Folia biologica (Kraków), vol. **53** (2005), No 3-4

Cytogenetic Variability of European Tettigoniinae (Orthoptera, Tettigoniidae): Karyotypes, C- and Ag-NOR-banding

Elżbieta WARCHAŁOWSKA-ŚLIWA, Klaus-Gerhard HELLER, and Anna MARYAŃSKA-NADACHOWSKA

Accepted September 6, 2005

WARCHAŁOWSKA-ŚLIWA E., HELLER K-G., MARYAŃSKA-NADACHOWSKA A. 2005. Cytogenetic variability of European Tettigoniinae (Orthoptera, Tettigoniidae): karyotypes, C-and Ag-NOR-banding. Folia biol. (Kraków): **53**: 161-171.

Karyotypes, C-banding pattern and localization of nucleolus organizer regions (NORs) in 34 European species and subspecies belonging to the subfamily Tettigoniinae are described (the karyotypes of 26 species for the first time). In the males chromosome numbers vary from 2n=33 to 2n=23 and Fundamental Numbers (FN) from 36 to 27. The highest number of chromosomes for this group, 2n♂=33 (FN=33), was found in *Psorodonotus illyricus macedonicus*. In species belonging to genera *Decticus*, *Metrioptera*, *Anterastes, Bucephaloptera*, *Parapholidoptera*, and *Eupholidoptera*, as well as in *Pholidoptera frivaldskyi* and *Pholidoptera griseoaptera*, a karyotype of 2n=30+X0 (FN=31) was found. *Ph. macedonica* and *Ph. aptera aptera* are characterized by 2n=28+X0 (FN=31). In species from genera *Drymadusa* (*D. dorsalis limbata*) 2n=26+X0: FN=30 and *Gampsocleis* (*G. abbreviata*), karyotypes of 2n=22+X0 (FN=36) were found. *Ph. macedonica* and *Ph. aptera aptera* as well as *G. abbreviata* differ in karyotypes from other representatives of these genera. New data confirmed that Robertsonian fusions and tandem fusions played the most important role in the evolution of the chromosome set in Tettigoniinae. Cytogenetic similarities and differences between particular species are discussed.

Key words: Orthoptera, Tettigoniidae, katydids, karyotype, cytotaxonomy, C-banding, NOR.

Elżbieta WARCHAŁOWSKA-ŚLIWA, Anna MARYAŃSKA-NADACHOWSKA, Department of Experimental Zoology, Institute of Systematics and Evolution of Animals, Polish Academy of Sciences, Sławkowska 17, 31-016 Kraków, Poland.
E-mail: warchalowska@isez.pan.krakow.pl
maryanska@isez.pan.krakow.pl
Klaus-Gerhard HELLER, Department of Zoology II, University of Erlangen-Nűrnberg,
E-mail: Heller.volleth@T-Online.de

The systematics of the subfamily Tettigoniinae, including the former Decticinae, also sometimes considered as the family Tettigoniidae (HELLER *et al.* 1998), are unsatisfactory at present. This refers to the level of subgenera and genera (e.g. the large genera *Metrioptera/Platycleis* or *Pterolepis/Rhacocleis*), but more importantly also to higher categories. Information concerning which morphological characters – if any – can be used to reliably define groups of related genera and tribes is lacking (e.g. RENTZ & COLLESS 1990). Of course, relationships have been established for some groups of genera, but for many they are missing. Careful morphological studies can sometimes improve the situation (see F. & L. WILLEMSE in preparation), but additional information would be highly welcome. Therefore the karyotypes of 26 species of this group, previously undescribed, have been examined in this study. Chromosomal information has been demonstrated to be valuable taxonomically in a large range of vertebrates (FONTANA & RUBINI 1990; SMITH 1990; VOLLETH & HELLER 1994; RUMPLER Y. 2000) and arthropods (BLACKMAN 1980; PETITPIERRE 1997; SHAPOSHNIKOV *et al.* 1998; WARCHAŁOWSKA-ŚLLIWA E. 1998).

The diploid number, chromosome morphology, and the type of sex determination system of Tettigoniinae in the Palaearctic have been described for about 50 species of 22 genera, including 30 species of 11 genera from Europe (JOHN & HEWITT 1968; CAMACHO *et al.* 1981; UESHIMA 1986; SOUTHERN 1967; SERGEEV & BUGROV 1988; AREFJEV 1989; WARCHAŁOWSKA-ŚLIWA 1984, 1988, 1998; BUGROV 1990; WARCHAŁOWSKA-ŚLIWA & MICHAILOVA 1993; WARCHAŁOWSKA-ŚLIWA & BUGROV 1996a; WARCHAŁOWSKA-ŚLIWA *et al.* 1987, 1992, 1994).

Table 1

General karyotypical features, C-heterochromatin patterns, and NORs in species of Tettigoniinae (acro- acrocentric, subacro- subacrocentric, submeta- submetacentric, meta- metacentric, * intraspecific variation of C-heterochromatin)

