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DESCRIPTIONS OF *HOMONEURA* FEMALES (DIPTERA, LAUXANIIDAE) NATIVE TO SLOVAKIA

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Abstract: Females of a number of species of *Homoneura* are not recognizable according to the present knowledge. One of them is *H. notata* group (in Europe comprising *H. dilecta* and *H. notata*). Therefore, a sample of 65 *H. notata* group males and females originating from western Slovakia was examined in greater detail. Within the females, two groups were recognized defined by the shape of 8th sternite. The tentative species identity of females was derived from the habitat preferences. To confirm the species identity of female forms, morphometric analysis of wing shape was performed. The raw coordinates of 12 landmarks were superimposed by Generalized Procrustes Analysis and resulting coordinates were analyzed by standard statistical tools. Both species significantly differed in wing size and shape; 77% of all specimens were correctly assigned to species by linear discriminant analyses, but if the identity of females was switched, the proportion of correct assignments decreased to 57%. The wing shape and habitat preferences thus confirm that the two species can be reliably identified by shape of the 8th sternite: *H. dilecta* bears slender projections, while *H. dilecta* bears short and obtuse projections.

Key words: *Homoneura notata*, *H. dilecta*, females, genitalia, wing, geometric morphometrics

INTRODUCTION

Differences between insect species are generally more pronounced in males, the differentiating structures are sclerotised and well accessible, making them very practical in species identification. In contrast, females are more uniform, the differentiating structures are less accessible and partly membranous (PUNIAMOORTHY et al. 2010). This is likely explaining why females are understudied comparing to males. By analysis of the literature, AH-KING et al. (2014) show that the male bias has worsened with time. AH-KING et al. (2014) further argue that combined male-female studies provide deeper insight in our understanding of evolution of animal genitalia.

Apart from the very interesting and much discussed topic of evolution of insect genitalia, there is a more practical point of view. Without knowing the females, determination of many insect species relies on the males. However, the sex ratio can be influenced e.g. by endosymbiotic *Wolbachia* bacteria, which can induce cytoplasmatic incompatibility, male killing or, most rarely, feminization (WERREN et al. 2008). For this reason, males are often the less common sex, on which the recognition of many insect species relies. So there are good practical reasons to pay more attention to the females.

Within lauxaniids, females of several species are considered as not recognizable with the present knowledge. One of the examples is the *notata* species group of genus *Homoneura* van der Wulp, 1891. The group is easy to recognize by spots on wings and spines on hind male femurs. The four species: *H. notata* (Fallén, 1820), *H. dilecta* (Rondani, 1868), *H. maghrebi* Papp, 1978 and *H. tunisica* Papp, 1978 (PAPP, 1978, MERZ, 2003) differ by the male terminalia and ornamentation on their hind legs. The spiny hind femurs possibly assist during copulation, as was suggested in anthomyzids by ROHÁČEK & BARBER (2016) and ROHÁČEK & TÓTHOVÁ (2014, see Fig. 2 for example of copulation).

H. notata and *H. dilecta* are widely distributed within Europe (SHATALKIN 2000), while *H. maghrebi* and *H. tunisica* are restricted to Northern Africa (PAPP, 1978). In the area of central Europe, *H. notata* and *H. dilecta* often co-occur and for faunistical research it would be helpful to know, how to discern females of the two species. Females of the *H. notata* and *H. dilecta* were carefully inspected for their terminalia by the author. Luckily, two forms were identified. But the problem arises, which form corresponds to which species. Possible methods include using the habitat preferences and wing shape.

Eleven more species of *Homoneura* (including *H. muscaria*, until recently placed in genus *Cnemacantha*, SEMELBAUER 2016a) are known from the territory of Slovakia (DVOŘÁKOVÁ & GAIMARI 2009, SEMELBAUER 2015). Females of H. *mediospinoza* and *H. interstincta* have been described by MERZ (2003), and females of *H. patelliformis* and *H. thalhammeri/H. consobrina* by SEMELBAUER (2017). The aim of the paper is to identify and describe females of *H. notata* and *H. dilecta*, and describe female terminalia of remaining species of *Homoneura*.

