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Anatomical and molecular studies of *Stenodema* Laporte genus (Heteroptera: Miridae)*

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Abstract. In this paper, the results of anatomical and molecular studies on some species of *Stenodema* genus have been presented. The results confirm separation of two subgenera: *Brachystira* and *Stenodema*.

Key words: entomology, taxonomy, Stenodemini, Miridae, Heteroptera, male reproductive system, 16S rRNA, COI.

INTRODUCTION

Stenodema Laporte is the most numerous genus within Stenodemini China tribe (Schuh 1995). 49 species occuring all over the world have been described so far, 6 of them occur in Poland (Kerzhner et Josifov 1999). All the species belonging to this genus are phytophagae, feeding on different kinds of grass (*Poaceae*) (Wachmann et al 2004).

There are two subgenera within this genus: *Brachystira* Fieber and a nominative subgenus. Initially, *Brachystira* has been determined as an individual genus with a typical species *Miris calcaratus* (Fallen 1807). Later, it has been considered as a subgenus within *Stenodema* (Kerhner et Josifov 1999).

Three species are included into *Brachistyra* subgenus: *Stenodema calcarata* (Fallen 1807)*, S. pilosa* (Jakovlev 1889), *S. trispinosa* Reuter,1904 (Kerzhner et Josifov 1999).

The above-mentioned subgenera have been determined basing on morphological features. All the representatives of *Brachystira* have distinctive spines on their meta-femora, while the representatives of *Stenodema* lack that feature.

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The objective of this research is to determine whether there are any anatomical and molecular differences between these both subgenera.

MATERIALS AND METHODS

Males of 3 species belonging to the *Stenodema* genus have been analysed: *S. (B.) calcarata* (Fallen, 1807), *S. (S.) holsata* (Fabricius, 1787), *S. (S.) laevigata* (Linnaeus, 1759). The individuals of *S.calcarata* species came from 5 countries: Spain, Japan, Kazakhstan, Korea and Poland. The *S. laevigata* individuals came from Spain and Poland.

In order to make anatomical analyses, gathered material and data were consolidated in Carnoy liquid. The paraffin method, together with standard histological methods, was used to make specimens.

These specimens were coloured by sour Erlich hematoxilin, oxygenated under running water and differentiated by 0,5% xylidine ponceau (in 1% acetic acid) (Bagiński1969).

Two species were used to make molecular analyses: of *S. (B.) calcarata* and *S. (S.) laevigata*.

The analysed species were consolidated in 96% of ethynol. Next, each individual was homogenized to isolate the whole DNA.

Samples of isolated DNA were treated with PCR (Saiki et al 1988; Simon et al 1991) to multiply and isolate the fragments of genes that were of interest to us , namely COI and 16S rRNA.

Primers used normally in such analyses among similar groups of insects were used to amplify both genes. The primers used for 16S rRNA gene were as follows: LR-K-13417, LR-J-12961 (Simon et al 1994; Hebsgaard et al 2004), while for COI we used the primers: C1J2183 and TL2-N-3014 (Simon et al 1994; Damgard et al 2001).

The sequences were obtained in the form of chromatograms, which were then analysed with the usage of Chromas 1.45 programme (McCarthy 1988), as well as Clustral X (Thompson et al 1997).

After adjusting the sequences, genetic distances between sequences of some species were calculated using BioEdit program DNADist (Hall 1999).

