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# Karyotypes of seven European aphid species of the genus *Cryptomyzus* (Hemiptera, Sternorrhyncha: Aphididae)

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Karyotypes of seven *Cryptomyzus* species collected in Lithuania (*C. alboapicalis*, *C. galeopsidis*, *C. ribis, C. korschelti, C. ulmeri, C. maudamanti* and *C. leonuri*) were analysed, two of them (*C. leonuri* and *C. ulmeri*) were karyotyped for the first time. Chromosome numbers in metaphase plates were detected, and the diploid chromosome number 2n = 12 proved to be dominant. Actual lengths were measured and relative lengths calculated to construct idiograms. The karyotype of *Cryptomyzus* aphids contained one pair of long, three pairs of medium-sized and two pairs of short chromosomes. To illustrate differences of relative chromosome lengths in haploid set between species, cluster analysis was performed using the matrix of Mahalanobis squared distances.

**Key words:** *Cryptomyzus,* karyotypes, idiograms, Lithuania

## INTRODUCTION

There are 18 species of the genus *Cryptomyzus* in the world (Remaudiere, Remaudiere, 1997; Кадырбеков, 2000); ten of them are present in Europe (Nieto Nafria, 2004). This genus is of Palaearctic origin, but *Cryptomyzus ballotae* Hille Ris Lambers, 1953, *Cryptomyzus galeopsidis* (Kaltenbach, 1843), *Cryptomyzus korschelti* Börner, 1938 and *Cryptomyzus ribis* (Linnaeus, 1758) are also recorded from other zoogeographical regions (Nieto Nafria, 2004). The genus *Cryptomyzus* is rather uniform both morphologically and biologically. However, four subgenera have been separated (Remaudiere, Remaudiere 1997), and a further subdivision of *Cryptomyzus sensu stricto* was also proposed (Hille Ris Lambers, 1953).

The intrageneric chromosome numbers of aphids are usually constant, though their karyotypes are not absolutely identical (Robinson, Chen, 1969; Turčinavičienė et al., 1997; Blackman et al., 2003). In some cases, the chromosome number within the genus is variable (Кузнецова, Шапошников, 1973; Blackman, 1980) and sometimes the structure of karyotype differs markedly, even in the same species like in *Trama* sp. (Czylok, 1990; Blackman et al., 2000).

Reference data (Robinson, Chen, 1969; Кузнецова, Шапошников, 1973; Blackman, 1980; Guldemond, 1991) show *Cryptomyzus* aphids to have the same chromosome numbers and their diploid set to consist of six chromosome pairs. Robinson and Chen (1969) were the first to detect the chromosome numbers of *Cryptomyzus* aphids (Кузнецова, Шапошников, 1973), reporting a 2n = 12 diploid chromosome set in *C. ribis*, which is typical of the tribe Macrosiphini (Кузнецова, Шапошников, 1973; Blackman, 1980). Blackman (1980) obtained the same number of chromosomes for *Cryptomyzus alboapicalis* (Theobald, 1916), *C. ballotae* and *C. galeopsidis*. Guldemond (1991) summarized all available data on *Cryptomyzus* chromosomes and added the chromosome numbers of *C. galeopsidis*, *C. korschelti*, *Cryptomyzus maudamanti* Guldemond, 1990 and *Cryptomyzus stachydis* (Heikinheimo, 1955).

Currently, chromosome numbers of most European *Cryptomyzus* species are known, except for *Cryptomyzus ulmeri* Börner, 1952, *Cryptomyzus heinzei* Hille Ris Lambers, 1953 and *Cryptomyzus leonuri* Bozhko, 1961 (Guldemond, 1991). We found no references concerning the karyotypes of East Palaearctic *Cryptomyzus*.

However, little is known about intrageneric variability in the karyotype structure of *Cryptomyzus*, as the only published idiogram of *C. ribis* comes from Robinson and Chen (1969), so data on *Cryptomyzus* karyotype structure still remain scarce.

The aim of this study was to examine the karyotype structure of seven *Cryptomyzus* (*C. alboapicalis*, *C. galeopsidis*, *C. korschelti*, *C. leonuri*, *C. maudamanti*, *C. ribis* and *C. ulmeri*) species collected in Lithuania.

