Attempt to define morphological parameters enabling sex determination of *Ips typographus* (Linaeus, 1758) (Coleoptera: Curculionidae: Scolytinae) using PCA and CLU

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ABSTRACT: There are only two known methods for determining the sex of *Ips typographus* (L.) – dissection that cannot be used with live beetles and by distinguishing between the density of the hairs on the head, a less reliable method that requires experienced personnel. As a result, we have sought to find a more reliable method of sex determination for *I. typographus* which can be used with live specimens but is still reliable and easy to conduct. The aim of the article is to explore the inner structure in data from measurements of morphological parameters of spruce bark beetles and to find correlations which could be used for sex determination. The number of beetles in our sample was 110, all from the first trapping of one pheromone trap. The statistical methods of principal components analysis (PCA) and cluster analysis (CLU) were used to support any correlation between the sex parameter and other morphological parameters (weight, elytra length, elytra width, pronotum length, pronotum width). As no correlation was found in the case of sex, we can claim that it is not possible to determine sex according to the examined morphological parameters. However, we have found an interesting inner structure in the data and it was confirmed that even weight is slightly correlated with other morphological parameters.

Keywords: spruce bark beetle; principal components analysis; cluster analysis

The necessity of sex determination of the spruce bark beetle – *Ips typographus* (Linnaeus, 1758) comes more from research than from the practice of forestry, although accurate methods for sex discrimination of this species have been poorly investigated. The only known reliable method is genital dissection (Annila 1971; Botterweg 1983; Lobinger 1996; Faccoli, Buffo 2004). The beetles are damaged during this process and it is not possible to use this method with live beetles. Determining the sex of spruce bark beetles according to the density of pronotal hair bristles is well documented in the literature (Schlyter, Cederholm 1981; Anderbrant et al. 1985; Anderbrandt 1990) but Bakke (1970) provided an alternative method for establishing the sex of live males. Bednarz and Kacprzyk (2012) used the higher weight of female beetles from galleries due to the presence of maturing eggs.

These reasons led us to investigating the use of statistical methods based on selected morphological measurements (weight, elytra length, elytra width, pronotum length, pronotum width). We attempted to find correlations between the parameters and internal structure of the data using the PCA and CLU statistical methods to establish if it is possible to use the selected characteristics to allow for the non-destructive sex determination of spruce bark beetles. Sex determination has no practical impact on forestry practice, but is important experimentally. The aim of our work was to find a non-destructive method for sex determination that would provide the possibility of using the same beetles for further research.

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MATERIAL AND METHODS

110 spruce bark beetles were randomly selected from the first trapping. To avoid possible differences due to the location, all 110 beetles in the sample were selected from a single pheromone trap. All of the beetles were measured for the following parameters: (*i*) shield width [mm]; (*ii*) shield length [mm]; (*iii*) elytra width [mm]; (*iv*) elytra length [mm]; (*v*) weight [mg]. Dissection and sex determination then took place. Following Zahradník (2013), the whole abdomen was broken off and subsequently boiled in a 10% potassium hydroxide (KOH) water bath. Measurements were performed using callipers and an OLYMPUS SZX16 microscope (Olympus, Tokyo, Japan). A statistical evaluation was made with STATISTICA 12 (SPSS, Tulsa, USA) software using PCA and CLU methods.

The first statistical evaluation method was principle component analysis (PCA). PCA is used extensively in many forms of analysis – from neuroscience to computer graphics – because it is a simple, non-parametric method of extracting relevant information from confusing data sets (Shlens 2003). Its aim is to transform the original characters $x_{i,j}$ = 1, ... , m, into a smaller number of latent variables y,j (the principal components). These latent variables are far fewer in number than the original variables, but still describe almost all of the variability of the original characters and are mutually uncorrelated (Meloun et al. 2005). The number of principal components will be determined by the Scree Plot (Fig. 1). When large multivariate datasets are analyzed, it is often desirable to reduce their dimensionality (Jolliffe 2005). PCA is able to reduce the number of characters while maintaining a small loss of information.

The other method, the analysis of clusters (CLU), examined the similarity of multidimensional objects. We can examine both objects (110 beetles) and variables (morphological parameters and sex) according to the correlation coefficients they create in clusters (Meloun et al. 2005).

