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An attempt to identification of nymphal instars of biting lice (*Mallophaga*) using discriminant function

Próba identyfikacji stadiów nimfalnych wszołów (*Mallophaga*) przy pomocy funkcji dyskryminacji

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ABSTRACT. Discriminant analysis was used to determine the most suitable metrical characters for separating the nymphal instars within and between the following biting lice — *A. p. megalosoma* and *U. phasiani* (suborder *Amplycera*: *Menoponoidea*) as well as *L. m. maculosus* and *R. m. colchicus* (suborder *Ischnocera*: *Lipeuridae*). It was found that dimensions of head, body length and in a few cases abdomen width were good discriminators. For the best pair of characters the equations of linear discriminant function were developed and the percentage of agreement with previous identification, based only on chaetotaxy, was estimated.

INTRODUCTION

Descriptions of nymphal instars of biting lice are scarce and based mainly on such morphological characters as the number and arrangment of setae on the body surface. Basing on chaetotaxy of pterothorax the individual nymphal stages were separated in a chosen few species from the suborder *Ischnocera* (WILSON 1939, CONCI 1956, MODRZEJEWSKA 1983). Only in one case (CLAY, 1958) chaetotaxy of nymphs was used to characterize the genus *Degeeriella* sensu lato. In the available literature the papers on the identification of lice nymphs from the suborder *Amblycera* have not been found. This gap was filled partly by MODRZEJEWSKA's (1983) study. The author found that in the nymphs of *Amyrsidea p. megalosoma* and *U. phasiani*, the ectoparasites of pheasants — *Phasianus colchicus* L., the number and com-

position of setae at the sides of ventral abdominal III–V segments change progressively. The valuable characters were the measurments earlier mentioned by previously cited WILSON (1939), as well as PIOTROWSKI and KADULSKI (1979) and KADULSKI (1978).

The identification of developmental stages, especially according to the closely related species, determines precisely taxonomic study by introducing a new set of diagnostic characters.

The aim of the present paper was the application of the discriminant function to find the best single character or combination of metrical characters which would provide the most reliable criteria for separating the nymphs within and between the species of biting lice. The study was carried out on the followong species: *Amyrsidea perdicis megalosoma* (OVERGAARD, 1943) and *Uchida phasiani* (MODRZEJEWSKA and ZŁOTORZYCKA, 1977) (suborder *Amblycera*: *Menoponoidea*) as well as *Lipeurus maculosus maculosus* CLAY 1938, and *Reticulipeurus mesopelios colchicus* (CLAY, 1938) (suborder *Ischnocera*: *Lipeuridae*) and also between I, II, III stages of *A. p. megalosoma* vs. I, II, III stages of *U. phasiani* and analogically — *L. m. maculosus* vs. *R. m. colchicus*.

MATERIALS AND METHODS

The nymphs of biting lice were obtained from 117 pheasants, collected in Lower Silesia, Poland. The whole collection was used for the measurements. The numbers of nymphs presumably belonging to the individual stages of *A. p. megalosoma* were: I = 35, II = 35, III = 45; *U. phasiani* I = 16, II = 26, III = 36; *L. m. maculosus* I = 13, II = 13, III = 21; *R. m. colchicus* I = 21, II = 25, III = 28.

The biting lice were preserved in 70% ethyl alkohol, macerated with 5–10% NaOH and mounted in Canada balsam according to the universally accepted method.

The following five characters were measured: length of head (x_1), width of head (x_2), length of thorax with abdomen (x_3), width of abdomen (x_4) and total body length (x_5).

Discriminant analysis was carried out using the linear discriminant function for two groups with selection of variables according to computer program dis2 (BARTKOWIAK, 1982). All the computations were made on Odra 1204 computer in the Computer Analysis Centre, University of Wrocław. Discriminant function is designed for paired comparisons and the nymphs were put into the groups as follows:

A_I vs. A_{II}, A_I vs. A_{III}, A_{II} vs. A_{III}, [1]
U_I vs. U_{II}, U_I vs. U_{III}, U_{II} vs. U_{III},
A_I vs. U_I, A_{II} vs. U_{II}, A_{III} vs. U_{III},
L_I vs. L_{II}, L_I vs. L_{III}, L_{II} vs. L_{III},
R_I vs. R_{II}, R_I vs. R_{III}, R_{II} vs. R_{III},
L_I vs. R_I, L_{II} vs. R_{II}, L_{III} vs. R_{III}.