MATERIAL AND METHODS

Collection of specimens

The specimens of *H. notata* group (Fig. 1, Tab. 1) were obtained via Malaise traps (MT) localized within the nature reserve Jurský Šúr and were collected by MAJZLAN & VIDLIČKA (2010) during years 2008-2009. Altogether, six MT were installed corresponding to certain habitat type, five of which were included in the present paper: Alder forest GPS: 48°13'55.62'' N; 17°12'31.80''. Biological

station GPS: 48°13'40.08'' N; 17°12'20.88'' E. Oak woodland GPS: 48°13'16.80'' N; 17°13'07.02'' E. Salt marsh, GPS: 48°13'12.72'' N; 17°13'20.76'' E. Wetland GPS: 48°13'17.34'' N; 17°13'04.44'' E. Data for remaining species: *H. biumbrata*: Slovakia, Ipeľské predmostie, 24.8.1994, Kozánek lgt.; *H. muscaria*: Slovakia, Bratislava-Rača, Vinohrady, 18.5.2013, Majzlan lgt.; *H. limnea*: 5.9.2008, Sv. Šúr, oak woodland GPS: 48°13'16.80'' N; 17°13'07.02'' E, Majzlan & Vidlička lgt.; *H. remmi*: 5.8.2007, Slovakia, Bučany, GPS: 48°25'3.33'' N; 17°41'47.01'' E, Vidlička lgt.

Fig. 1. *Homoneura notata* group specimen. Photo: Milan Kozánek.

Tab. 1. Counts of *H. notata* and *H. dilecta* from diferrent habitats.

	H. notata	***H. dilecta***
alder forest	-	9♂, 6♀
oak woodland	4♂, 4♀	1♀, 1♂
saltmarsh	12♂, 11♀	4♀
wetland	7♂, 4♀	-
biol. station	1♂	-

Collection of landmarks

For the analysis of wing shape, following numbers of specimens were used: *H. dilecta*: 11♀, 10♂, *H. notata*: 20♀, 24♂. The wings were mechanically removed, immersed in alcohol, mounted on slides with glycerol and photographed in 16x magnification under Stemi 2000-C stereomicroscope with a mounted Micrometrics SE camera. Each image was labelled so that the first three characters correspond with the unique specimen number, the fourth one to the species (n – notata, d – dilecta), the fifth one to the sex (m – males, f – female) and the last one to the locality (a – alnetum, b – biological station, q – oak woodland, s – salt marsh, w – wetland). A tps file was created using the tpsutil (ROHLF 2010); the landmarks were digitized using tpsdig (ROHLF 2009). The position and order of landmarks is depicted in Fig. 2. To asses the measurement error, the digitization was repeated twice.

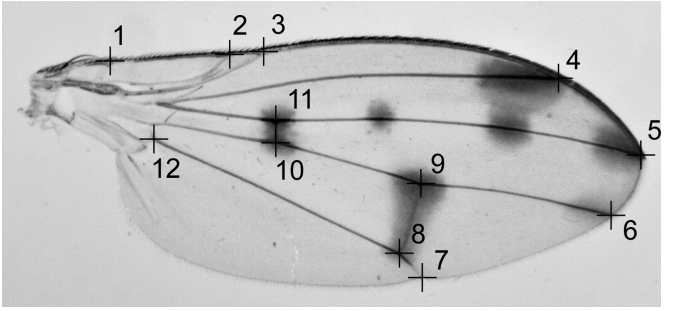


Fig. 2. Wing of *Homoneura notata* group with indicated positions of landmarks (LM). LM1 and LM3 were omitted from the statistical analysis.

Statistical analysis

The raw landmark coordinates were superimposed by the Generalized 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 are filtered out. During the superimposition, four degrees of freedom are lost, which complicates computing the degrees of freedom in statistical tests. One possibility of coping with this problem is to use a principal component analysis (PCA) (ZELDITCH et al 2004). The last four principal components (PCs) of Procrustes coordinates have zero eigenvalues and can be omitted, i.e. 20-4 PCs were used for statistical tests. The reconstruction of the shape change along the principal components was performed by tpsrelw (RHOLF 2008). More information on the GPA method can be found in CLAUDE (2008, 2013), WEBSTER & SHEETS (2010) and VISCOSI & CARDINI (2011). The data manipulation and analysis was performed in R using functions available in the package geomorph (ADAMS & OTÁROLA-CASTILLO 2013), CLAUDE (2008, 2013) and generic functions of program R (R CORE TEAM 2013). The original landmark coordinates, table of factors and R code are available at the author.