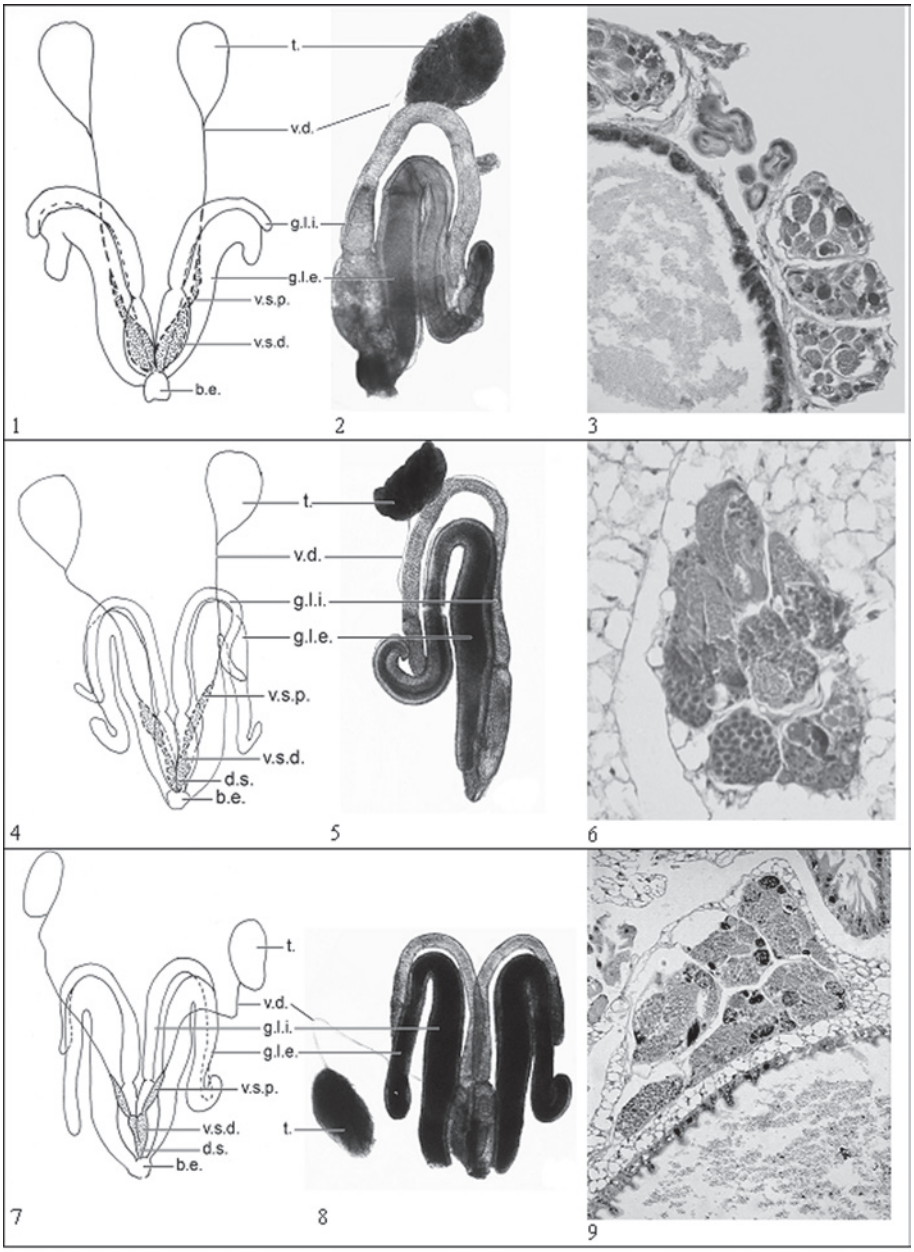
RESULTS

Anatomical researches

In the course of the research it has been proved that the species of the *Stenodema* genus are characterised by similar features of structure of the male reproductive system (Figs 1,2,4,5,7,8), but essential differences were found in the amount of testis follicles within individual subgenera. *S. (B.) calcarata* has 3 testis follicles (Fig 3), while species of *S. (S.) laevigata* (Fig 6) and *S. (S.) holsata* (Fig 9) have 6 testis follicles each.

Molecular researches

The alcali sequences obtained in the case of 16S rRNA were of the folloving lengths: *S. (B.) calcarata* 442p, while *S. (S.) laevigata* - 440pz . In the case of COI, the length of the sequences for both species was 839pz.



1- 9. Male reprodactive system: 1-3 *Stenodema (B.) calcar*ata, 4-6 *Stenodema (S.) laevigata*, 7-9 *Stenodema (S.) holsata*: t.- testes, v.d. – vas deferens, g.l.i - internal lateral gland, g.l.e. - external lateral glands, v.s.p.- proximal seminal vesicles, v.s.d.- distal seminal vesicles ,d.s.- ductus seminis, b.e. - bulbus ejaculatorius. 3, 6, 9. Testicular follicle of adult male in cross-section (x100)

Both markers had the majority of rules characteristic of insects, namely A+T 74,21-74,32 16S rRNA, 70,08-68,89 COI. The genetic distances calculated between these two species in the case of 16S rRNA were 0.0574, while in the case of COI they were 0.1231-0,1254.

The anasyses proved that the sequences of COI gene are far less conservative that those of 16S rRNA. Little differences in the sequence of this gene between two individuals belonging to one species were observed. Such differences were not noticed at the 16S rRNA marker, where the analysis was based on 3 individuals of 1 species. The value of the distances in these sequences was always 0,0000.

DISCUSSION

The amount of testis is one of the most frequently analysed features when taking a closer look at the build of a reproductive system. The basic amount of testis follicles in a nucleus of insects is in agreement with the amount of pregenital sequences and equals 7 (Sharov 1966). It can be reduced in the process of oligomerization, which happens independently in individual families (Pendegrast 1957, Grozeva et Kuznetzova 1992).

The older taxons usually have more testis. The analysis of 72 species of the Mirinae subfamily proved that 83,33% of the analysed species have 7 testis follicles (Akingbohungbe 1983). We can, therefore, conclude that the amount of 7 testis follicles in the Mirinae subfamily is a phlesiomorphic feature.

Basing on the obtained results, we can say that the build of the reproductive system of males of *Stenodema* genus is characterised by homogeneity and similarity of build between individual species. The changes are mainly comprised of reduction of the amount of testis follicles, the so-called oligomerization process at *Brachystira* subgenus. The differences are, nevertheless, essential, as such a big difference in the amount of testis follicles has never been observed at species belonging to one genus, before so far in no group of analysed Heteroptera (Akinbougue 1983).

In all the individuals belonging to *Stenodema calcarata* species the build of the system has always been identical (Figs 1, 2), and the amount of testis follicles was 3 (Fig 3), while in species belonging to *Stenodema* subgenus the amount of testis was 6 (Figs 6, 9).

The molecular analyses based on two mitochondrial markers of COI and 16S rRNA show a bigger difference in nucleotide sequences between species belonging to these two subgenera than between different genera within this tribe. The values of genetic distances between these two species obtained in the case of a 16S rRNA marker are ex. three times bigger than the obtained value of distances between species belonging to *Notostira* genus,while in the case of a COI marker-two times bigger.

The are therefore faced with the question: the *Brachystira* subgenus be promoted to a genus?

In order to answer this question, similar researches on a bigger amount of species from both subgenera need to be conducted.

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