1	0.859***
PP				1

Significance levels: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , NS (not significant)  $p > 0.05$ .

pronotum width, showed their simultaneous change from site to site which may hint at a very strong intercorrelation and therefore a similar proportional plasticity.

From this point of view, correlation between morphometric variables refers to any statistical relationship involving dependence between the analysed characters. This fact may show a parallel response to the ecological factors.  $p$ -Values (Figs. 3 and 4) are present only for statistically significant cases. A significant relationship has been observed between the head width and the anterior pronotum width (0.729 for females and 0.822 for males), as well as AP and PP (0.859 for males), while the lowest degree of correlation occurs between HL and HW (0.277 in females) (Tables 2 and 3).

### 3.2. Morphometric response to the ecological variables

Evaluation of data based on our analysis disproves a random distribution and indicates a dependence on ecological factors, since our results suggest that the ecotonal environment corresponds with the smallest specimens as well as with a higher variability of the body characteristics measured (Figs. 3 and 4). Along the transect into the forest interior (study sites E, S, L) with a decline in soil temperature and increase in humidity (Table 1) we noticed an increase in thrips morphometric variables (Figs. 3 and 4).

According to Pillai's statistics based on a MANOVA, it is clear that soil temperature and humidity as well as food supply determine phenotypic variability. MANOVA revealed only an interaction of soil humidity and soil temperature, which may indicate the dependence of female morphology on habitat conditions ( $p = 0.011$ ). Univariate testing indicated this interaction to be significant. This method further showed following multivariate main effects to be significant. Female abundance was correlated to soil temperature ( $p = 0.018$ ) and to the quantity of their food source (Acarina,  $p = 0.026$ ). As Pillai's  $p$ -values showed that soil temperature itself may have an effect on morphology of *H. subtilissimus*, but soil humidity must interact with soil temperature as a synergic complex to have such an effect (Fig. 5).

Soil humidity (Fig. 6) accounted for male morphology ( $p = 0.038$ ), while the relationship to food quantity (abundance of Acarina) was not significant ( $p = 0.350$ ). Soil temperature ( $p = 0.112$ ) did not play an important role in the environment-morphology assessment, even in connection with soil humidity ( $p(\text{Soil Humidity: Soil Temperature}) = 0.315$ ).

The analysis of the individual morphometric variables in connection with the ecological characteristics measured showed

that head length was neither affected by the selected ecological conditions nor by food, within females ( $p(\text{Soil Humidity}) = 0.722$ ;  $p(\text{Soil Temperature}) = 0.304$ ;  $p(\text{Acarina}) = 0.694$ ) as well as within the male population ( $p(\text{Soil Humidity}) = 0.448$ ;  $p(\text{Soil Temperature}) = 0.815$ ;  $p(\text{Acarina}) = 0.278$ ).

Besides the analysis of ecological conditions affecting the *H. subtilissimus* body parameters, Kruskal–Wallis test was used to show a difference between study sites with different anthropogenic impact according to the measured characters. The score 1 study sites (E, P) were pointed as different to the others only for female HW ( $p < 0.001$ ) and AP ( $p < 0.01$ ) (Figs. 7 and 8). However, this might be caused by the other variables (above) consequencing to tiny specimens in the ecotone, rather than anthropogenic impact.