Species	Numbers of individuals, collection locality	2n, FN	C-bands	NOR	References other than this paper
Psorodonotus illyricus macedonicus	4 males, Greece	33, 33 all acro	All paracentromeric thin, M_2, M_3 interstitial, M_2, M_3, most of small bivalents telomeric		
Decticus verrucivorus	2 males, Poland	31,31 all acro	All paracentromeric thin $M_{4/5}$ interstitial, M_3-M_6 terminal thick	$M_{4/5}$	present paper & WARCHAŁOWSKA-ŚLIWA 1984
Platycleis (*Parnassiana*) *tenuis*	1 male, Greece	31, 31 all acro	very small division, without C-bands		
Metrioptera roeselii ambitiosa	1 male, 1 female, Greece	31, 31 all acro	All paracentromeric thin, L_1, L_2, M_3, X telomeric thin		
Metrioptera roeselii roeselii	6 males, Poland	31, 31 all acro	All paracentromeric thin, L_2*, M_3, M_4, M_5*, X telomeric,	$M_{3/4}$	WARCHAŁOWSKA-ŚLIWA 1984
Metrioptera oblongicollis	2 males, Greece	31, 31 all acro	All paracentromeric thin, $L_{1/2}$ telomeric		
Metrioptera brachyptera	5 males, Slovakia	31, 31 all acro	All paracentromeric thin, L_2, M_3, M_4 *interstitial, L_2,(thin), M_3, M_4 (thick) telomeric, X near centromeric and telomeric		present paper & WARCHAŁOWSKA-ŚLIWA 1984
Metrioptera bicolor	2 males, Poland	31, 31 all acro	All paracentromeric thin, L_1*, L_2, M_3, M_4, M_5 telomeric		present paper & WARCHAŁOWSKA-ŚLIWA 1984
Anterastes serbicus	3 males, 1 female, Greece	31, 31 all acro	All paracentromeric thin, X thick centromeric and thin telomeric		
Bucephaloptera bucephala	1 male, Turkey	31, 31 all acro	All paracentromeric thin,		
Parapholidoptera signata	1 male, Turkey	31, 31 all acro	Two medium size with thick paracentromeric C-band, the rest thin		
Eupholidoptera epirotica	6 males, 1 female, Greece	31, 31 all acro	All paracentromeric thin, $M_{3/4}$ interstitial (subtelomeric) L_1 telomeric heterozygous (two males)	$M_{3/4}$	
Eupholidoptera tauricola	2 males, Turkey	31, 31 all acro	All paracentromeric thin, $M_{3/4}$ interstitial , L_1* telomeric (both males)		
Eupholidoptera smyrnensis	5 males, 1 female, Bulgaria	31, 31 all acro	All paracentromeric thin, $M_{3/4}$ interstitial, L_1 *telomeric (two males)		
Eupholidoptera chabrieri garganica	3 males, Greece	31, 31 all acro	All paracentromeric thin, $M_{3/4}$ interstitial L_1*telomeric (one males 20/04)		
Eupholidoptera megastyla	4 males, Greece	31, 31 all acro	All paracentromeric thin, $M_{3/4}$* interstitial (in one male (25 /03)		
Eupholidoptera annulipes	2 males, Turkey	31, 31 all acro	All paracentromeric thin, $M_{3/4}$ interstitial		
Eupholidoptera sp. (ex Andros)	2 males, 2 females Greece	31, 31 all acro	All paracentromeric thin, $M_{3/4}$ interstitial (subtelomeric) L_1, L_2 telomeric		
Eupholidoptera prasina	5 males Turkey	31, 31 all acro	All paracentromeric thin, $M_{3/4}$, M_5 interstitial L_1, L_2, $M_{3/4}$, M_5 telomeric, X interstitial and telomeric		

Table 1 cont.

Eupholidoptera anatolica	2 males, Turkey	31, 31 all acro	All paracentromeric thin, $M_{3/4}$, M_5 interstitial L_1, L_2, $M_{3/4}$, M_5 telomeric X interstitial?		
Eupholidoptera mersinensis	2 males, Turkey	31, 31 all acro	All paracentromeric thin, $M_{3/4}$ interstitial, L_1 – $M_{7/8}$ telomeric	$M_{3/4}$	
Eupholidoptera karabagi	3 males, Turkey	31, 31 all acro	All paracentromeric thin, $M_{4/5}$ interstitial L_1, L_2, M_3, M_4, $M_{6/7}$ telomeric X interstitial	$M_{3/4}$	
Eupholidoptera icariensis	1 male, Greece	31, 31 all acro	very small division, without C bands		
Pholidoptera frivaldskyi	1 male, Greece	31, 31 all acro	All paracentromeric thin, M_3 interstitial thin	M_3	present paper & WARCHAŁOWSKA-ŚLIWA, MICHAILOVA 1993
Pholidoptera giseoaptera	6 males, Poland	31, 31 all acro	All paracentromeric thin, M_3 interstitial thin	M_3	present paper & WARCHAŁOWSKA-ŚLIWA 1984
Pholidoptera macedonica	2 males, Greece	29, 31 L_1 submeta; L_2-S_{14}, X acro	All paracentromeric thin, L_1 telomeric		
Pholidptera aptera aptera	4 males, Poland	29, 31 L_1 submeta; all acro	All paracentromeric thin	L_1	present paper & WARCHAŁOWSKA-ŚLIWA 1988
Drymadusa dorsalis limbata	1 male, Turkey	27, 30 L_1 meta/ /submeta; L_2-S_{13} acro; X submeta/ /subacro	All paracentromeric thin, M_2 interstitial near centromeric L_1, M_3-S_{13} telomeric ($M_{3,4,5}$ thick) X telomeric in longer arm		
Pterolepis ferdinandi	1 male, Greece	25, 27 L_1 meta; M_2-S_{12}, X acro B chromosome	All paracentromeric medium size, L_1 interstitial + telomeric (in both arms)		
Pterolepis germanica	2 males, Greece	25, 27 L_1 meta; M_2-S_{12}, X acro	All paracentromeric medium size, L_1 interstitial + telomeric (in both arms)		
Pterolepis insularis	3 males, Greece	25, 27 L_1 meta; M_2-S_{12}, X acro	All paracentromeric medium size. L_1 interstitial+ telomeric (in both arms); M_2, M_3, M_4 interstitial, all telomeric thin		
Pterolepis sp. *nova*	2 males, 1 female, Greece	25, 27 L_1 meta; M_2-S_{12}, X acro	All paracentromeric medium size, M_2, M_3, M_4 interstitial and telomeric		
Pterolepis edentata	3 males, Greece	25, 27 L_1 meta; M_2-S_{12}, X acro	All paracentromeric medium size, L_1 interstitial + interstitial (both arms), $M_{2/3}$ interstitial, $M_{4/5}$ thick telomeric, X thin telomeric		
Gampsocleis abbreviata	3 males Greece	23, 36 L_1 submeta; M_2, M_6, M_7, S_9, S_{11} X meta; M_3-M_5, S_8, S_{10} acro	All paracentromeric thin (excluding pair $S_{7/8}$) X, S_9 telomeric thin	M_6	WARCHAŁOWSKA-ŚLIWA 1998

The great majority of cytotaxonomic analyses have been based on the conventional staining technique. Banding techniques such as C-banding and Ag-NOR-banding allow for a better characterization of tettigonid karyotypes and selectively reveal chromosome regions consisting of constitutive heterochromatin and NOR-sites of the RNA genes. New cytological markers are useful for better insight into the pathways by which the various karyotypes have evolved in Tettigoniidae. Constitutive heterochromatin shows characteristic distribution patterns in karyotypes and contributes to the broad scattering of genome sizes throughout biological taxa (REDI *et al*. 2001).

Data on C-banding patterns in mitotic and meiotic cells, and information on the nucleolus organizer region (NOR), are limited in the European Tettigoniinae (WARCHAŁOWSKA-ŚLIWA & BUGROV 1996a; WARCHAŁOWSKA-ŚLIWA *et al*. 1992, 1994). Here a further study on the karyotypes of 34 taxa of the subfamily is provided in order to obtain more information on the genome organization of these orthopteran species.

Material and Methods

Adult males and females were collected in Greece, Turkey, Poland, and Slovakia for the cytogenetic study (see Appendix for taxonomic data and specific localities). Specimens from Greece and Turkey are deposited in Collectio Heller (CH + reference number), the others – in the Institute of Systematics and Evolution of Animals PAS (Kraków).

