

Genital Morphology of the *Meiosimyza illota* Species Group (Diptera: Lauxaniidae) Suggests that Females Are Species-specific while Males Are Highly Variable

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Abstract. The females of *Meiosimyza illota* (Loew, 1847), *M. mihalyi* Papp, 1978 and *M. subfasciata* (Zetterstedt, 1838) have not been distinguishable from each other based on the previous knowledge. However, two morphological forms can be detected among them. The males of *M. illota* and *M. mihalyi* differ by shape of genitalia but intermediate specimens are also observed. To resolve these identification problems, an outline analysis of epandrial projections combined with the landmark based approach applied to wing shape were employed. Wings of 207 specimens and epandrial projections of 86 males were used. Multivariate statistical analyses, in combination with generalised Procrustes Superimposition (landmark based approach) and Elliptical Fourier Analyses (outlines) were performed in R. Females are, based on the wing shape, conclusively ascribed to either the *M. illota* group or *M. subfasciata*. Rather surprisingly, *M. illota* and *M. mihalyi* form a single continuum in shape of epandrial projection and do not differ in wing shape. *M. mihalyi* is therefore proposed as a new synonym of *M. illota*.

Key words: outline analysis; wing shape; geometric morphometrics; synonymy

Introduction

The insect taxonomists often rely on well-sclerotised and accessible structures such as the male intromittent organ. In contrast, the female reproductive tract is membranous and fairly inaccessible, resulting into the presence of less comparative data for females than for males (PUNIAMOORTHY et al. 2010, AH-KING et al. 2014). As a result, females of many insect species are considered unidentifiable. However, occasional detailed inspections of female terminalia reveal clear morphological differences and this raises the question about the differential characters to define the proper species.

An opportunity to associate the sexes is to observe copulation in nature. If we exclude the rare possibility of interspecific copulation, we still have to rely on chance, which can be low, even for common species. The examination of the wing geometric morphometrics is another option. The wing shape

has repeatedly been shown to be a very sensitive tool in taxonomic studies, e.g. in hymenopterans (VILLEMANT et al. 2007, MIGUEL et al. 2010) as well as in the Diptera, e.g. in the families Ceratopogonidae (MUÑOZ-MUÑOZ et al. 2011), Psychodidae (DE LA RIVA et al. 2001), Syrphidae (LUDOSKI et al. 2008, FRANCUSKI et al. 2009, MILANKOV et al. 2009, FRANCUSKI et al. 2011, NEDELJKOVIĆ et al. 2013, VUJIĆ et al. 2013), Tephritidae (SCHUTZE et al. 2012, KROSCH et al. 2013), Drosophilidae (MORAES et al. 2004) and Glossinidae (BOUYER et al. 2007, PATTESON & SCHOFIELD 2005). In all these studies, the results obtained by wing geometric morphometrics are congruent with those from DNA sequencing. The effects of sex, temperature (AYTEKIN et al. 2009, DEBAT et al. 2003) and even laboratory lines (Culicidae, see JIRAKANJANAKIT & DUJARDIN 2005) are often detectable by the wing shape. Most of the

available evidence justifies the use of wing shape in insect taxonomy at the level of the species or the population.

The rationale behind using the wing shape to identify the female of a species is based on the assumption that the wing shape is more similar in the conspecific sexes as demonstrated, e.g., in bees (FRANCOY et al. 2009). The wing shape, at least in some cases, is sexually dimorphic (PRETORIUS 2005, BREUKER et al. 2007, BENÍTEZ et al. 2011) but this does not necessarily disprove its usability. On the other hand, though many insect species have been distinguished on the basis only of the morphology of male genitalia and associated parts, this does not imply that male genitalia are constant (HUBER 2003, HUBER & GONZALEZ 2001, JOCQUÉ 2002).

An interesting insight in using characters of both sexes for taxonomic purposes may be provided by the examination of three species of the lauxaniid genus *Meiosimyza* Hendel, 1925: *M. subfasciata* (Zetterstedt, 1838), *M. illota* (Loew, 1847) and *M. mihalyi* (Papp, 1978). All three species are commonly distributed throughout the Europe and the Caucasus (MERZ 2002). While *M. subfasciata* prefers mesic habitats from highlands to mountains, *M. illota* and *M. mihalyi* are more restricted to the mountains (MERZ 2002). The latter two species have almost identical structure of the male genitalia and are further referred to as the *M. illota* group. In the keys by SHATALKIN (2000) and PAPP (1979), females of all three species are considered indistinguishable from each other. In contrast, the terminalia of *M. illota* and *M. subfasciata* were depicted by REMM & ELBERG (1979) and their female terminalia were considered distinguishable. However, the male terminalia of the putative *M. subfasciata* suggest that REMM & ELBERG (1979) in fact depicted another species, probably *M. conjugata* (Becker 1895), since its surstylus is obtuse but PAPP (1978) has described *M. subfasciata* with a pointed surstylus. Despite the fact that *M. subfasciata* has been described almost two centuries ago, its female terminalia remain undescribed.

The characters of males of the *M. illota* group appear problematic, too. SHATALKIN (2000) described the wing apex with a dark costal area for *M. illota* and a clear apex for *M. mihalyi*. PAPP (1979) differentiated them on the basis of the shape of gonites and also by the thickness of polinosity on the tergum: high for *M. illota* and low for *M. mihalyi*. According to our experience, only the shape of the gonites is a reliable character. The right gonite is long and scimitar-like in *M. illota* while in *M. mihalyi* it is with the shape of a boot with a high heel.

However, MERZ (2002) mentioned the presence of specimens with an intermediate gonite shape.

The taxonomic relationships of the species complex are further complicated by the inclusion of *M. conjugata* (Becker, 1895) in it. *M. conjugata*, *M. subfasciata*, *M. illota* and, presumably, *M. mihalyi* were considered closely related (SEMELBAUER 2016). MERZ (2002) proposed that *M. conjugata* and *M. subfasciata* do not differ in male terminalia and that the two species reliably differ only in the colour of postpedicel: yellow for *M. conjugata* and yellow with dark apex for *M. subfasciata*. However, according to MERZ (2002), the situation is complicated by the presence of specimens with intermediate characters. Therefore, to confirm the identity of *M. subfasciata*, the type specimen should be studied.

The present article has two aims: to recognise and describe the females of *M. subfasciata* and the *M. illota* group and to characterise the pattern of variation of male genitalia of *M. mihalyi* and *M. illota* in detail.