For each pair of groups the most differentiating single character, a pair of, three and four characters were determined after studing all possible combinations of characters. The indications of the discriminant power of characters were: coefficient Mahanolobis D^2, R^2 — square of multiple correlation coefficient and se — linear dispersion.

Table 1. The best characters for discriminating of nymphal instars within and between the species of biting lice

Paired comparisons	Sets of the best characters			
	single	pair	three	four
A_I vs. A_{II}	x_1	x_1, x_2	x_1, x_2, x_3	x_1, x_2, x_3, x_5
A_I vs. A_{III}	x_1	x_1, x_2	x_1, x_2, x_3	x_1, x_2, x_3, x_5
A_{II} vs. A_{III}	x_1	x_1, x_2	x_1, x_2, x_3	x_1, x_2, x_3, x_5
U_I vs. U_{II}	x_2	x_1, x_2	x_1, x_2, x_4	x_1, x_2, x_3, x_4
U_I vs. U_{III}	x_2	x_2, x_4	x_1, x_2, x_4	x_1, x_2, x_3, x_4
U_{II} vs. U_{III}	x_2	x_2, x_4	x_1, x_2, x_4	x_1, x_2, x_4, x_5
A_I vs. U_I	x_2	x_1, x_2	x_1, x_2, x_3	x_1, x_2, x_3, x_5
A_{II} vs. U_{II}	x_2	x_1, x_5	x_1, x_3, x_5	x_1, x_2, x_3, x_5
A_{III} vs. U_{III}	x_2	x_3, x_5	x_2, x_3, x_5	x_2, x_3, x_4, x_5
L_I vs. L_{II}	x_5	x_1, x_5	x_1, x_2, x_5	x_1, x_2, x_3, x_5
L_I vs. L_{III}	x_5	x_2, x_5	x_2, x_3, x_5	x_2, x_3, x_4, x_5
L_{II} vs. L_{III}	x_5	x_1, x_5	x_1, x_4, x_5	x_1, x_2, x_4, x_5
R_I vs. R_{II}	x_1	x_1, x_5	x_1, x_4, x_5	x_1, x_2, x_4, x_5
R_I vs. R_{III}	x_1	x_1, x_5	x_1, x_4, x_5	x_1, x_2, x_4, x_5
R_{II} vs. R_{III}	x_1	x_1, x_5	x_1, x_4, x_5	x_1, x_3, x_4, x_5
L_I vs. R_I	x_2	x_2, x_5	x_1, x_2, x_5	x_1, x_2, x_4, x_5
L_{II} vs. R_{II}	x_1	x_1, x_4	x_1, x_4, x_5	x_1, x_2, x_4, x_5
L_{III} vs. R_{III}	x_4	x_1, x_4	x_1, x_4, x_5	x_1, x_2, x_4, x_5

On the ground of discriminant equations for the best pair of characters, the specimens previously classified to other groups were discovered (the value b_o is constructed in such way, that z is positive if we substitute the

[1] The full systematic names of biting lice are limited to initials of genera. Roman letters mean I, II, III nymphal instars of lice.

data from the group I and it is negative if we put data from the second one). The percent of misclassification was estimated according to the formula:

$$\text{percent} = \frac{n_i + n_j}{n_1 + n_2} \cdot 100,$$

where, n_i and n_j stand for the numbers of specimens classified to the other groups, and n_1, n_2 mean the size of samples.

RESULTS

The means and standard deviations of five variables measured and included in the discriminant function analysis as well as another statistical parameters (ranges, standard errors of mean, coefficients of varation %) are graphically presented in Figures 1–3. The statistically significant differences occurred between the arithmetic means of nearly all characters of nymphs of I and II stage as well as of II and III stage. Only the abdomen width of nymphs of I and II stages of *L. m. maculosus* is not statistically different. For the most characters, variation defined as the standard deviation in the percent of mean, is not higher than 10%. The measurments of head show the smallest coefficient of variation (3–8%); body length has the higher (4–11%) and the greatest — the length of thorax with abdomen (6–19%).