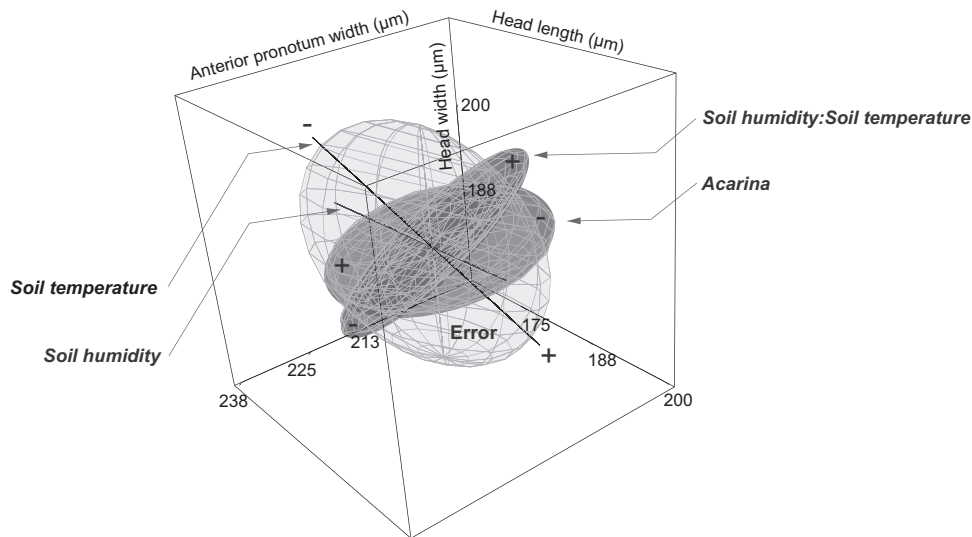
## 4. Discussion

This study has approved the effect of ecological variables in *H. subtilissimus* population phenotypic variability under mild climate oak forest conditions. In the wide range of taxa, individuals are able to express strikingly different morphologies in response to the habitat conditions encountered during development (Moczek, 1998). Therefore, phenotypic plasticity describes the capacity of a genotype to exhibit a range of phenotypes in response to variation in the environment (Garland and Kelly, 2006; West-Eberhard, 1989; Whitman and Agrawal, 2009); in fact it represents the change in the expressed phenotype of a genotype as a function of the environment (Scheiner, 1993). Here, plasticity represents a complex, evolved response in dealing with important ecological changes, allowing organisms to maintain high fitness in the face of environmental variability (Schlichting and Pigliucci, 1998). This undisputedly corresponds with higher variance in *H. subtilissimus* intraspecific variability (Figs. 3 and 4) under the ecotonal conditions between two major biotopes (forest and field) where heterogenous conditions require higher morphometric plasticity as a predisposition for alternative ontogenies (Balon, 2004; Komers, 1997; Schlichting and Pigliucci, 1998).

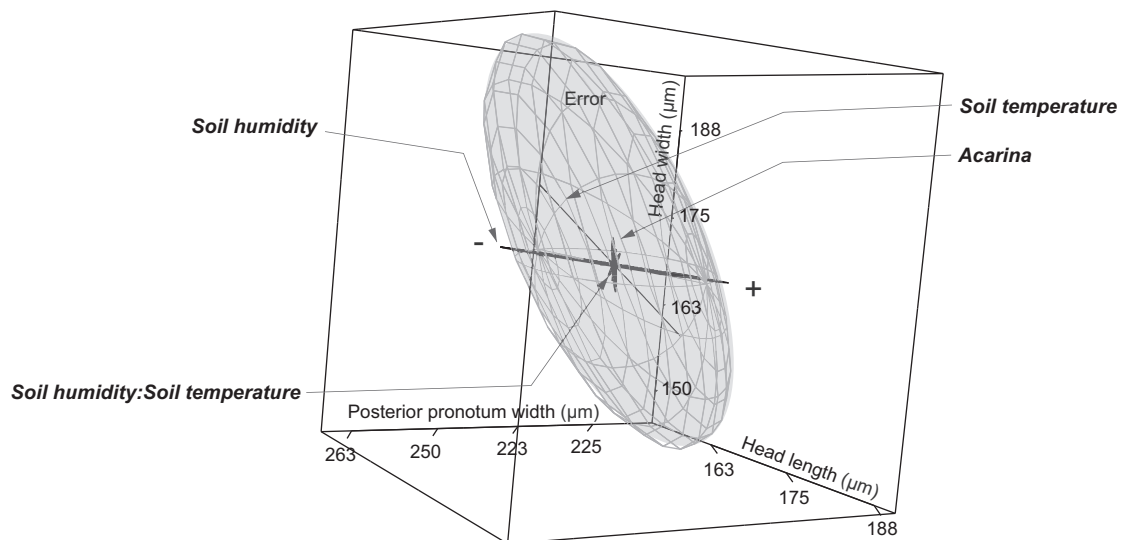
Consequently, the analysed ecological variables affect *H. subtilissimus* morphology (Section 3.2). Blanckenhorn (2009) claims that any external factor in principle can produce plastic responses in organisms, with the most prominent factors being food availability, predators, temperature, season length, photoperiod and humidity. A single population can display annual or even seasonal variation in life-history and metabolic traits, and differences among a set of populations may be observed in some surveys but not in others (Lardies and Bozinovic, 2008). As shown by Sukhodolskaya and Saveliev (2014) or Laparie et al. (2010), variability cannot be explained by a set of general rules and should be studied more in specific detail using modern statistical methods. By measuring *H. subtilissimus* morphometric characters we have discovered that there were interactions between the environment and their body parameters (Section 3.2).