Materials and Methods

Acquisition of specimens

The specimens were obtained by Malaise traps installed in High Tatras National Park. They originate from five study sites (Fig. 2): Kežmarské Žľaby (2010), young spruce forest, 49°10'55.62" N, 20°19'57.90" E; Podbanské (2008), alder forest, 49°08'04.80" N, 19°53'58.62" E; Stará Lesná (2011), young spruce forest, 49°09'06.48" N, 20°16'47.76" E; Tatranké Zruby (2006-2009), burnt spruce forest, 49°08'10.86" N, 20°11'57.00" E; Zuberec (2011), spruce forest, 49°14'49.50" N, 19°42'49.68" E. More detailed description of the study sites is given by VIDLIČKA (2015). Collection of materials was allowed by Regional Environmental Office, permission number 1/2009/00324-005/SJ.

The specimens were mounted on paper strips and the species were identified on the basis of the keys by PAPP (1979) and SHATALKIN (2000, in the version of its English translation by SCHACHT et al. 2004). Specimens for analysis of genitalia and wings were chosen from the same pool, though they do not overlap entirely, since some specimens had damaged wings or the preparation of male genitalia was unsuccessful.

The holotype of *M. subfasciata*, loaned from the Museum of Zoology, Lund University, Sweden, was also examined.

Terminology

Papp (1978), in his description of *M. mihalyi*, men-

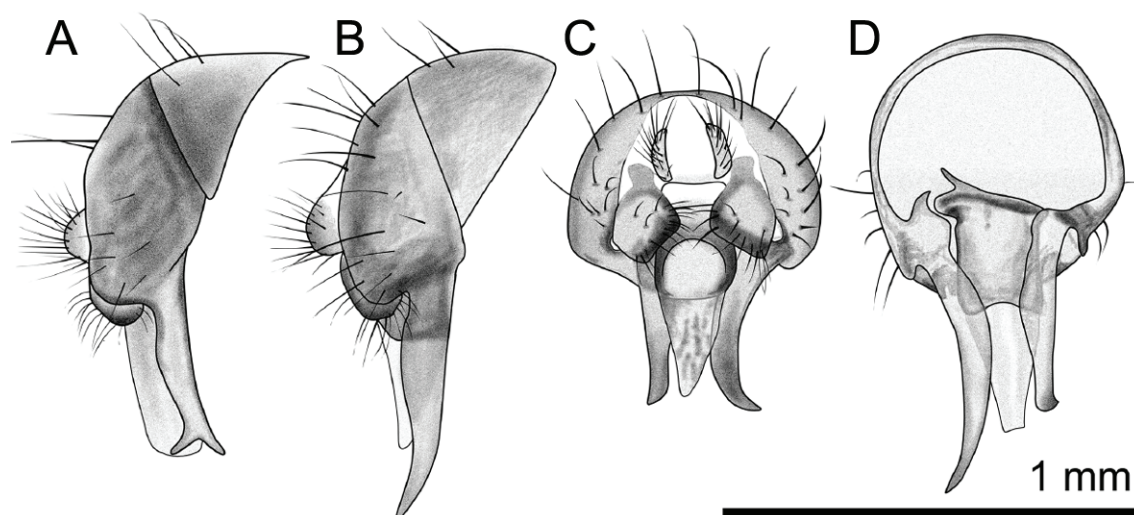


Fig. 1. Male terminalia of *Meiosimyza illota*, *M. mihalyi* and *M. subfasciata*; A, C – *M. illota* in lateral and posterior view; B – terminalia of *M. mihalyi*, lateral view; D, E – terminalia of *M. subfasciata* in lateral and ventral view.

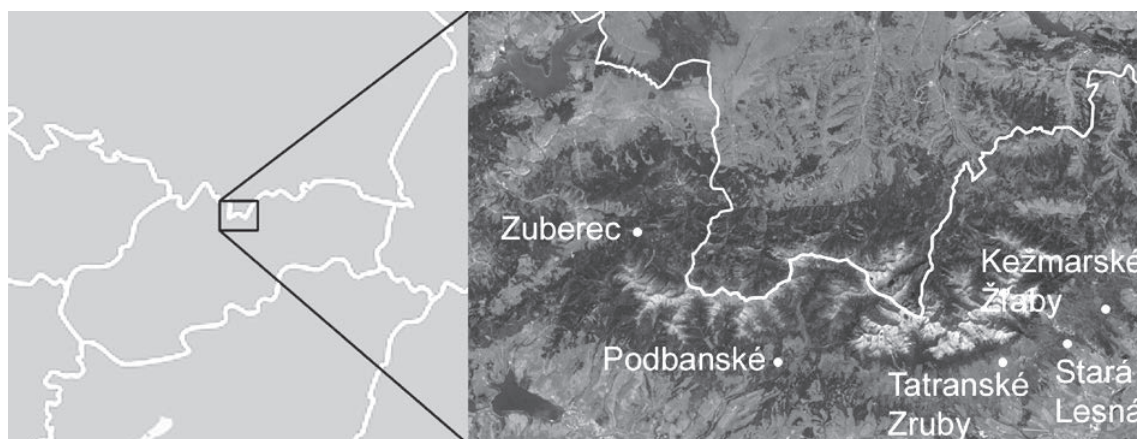


Fig. 2. Distribution of study sites.

tioned the presence of two asymmetrical gonites. Either pre- or postgonites are connected to the hypandrium. The inspection of the male genitalia clearly shows that the putative gonites are firmly connected to the epandrium and hence can be considered as outgrowths of it (Fig. 1). Therefore, structures named gonites by PAPP (1978) will be referred to as epandrial projections in the present paper.

Collection of landmarks on wings, statistical analysis

The specimens were divided into the following groups: *M. illota* ♂, *M. mihalyi* ♂, *M. subfasciata* ♂, assumed *M. subfasciata* ♀ and assumed the *M. illota* group ♀. The wings were mechanically removed, immersed in alcohol, mounted on slides with glycerol and photographed in 10x magnification under Stemi 2000-C stereomicroscope equipped with Micrometrics SE camera. Each image was la-

belled in a way allowing the first three characters corresponding to the specimen number, the fourth one reflecting the locality, the fifth one representing the group (F=female *M. subfasciata*, E=female *M. illota*, S=male *M. subfasciata*, M=male *M. mihalyi*, L=male *M. illota*) and the last one corresponding to the session number. A tps-file was created from the images using the tpsutil (ROHLF 2010); the landmarks were digitised using tpsdig (ROHLF 2009).

The raw coordinates were superimposed by the Generalised Procrustes Analysis (GPA), using the function gpagen from package geomorph (ADAMS & OTÁROLA-CASTILLO 2013) implemented in R (R CORE TEAM 2013). In the GPA, differences due to position, rotation and scale were filtered out. During the superimposition, four degrees of freedom were lost (two for translation, one for rotation and one for scaling), what complicated computing the degrees of freedom in statistical tests. One possibility to cope

with this problem was to use Principal Component Analysis (PCA) (ZELDITCH et al. 2004). The last four principal components (PCs) of Procrustes coordinates had zero eigenvalues and could be omitted, i.e. 24 – 4 PCs were used for statistical tests. The reconstruction of the shape change along the principal components was performed by *tpsrelw* (RHOLF 2008). The centroid size was used as an estimator of the size. Practical information on the GPA can be found in CLAUDE (2008, 2013), WEBSTER & SHEETS (2010) and VISCOSI & CARDINI (2011). The theoretical background with recent advances can be found in some of numerous reviews (e.g. ROHLF & MARCUS 1993, ADAMS et al. 2004, 2013, MITTEROECKER & GUNZ 2009).