Testes and ovarioles were excised, incubated in a hypotonic solution (0.9% sodium citrate), and then fixed in ethanol:acetic acid (3:1). The fixed material was squashed in 45% acetic acid. Coverslips were removed by the dry ice procedure and then preparations were air dried. The C-banding examination was carried out according to SUMNER (1972) with slight modification. The silver staining method for NORs was performed as previously reported (WARCHAŁOWSKA-ŚLIWA & MARYAŃSKA-NADACHOWSKA 1992). The fixed material is deposited in the Institute of Systematics and Evolution of Animals PAS (Kraków).

Results

The chromosome number (2n), morphology of chromosomes (Fundamental Number = FN), the C-banding patterns, and NOR locations in the species studied are reported in Table 1. The diploid chromosome number of 26 species representing 10 genera was investigated for the first time.

Most of the European species of Tettigoniinae analyzed in this paper in the genera *Decticus*, *Platycleis*, *Metrioptera*, *Anterastes*, *Bucephaloptera*, *Parapholidoptera*, *Eupholidoptera*, and some species of *Pholidoptera*, have 2n♂=31 (FN=31; only acrocentric chromosomes) (Fig. 1). However, in *Pholidoptera macedonica*, the chromosome number is reduced to 2n♂=29 (FN=31) with one submetacentric pair (L_1), similar to *Ph. aptera* (Fig. 2) described earlier (WARCHAŁOWSKA-ŚLIWA 1988).

The highest number of chromosomes for this group, 2n♂=33, was found in *Psorodonotus illyricus macedonicus* (Fig. 3).

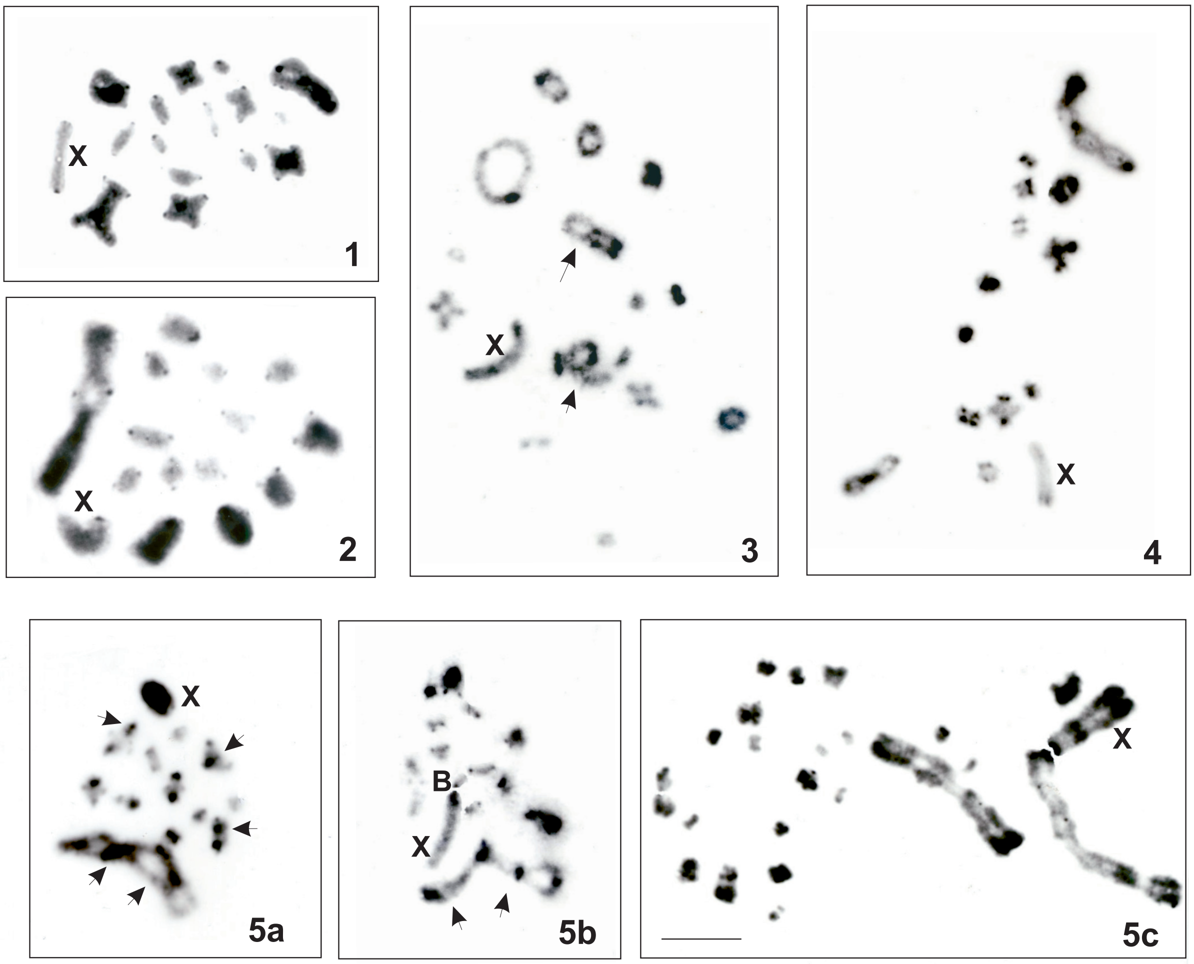
In *Drymadusa dorsalis limbata* the complement is reduced to 2n♂=27, FN=30 with a meta/submetacentric L_1 pair and the submeta/subacrocentric X chromosome (Fig. 4).

Five species of the genus *Pterolepis* are characterized by 2n♂=25. This is a chromosomal set with one extremely long metacentric L_1 (FN=27) (Fig. 5a,b,c). The lowest chromosome number in this group was found in *Gampsocleis abbreviata* 2n♂=23 (FN=36) with L_1 submetacentric, five pairs metacentric, and five pairs acrocentric. The metacentric X chromosome is the largest element in the karyotype as in *G. glabra* (WARCHAŁOWSKA-ŚLIWA *et al*. 1992).

All analyzed species show the XO♂ and XX♀ type of sex chromosome determination.

Table 1 shows the C-banding patterns of 34 species, and Figures 1-13 give some examples of the results. Most of the species of Tettigoniinae have paracentromeric C-bands in the vicinity of the centromeric regions. In most cases, the paracentromeric C-bands are restricted to the centromeric region (thin C-bands), e.g. in the whole autosome complement and the X chromosome of *Metrioptera roeselii* (Figs 1, 6), *Eupholidoptera mersinensis* (Fig. 7), and *Pholidoptera aptera* (Fig. 2). In other cases, C-bands occupy the region next to the centromere (thick C-bands) as in the two medium-sized pairs of *Parapholidoptera signata*, and in all chromosomes of *Pterolepis* sp.n.