The above variables can show the influence of various units and provide the first step towards data standardization. The original data are standardized according to the formula $(x - \overline{x})/s$.

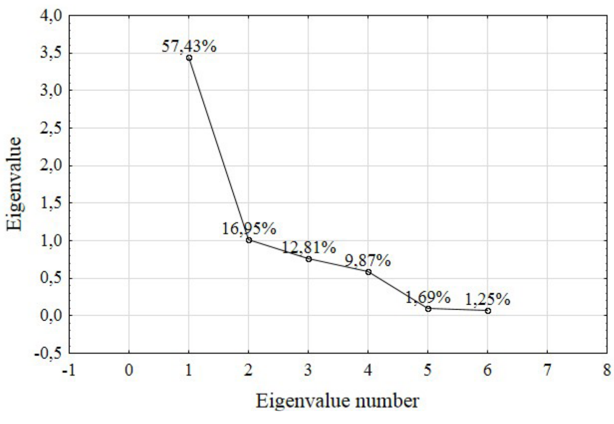

Fig 1. Assessment of the necessary number of main components (scree plot)

RESULTS

Initial familiarization with the source data matrix allows for a graphical representation (Fig 2). The similarity of the characters provides icon plots for all of the parameters for all of the characters. For example, Fig. 2 shows similar star shapes which represent similar beetles. Every single vector presents single variable – higher value = longer vector. The Scree Plot (Fig. 1) shows that the eigenvalue number is greater than 1 (axis y) for the first two components (Kaiser criterion), which provide together the information of 74.38% (57.43% and 16.95%). The other components are noise, therefore we will focus on the first two components.

The matrix graph (Fig. 3a) shows that there were almost zero correlations with other variables that indicate variable sex. The interpolating line is nearly parallel to the axis x. The correlations between sex and: weight (0.06), pronotum width (–0.06), pronotum length (0.012), elytra width (–0.03), elytra length (–0.003) are not statistically significant (coefficient presented in parentheses). Some other variables, the length of pronotum and width of elytra (0.79), width and length of elytra (0.62), width of pronotum and width of elytra (0.91), are intersecting nearly diagonally (high correlation), but weight is less correlated with other variables – pronotum width (0.59), pronotum length (0.51), elytra width (0.48), elytra length (0.39), with the line making a sharper angle with the axis x.

The scatter diagram shows the component score information by the first and second main component (Fig. 3c). The first and second main component provides the largest percentage of information, therefore the greatest weight is given to them. The radius vectors of all of the measured values are close to the circle, therefore they are important and we will not remove them. The radius vector of weight is shorter and forms an obtuse angle with the other morphological parameters, meaning they are less correlated. The radius vector of weight is also shorter, making the resulting information less

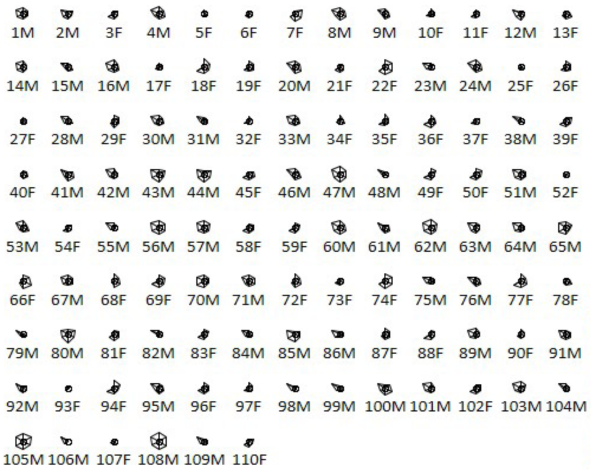

Fig. 2. Icon plot – the similarity of assessed beetles substituted by stars (F – female, M – male, clockwise: weight; protonum W, L; elytras W, L; sex)

important. The other radius vectors of morphological parameters together form an extremely sharp angle and almost coincide, indicating a high correlation. It is possible to omit the variables with the shorter radius vectors (length of elytra and pronotum) from the data structure survey. The only radius vector orthogonal to the axis x and vectors of other characters is the Sex parameter, which means that it has minimal to no correlation with other characteristics. The radius vector touches the circle and is parallel to the axis y, therefore it is highly correlated and influences the second main component.