## MATERIALS AND METHODS

Clones of seven species of the genus *Cryptomyzus* were used in this study. Aphids were sampled in the field and reared on their typical host plants, potted and isolated, under outdoor conditions in Pakalniškės (Vilnius distr., Lithuania). Rearing methods were the same as described in Emden (1972). Information on the aphid material used is given in Table 1.

Modified cell suspension technique (Blackman, 1985; Turčinavičienė et al., 1997) was applied to make preparations of chromosome spreads. Apterous viviparous females were dissected in Rhinaldini's salt solution (citric acid 0.676 g/l, KCl

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Table 1. **Aphid material used for cytogenetic study**

| Aphid species | Clone No, life cycle | Host plant | Sampling locality |
|---|---|---|---|
| *C. alboapicalis* | H1, monoecious | *Lamium album* L. | Vingis park, Vilnius |
| *C. galeopsidis* | F2, heteroecious, with *R. nigrum* L. as winter host | *Galeopsis tetrahit* L. | Pakalniškės, Vilnius distr. |
| *C. korschelti* | K2, heteroecious, with *R. alpinum* L. as winter host | *Stachys sylvatica* L. | Čiurlionio str., Vilnius |
| *C. leonuri* | L1, presumably monoecious | *Leonurus cardiaca* L. | Truskava, Kėdainiai distr. |
| *C. maudamanti* | J2, heteroecious, with *R. rubrum* L. as winter host | *Lamiastrum galeobdolon* (L.) Ehrend. et Polatschek | Skaidiškės, Vilnius distr. |
| *C. ribis* | D3, heteroecious, with *R. rubrum* L. as winter host | *Stachys palustris* L. | Pakalniškės, Vilnius distr. |
| *C. ulmeri* | I1, monoecious | *Lamium maculatum* L. | Vingis park, Vilnius |

Table 2. **Chromosome numbers of seven *Cryptomyzus* species**

| Aphid species | Total number of metaphase plates analysed | Number (percentage) of metaphase plates having certain numbers of chromosomes | | |
|---|---|---|---|---|
| | | 2n = 11 | 2n = 12 | 2n = 13 |
| *C. alboapicalis* | 172 | 23 (13.37%) | 137 (79.65%) | 12 (6.98%) |
| *C. galeopsidis* | 68 | 9 (13.24%) | 55 (80.88%) | 4 (5.88%) |
| C. korschelti | 180 | 47 (26.11%) | 129 (71.67%) | 4 (2.22%) |
| *C. leonuri* | 256 | 46 (17.97%) | 200 (78.13%) | 10 (3.91%) |
| C. *maudamanti* | 243 | 24 (9.88%) | 214 (86.83%) | 8 (3.29%) |
| *C. ribis* | 332 | 33 (9.94%) | 283 (85.24%) | 16 (4.82%) |
| C. *ulmeri* | 121 | 14 (11.57%) | 99 (81.82%) | 8 (6.61%) |

Table 3. **Relative lengths, % (absolute lengths, µm) of chromosome pairs, mean ± standard deviation, n = 25 for each species**