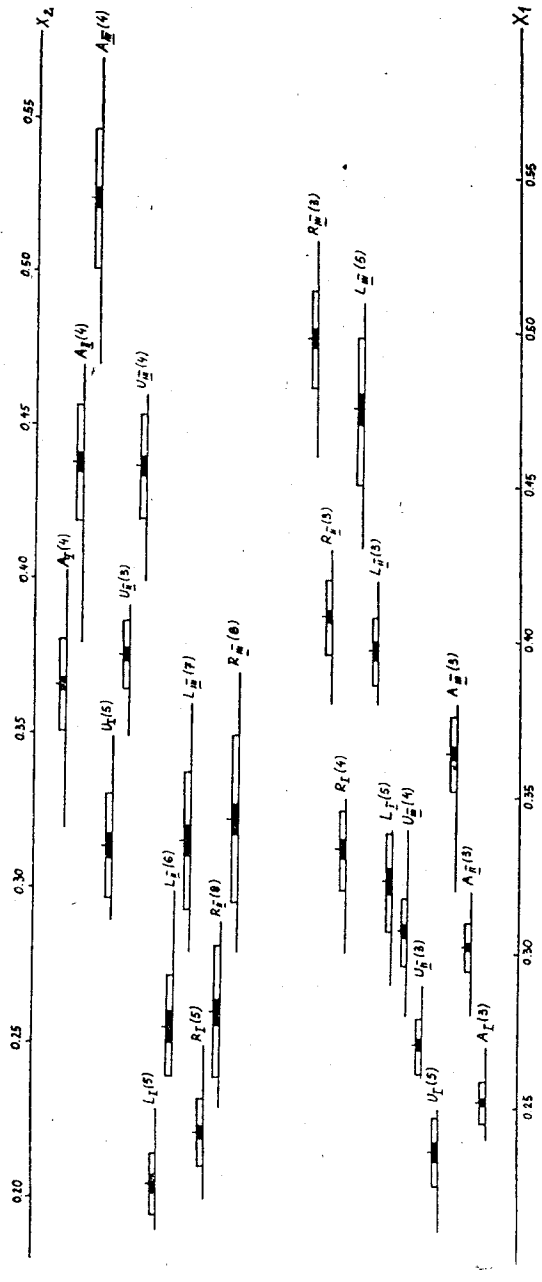
The different sets of metrical characters chosen to be used as good discriminators within and between the individual nymphal instars are presented in Table 1.

The discriminant function equations, based on the best pair of characters were developed to classify a nymph into one of two compared groups (Table 2). For instance, if the value $z \geqslant 0$, the specimen is most probably A_I; otherwise ($z < 0$) the nymph is identified as A_{II}. In this case the percent of misclassification was 99.

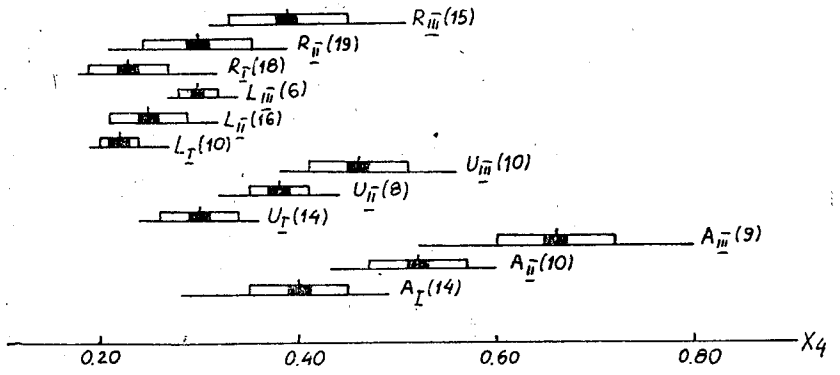
The pair of characters, x_1 and x_5, plotted in Figure 4, show the example of the best discrimination between the I and II stages of *R. m. colchicus* nymphs. The ranges of these characters do not overlap and there is a 100% agreement with the previous classification based only on the chaetotaxy. Figure 5 illustrates 88% agreement according to L_I vs. R_I.