Analogously, due to the importance of the previous chart projections of the characters in the factor plane 1 × 2 (the first two principal components save the biggest amount of information) we will prefer this plane also in the projection of cases (Fig. 3d). The second component divides the beetles into males and females. Females are shown in the graph above zero, males below zero. Clusters of male and female beetles are stretched to the sides according to the morphological characteristics of individual beetles. Larger beetles are on the left; smaller beetles are on the right.

Clusters of variables show similarity between measured parameters and sex (Fig. 3b). A higher correlation coefficient means a shorter distance in the graph and greater similarity. The greatest distance from the other variables, so the smallest

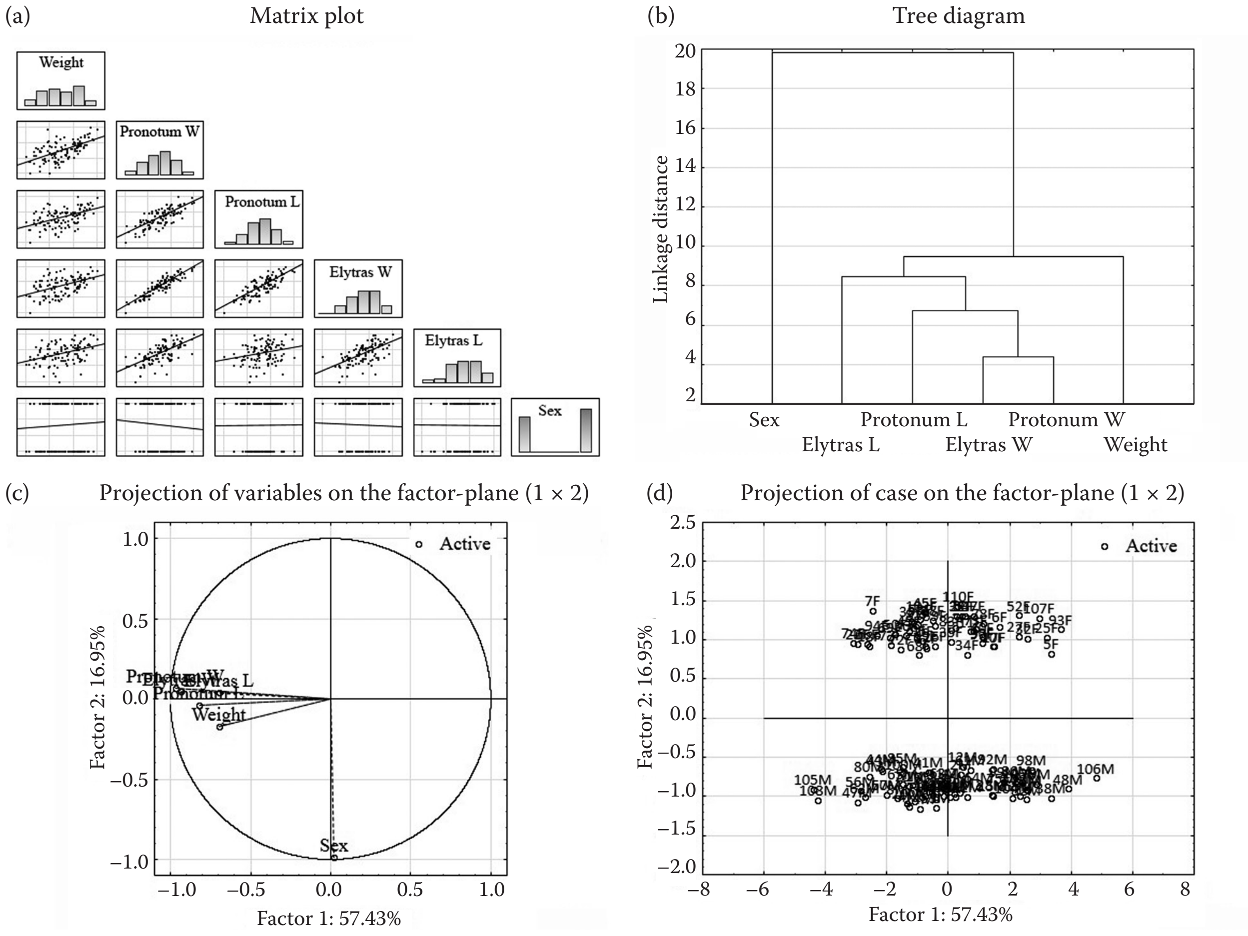

Fig. 3. Correlation between morphological parameters (a); joined similar variables making clusters (b); morphological parameters and sex (1 × 2) (c); beetles split into two clusters according to sex (d)