The data manipulation and analysis was performed in R using R generic functions, functions available in the package *geomorph* (ADAMS & OTÁROLA-CASTILLO 2013) and CLAUDE (2008). The significance of factors on centroid size was evaluated via linear models. The shape variables were processed via standard statistical tools, namely Multivariate Analysis of Variance (MANOVA), Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA). Assumptions of linear models were evaluated by validation plots rather than formal statistical tests, as recommended by ZUUR et al. (2009). In case of violation of one or more of the assumptions (normality and independence of the residuals, homogeneity, no outliers), generalised least squares model was used (from package *nlme*, PINHEIRO et al. 2017), as it allowed to include e.g. correlation between data points or heteroscedasticity in the model.

Original data are available at the author: the *tps*-file containing original wing landmarks, table of factors and the R code.

Collection of outlines on epandrial projections of the *M. illota* group. Statistical analysis

The male genitalia were prepared in the following way: the abdomen was removed and immersed in alcohol; the right outgrowth of the epandrium was mechanically removed and photographed by camera mounted on binocular microscope in 50x magnification. The outgrowth was positioned so that the distal tip was directed to the left. The images were edited in Adobe Photoshop CE. The images were converted to *tps* format by *tpsutil* (RHOLF 2010). The *tps*-file was opened in *tpsdig* and coordinates of the outlines were acquired with the function *Outline object* (click by the left mouse button right from the object automatically creates curve, click by the right mouse button opens pop-up menu, from which was chosen

“save as XY coords”). The saved *tps*-file was opened in notepad and “OUTLINES=” was replaced by “CURVES=”, then *tps*-file was reopened in *tpsdig*, with the cursor positioned over the curve, click with right mouse button opens pop-up menu, from which was chosen “resample curve”, number of points was set to 250 “by length”. The file was reopened in notepad and adjusted (LM=0 was replaced by LM=250, CURVES=1 and POINTS=250 were deleted) so that the function *readland.tps()* of the package *geomorph* could read in the data and transform them automatically in 3D array (required by function *NEF*, see below).

The data were transformed into the shape variables by Fourier Elliptical Series. At the assumption that a point was passing in regular speed along a closed outline and x and y coordinates were explained as a function of time, we got a periodic function. The Fourier series decomposed a complex periodic function into a series of goniometric functions such as sine and cosine. These simple functions had frequencies that were integer multiples (=harmonics) of one another. The lower harmonics provided a coarse description of the original periodic function, while the high-frequency harmonics fitted its fine-scale variations (BONHOMME et al. 2014). The Fourier series was originally designed for continuous functions; therefore, a discrete equivalent of the Fourier series was used in the morphometrics. The maximal number of harmonics was half the points collected along the outline (pseudolandmarks). There were four coefficients for each harmonics (two for x and two for y coordinates). In the case of 250 pseudolandmarks, the maximum number of harmonics was 125, resulting in $4 \times 125 = 500$ coefficients representing each specimen.

The Fourier Coefficients were extracted by function *NEF* (CLAUDE 2008), which performs normalised elliptic Fourier Transformation (removed differences due to rotation and translation). The shape variables acquired by the Fourier series were highly redundant and the coefficients of the high order harmonics added very little information to the description of shape. Therefore, only several first coefficients were retained for the analysis. Selected Fourier coefficients were used to perform PCA, MANOVA and LDA using the functions programmed in R (R CORE TEAM 2013). The size of the first ellipse was used as the estimator of size (computed automatically by *NEF*). The other R scripts were adapted from CLAUDE (2008, 2013).

Original data are available at the author: the *tps*-file containing pseudolandmark configurations, table of factors and the R code.

Results

Females

The females of *Meiosimyza* determined as either *M. subfasciata* or the *M. illota* group were inspected for the terminalia and separated into two groups. The one group, presumably the *M. illota* group, was larger and had yellowish wings with smoked apex. The other one, presumably *M. subfasciata*, was smaller and had more hyaline wings without smoked apex (Fig. 3). For morphology of their Terminalia, see Fig. 4.

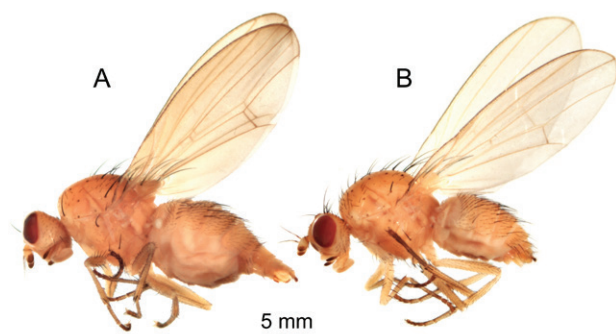


Fig. 3. A – female of *Meiosimyza illota* (Loew, 1847); B – female of *M. subfasciata* (Zetterstedt, 1838)

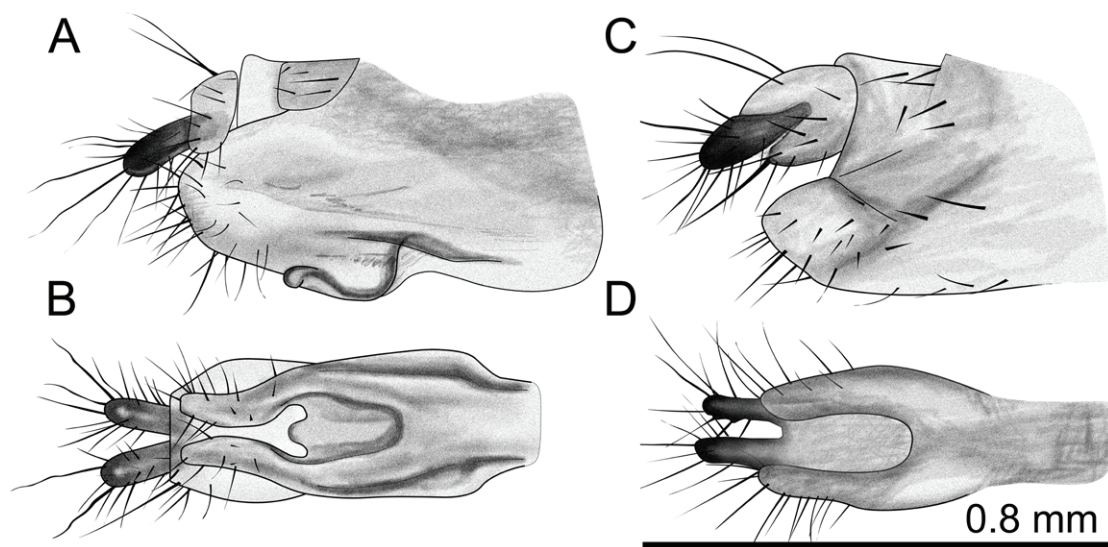


Fig. 4. Terminalia of *Meiosimyza* females; A, B – terminalia of *Meiosimyza illota* (Loew, 1847); A – lateral; B – ventral. C, D – terminalia of *M. subfasciata* (Zetterstedt, 1838); C – lateral; D – ventral.