DISCUSSION

It was found that the analyses of the discriminant functions applied to separate the individuals of nymphal instars within species as well as between respective stages of closely related lice — *A. p. megalosoma* and *U. phasiani* (*Menoponoidea*) as well as *L. m. maculous* and *R. m. colchicus* (*Lipeuridae*) —



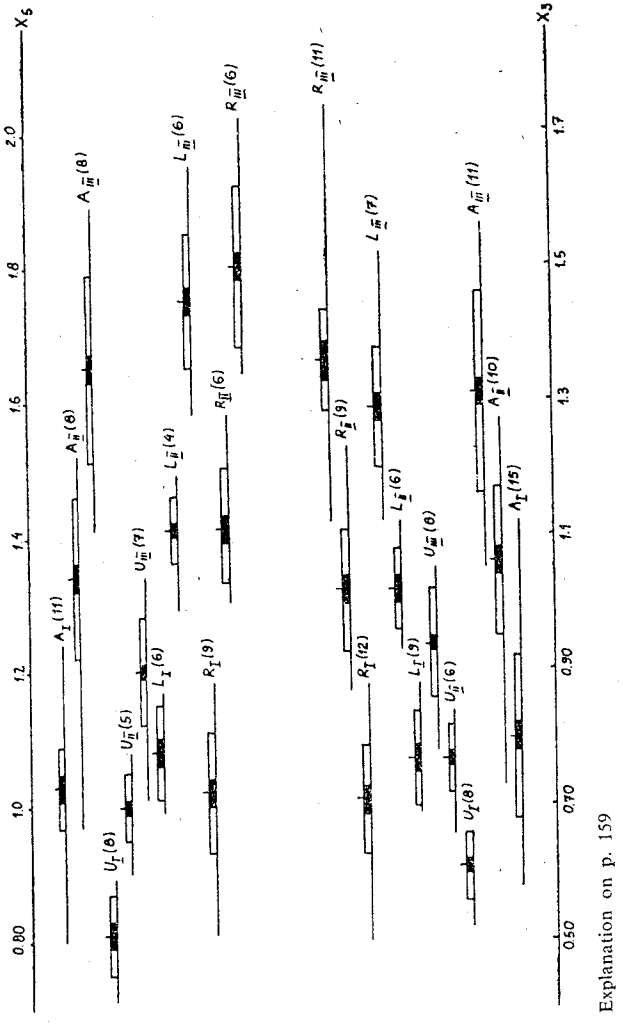
Explanation on p. 159



1–3. Variation of characters in individual nymphal instars of biting lice. Horizontal line equals range; vertical line sample mean; black bar twice the standard error of the mean; onehalf of each black bar plus the white bar at either end one sample standard deviation; coefficient of variation (%) in brackets

Table 2. The equations of discriminant function based on the best pair of characters

Paired comparisons	The coefficients of the discriminant function and value $z = b_0 + b_i x_i + b_j x_j$ for the best pair of characters	The degree of conformity with previous identification (%)
1	2	3
A_I vs. A_{II}	$z = 270.32 + (-658.53)x_1 + (-213.98)x_2$	99
A_I vs. A_{III}	$z = 449.06 + (-1087.92)x_1 + (-249.22)x_2$	100
A_{II} vs. A_{III}	$z = 263.88 + (-624.23)x_1 + (-115.63)x_2$	100
U_I vs. U_{II}	$z = 146.00 + (-206.68)x_1 + (-271.60)x_2$	98
U_I vs. U_{II}	$z = 172.22 + (-497.37)x_1 + 38.76x_4$	100
U_{II} vs. U_{III}	$z = 122.20 + (-323.38)x_2 + 22.07x_4$	97
A_I vs. U_I	$z = -104.77 + 148.65x_1 + 200.26x_2$	94
A_{III} vs. U_{II}	$z = -170.47 + 421.32x_1 + 42.57x_5$	98
A_{III} vs. U_{III}	$z = -111.12 + (-180.34)x_3 + 219.65x_5$	100
R_I vs. R_{II}	$z = 231.86 + (-520.59)x_1 + (-31.72)x_5$	100
R_I vs. R_{III}	$z = 434.25 + (-849.71)x_1 + (-57.12)x_5$	100
R_{II} vs. R_{III}	$z = 262.64 + (-479.06)x_1 + (-28.26)x_5$	100
L_I vs. L_{II}	$z = 176.61 + (-260.52)x_1 + (-66.67)x_5$	100
L_I vs. L_{III}	$z = 143.4 + (-175.66)x_2 + (-68.9)x_5$	100
II vs. L_{III}	$z = 105{,}25 + (-126.91)x_1 + (-31.6)x_5$	100
I vs. R_I	$z = -21.53 + 167.80x_2 + (-13.63)x_5$	88
II vs. R_{II}	$z = -35.56 + 78.38x_1 + 14.68x_4$	76
L_{III} vs. R_{III}	$z = -42.07 + 58.60x_1 + 39.51x_4$	86