Determination and preparation of female terminalia

For determination of *H. notata* group males was used key of PAPP (1979) with emendation by MERZ (2003). For the remaining species, the key of SHATALKIN (2000) with combination with its English translation (SCHACHT et al. 2004) was used. The females were inspected for their terminalia and divided in two groups according to their shape. In specimens, where the terminalia were retracted, whole abdomen of female was mechanically removed and macerated in NaOH for 1-3 hours. The terminalia were photographed immersed in glycerol by Micrometrics camera mounted on ZEISS Stemi 2000-C binocular microscope and the pictures were redrawn in Photoshop. The forms were tentatively ascribed to species based on habitat preferences.

RESULTS

Wing shape and size

Prior to the analysis, the repeatability of landmarks was assessed. For each landmark and specimen the variance between digitization sessions was calculated separately for x and y coordinates and then summed. The resulting values showed that landmarks 1 and 3 had much higher variance (i.e. were less precisely shot on average) and were removed. The configurations of 10 remaining landmarks were superimposed by GPA. The measurement error (ME) was assessed by ANOVA approach (YEZERINAC et al. 1992). Two linear models were fitted to both centroid size and first 16 principal components based on Procrustes coordinates. In the first model the explaining variable was individual (65 levels), while in the second model

it was the session (2 levels). In both variables the session factor was non-significant (p-value>0.964), while the individual factor was highly significant (p-value<0.0001), suggesting that the variability due to ME is negligible. Once the ME was assessed, the replicates were averaged.

A linear model was fitted to centroid size with sex and species as explaining variables. Both factors appear to be significant (Tab. 2), with males and *H. dilecta* being larger on average (Fig. 3). A PCA was performed on Procrustes coordinates. The first two components explain 33.8% and 22.5% of variance respectively. In a space defined by the first two PCs the two species appear to be fairly well separated (Fig. 4). 20-4 = 16 PCs were selected for the subsequent analysis. A linear model was fitted to the PCs with species, sex and centroid size as explaining variables. All of the main effects and number of interaction terms appear to be significant (Tab. 3). Finally, to asses how efficiently the wing shape is discriminating between the species, linear discriminant analysis (LDA) was applied to the original Procrustes coordinates; about 77% of all specimens were correctly assigned to the species. On the other hand, if the identity of females was switched, only about 57% of all specimens were correctly assigned.

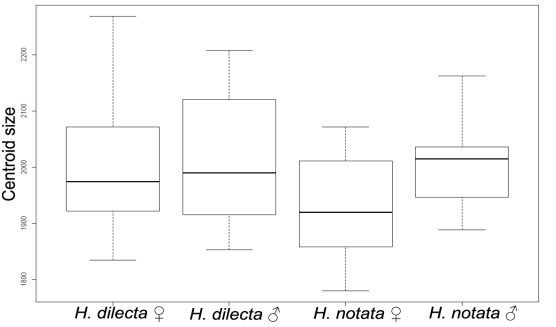


Fig. 3. Box plot of wing centroid sizes.

Tab. 2. ANOVA table of wing centroid size.

	Df	SS	MS	F-value	p-value
species	1	115745	115745	15.6321	0.0002028 ***
sex	1	72611	72611	9.8065	0.0026698 **
species:sex	1	9	9	0.0012	0.9722539
Residuals	61	451665	7404		

Tab. 3. Results od MANOVA based 20-4 principal components based on Procrustes coordinates. The nonsignificant three way interaction term was removed from the model.

	Df	Pillai	approx F	Num Df	Den Df	p-value
species	1	0.70036	4.5578	20	39	2.568e-05 ***
sex	1	0.40587	1.3321	20	39	0.2168463
size	1	0.64316	3.5147	20	39	0.0003838 ***
species:sex	1	0.55375	2.4198	20	39	0.0089414 **
species:sex	1	0.63682	3.4192	20	39	0.0004991 ***
sex:size	1	0.49117	1.8823	20	39	0.0448620 *
Residuals	58					

Female terminalia

The original suggestion of species identity of *H. notata* group females appears to be corroborated by the analysis of wing shape, so now the females can be formally described.