Table 1. Number of specimens included in the analysis of wing shape.

	Zuberec	Podbanské	Tatranské Zruby	Stará Lesná	Kežmarské Žľaby	Total
<i>M. subfasciata</i> ♀	-	-	-	10	13	23
<i>M. subfasciata</i> ♂	-	-	-	12	12	24
<i>M. illota</i> ♀	8	8	8	8	-	32
<i>M. illota</i> ♂	15	6	12	22	-	55
<i>M. mihalyi</i> ♂	20	24	23	6	-	73
Total	43	38	43	59	25	207

Males

The holotype of *M. subfasciata* is in very good condition, only the right wing has a broken distal part. The abdomen was convoluted and the terminalia were not visible. It was necessary to dissect the specimen. The terminalia (Fig. 5) clearly corresponded to those of the specimens identified as the same species in the course of the present study.

Wing shape (landmarks)

For the analysis of wings, 207 specimens were studied (Table 1). For the location and order of the landmarks, see Fig. 6. The table of factors contained four variables: ID (unique code for each specimen), sex, species (*M. subfasciata*, *M. illota* and *M. mihalyi*) and locality (5 levels).

Digitization of all specimens was repeated twice. Prior to analysis, the repeatability of landmark data was assessed. For each landmark and specimen, variance (separately for x and y axis and then summed) was calculated. Specimens with exceedingly high variance for a certain landmark were visually inspected in tps dig and the respective landmarks were adjusted. The landmarks 3 and 12 had the largest average variance, obviously less clearly defined.

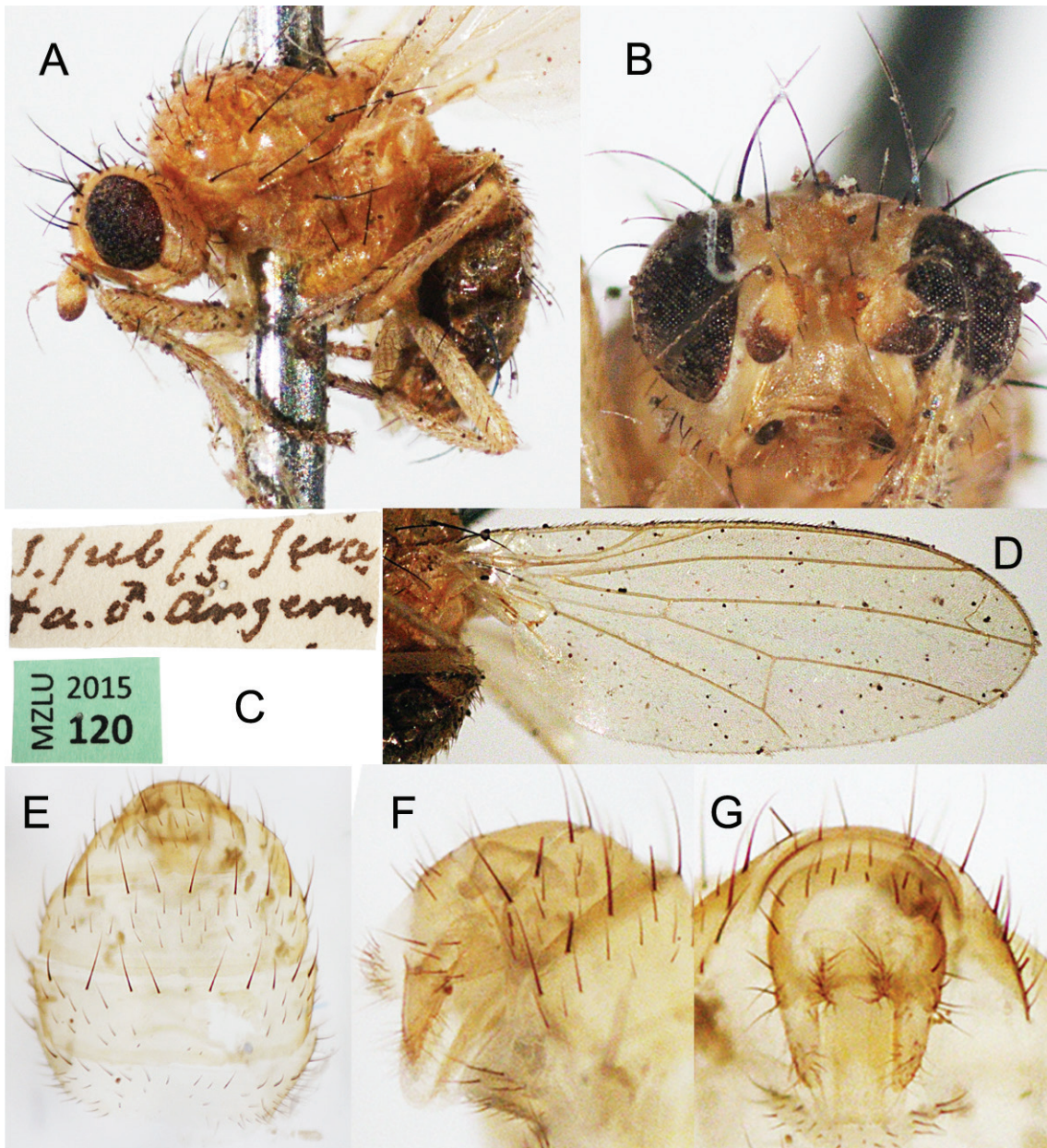


Fig. 5. The type specimen of *M. subfasciata*, images are not in scale; A – body, lateral view; B – head, frontal view; C – labels pinned below the specimen; D – left wing, ventral view; E – abdomen, dorsal view; F – terminalia, lateral view; G – terminalia, ventral view.