In 22 species of Tettigoniinae, shown in Table 1, interstitial C-bands on one or more chromosomes are present. Interstitial C-bands are located for instance near the paracentromeric region of the X chromosome in *Eupholidoptera karabagi* (Fig. 8), in the interstitial region of pairs M_2, M_3 in *Psorodonotus illyricus macedonicus* (Fig. 3), in the pair $M_{4/5}$ of *Decticus verrucivorus* (Fig. 9), in the pairs $M_{3/4}$ and M_5 of *Eupholidoptera anatolica* (Fig. 10), in the interstitial parts of both arms in pair L_1 of *Pterolepis ferdinandi* as well as in pairs M_2-M_4 of *Pterolepis insularis* (Fig. 5a), and of *Pterolepis* sp. n. Only in *Metrioptera brachyptera* (in one indi-



Figs 1-5. C-banded karyotypes of males. Fig. 1. *Metrioptera roeselii* and Fig. 2. *Pholidoptera aptera aptera* – 2n=31, FN=31 and 2n=29, FN=31 respectively, metaphase I, both species with very thin paracentromeric C-bands. Fig. 3. *Psorodonotus illyricus macedonicus* – 2n=33, FN=33, metaphase I, arrows indicate interstitial C-bands. Fig. 4. *Drymadusa dorsalis limbata* – 2n=27, FN=30, metaphase I. Figs 5a, b. (a) *Pterolepis insularis* and (b) *Pterolepis ferdinandi* – 2n=25, FN=27, metaphase I with interstitial C-bands (arrows), B (supernumerary chromosome), Fig. 5c. *Pterolepis edentata*, mitotic metaphase with extremely long metacentric pair L_1. Bar = 10 μm.

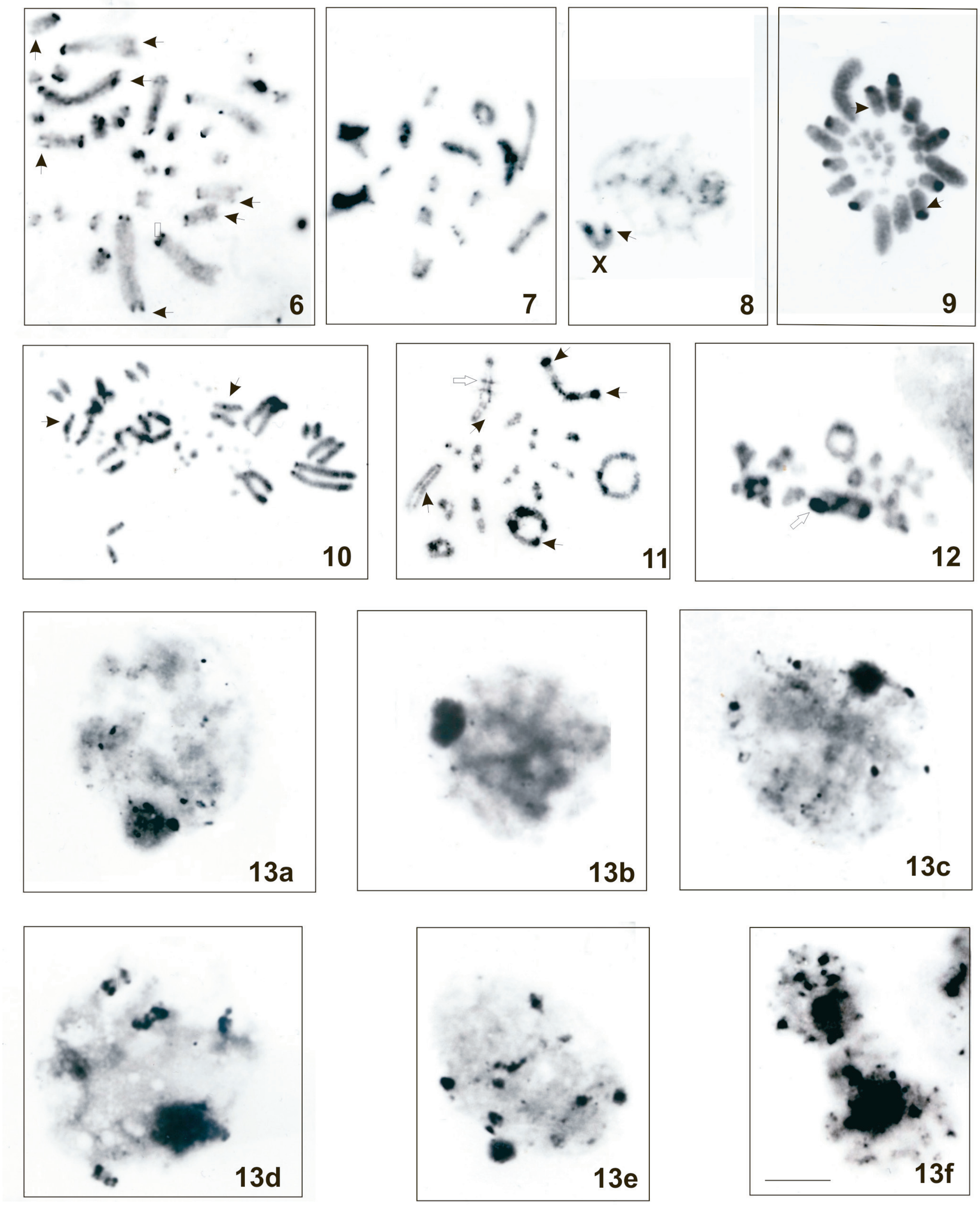
vidual) variation in M_4 due to the presence or absence of interstitial C-bands is observed (Fig. 11).

When telomeric C-bands are present, they are located in chromosomes of different sizes and in most cases they are thin (Table 1, Fig. 6). However, in three pairs of autosomes (M_3-M_6) of *Decticus verrucivorus* and in M_2, M_3 of *Metrioptera brachyptera,* these bands are thick (Figs 9, 11). It is also worth mentioning that sometimes size variation of telomeric C-bands of two homologous chromosomes may be present, e.g. in two pairs (L_2, M_5) of *Metrioptera roeselii*, or in some individuals in four species of *Eupholidoptera* (Fig. 12).

To sum up information connected with heterochromatin, the C-banding technique revealed that most of the examined species with 2n=31 in males show very small (thin) segments of constitutive heterochromatin in all autosomes and in X chromosomes (excluding terminal C-bands in *Decticus verrucivorus*). However, in some species of the genera *Pterolepis* and in *Drymadusa dorsalis* these segments are larger (thick). Differences in the amount of heterochromatin are clearly visible in the interphase nuclei and in the early prophase as e.g. in the genera *Psorodonotus*, *Metrioptera*, *Pholidoptera*, *Decticus*, *Drymadusa*, and *Pterolepis* (Figs 13a-f).