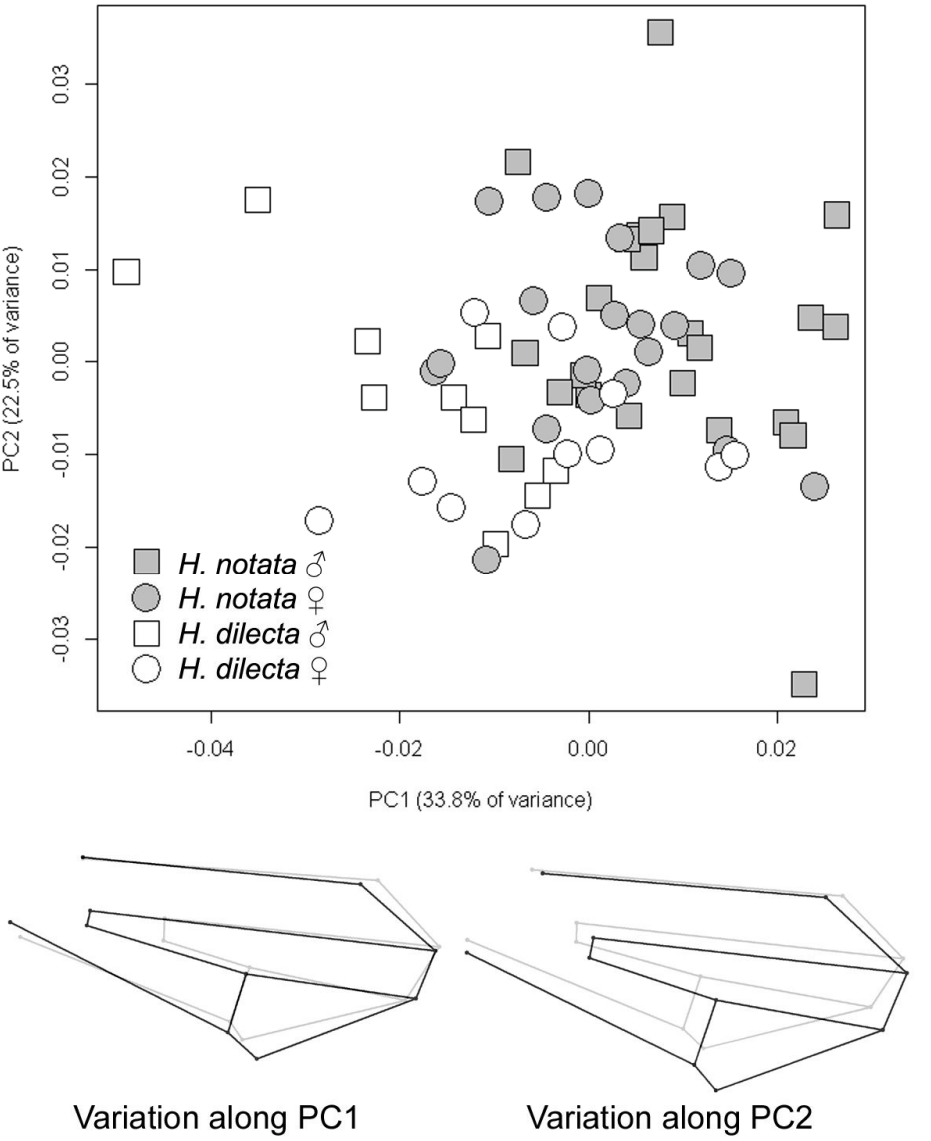
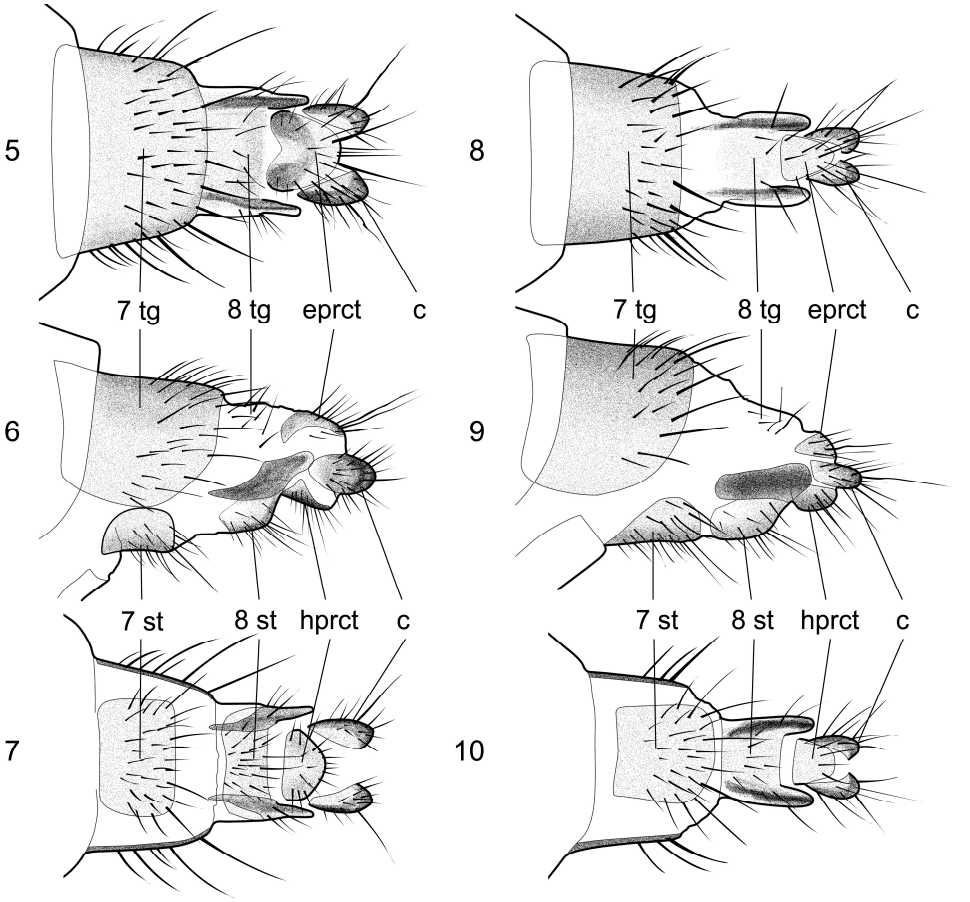