Measurement error (ME). The ME was estimated by the ANOVA approach described by YEZERINAC et al. (1992); practical application in R environment was adapted from CLAUDE (2008, 2013). Within the data set, we considered two sources of variation: variation due to individuals and variation due to repeated measurements. Two linear models were fitted to the centroid size: one with the session factor and one with the individual factor. The session factor appeared as non-significant ($df=1$, $F=0.003$, $p\text{-value}=0.956$), while individual factor was highly significant ($df=206$, $F=11796$, $p\text{-value}<0.0001$), implying that the inter-individual variability was much larger

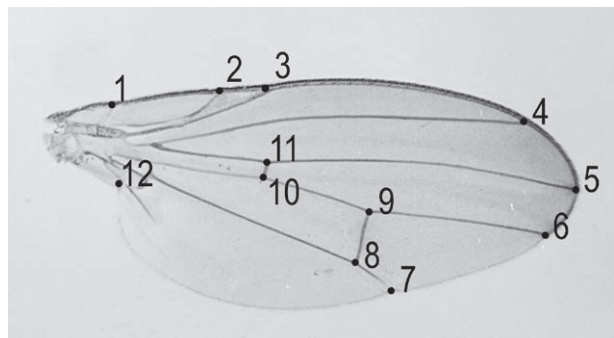


Fig. 6. Wing of *M. mihalyi* with indicated position of landmarks.

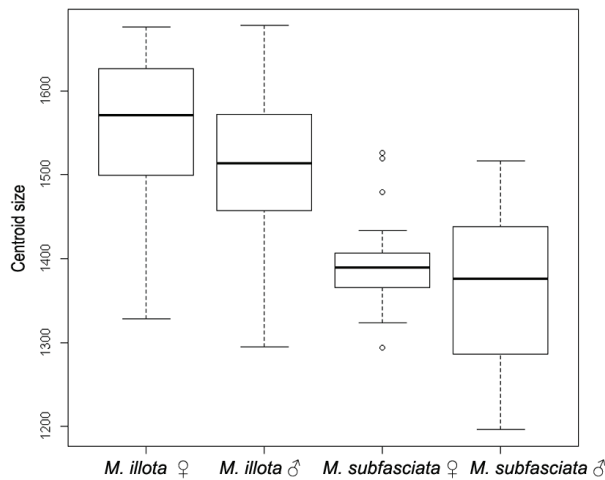


Fig. 7. Boxplot of wing centroid sizes of *Meiosimyza* species.

Table 2. ANOVA of wing centroid size, whole dataset, species factor set to two levels (*M. subfasciata* and the *M. illota* group). The non-significant terms were excluded.

	Df	F value	p-value
species	1	115.50	< 0.0001 ***
sex	1	6.17	0.0138 **

Table 3. ANOVA table, wing centroid size of *M. illota* and *M. mihalyi* males.

	Df	Sum Sq	Mean Sq	F value	p-value
Species	1	15088	15088.9	2.2923	0.13264
Locality	3	72780	25260.1	3.6857	0.01397*
Species: locality	3	17237	5845.7	0.8729	0.45727
Residuals	120	789875	6582.3		

Table 4. MANOVA of wing 20 principal components, whole dataset; the factor “species” was set to two levels (*M. subfasciata* and the *M. illota* group). Non-significant interaction terms were excluded.

							Random Intercept Model			
	Df	Pillai	approx F	num Df	den Df	p-value	approx F	num Df	den Df	p-value
Species	2	0.81938	6.1422	40	354	< 2.2e-16 ***	6.1422	1	199	<.0001***
Sex	1	0.20631	2.2874	20	176	0.002249 **	2.2874	1	199	0.0463 *
Locality	4	0.58093	1.5207	80	716	0.003465 **				
Size	1	0.39035	5.6344	20	176	4.349e-11 ***	5.6344	1	199	0.0005 ***
Sex: Locality	5	0.56169	1.4621	80	716	0.007370 **				
Residuals	195									

Table 5. MANOVA of wing 20 principal components of *M. illota* and *M. mihalyi* males. Non-significant interaction terms were removed from the model.

	Df	Pillai	approx F	num Df	den Df	p-value
Species	3	0.18936	1.2030	20	103	0.2673538
Locality	1	0.70696	1.6186	60	315	0.0048029 **
Size	3	0.35248	2.8034	20	103	0.0003654 ***
Residuals	122					

in comparison to variability due to the repeated measurement of the same specimen. For the Procrustes coordinates, Procrustes ANOVA (GOODALL 1991, KLINGENBERG & MCINTYRE 1998) was adapted following CLAUDE (2008, 2013). Again, the session factor was non-significant (df=1, F=0.567, p=0.934), while the individual factor was highly significant (df=206, F=23.76, p-value<0.0001). The results suggested again that the variation due to repeated measurements was negligible comparing to the variation between individuals. After assessing ME, the data were averaged over the replicates.

Centroid size. Two separate analyses were performed. In the first model, factors locality, sex and species were used as explaining variables, and the species factor was set to two levels (*M. subfasciata* vs. the *M. illota* group). The model validation plots

suggested violation of homogeneity, therefore GLS model was applied. Argument “weights” allowed the variances to differ per species and sex. Both species and sexes significantly differed in wing centroid size (Table 2, Fig. 7). In the second model, only males of the *M. illota* group were considered. The two species did not significantly differ in wing centroid size (Table 3).

Shape. A PCA was performed on Procrustes coordinates (Fig. 8). The first two principal components explained 35.8% and 19.1% of variance, respectively. *M. subfasciata* and the *M. illota* species group were fairly good separated along the PC1. 24-4 PCs were selected for subsequent analysis. A linear model was applied to the PCs with species, sex, locality and centroid size as explaining variables. The factor species was set to 2 levels (the *M. illota* group and

Table 6. Numbers of males of *M. illota* and *M. mihalyi* per locality included in the analysis of outlines of epandrial projections.