| Aphid species (total length of diploid set, in µm) | 1st pair | 2nd pair | 3rd pair | 4th pair | 5th pair | 6th pair |
|---|---|---|---|---|---|---|
| *C. alboapicalis* | 14.99 ± 1.14 | 10.43 ± 0.75 | 9.02 ± 0.48 | 8.04 ± 0.80 | 4.22 ± 0.62 | 3.31 ± 0.62 |
| (30.00 ± 5.61) | (4.52 ± 1.01) | (3.13 ± 0.62) | (2.70 ± 0.41) | (2.41 ± 0.47) | (1.26 ± 0.27) | (0.99 ± 0.26) |
| *C. galeopsidis* | 14.59 ± 0.97 | 10.53 ± 0.78 | 9.12 ± 0.59 | 7.99 ± 0.92 | 4.28 ± 0.67 | 3.49 ± 0.54 |
| (25.98 ± 3.68) | (3.79 ± 0.57) | (2.74 ± 0.50) | (2.38 ± 0.41) | (2.08 ± 0.42) | (1.10 ± 0.18) | (0.90 ± 0.13) |
| *C. korschelti* | 13.14 ± 0.93 | 9.88 ± 0.51 | 8.88 ± 0.37 | 8.18 ± 0.45 | 5.36 ± 0.56 | 4.55 ± 0.64 |
| (20.71 ± 2.18) | (2.73 ± 0.37) | (2.05 ± 0.26) | (1.84 ± 0.24) | (1.70 ± 0.22) | (1.11 ± 0.12) | (0.94 ± 0.12) |
| *C. leonuri* | 15.48 ± 1.26 | 10.48 ± 0.88 | 8.72 ± 0.66 | 7.68 ± 0.57 | 4.29 ± 0.72 | 3.35 ± 0.65 |
| (31.09 ± 5.18) | (4.83 ± 0.95) | (3.26 ± 0.60) | (2.72 ± 0.53) | (2.40 ± 0.49) | (1.32 ± 0.23) | (1.03 ± 0.20) |
| *C. maudamanti* | 13.79 ± 1.19 | 10.06 ± 0.73 | 8.74 ± 0.37 | 8.02 ± 0.44 | 5.09 ± 0.59 | 4.30 ± 0.50 |
| (21.76 ± 2.93) | (3.01 ± 0.53) | (2.19 ± 0.36) | (1.90 ± 0.27) | (1.75 ± 0.27) | (1.10 ± 0.12) | (0.93 ± 0.12) |
| *C. ribis* | 13.42 ± 1.15 | 10.36 ± 0.66 | 9.25 ± 0.44 | 8.47 ± 0.50 | 4.75 ± 0.69 | 3.76 ± 0.62 |
| (24.24 ± 3.82) | (3.26 ± 0.63) | (2.52 ± 0.48) | (2.25 ± 0.43) | (2.06 ± 0.38) | (1.13 ± 0.13) | (0.90 ± 0.12) |
| *C. ulmeri* | 15.05 ± 1.22 | 9.85 ± 0.70 | 8.51 ± 0.48 | 7.77 ± 0.47 | 4.86 ± 0.54 | 3.95 ± 0.66 |
| (24.47 ± 2.83) | (3.69 ± 0.57) | (2.41 ± 0.35) | (2.08 ± 0.24) | (1.90 ± 0.21) | (1.19 ± 0.18) | (0.96 ± 0.18) |

0.2 g/l, $NaHCO_3$ 1.0 g/l, NaCl 8.0 g/l, $NaH_2PO_4$ 0.05 g/l, glucose 1 g/l). Embryos without pigmented eyespots were selected and transferred into 0.75% KCl solution for 10 min and then centrifuged for 10 min. The solution was discarded and replaced with a freshly prepared fixative (3 parts of methanol and one part of glacial acetic acid) and left for 20 min in refrigerator. The fixative was changed two times, and some drops of cell suspension were applied on a clean wet microscope slide. The slides were then flame-dried, stained with 5% Giemsa solution for 10 min and analysed with a 100× objective under immersion oil without a cover slip. Metaphase plates were photographed, and 25 metaphase plates of each species were selected to perform measurements from digital photographs using the MicroImage interactive measurement system (OlympusOptical Co. GmbH).

As aphid chromosomes are holocentric and lack clear morphological features, they are grouped in descending order by relative lengths (chromosome length*100% / total length of chromosomes in a diploid set): the pair of longest chromosomes was named as 1st and so on. Mean chromosome pair lengths were used to construct the idiograms of presumably homologous pairs.

To illustrate the differences of relative chromosome lengths among the species, cluster analysis was performed. The matrix of squared Mahalanobis distances between group centroids for cluster analysis (linkage method: unweighted pair group average, UPGA) was obtained by a forward stepwise discriminant analysis. Statistica for Windows 5.5 version software (Statsoft 2000) was exploited for these calculations.

## RESULTS AND DISCUSSION

Metaphase plates with the diploid chromosome number 2n = 12 proved to be dominant (Fig. 1 and Table 2). Karyotypes of seven *Cryptomyzus* species were made up of one pair of long, three pairs of medium-sized and two pairs of short chromosomes (Fig. 1). Absolute and relative chromosome pair lengths of a haploid set together with the total length of a diploid set are given in Table 3.