Unequivocally, there was a higher intraspecific variability among the specimens from the ecotonal site when compared to the forest with lower temperature and higher humidity of the soil. Moreover, the ecotone site was inhabited by the specimens with lower morphometrical characteristics, both males (Fig. 4) and females (Fig. 3) (Section 3.1). In accordance with a general temperature-size rule, body size in a large majority of ectotherm organisms is larger when grown at lower temperatures, although the developmental rate is slower (Atkinson, 1994).

However, occurrence of smaller individuals in the ecotone site may rather correspond with higher interspecific competition of rich thrips assemblages which inhabit open habitats (fields). For instance, the effect of habitat on the size of *Carabus granulatus*



**Fig. 5.** *Haplothrips subtilissimus* female morphometric variables according to the selected ecological variables (Pillai's trace statistic based on the multivariate analysis of variance).

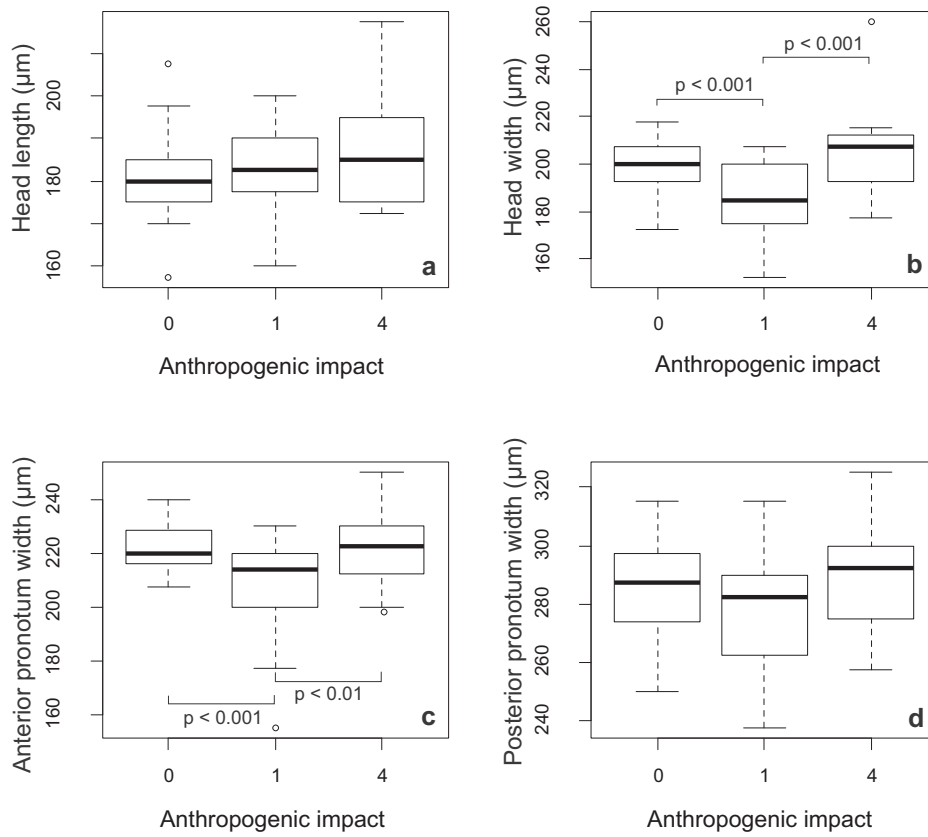


**Fig. 6.** *Haplothrips subtilissimus* male morphometric variables according to the selected ecological variables (Pillai's trace statistic based on the multivariate analysis of variance).