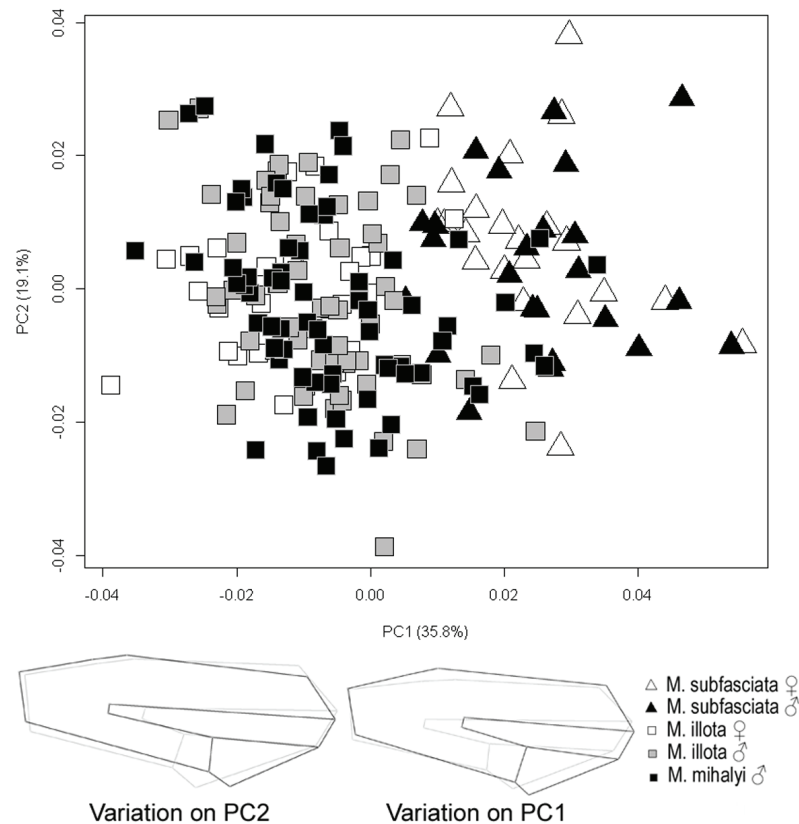
	Podbanské	Stará Lesná	Tatranské Zruby	Zuberec	Total
<i>M. illota</i>	6	18	8	11	43
<i>M. mihalyi</i>	11	10	12	10	43
Total	17	28	20	21	86

Table 7. ANOVA of size of the epandrial projections; the non-significant interaction term was removed from the model.

	Df	Sum Sq	Mean Sq	F value	p-value
species	1	38618	38618	89.197	1.052e-14 ***
locality	3	7119	2376	5.4813	0.001765 **
Residuals	81	35069	433		

Table 8. MANOVA of Fourier coefficients; the non-significant interaction terms were removed.

	Df	Pillai	approx F	num Df	den Df	p-value
species	1	0.98142	10.1	68	13	2.603e-05 ***
locality	3	2.51803	1.2	204	45	0.2916
size	1	0.89929	1.7	68	13	0.1428
Residuals	80					

**Fig. 8.** PCA plot, first two principal components of PCA performed on wing Procrustes coordinates, shape variation along the axis is depicted below the PC1.

M. subfasciata). All main terms and one interaction term were statistically significant (Table 4). A separate model was fit to the *M. illota* group males, using the species, locality and centroid size as explaining variables. Surprisingly, *M. illota* and *M. mihalyi* did not differ in mean wing shape (Table 5).

A linear discriminant analysis (LDA) using the leave-one-out cross-validation procedure was performed on the Procrustes coordinates. At first, two groups were tested: the *M. illota* group and *M. subfasciata*; 97.58% of all individuals were correctly assigned. On the other hand, if the identity of females

was switched, the percentage of correctly assigned specimens decreased to 68.59%. The females create roughly 1/4 of the whole dataset, suggesting that part of males and almost all or all females were incorrectly assigned. In contrast, if the LDA was applied

to the males of the *M. illota* group, only 53.12% of specimens were correctly assigned.

Shape of the epandrial projection (outlines)

For the analysis of epandrial projections, 86 males from 4 localities were included (Table 6). Measurement error was assessed in similar way as in wings. Each specimen was processed two times. Two linear models were fitted to the size: one with the session factor and one with the individual factor. The session factor appears as non-significant (df=1, F=0.19, p-value=0.664), while individual factor was highly significant (df=85, F=49.63, p-value<0.0001), implying that the variation due to repeated measurements is negligible compared to the inter-individual variation. In the Fourier Coefficients, the measurement error was rising from about 0.001% for lower harmonics to about 80% for high rank harmonics. Subsequent analysis was performed on the averaged values.

Size. A linear model was applied to the size variable with species and locality as explanatory

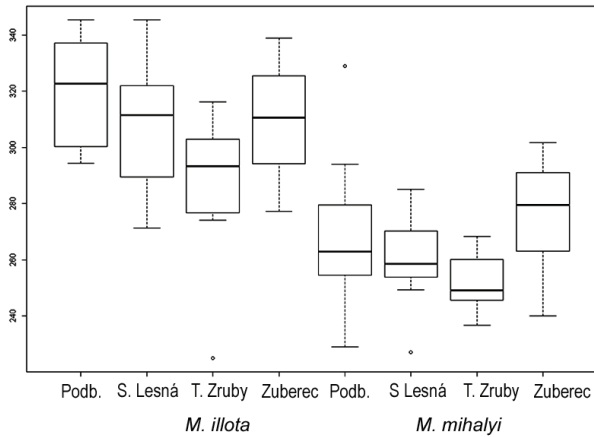


Fig. 9. Boxplot of epandrial projections sizes of *M. illota* and *M. mihalyi* from the study sites.

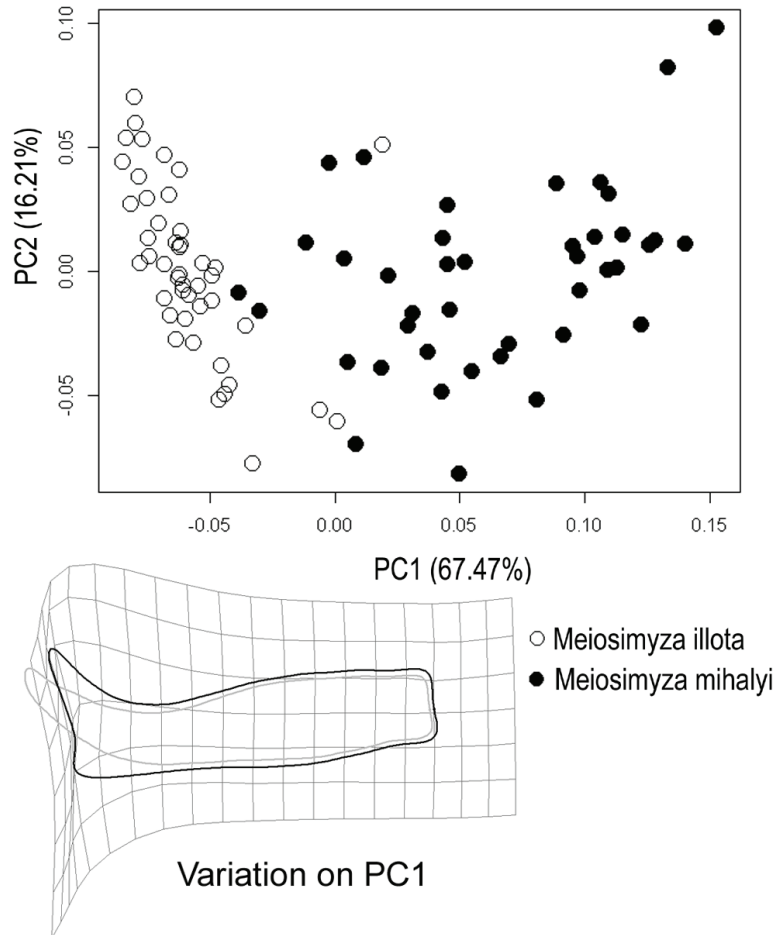


Fig. 10. PCA plot, first two principal components of PCA performed on first 18 Fourier harmonics calculated from the pseudolandmarks collected along the outline of the epandrial projections. Shape change associated with the PC1 is depicted below the PCA plot.