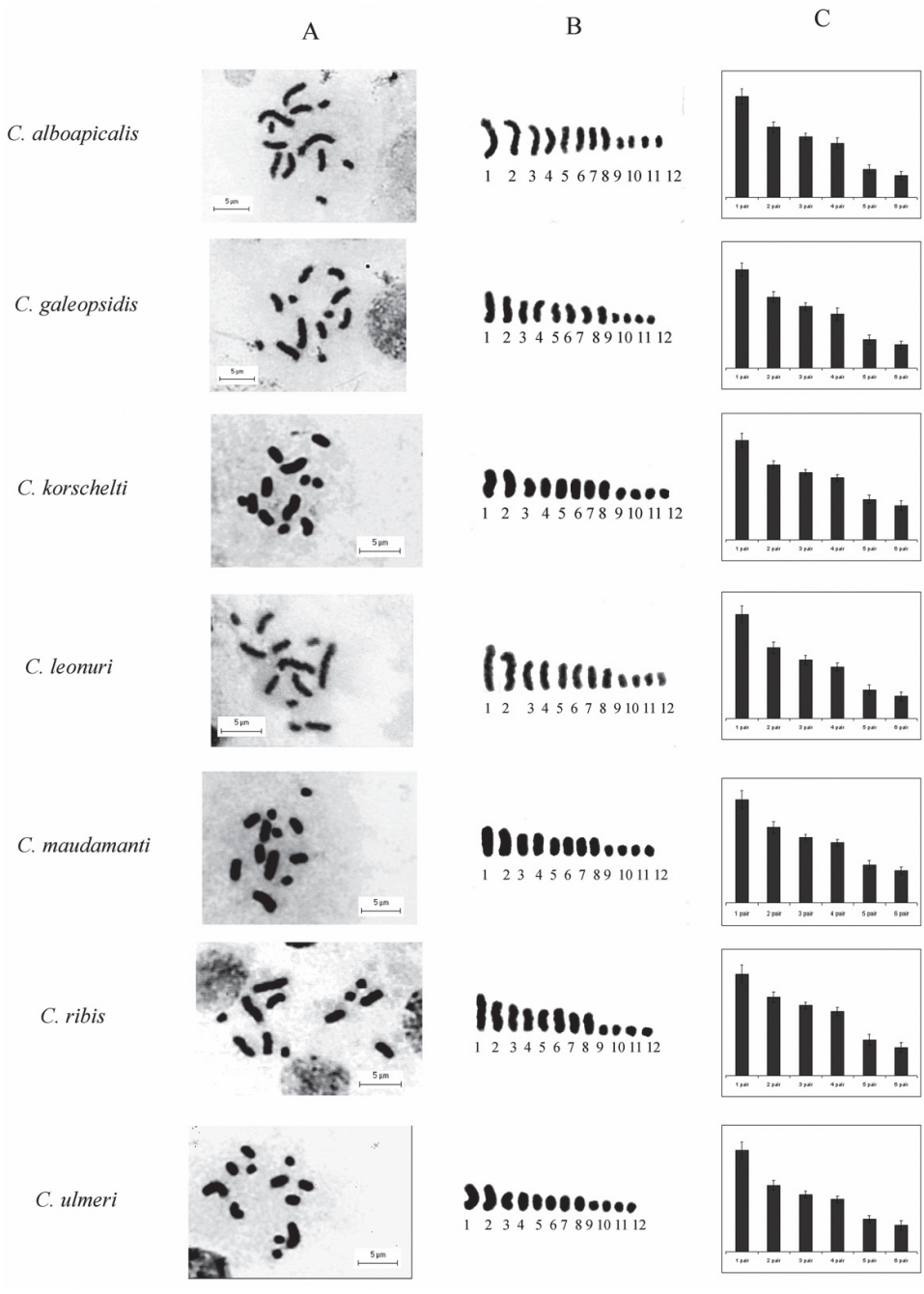


**Fig. 1.** Metaphase plates and idiograms of seven *Cryptomyzus* species. *A* – metaphase plates; *B* – chromosomes grouped by their relative lengths in descending order; *C* – idiograms of haploid sets based on relative chromosome lengths (column height indicates the mean value, and whiskers show standard deviation)

