The variability of chaetotaxy of *Lipoptena fortisetosa* Maa, 1965 (Diptera: Hippoboscidae)

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Abstract

Keds (Diptera: Hippoboscidae) are a very specific and undoubtedly interesting family of ectoparasitic dipterans. A morphometric analysis of the setae of *Lipoptena fortisetosa* (Maa, 1965) revealed considerable variability, with many setae often missing in each specimen. Therefore, it is advisable to select a smaller number of landmarks that are consistently present in all individuals. The analysis of the location of such head and thorax landmarks in *L. fortisetosa* indicated a significantly higher variability in females. The "ideal" individual is always characterized by 6 setae on the head and 22 on the thorax (4 humeral setae, 4 laterocentral setae, 8 postalar setae and 6 scutellar setae).

Key words: louse flies, ectoparasites, morphometry, setae, thorax, head

Introduction

The dipteran family Hippoboscidae (louse flies ectoparasites of birds or keds - ectoparasites of mammals) is an interesting object of research (e.g., Dibo et al. 2022; Andreani et al. 2022; Yatsuk et al. 2023; Tiawsirisup et al. 2023, etc.). The most important defining features of this family include host affinity and morphological characteristics, such as the presence, absence or reduction of the wing, veins and microtrichia on the wing, body shape and colour, and the presence of setae and other structures on the body, etc. (Povolný & Rosický 1955; Maa 1965; Chalupský 1980; Chalupský & Povolný 1983; Hutson 1984; Dosnažov 1987; Oboňa et al. 2022, etc.). The genus Lipoptena Nitzsch, 1818, is represented by two species in Slovakia (Sychra 2009; Oboňa et al. 2019a). The first, L. fortisetosa Maa, 1965, is a relatively common but non-native species in Slovakia (Figure 1, right), distributed in the eastern Palaearctic region. In the past, it was frequently misidentified with L. cervi (Linnaeus, 1758), and appears to have a western distribution limit in Central

Europe. This species is an ectoparasite of Cervidae and also attacks humans and birds (e.g., Ducháč & Bádr 1998; Oboňa et al. 2019a, 2022). The second species is *L. cervi*, a relatively common species native to Central Europe, distributed in the Palaearctic region and introduced into the Nearctic region. Similarly as *L. fortisetosa*, it is an ectoparasite of Cervidae and can also attacks humans (Krištofík 1998; Oboňa et al. 2019a).

To a large extent, the main differences between the two species are in the chaetotaxy of the head and thorax (mesonotum) (Maa 1965; Chalupský 1980; Dosnažov 1987; Ducháč & Bádr 1998; Salvetti et al. 2020; Oboňa et al. 2022). The chaetotaxy, especially on the thorax, is relatively variable; therefore, we decided to analyse this variability in more detail.

The main aims of this work are i) to analyse the chaetotaxy of

L. fortisetosa and ii) to evaluate the differences in chaetotaxy

between the sexes of this species in Slovakia.

Figure 1. Photographs of specimens of Lipoptena cervi (Linnaeus, 1758) (left) and Lipoptena fortisetosa Maa, 1965 (right).

Material and methods

The majority of the material was collected during field excursions in different parts of Slovakia (mainly from humans). The samples are deposited in the Laboratory and Museum of Evolutionary Ecology, Department of Ecology, University of Prešov (LMEE PO), see Oboňa et al. (2019a). Chaetotaxy analysis was performed on L. fortisetosa specimens deposited at LMEE PO. All specimens were photographed from the back under the same conditions using a Motic trinocular microscope with a camera (10x magnification). The photographs were then sorted by location, collection date and sex. Prior to data collection, tps files were created from the photographs using the program tpsUtil (ver. 1.75). All setae on the thorax (20 landmarks) were first selected. Consequently, the data were standardized in MorphoJ (ver. 1.06d) and analysed in the same program using the PCA (Principal Component Analysis) method, with graphical output. Landmarks were highly variable and several were often absent in many individuals. Their number was reassessed and the procedure was repeated only on the landmarks present in all individuals (10 landmarks on the head and 15 landmarks on the thorax, Fig. 2), so that they could be evaluated in the tpsDIG1 program (ver 1.40). In the PAST software (ver. 3.18; Hammer et al. 2001), the data were standardized to Procrust coordinates, thus eliminating the influence of position, size and rotation. In the same software, the data were analysed using the Principal Component Analysis (PCA) method, generating a graphical output of the analysis (scatterplot) with 95% ellipses (the probability that landmarks placed on other specimens of the sampled population will be within the ellipse is 95%).