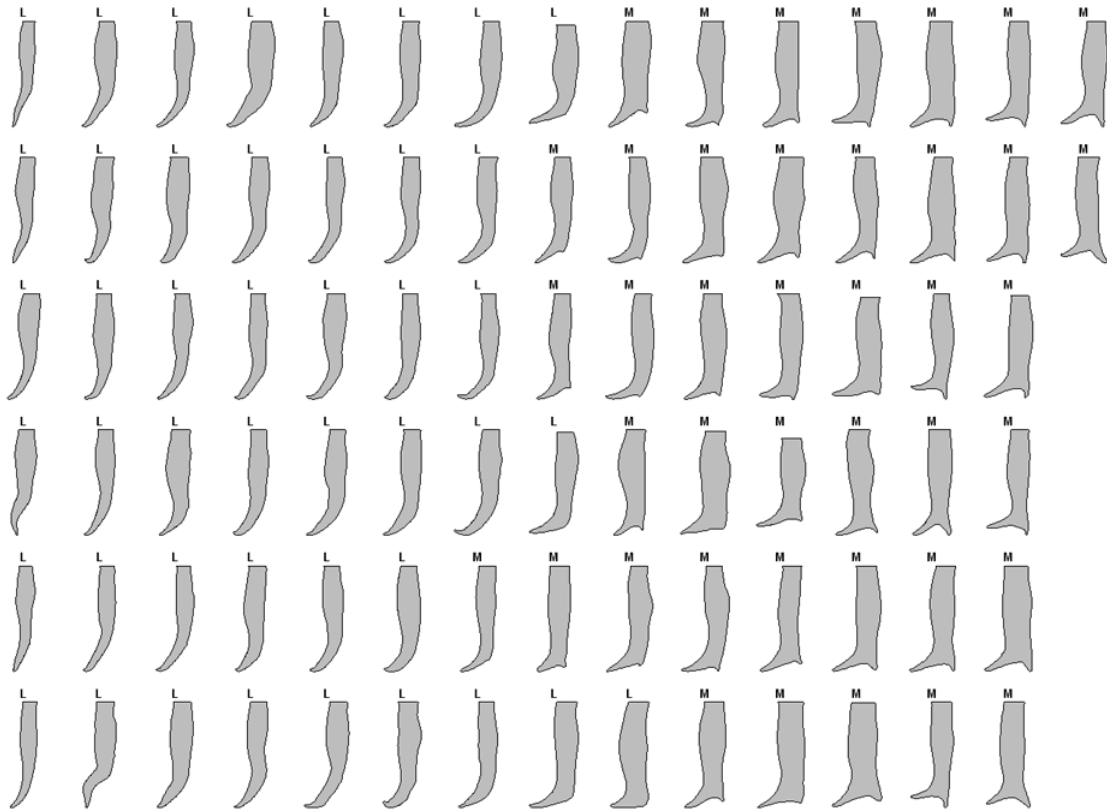


Fig. 11. Epandrial projections of all males of the *Meiosimyza illota* group; the projections are ordered according to the PC1 scores; L refers to *M. illota*, M. to *M. mihalyi*.

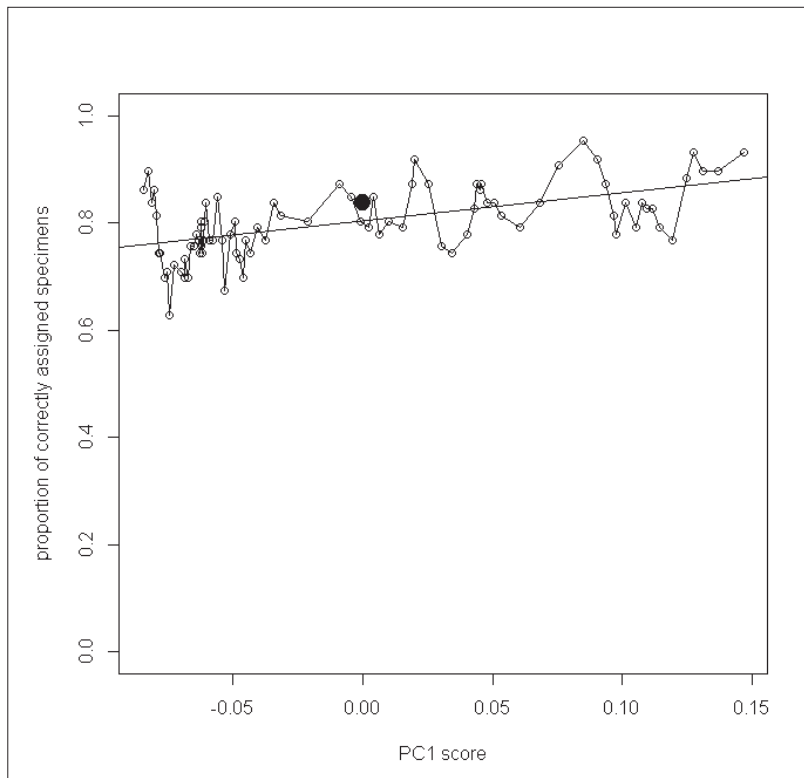


Fig. 12. PCA1 scores plotted against LDA results based on artificial grouping factors based on PCA1 scores (blank points connected with lines). The large black dot is result of PCA based on the original grouping factor. The line was added to accentuate the linear trend, suggesting that on average the LDA scores better as we move along the PC1.

variables. Both factors were statistically significant (Table 7, Fig. 9).

Shape. As far as the shape of some specimens has fine scale shape characteristics (very small “heel”), the higher number of harmonics was preferred. After evaluating the harmonic power, first 18 coefficients were selected, which included more than 99.9% of total variability. A PCA was performed on these coefficients (Fig. 10). First two principal components explained 67.78% and 16.34% of variance, respectively. *M. illota* and *M. mihalyi* were clearly separated along the PC1. A linear model was applied to Fourier Coefficients, with factors species, locality and size (Table 8). Only factor species appeared to be significant. A linear discriminant analysis (LDA) using the leave-one-out cross-validation procedure was performed; almost 84% of specimens were correctly assigned to the species.