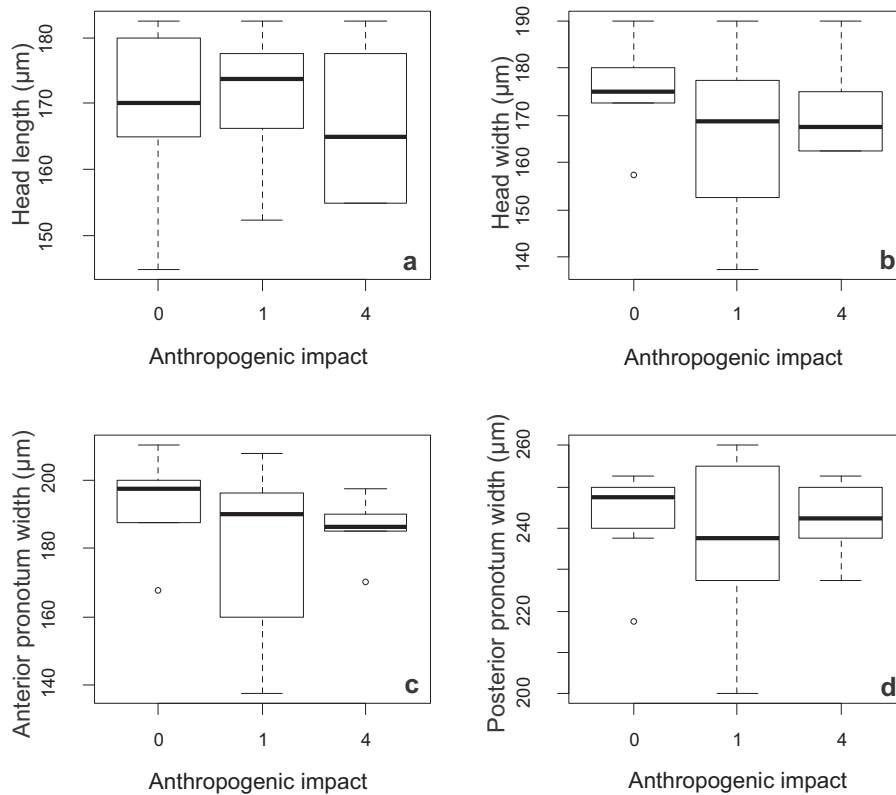
was related to interspecific competition rather than to ecological conditions (Sota et al., 2000; Sukhodolskaya and Saveliev, 2014).

Finally, humidity, as well as quantity and quality of food supply, may have some effect on the adult body size and colour of *Haplothrips* specimens (Minaei and Mound, 2008). Putman (1942) claims that relatively high moisture appears to be essential for the survival mainly of the larval stages. Some ecological factors produce well-known evolutionary patterns of phenotypic plasticity in body size within species. In general, specimens with smaller body size predominantly occur in conditions which constrain growth (Berrigan and Charnov, 1994). This definitely holds true for food limitation, which increases intra-specific as well as inter-specific competition. Body size is mediated by physiological and behavioural plasticity at the juvenile stage (West-Eberhard, 2003). Blanckenhorn (2009) claims that much of the phenotypic variation in body size is due to variation in per capita food availability at the larval stage. Furthermore, plasticity of body size is itself

adaptive, enabling growing animals to survive in environments prone to fluctuations in the quantity and quality of food (Parker and Johnston, 2006). That is what we suppose for *H. subtilissimus* variability. As Putman (1942) states, *H. subtilissimus*, both larvae and adults, feed on *Paratetranychus pilosus* (Acarina). According to our results (Table 1), there is higher food availability in the forest than in its ecotone stands. However, as the proper conditions for suitable larval development are not precisely known, we suspect food availability as the determinant in females. Kindlmann et al. (2001) claim that increased growth rate associated with improved food conditions result in larger adults, whereas an increased growth rate associated with higher temperature results in reduced adult size. Differences between males (Fig. 6) and females (Fig. 5) in food source demands are probably connected with their different life span. It is known that individuals of some thrips species (e.g. *Hoplothrips pedicularius*, *Ecacanthothrips tibialis*) do exhibit such variation in body forms and sizes, expressed by various projections



**Fig. 7.** Box and whisker plots of female morphometric characters measured in populations from study sites with different anthropogenic impact; *p* values from Kruskal–Wallis tests are given for significant differences between sites; a – length of the head, b – width of the head, c – anterior pronotum width, d – posterior pronotum width.



**Fig. 8.** Box and whisker plot of male morphometric characters measured in populations from study sites with different anthropogenic impact; *p* values from Kruskal–Wallis tests are given for significant differences between sites; a – length of the head, b – width of the head, c – anterior pronotum width, d – posterior pronotum width.

or enlargements of some body parts, that they are often considered to be more than a single species. Although we did not record any projections (nor enlargement) our results hint at the thesis of generally smaller specimens in ecotone. Besides the availability of food sources and different temperature and humidity conditions, there is probably a connection between the fecundity and mating frequency of large females (Ananthkrishnan, 2005).

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