PCA searches for hypothetical variables (components) that explain most of the variance in the measures (Davis 1986; Harper 1999). These variables are linear combinations of the original variables and this method is mainly used to reduce the data set to two components and to produce a graphical output (Peres-Neto et al. 2003). In order to visualize the relative deformations, the deformation energy matrices were calculated from the eigenvectors and then represented as deformations of the mesh, showing how the object would look if its relative deformation values (relative wraps-score) were at opposite ends of one of the relative deformation axes and at zero on the other axes. The percentage of the total variance expressed by each component was also calculated. The graphical output of the eigenvalues for each component was complemented by a "broken stick" - a curve showing the predicted eigenvalues in a random model, which helps to assess the number of significant components (above the intersection point). The XY plot, supplemented by a 95% ellipse, was used to illustrate the location of landmarks and their variability within the set groups.



Figure 2. Scheme of the most suitable landmarks on the head (h1 – h10) and thorax (t1 – t15) of *L. fortisetosa*.

Results and Discussion

A total of 48 specimens (26 females and 22 males) of *L. fortisetosa* were analysed. The number of landmarks varied among specimens, as many were missing many of the setae. This may have biased the analysis, and no significant relationship was found between morphometrics and collection time. Variable landmarks were therefore excluded from the analysis. The generated chart, completed with 95% ellipses (Figure 3 head, Figure 4 body), confirmed the appropriate selection of landmarks. The placement of landmarks was almost identical in both sexes (Figures 5, 6), although the variability was higher in females, as indicated by the red ellipse consistently being larger than black one).



Figure 3. XY plot supplemented by 95% ellipses showing the location of landmarks and their variability within defined head groups of *L. fortisetosa* (red dots – female, black – male).



Figure 4. XY plot supplemented by 95% ellipses showing the location of landmarks and their variability within defined thorax groups of *L. fortisetosa* (red dots – female, black – male).

The graphical output showed that despite the higher variability of females (Figure 5 head, Figure 6 body), males and females did not differ significantly in the morphometric features observed. A greater difference was found for the head landmarks, where 4 points (18%) of the analysed female specimens were outside 95% of the male ellipse.



Figure 5. Scatter plot with 95% ellipse on the head of *L. fortisetosa* (red dots – female, black dots – male).



Figure 6. Scatter plot with 95% ellipses on the thorax for *L. fortisetosa* (red dots – female, black dots – male).

The percentage of total variance accounted for by each component was also evaluated (Figures 7 and 8). The graphical output was supplemented by a curve showing the predicted values of the significant components (above the intersection point).



Figure 7. Percentage of the total variance with a curve showing the predicted values of significant components (above the crossing point) on the head of *L. fortisetosa*.

The chaetotaxis of the species *L. fortisetosa* is quite variable, as shown in the above results. However, when suitable landmarks (present in all individuals) are selected and a sufficient amount of material is processed, the results clearly confirm that males and females are similar in chaetotaxy, but greater variability was noted in females of *L. fortisetosa*. For the species *L. fortisetosa*, the landmarks in Figure 9 appear to be the most suitable for further possible analysis.







Figure 8. Percentage of the total variance with a curve showing the predicted values of significant components (above the crossing point) on the thorax of *L. fortisetosa*.

The chaetotaxy of the head and thorax of the species *L*. *fortisetosa* is quite variable (even in terms of length and thickness of individual setae); the "ideal" individual has 6 setae on the head and 22 on the thorax. At the front of the mesonotum 2 setae (on each side – humeral setae), below them, approximately in the middle of the mesonotum – 2 laterocentral setae on each side. On the dorsum of the mesonotum 8 posterior setae, and on the scutellum with 6 scutellar setae (Figure 9).

Similar results were published by Maa (1965), Chalupský (1980), Dosnažov (1987) and Ducháč & Bádr (1998). Therefore, it is necessary to select appropriate landmarks that are present in all specimens examined. The ideal landmarks for *L. fortisetosa* are the points in Fig. 2 or the setae in Fig. 9. These points were present in both sexes, although greater variability was observed in the females. It is interesting to note that certain shape variables were also recorded in the photographed specimens (e.g., in the scutellum).

Acknowledgements

We are particularly grateful to the editor and anonymous reviewers for their constructive comments, which helped to improve the manuscript. This study was partially supported by the Slovak Research and Development Agency under contract no. APVV-22-0440 and by VEGA 1/0876/21.

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