The pattern of variation along the PC1 appeared to be rather continual (Fig. 11). To confirm this impression, the LDA was re-run with grouping factor based on PC1 scores; 85 grouping factors were obtained as averages between two neighbouring specimens along the PC1. The proportion of correctly assigned specimens was plotted against the respective PC1 score (Fig. 12). On average, the artificial factors were as efficient as the original factor, supporting the assumption that division of the specimens in two or more clusters was artificial at any point.

Discussion

Females

The wing shape conclusively assigned females to species, in accordance with other morphological characters. Most notably, members of the *M. illota* group have wings with smoked apex, while *M. subfasciata* has a clear apex. It must be pointed out that the characters such as wing coloration are not reliable in separating the species, as in freshly emerged specimens it may not be fully developed. Furthermore, characters like colour of the wing can vary within species (e.g. along altitude or latitude), and other populations can have distinctly smoked wing.

M. subfasciata and *M. conjugata*

According to MERZ (2002), *M. conjugata* is not distinguishable from *M. subfasciata* on the basis of its male terminalia. The only reliable character, in which they should differ, is the colour of postpedicel (SHATALKIN 2002, PAPP 1979), thought tip of the postpedicel of *M. conjugata* may be slightly infuscated as well (MERZ 2002, confirmed also by the present study) and thus it can be misidentified as *M. subfasciata*. Local populations of *M. conjugata*

with dark tip of postpedicel exist (personal observation), possibly misleading REMM & ELBERG (1979) to wrong identification of *M. conjugata* as *M. subfasciata*. To recognise *M. conjugata*, the terminalia seem to provide safe characters – both for male (surstyli with blunt tip) and female (subgenital plate without ventral incision). However, the identity of *M. conjugata* should be confirmed by examination of its type specimen.

Meiosimyza illota-group males

There is no difference in mean wing shape between *M. illota* and *M. mihalyi*. In contrast, some populations of these species may significantly differ in mean wing shape. The absence of wing shape difference between the males thus cannot be attributed to the lack of variation or to the low sample size. In the linear discriminant analysis, roughly half of the specimens have been correctly assigned – equivalent to a random assignment. This is striking when compared to over 70% of successful assignments of sexes within species and to almost 100% successful assignment to either *M. subfasciata* or the *M. illota* group. Based on the wing shape, there is no evidence to consider *M. illota* and *M. mihalyi* as two species.

On the other hand, *M. illota* and *M. mihalyi* clearly differ in mean shape of the right epandrial projection. The pattern of variation of diagnostic characters is expected to be discrete rather than gradual. Multivariate test compares mean shapes and significant result does not exclude gradual variation. To admit that the variation in shape of projections is gradual seems to better reflect the pattern of variability in the *M. illota* group. This intuitive conclusion was supported by (rather *ad hoc*) statistical test. If there is a discrete variability, we would expect to find local optimum where the LDA scores better (but no such optimum was observed).

The distinction between the two species is based on rather arbitrary (thought intuitively appealing) point, where the scimitar-like shape melts into boot-like. Combined with the analysis of wing shape, the results provide convincing evidence that *M. mihalyi* is only part of morphological continuum of *M. illota* and is proposed as a new synonym of *M. illota*.

The case of the *M. illota* group represents an example of genitalic polymorphism. Intraspecific variation of male genitalia has been documented by OTRONEN (1998), KAWANO (2004), HOUSE & SIMMONS (2005), MUTANEN et al. (2005), MUTANEN & KAITALA (2006), MUTANEN & PRETORIUS (2007), ANDRADE et al. (2009) and HIGGINS et al. (2009). Experimentally, it has been demonstrated by SIMMONS et al. (2005). The genitalic polymorphism has been documented also in spiders (SCIOSCIA 1995, HUBER & GONZALEZ

2001, JOCQUÉ 2002). HUBER (2003) argues that the low number of known examples might be a result of the conceptual approach: if we consider every population of a certain insect or spider with different shape of genitalia as a separate species, then discovering polymorphic species is impossible.

Currently, the sexual selection is recognised as the major driver in the evolution of insect genitalia and the rate of genital evolution is believed to exceed that of other body parts (ARNQVIST 1998, HOSKEN & STOKLEY 2004, SIMMONS 2014). Thus, in the recently separated species, we may find out that they differ in genitalia but are otherwise identical. This argumentation seems to disprove the conclusion drawn. However, the hidden assumption made is that all changes that occur are apparent to the naked human eye. This assumption is easily disproved as arrive of modern morphometric tools revealed small but detectable changes within single species (sexes, populations, even laboratory lines; see papers cited in the introduction), which nobody has noticed before. Therefore, recently separated species, which are identical for the observer, may not be identical in reality.

We can assume that the species is a reproductively isolated unit (the biological species concept), the wing shape is only minimally affected by the environment (BIRDSALL et al. 2000, BITNER-MATHÉ & KLACZKO 1999, MORAES & SENE 2004) and the wing shape fluctuates in time and space practically inexhaustibly (but within reasonable limits). From these assumptions, we can conclude that it is highly unlikely to find two or more reproductively isolated units (species) with identical wing shape. The present paper thus provides evidence for polymorphism in insect male genitalia and supports the idea of HUBER (2003) that it is much more common than previously believed. A legitimate objection can be proposed. The sampled localities represent a tiny portion of the range of the *M. illota* group. Thus, it is possible that the sampling sites accidentally stroked to a hybrid zone of the two species and there is no surprise that there are intermediate forms. However, this objection is not in accordance with data about the distribution of the studied species. The ranges of the two species are practically identical, covering most of the western and central Europe and reaching to Scandinavia and the Ukraine (MERZ 2007). Overlap in distribution can be seen even if we are looking on a much finer geographical scale. The two species occupy almost always the same localities or regions, as can be seen from the dataset used in the present paper that includes faunistic data from the Czech Republic (DVOŘÁKOVÁ & VONIČKA 2009), Germany (STUKE & MERZ 2007), Norway (GREVE

2009), Slovakia (DVOŘÁKOVÁ & ROHÁČEK 2009, ROHÁČEK et al. 1995) and Switzerland (MERZ 2002).

Summing up all the available evidence, i.e. the largely overlapping ranges, the continual pattern of terminalia variation and the significant differences in mean wing shape (between *M. subfasciata* and *M. illota*, between sexes of *M. illota*, between populations of *M. illota*, but not between males of *M. illota* and *M. mihalyi*), then the most parsimonious conclusion is that this is a single variable species. The results also demonstrate that the females have been overlooked for almost two centuries. Study of insect genitalia is male-biased and the trend is continuing and even strengthening (AH-KING et al. 2014). However, abandoning characters of females seriously hampers our understanding of genital evolution (MENDEZ & CORDOBA-AGUILAR 2004) as well as the information on the distribution, ecology and conservation status: females are often much more common than males and sometimes the only available.

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