A B chromosome was detected in one male of *Pterolepis ferdinandi*. It was a acrocentric small element in the complement (Fig 5b).

The location of chromosome NOR's was shown in only 9 of the species studied. For other species the NOR was not revealed owing to the absence of diplotenes in the studied cells. Eight species, namely *Decticus verrucivorus*, *Metrioptera roeselii*, *Eupholidoptera epirotica, E. mersinensis, E. karabagi, Pholidoptera griseoaptera*, and *Ph. aptera* each showed a single active NOR located on



Figs 6-13. Fig. 6. *Metrioptera roeselii*, mitotic metaphase I, arrows indicate telomeric C-bands. Fig. 7. *Eupholidoptera smyrnensis*, metaphase I. Fig. 8. *Eupholidoptera karabagi*, meiotic prophase, the X chromosome with interstitial C-band (arrow). Fig. 9. *Decticus verrucivorus*, mitotic metaphase, arrows indicate telomeric C-bands. Fig. 10. *Eupholidoptera anatolica*, mitotic with interstitial C-bands. Fig. 11. *Metrioptera brachyptera*, diakinesis, ⇨ indicates variability of interstitial heterochromatin, arrows indicate telomeric C-bands, thick in autosomes and thin in the X chromosome. Fig. 12. *Eupholidoptera chabrieri garganica*, metaphase I, ⇨ indicates variability in telomeric C-band. Figs 13a-f. Nuclei with heterochromatin: (a) *Metrioptera roeselii*, (b) *Pholidoptera aptera aptera*, (c) *Psorodonotus illyricus macedonicus*, (d) *Decticus verrucivorus*, (e) *Drymadusa dorsalis limbata*, (f) *Pterolepis insularis*. Bar = 10 μm.

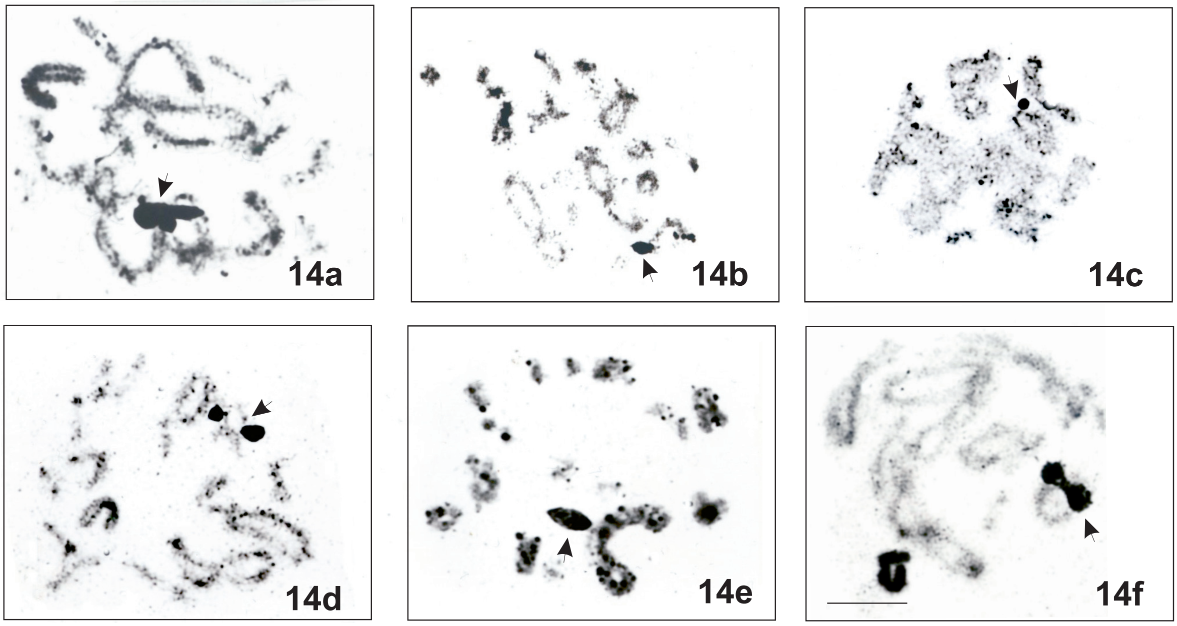


Fig. 14a-f. Ag-staining areas of the NORs, diplotene/diakinesis: (a) *Decticus verrucivorus*, (b) *Metrioptera roeselii,* (c) *Eupholidoptera karabagi*, (d) *Pholidoptera griseoaptera*, (e) *Pholidoptera aptera aptera*, (f) *Gampsocleis abbreviata* (arrows). Bar = 10 μm.

the medium-sized bivalents ($M_{3/4}$ and $M_{4/5}$), and in *Gampsocleis abbreviata* on the M_6 bivalent. Active NORs were detected distally in *D. verrucivorus*, *M. roeselii*, *G. abbreviata*, and in a near distal position in *Eupholidoptera epirotica,* whereas they are located interstitially in *Eupholidoptera mersinensis* , *E. karabagi*, *Pholidoptera frivaldsky*, *P. griseoaptera*, and *Gampsocleis. Pholidoptera aptera aptera* showed one NOR located on L_1 probably near a distal position. (Figs 14a-f).

Discussion

Most species of the Palearctic Tettigoniinae have been found to have 2n=31 with only acrocentric chromosomes (FN=31) (see review WARCHAŁOWSKA-ŚLIWA 1998). The same is true of the species sampled in this study in the genera *Anterastes*, *Bucephaloptera*, *Decticus*, *Eupholidoptera*, *Parapholidoptera*, *Platycleis*, and *Metrioptera.* For these species, karyology gives no clues as to their systematic relationships. There are, however, some species which differ from this basic pattern and which have to be discussed separately. Only one of the examined species has a chromosome number higher than the modal number of this subfamily. *Psorodonotus illyricus macedonicus*, like its congeneric species *P. specularis* Fischer de Waldheim*,* 1839 (WARCHAŁOWSKA-ŚLIWA 1998), shows 2n=33?. One of the possible mechanisms producing this unusually high number rests in the relatively complicated chromosomal reorganization that could be connected with chromosome aneuploidy. Taxonomically this character can in the future be used to clarify the problematic relationships between *Psorodonotus* and the genus *Uvarovina* (*Peltastes*) (see STOROZHENKO 2004).

Nine species have chromosome numbers lower than the modal number of the subfamily. They may have their origin in independent Robertsonian fusions of acrocentric chromosomes. Interestingly, the simplest case, the fusion of two pairs of chromosomes resulting in 2n=29, was only found here in the closely related *Pholidoptera aptera* and *P. macedonica*, but not in other species of this genus (see WARCHAŁOWSKA-ŚLIWA 1998 for review). This character could help the taxonomy if it is used to define the morphologically difficult *P. aptera* group (for characteristics of the song pattern see HELLER 1988).