Fig. 4. PCA plot of wing Procrustes coordinates. Below the plot is depicted shape change associated with the first two principal components (PCs).

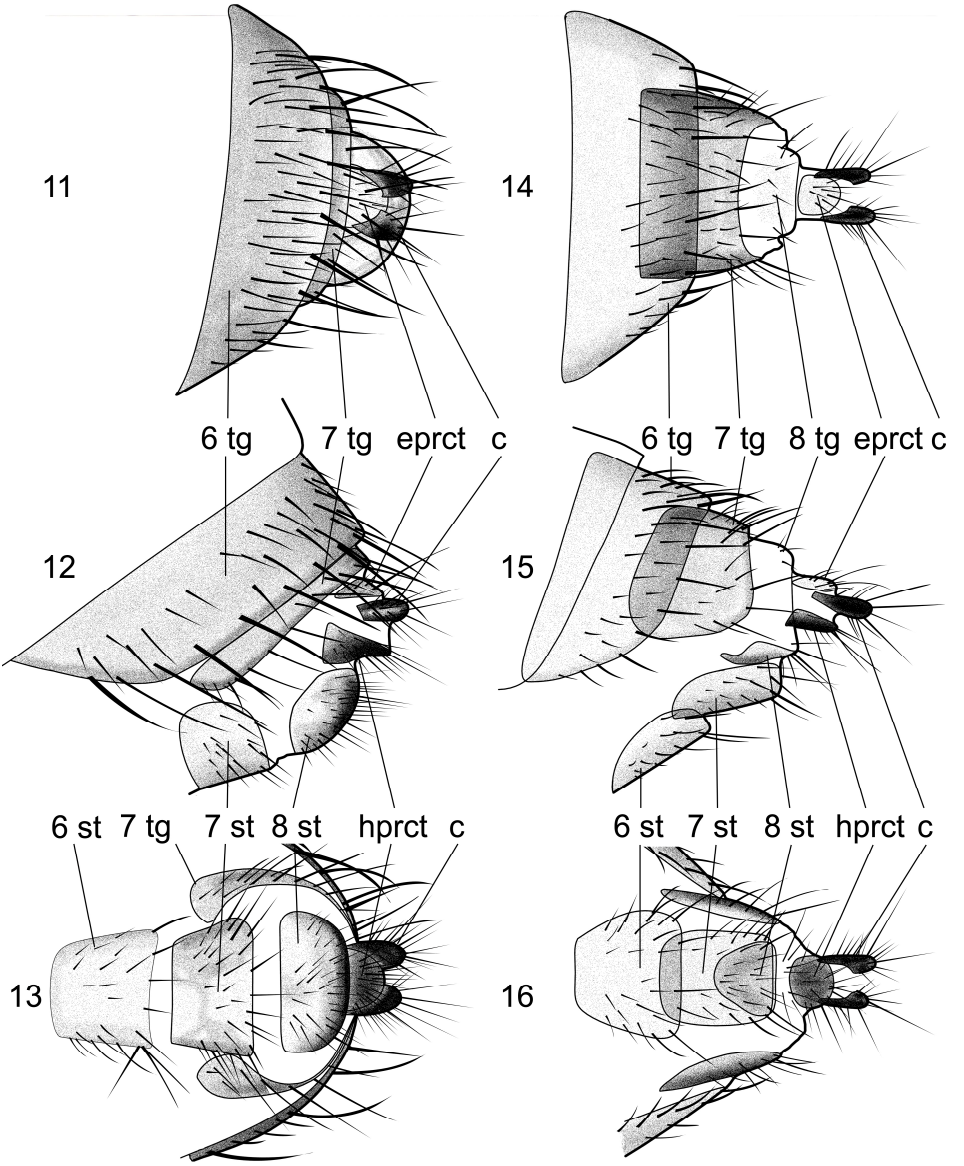
H. dilecta and *H. notata*. The abdomen tapering to the distal end; the 8th sternite convex, with a pair of projections; the 8th tergite not differentiated, indicated only by few setae. Cerci are oval and dark, with moderately long setae, epiproct pale, hypoproct dark. The shape of projections of 8th sternite seems to be the only clear differentiating character between females of the two species. In *H. notata*, the projections are thin and elongated, while in *H. dilecta*, the projections are short and blunt. It must be pointed out, that although the projections appear as natural continuation of the sides of sternite, they outgrow from the inner side of the sternite (Figs 5-10).