similarity, is for the variable sex. In contrast, the variables width of elytra and width of pronotum are the most similar (0.91). Thus it is evident that the parameter dependent on inheritance (sex) is allocated from the parameters dependent on the source of food.

DISCUSSION

For European species of the genus *Ips* (DeGeer, 1775) there are distinct sexual dimorphisms in only two species – *I. acuminatus* (Gyllenhal, 1827) and *I. duplicatus* (C.R. Sahlberg, 1836), which manifests itself in both cases in the shape of the teeth on the rear of the elytra (Pfeffer 1995). Unfortunately, this is not true of all species of the genus *Ips*, including the most important European representative – *I. typographus* L. although similar sexual dimorphisms are known in some North American species of this genus.

Differences between the sexes can be found on the frons – variously dense setation and/or different punctuation [e.g. the species *Ips confusus* (LeConte, 1876) or *Ips interruptus* (Mannerheim, 1852)], on the anterior tibiae – variously arranged teeth – again *I. confusus* (LeC.) or *I. interruptus* (Mann.) or the same as for the aforementioned European species for declivital armature – e.g. *Ips mexicanus* (Hopkins, 1904) and *I. confusus* (LeC.) (Hopping 1963, 1965a,b). Lanier and Cameron (1969) used a single secondary sexual character which permits accurate separation of the sexes for most American *Ips* species but for other species, a combination of characters is required.

Similar differences can be found in some other representatives of the tribe Ipini, for example in the genera *Pityogenes* Bedel, 1888, *Orthotomicus* Ferrari, 1867 or *Pityokteines* Fuchs, 1911 (Pfeffer 1995). The issue of sex determination of the spruce bark beetle is rarely clear. Currently, only two methods are used, and genital dissection remains the only entirely reliable method (Zahradník 2013). According to the other methods described by Schlyter and Cederholm (1981), the sexes of *I. typographus* can be reliably distinguished by the greater density of bristles on the pronotum of the female. They used laboratory bred beetles and determined the density of their bristles which depends on the condition of the animals and also the experience of the observer. Older specimens with damage to the bristles as along with dirt and residues can make this method of sex determination unreliable and sometimes impossible.

For field trials where it is necessary to only use male beetles, Bakke (1970) described how males can be distinguished by the fact that only males gnaw the entrance holes of logs. Sexually mature male beetles produced considerably more borings in a fresh spruce log over 24 hours than females (Birgersson et al. 1984). Rudinsky (1979) made microscopic examinations of the possible presence of stridulatory organs in the usual vertex and gular regions with subsequent sex confirmation by dissection.

As Grodzki (2004) mentioned in his work, the body size of *I. typographus* is influenced by both intraspecific competition and stand conditions. Avoiding any statistically significant differences in morphological parameter measurements we used beetles only from one trapping to a single pheromone trap.

Bednarz and Kacprzyk (2012) determined the sex of beetles caught in trap trees. Fresh weight as well as dry weight of females was significantly higher than fresh and dry weight of males. Unfortunately, this parameter discriminating the sex of *I. typographus* cannot be used for beetles from pheromone traps, because females are not probably fertilized, so they do not have maturing eggs. In this case the weight has a very slight correlation with sex.

CONCLUSION

Using the PCA approach, we partly found the expected structure in the data where beetles were clearly divided by sex (first principal component), and then by the size of morphological parameters (second principal component). Cluster analysis, however, revealed a more detailed structure in which beetles were not associated by sex and we can conclude that the sex of spruce bark beetle can still be determined reliably only by dissection. The sex parameter had no correlation with any other parameter (weight, elytra length, elytra width, pronotum length, pronotum width). Both methods confirm statistically insignificant correlations between sex and all other parameters.

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