For *Drymadusa dorsalis limbata* a Robertsonian fusion and an additional tandem fusion followed by an inversion, resulting in 2n♂=27 (FN=30) (one metacentric pair and submeta/subacrocentric X chromosome originated by pericentric inversion) have to be assumed. A very similar karyotype is known from *Bergiola montana* (WARCHAŁOWSKA-ŚLIWA & BUGROV 1996a), another genus of the

Drymadusini. Several other species (from the genera *Atlanticus*, *Anatlanticus*, *Tadzhikia*, *Anadrymadusa*, *Ceraeocercus*, *Paratlanticus*) from this tribe have karyotypes with a reduced number of chromosomes (2n = 25-29) (WARCHAŁOWSKA-ŚLIWA & BUGROV 1996a).

The five species of the genus *Pterolepis* (formerly *Rhacocleis*, HELLER *et al*. 1998) showed a karyotype with 2n = 25 (FN=27). In this case, the first pair L_1 occurs probably as a result of one centric fusion between two autosome pairs and two other pairs as a result of tandem fusions. From chromosome data, nothing can be said about the phylogenetic relationships of this genus. However, in no species of the Platycleidini s.str. such low chromosome numbers are found, whereas they occur quite frequently in genera characterized morphologically by two spines at the prosternum, e.g. Drymadusini, *Pterolepis/Rhacocleis*, *Gampsocleis*.

Within the genus *Gampsocleis*, however, species with very different karyotypes were found. Several species (see WARCHAŁOWSKA-ŚLIWA 1998 for review) show chromosomes which do not differ in number nor in shape from the typical ones of the subfamily (2n =31, FN=31). On the other hand, two species, *G. glabra* and *G. abbreviata*, have only 23 chromosomes with FN=26. Since these two species are the most western representatives of the genus, a common origin of this highly derived karyotype can be assumed and the reduction in chromosome number happened certainly independently from that in other genera mentioned above.

An interesting feature of the *Pterolepis ferdinandi* karyotype is the sporadic occurrence of B chromosomes. According to many authors (e.g. BATTAGLIA 1964; JOHN & LEVIS 1968; WHITE 1973; HEWITT 1979; CAMACHO *et al.* 1981) the existence of B chromosomes, previously noted in few species of tettigoniids, indicates that these elements are rare in this group and often unstable (WARCHAŁOWSKA-ŚLIWA *et al.* 1992).

In tettigoniids (similar to other Orthoptera) the C-banding technique is used, among others, in comparative studies of populations, species and genera of the subfamilies Bradyporinae (NAVAS CASTILLO *et al*. 1986; WARCHAŁOWSKA-ŚLIWA & BUGROV 1996b, 1998) and Phaneropterinae (WARCHAŁOWSKA-ŚLIWA & HELLER 1998; WARCHAŁOWSKA-ŚLIWA & MARYAŃSKA-NADACHOWSKA 1992; WARCHAŁOWSKA-ŚLIWA *et al.* 1992, 1995, 1996, 2000). Differences between species belonging to the same genus and between different genera are the result of the heterochromatin differentation as is reflected by the existence of intraspecific variation for many species (SANTOS *et al*. 1983; CABRERO & CAMACHO 1986a; VISERAS *et al*. 1991). However, C-banding patterns of European Tettigoniinae have been described only in a few genera: *Gampsocleis* (WARCHAŁOWSKA-ŚLIWA *et al*. 1992), *Montana* (WARCHAŁOWSKA-ŚLIWA *et al*. 1994), *Tettigonia* (WARCHAŁOWSKA-ŚLIWA & MARYAŃSKA-NADACHOWSKA 1995), *Anadrymadusa*, as well as in some genera of Drymadusini from Asia (WARCHAŁOWSKA-ŚLIWA & BUGROV 1996a). In most of these genera, and in most described in the present paper, thin paracentromeric C-bands were uniformly present in all autosomes and in the X chromosome. Exceptionally, in *Parapholidoptera* (on three medium-sized pairs), *Gampsocleis* (on one small-sized pair) and the earlier described (WARCHAŁOWSKA-ŚLIWA & BUGROV 1996a) *Tadzhikia* (three small pairs), these C-blocks occupy the region next to the centromere (thick C-blocks). However, in *Pterolepis* all chromosomes are characterized by medium-sized paracentromeric C-bands, while the metacentric X chromosomes in the latter genus have a thick (double) block in this region. Similarly to the above mentioned species, most genera and species of Phaneropterinae (*Poecilimon*, *Isophya*, *Metaplastes*, *Polysarcus*, *and Andreiniimon*) possess thick paracentromeric C-bands on one or on almost all chromosomes (WARCHAŁOWSKA-ŚLIWA & BUGROV 1998; WARCHAŁOWSKA-ŚLIWA & HELLER 1998; WARCHAŁOWSKA-ŚLIWA *et al.* 2000).

The C-banding patterns and distribution of interstitial and telomeric C-bands in autosomes and the X chromosomes are usually found to vary among genera and between species of one genus. For instance, qualitative and quantitative variation of C-bands was observed in *Metrioptera* and *Eupholidoptera* (present paper), as well as in *Montana* (WARCHAŁOWSKA-ŚLIWA *et al.* 1994). Only *Pholidoptera frivaldsky* and *P. griseoaptera* as well as *Pterolepis ferdinandi* and *P. germanica*, possess the same C-banding patterns similar to *Anadrymadusa picta* and *A. robusta* (WARCHAŁOWSKA-ŚLIWA & BUGROV 1996a).

Intraspecific polymorphism mainly connected with different numbers of additional heterochromatin blocks (indicated in Table 1 as *) was described in a few species as in earlier studies of representatives of the genus *Gampsocleis* (WARCHAŁOWSKA-ŚLIWA *et al.* 1992).

The silver Ag-staining of the NORs is one of methods used for demonstrating the position of the gene complex at 18S and 28S ribosomal DNA in the chromosome set (GOODPASTURE & BLOOM 1975). In mammals and plants NOR studies are provided on mitotic divisions, however, in Orthoptera these regions may be stained only in meiotic cells. The location of NORs in six genera of tettigoniids is presented in Table 1. The distribution of

NORs often characterizes species within one genus, but sometimes shows variation even between specimens of the same species (WARCHAŁOWSKA-ŚLIWA & MARYAŃSKA-NA- DACHOWSKA 1995). In some Tettigoniinae the NOR occurs in the large bivalents as in the genus *Tettigonia* and in representatives of Drymadusini (WARCHAŁOWSKA-ŚLIWA & MARYAŃSKA-NADACHOWSKA 1995; WARCHAŁOWSKA-ŚLIWA & BUGROV 1996a, respectively), or in the middle-sized bivalents, e.g. in *Decticus*, *Eupholidoptera*, *Metrioptera* (the present paper) as well as in *Gampsocleis* and *Montana* (the present paper and WARCHAŁOWSKA-ŚLIWA *et al.* 1992, 1994). In species from the genera *Eupholidoptera*, *Pholidoptera* (only with 2n=31), or *Gampsocleis* (the present paper and WARCHAŁOWSKA-ŚLIWA *et al.* 1992), the NORs are located in most cases probably on the same bivalent (at least according to size).