Figs 5-10. 5-7 *H. notata*; 8-10 *H. dilecta*; c – cerci; eprct – epiproct; hprct – hypoproct; 7 st – 7th sternite; 8 st – 8th sternite, 7 tg – 7th tergite; 8 tg – 8th tergite.

H. biumbrata. The abdomen obtuse in distal end; the 7th and 8th tergites are reduced to narrow stripes; the 8th sternite oval, convex and covered in fine setae; hypoproct darker than epiproct, cerci dark and robust (Figs 11-13).

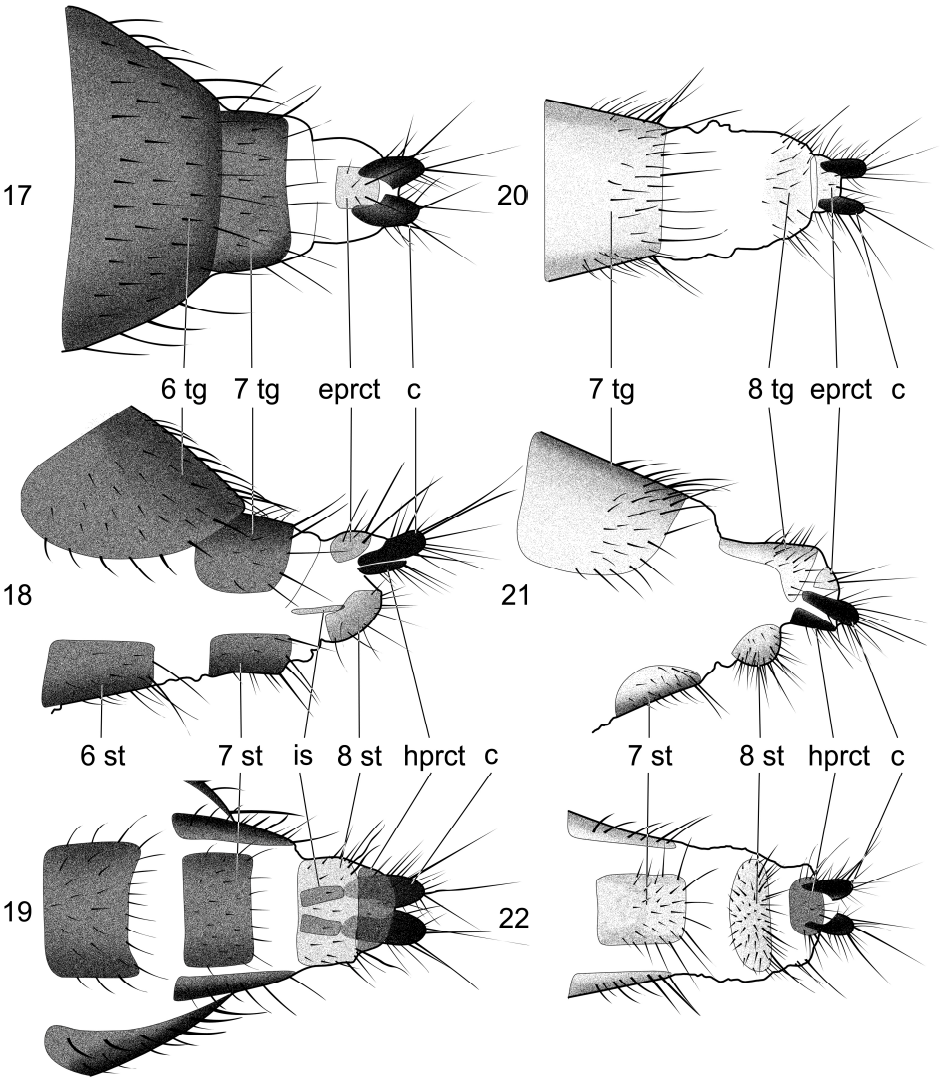
H. limnea. Abdomen tapering to distal end; 8th sternite triangular and retracted below the 7th sternite; 8th tergite not sclerotised, covered in few weak setae; epiproct paler than hypoproct, cerci dark (Figs 14-16).