The earlier published idiogram of *C. ribis* (Robinson, Chen, 1969) presented one chromosome pair distinctly longer than others, approximately twice the length of the second pair, while other chromosomes decreased gradually in size. In our idiograms, the longest chromosome pair of *C. ribis* only slightly differs from medium-sized ones, and two pairs of short chromosomes are clearly separating (Fig. 1). Such inconsistence could be explained by differences in the methods used for idiogram construction. We used the mean values of relative chromosome lengths, whereas the idiogram in Robinson and Chen (1969) seems to be based on direct measurements from photomicrographs. Differences might also reflect the geographical differences of *C. ribis* populations studied. Also, the life-cycle strategy of earlier studied populations (e. g. host-alternation) remains unclear (we analysed a host-alternating clone of *C. ribis*). More detailed studies of chromatin structure, including various banding techniques, FISH, as well as the analysis of DNA sequences would elucidate the infraspecific differences in *Cryptomyzus* species and help to reveal the possible correlates between the karyotype structure and various life cycle modifications.

Cluster analysis was performed to reveal differences of relative chromosome lengths in haploid sets. Two main clusters were obtained (Fig. 2). One cluster consisted of one species heteroecious between *R. nigrum* and *Galeopsis* and three monoecious species from herbaceous plants, *Lamium* and *Leonurus* (Table 1). The other cluster contained three species related to *R. rubrum* and / or *R. alpinum* as winter hosts and *Stachys* and *Lamiastrum* as summer hosts (Table 1). This is in some consistence with the available phylogenetic schemes of the genus *Cryptomyzus*, based on the morphological, host specificity, life-cycle and certain en-

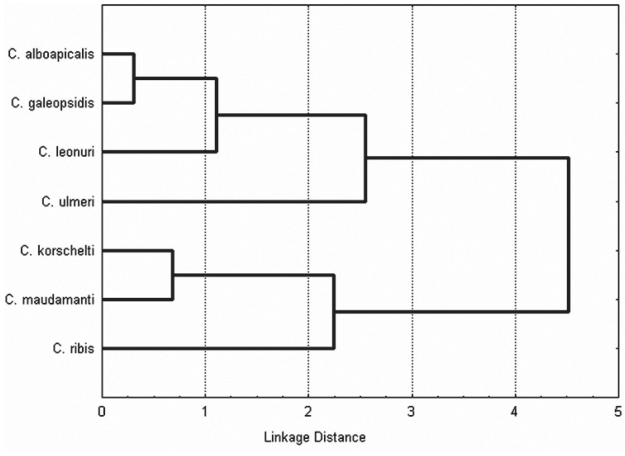


**Fig. 2.** Differences of relative chromosome lengths in haploid sets among seven *Cryptomyzus* species (squared Mahalanobis distances between group centroids, linkage method: UPGA)

zyme electrophoresis data (Guldemond, Eggers-Schumacher, 1989; Guldemond, 1990). These schemes revealed two distinct clades: the *C. galeopsidis* – *C. alboapicalis* species group appeared to be a sister group to *C. ribis* – *C. heinzei* complex. This version is consistent with the earlier attempt to exclude the *Myzella* subgenus from *Cryptomyzus s. str*. based on the morphology of newborn larvae, the number of hairs on the first tarsal segment and the morphology of antennal hairs (Hille Ris Lambers, 1953; Guldemond, 1990).

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## SEPTYNIŲ EUROPINIŲ *CRYPTOMYZUS* GENTIES AMARŲ (HEMIPTERA, STERNORRHYNCHA: APHIDIDAE) RŪŠIŲ KARIOTIPAI

*S a n t r a u k a*

Išanalizuoti Lietuvoje surinktų septynių *Cryptomyzus* genties rūšių amarų kariotipai. Dviejų iš jų, *C. leonuri* ir *C. ulmeri*, kariotipai aprašyti pirmą kartą. Metafazinėse plokštelėse dažniausiai pasitaikė diploidinis chromosomų skaičius 2n = 12. Išmatavus chromosomų ilgius, apskaičiuoti santykiniai jų ilgiai ir sukonstruotos idiogramos. Tirtų *Cryptomyzus* genties amarų kariotipus sudaro viena pora ilgų, trys poros vidutinio dydžio ir dvi poros trumpų holocentrinių chromosomų. Haploidinių rinkinių, sudarytų pagal chromosomų santykinius ilgius, skirtumai pateikti atlikus klasterinę analizę, jai panaudojus Mahalanobio atstumų kvadratų matricą.

**Raktažodžiai:** *Cryptomyzus*, kariotipai, idiogramos, Lietuva