The heterochromatin associated with NORs sometimes shows a positive C-banding response. In ten species described in this paper there are NORs which correspond to a C-band in the equivalent position (Table 1). On the other hand, NORs in *Gampsocleis abbreviata* do not show correspondence with C-bands; the nature of heterochromatin associated with these NORs requires further investigation using other banding techniques (CMA_3 and FISH, e.g. SOUZA *et al.* 2003). The present study demonstrates a high degree of conservatism of the NOR location pattern, agreeing with observations on the genera *Montana* and *Gampsocleis* (WARCHAŁOWSKA-ŚLIWA *et al.* 1992, 1994). Thus, NORs are useful chromosome markers for interspecific comparison in the Tettigoniinae, like in the acridid subfamilies Oedipodinae (VISERAS *et al.* 1991) and Gomphocerinae (CAMACHO & CABRERO 1986a,b).

In summary, the present results show some similarities and differences between karyotypes, C-banding patterns, and NOR patterns within European species of Tettiginiinae. It is worth stressing that modification of morphology of chromosomes caused a decrease in chromosome number in the genus *Pterolepis* and in some species of *Drymadusa*, *Gampsocleis* and *Pholidoptera*. The further employment of molecular techniques will probably provide additional information on the evolution of the genome in e.g. *Pterolepis*, *Gampsocleis*, *Pholidoptera*, and Drymadusini.

References

AREFJEV V. A. 1989. Karyotypes of three acridid species and one grasshopper species from the south European part of the USSR. Citologia **31**: 582-591. (In Russian).

BATTAGLIA E. 1964. Cytogenetics of B-chromosomes. Caryologia **17**: 245-299.

BLACKMAN R. L. 1980. Chromosome numbers in the Aphididae and their taxonomical significance. Systematics Entomology **5**: 7-25.

BUGROV A. G. 1990. Karyotypes of some rare katydids (Orthoptera, Tettigoniidae) from Siberia. (In: Redkije Gelminty Kleshchi i Nasekomyje. Nauka, Novosibirsk): 54-60. (In Russian).

CABRERO J., CAMACHO J. P. M. 1986a. Cytogenetics studies in gomphocerine grasshoppers. I. Comparative analysis of chromosome C-banding pattern. Heredity **56**: 365-372.

CABRERO J., CAMACHO J. P. M. 1986b. Cytogenetic studies in gomphocerine grasshoppers. II. Chromosomal location of active nucleolar organizing region. Canadian Journal of Genetics and Cytology **28**: 540-544.

CAMACHO J. P. M., OROZCO J. C., PASCUAL F. 1981. Chromosomal rearrangements and karyotypic evolution in Decticinae (Orthoptera: Tettigonioidea). Cytologia **46**: 209-215.

FONTANA F., RUBINI M. 1990. Chromosomal evolution in Cervidae. BioSystems **24**: 157-174.

GOODPASTURE C., BLOOM S. E. 1975. Visualization of nucleolar organizer regions in mammalian chromosomes using silver staining. Chromosoma **53**: 37-50

HELLER K.-G. 1988. Bioakustik der europäischen Laubheuschrecken (Ökologie in Forschung und Anwendung (1). Verlag Josef Margraf, Weikersheim. 358 pp.

HELLER K.-G., KORSUNOVSKAYA O., RAGGE D. R., VEDENINA V., WILLEMSE F., ZHANTIEV R. D., FRANTSEVICH L. 1988. Check-list of European Orthoptera. Articulata **7,** Beiheft: 1-61.

HEWITT G. M. 1979. Grasshoppers and crickets. Animal Cytogenetics, 3. Insecta I. Orthoptera. Borntraeger, Berlin, Stuttgart.

JOHN B., HEWITT G. M. 1968. Patterns and pathways of chromosome evolution the Orthoptera. Chromosoma **25**: 40-47.

JOHN B., LEVIS K. R. 1968. The chromosome complement. Protoplasmatologia. Vol. 6. Springer, Vienna, New York.

PETITPIERRE E. 1997. The value of cytogenetics for taxonomy and evolution of Leaf Beetles (Coleoptera, Chrysomelidae). Miscellania Zoologica **20**: 9-18.

REDI C. A., GARAGNA S., ZACHARIAS H., ZUCCOTTI M., CAPANNA E. 2001. The other chromatin. Chromosoma **110**: 136-147.

RENTZ D.C. F. COLLESS D. H. 1990. A classification of the shield-backed katydids (Tettigoniinae) of the world. (In: The Tettigoniidae: biology, systematics and evolution. Bailey W. J. & Rentz D. C. F. eds. Bathurst, Crawford House Press): 352–377.

RUMPLER Y. 2000. What cytogenetic studies may tell us about species diversity and speciation of lemurs. Int. J. Primatology **21**: 865-881.

SANTOS J. L., ARANA P., GIRALDEZ R. 1983. Chromosome C-banding patterns in Spanish Acrididae. Genetica **61**: 65-74.

SHAPOSHNIKOV G., KUZNETSOVA V., STEKOLSHCHIKOV A. 1988. (In: Aphids Natural and Managed Ekosystems. Nieto Nafria J. M., Dixon A. F. G. eds): 481-487.

SERGEEV M. G., BUGROV A. G. 1988. Katydids of subfamily Deracanthinae (Orthoptera, Bradyporidae) from Siberia. (In: Taksonomiya Zhivotnych Sybiri, Russian Acad. Sci., Novosibirsk): 46-53. (In Russian).

SOUTHERN D. I. 1967. Pseudo-multiple formation as a consequence of prolonged non-homologous chromosome association in *Metrioptera brachyptera*. Chromosoma **21**: 272-284.

SMITH S. A. 1990. Cytosystematic evidence agsinst monophyly of *Peromysces boylii* species group (Rodentia: Cricetida). J. Mammal. **71**: 645-667.

SOUZA M. J., O. HAVER P. R., MELO N. F. 2003. Karyotype, C- and fluorescence banding patterns, NOR location and FISH in the grasshopper *Xestotrschelus robustus* (Romaleidae). Caryologia **56**: 261-267.

STOROZHENKO S. Yu. 2004. Long-horned orthopterans (Orthoptera: Ensifera) of the Asiatic part of Russia. Vladivistok: Dalnauka. 280 pp.