Figs 11-16. 11-13 *H. biumbrata*; 14-16 *H. limnea*; c – cerci; eprct – epiproct; hprct – hypoproct; 6-8 st – 6-8th sternite; 7-8 tg – 6-8th tergite.

H muscaria. The abdomen tapering to distal end; the 8th segment adjoined to the 7th segment by membranous tube; within the tube is visible pair of fine sclerites; 8th sternite convex, rectangular in ventral view, 8th tergite not differentiated, cerci dark (Figs 17-19).

H. remmi/H tesquae. The abdomen tapering to distal end; the 8th segment adjoined to the 7th segment by membranous tube; 8th sternite transversal, convex, covered in fine outstanding setae; epiproct paler than hypoproct, cerci dark (Figs 20-22).



Figs 17-22. 17-19 *H. muscaria*; 20-22 *H. remmi*; c – cerci; eprct – epiproct; hprct – hypoproct; is – internal sclerites; 6-8 st – 6-8th sternite; 7-8 tg – 6-8th tergite.

DISCUSSION

The species identity of *H. notata* group females was credibly confirmed by the wing shape, and is also in agreement with the habitat preferences. The preferred habitat of *H. notata* were open places (salt marsh, wetland) while *H. dilecta* preferred shady alder forest. The shape differences between females are quite subtle, but seem to be reliable. However, it must be pointed out that the shape of terminalia may vary with geographic region. If the shape differences are reliable also for other regions remains to be confirmed by future research.

The female terminalia are in comparison to males much more uniform but in most species they provide clear differentiating characters, making them useful in determining e.g. damaged specimens. The only exception is the *H. remmi* - *H. tesquae* pair. The differences in shape of male terminalia of the two species are very pronounced (SEMELBAUER 2016b) and the two species can be recognized even by colour pattern of the wing (pigmented/non-pigmented cross veins). Surprisingly, no suggestion of difference in female terminalia was found. This of course does not imply that the female terminalia are identical. Possibly more detailed look and larger sample size could provide more objective view.

H. muscaria was recently reclassified from genus *Cnemacantha* to genus *Homoneura* based on the DNA sequences (SEMELBAUER 2016a). *H. thalhammeri* appears to be the closest relative of *H. muscaria*, and the two species share also the structure of male genitalia (SEMELBAUER 2016b). As revealed in the present paper, the two species have also similar structure of the female terminalia (see SEMELBAUER 2017 for *H. thalhammeri* drawing). Most notably, both species bear a pair of fine internal sclerites and the membranous tube connects the 7th and 8th segments. These findings corroborate the synonymy of *Cnemacantha* and *Homoneura*.

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