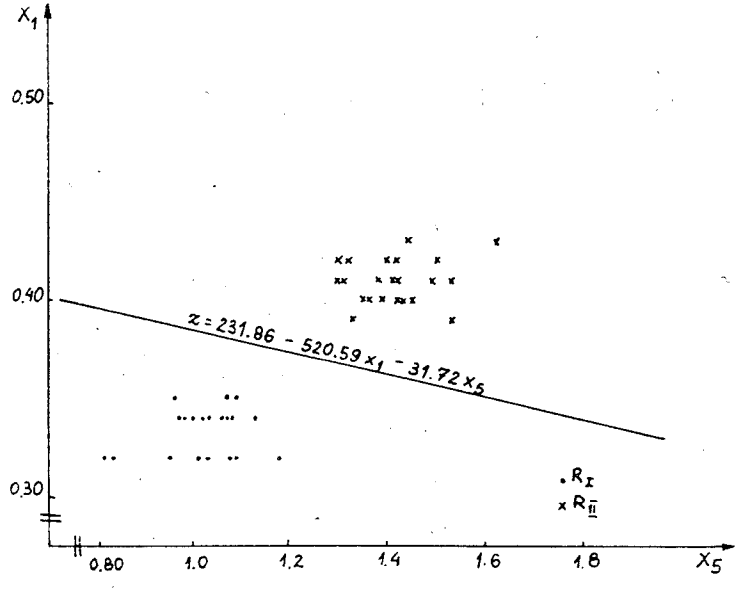


Explanation on p. 159

in general were consistent with the previous identification made by MODRZEJEWSKA (1983) and based only on the chaetotaxy.

The best single character which distinguishes the nymphal instars of *A. p. megalosoma* was the length of head and width of head for *U. phasiani*. Likewise, the nymphs of both species can be separated on the ground of the head width. However, the pairs of characters with the greatest discrimination power of the nymphs of I stage of these species were dimensions of head, II — head length together with body length, III — length of thorax with abdomen and body length.

According to CLAY (1968) the head width is the most stable diagnostic metrical character for the nymphal lice of the genus *Degeeriella* (*Degeeriellidae*). On the contrary, in the present research the important characters for diagnosis of nymphs within the genus *Lipeurus* and *Reticulipeurus* (*Lipeuridae*) were mostly lengths of head and body (100% agreement with previous identification). The head width together with body length were the best to differentiate only I stage from II of *L. m. maculosus* and I stage *L. m. maculosus* from I stage *R. m. colchicus*. The other combination of the best pair of characters:



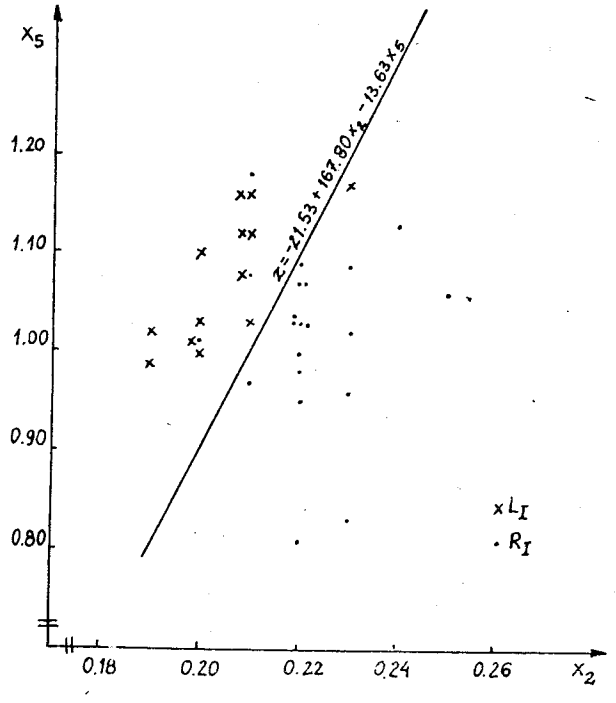
4. Discrimination between I and II nymphal instars of *R. m. colchicus*

the head length and abdomen width differ the next instars: II and III of both species with the smaller agreement (76 and 86%).

It has been stated that discriminant analysis used by many entomologists: BIGELOW and REIMER (1954), MCGUIRE and WIRTH (1958), KIM et al. (1963), GODWIN et al. (1982) to separated taxa of species level may be helpful also for identification of individuals of nymphal instars of lice.

CONCLUSION

Metrical characters of nymphs of biting lice, particularly dimensions of head and body length, like as the chaetotaxy, make a set of diagnostic characters and allow nearly a 100% separation of the individual nymphal instars within species as well as between closely related taxa.



5. Discrimination between I nymphs of *L. m. maculosus* and I nymphs of *R. m. colchicus*

STRESZCZENIE

Za pomocą funkcji dyskryminacyjnej wyznaczono cechy metryczne najlepiej różnicujące śródgatunkowo i międzygatunkowo poszczególne stadia rozwojowe wszołów — *A. p. megalosoma* i *U. phasiani* (*Amblycera*: *Menoponoidea*) oraz *L. m. maculosus* i *R. m. colchicus* (*Ischnocera*: *Lipeuridae*). Cechami najlepiej różnicującymi okazały się wymiary głowy, długość ciała i w nielicznych przypadkach szerokość odwłoka nimf. Dla najprzydatniejszej pary cech obliczono równanie funkcji dyskryminacyjnej oraz procent zgodności względem uprzedniej identyfikacji opartej o chetotaksję.

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Three new *Charletonia* (*Acari*, *Erythraeidae*) from Vietnam

Trzy nowe gatunki *Charletonia* (*Acari*, *Erythraeidae*) z Wietnamu

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ABSTRACT. Three new species: *Charletonia jolantae*, *Charletonia huensis* and *Charletonia danangensis*, collected in Vietnam are described and illustrated.

25 larvae of the genus *Charletonia* were collected on indetermined *Orthoptera*, noctulid moths and plants in Danang and Hue, middle Vietnam, in February 1985. All the specimens belong to species new for science. Measurements given in microns (μ); terminology after SOUTHCOTT (1966).

Charletonia huensis n.sp.

Colour in life red. Animal 992μ long to the tip of cheliceral blades (a moderately engorged specimen). Dorsal scutum broader than its length, AL scutalae arise near the rounded angles of scutum. PL scutalae almost on the level of scutum equator. The ML scutalae arise almost in the half-way from AL to PL scutala on each side. Scutalae PL and ML long, AL short ciliated. The A Sens arise a little posteriorly to the level of the AL scutalae, P Sens arise in the posterior end of scutum. Scutal sensillae long, filiform, distally ciliated (fig. 1, 3).

Dorsal idiosomalae similar (fig. 2) to the scutalae in the anterior post of idiosoma, 50–56μ long, in posterior end of idiosoma 60–64μ long. DS = 131; VS = 65.

Measurements in μ: AW 64, MW 68, PW 92, SBa 14, SBp 18, ASBa 18, ISD 66, L 88, W 98, AP 28, AM 18, AL 36, ML 60, PL 78, A Sens 68, P Sens 76, ASba/ISD 0.21.