SUMNER S. G. 1972. A simple technique for demonstrating centromere heterochromatin. Exp. Cell Res. **75**: 304-306.

UESHIMA N. 1986. Chromosome systems of some Japanese Tettigoniidae (Orthoptera). J. Matsusaka Univ. **4**: 13-20.

VISERAS E., CABRERO J., TALAVERA M., CAMACHO J. P. M. 1991. C-banding and NOR location variations in oedipodinae grasshoppers. Cytobios **68**: 165-177.

VOLLETH M., HELLER K.-G. 1994. Phylogenetic relationships of vespertilionid genera (Mammalia: Chiroptera) as revealed by karyological analysis. Z. zool. Evolut.-forsh. **32**: 11-34.

WARCHAŁOWSKA-ŚLIWA E. 1984. Karyological studies on polish Orthoptera species of the Tettigonioidea superfamily. II. Karyotypes of families Tettigoniidea and Decticidae. Folia biol. (Kraków) **32**: 311-325.

WARCHAŁOWSKA-ŚLIWA E. 1988. Karyotype of *Pholidoptera aptera karnyi* Ebner. from Bulgaria and its comparison with that of *Pholidoptera aptera aptera* (Fabr.) from Poland (Orthoptera, Decticidae). Caryologia **41**: 161-168.

WARCHAŁOWSKA-ŚLIWA E. 1998. Karyotype characteristics of katydid orthopterans (Ensifera, Tettigoniidae) and remarks on their evolution at different taxonomic levels. Folia biol. (Kraków) **46**: 143-176.

WARCHAŁOWSKA-ŚLIWA E., BUGROV A. G. 1996a. Karyotype evolution of Drymadusini (Decticinae, Orthoptera). Cytologia **61**: 33-39.

WARCHAŁOWSKA-ŚLIWA E., BUGROV A. G. 1996b. Karyotypes and C-banding patterns of Bradyporinae (Orthoptera, Tettigoniidae) Folia biol. (Kraków) **44**: 95-98.

WARCHAŁOWSKA-ŚLIWA E., BUGROV A. G. 1998. Karyotypes and C-banding patterns of some Phaneropterinae katydids (Orthoptera, Tettigonioidea) with special attention to a post-reductional division of the neo-X and the neo-Y sex chromosomes in *Isophya hemiptera*. Folia biol. (Kraków) **46**: 47-54.

WARCHAŁOWSKA-ŚLIWA E., BUGROV A. G., MARYAŃSKA-NADACHOWSKA A. 1995. Karyotypes of three species of the genera *Poecilimon* Fisch. and *Isophya* Br.-W. (Orthoptera, Tettigonioidea, Phaneropterinae) from the North Caucasus. Caryologia **48**: 27-34.

WARCHAŁOWSKA-ŚLIWA E., BUGROV A. G., MARYAŃSKA-NADACHOWSKA A. 1996. Karyotypes and C-banding patterns of some species of Phaneropterinae (Orthoptera, Tettigonioidea). Folia biol. (Kraków) **44**: 5-10.

WARCHAŁOWSKA-ŚLIWA E., HELLER K.-G. 1998. C-banding patterns of some species of Phaneropterinae (Orthoptera, Tettigoniidae) of Europe. Folia biol. (Kraków) **46**: 177-181.

WARCHAŁOWSKA-ŚLIWA E., HELLER K.-G., MARYAŃSKA-NADACHOWSKA A., LEHMANN A. 2000. Chromosome evolution in the genus *Poecilimon* (Orthoptera, Tettigonioidea, Phaneropteridae). Folia biol. (Kraków) **48**: 127-136.

WARCHAŁOWSKA-ŚLIWA E., MARYAŃSKA-NADACHOWSKA A. 1992. Karyotypes, C-bands, NORs location in spermatogenesis of *Isophya brevipennis* Brunner (Orthoptera: Phaneropteridae). Caryologia **45**: 83-89.

WARCHAŁOWSKA-ŚLIWA E., MARYAŃSKA-NADACHOWSKA A. 1995. Cytogenetic studies of the genus *Tettigonia* (Orthoptera, Tettigonioidea, Tettigoniinae). II. Heteromorphism of C-bands. Folia biol. (Kraków) **43**: 99-106.

WARCHAŁOWSKA-ŚLIWA E., MICHAILOVA P. 1993. Cytological study of *Pholidoptera frivaldsky* (Herm.) (Decticidae, Orthoptera). Cytobios **74**: 155-162.

WARCHAŁOWSKA-ŚLIWA E. MICHAILOVA P., PESHEV G. 1987. Karyological studies of *Metrioptera arnoldi* Rme (Decticidae) and *Ancistrura nigrovittata* (Br.) (Phaneropteridae: Tettigonioidea: Orthoptera). Caryologia **40**: 369-380.

WARCHAŁOWSKA-ŚLIWA E., MARYAŃSKA-NADACHOWSKA A., BUGROV A. G. 1992. Karyotypes, C- heterochromatin, and NOR in three species of the genus *Gampsocleis* Fieb. (Orthoptera: Tettigonioidea: Decticinae). Folia biol. (Kraków) **40**: 119-127.

WARCHAŁOWSKA-ŚLIWA E., BUGROV A. G., MARYAŃSKA-NADACHOWSKA A. 1994. Karyotypes, C- banding pattern, and NORs of the genus *Montana* Zeuner 1941 (Orthoptera, Tettigonioidea, Decticinae). Folia biol. (Kraków) **42**: 89-94.

WHITE M. J. D. 1973. Animal Cytology and Evolution, 3rd ed. Cambridge Univ. Press, London.

Appendix:

Species in alphabetical order: *Anterastes serbicus* Brunner v. Wattenwyl, 1882, CH6403-5, males, CH6407, female, GREECE: Kavalla, Mt. Pangaion (40°53'N, 24°3'E), 1700 m, 21 vii 2004, coll. K.-G. & M. Heller; *Bucephaloptera bucephala* (Brunner v. Wattenwyl, 1882), CH6073, male, TURKEY: Mersin, Anamourion (near Anamur) (36°2'N, 32°47'E), 3 m, 9 vii 2002, coll. K.-G. Heller; *Decticus verrucivorus* (Linnaeus, 1758), 2 males, POLAND: Pieniny National Park, Palenica, (49°25'N, 20°30'E), 13 vii 1996, coll. E. Warchałowska-Śliwa, L. Śliwa; *Drymadusa dorsalis limbata* Brunner v. Wattenwyl, 1882, CH6080, male, TURKEY: Antalya, above Gazipasa (near Ilýca) (36°24'N, 32°23'E), 800 m, 10 vii 2002, coll. K.-G. Heller; *Eupholidoptera anatolica* (Ramme, 1930), CH5380, CH5715, males, TURKEY: Antalya, Termessos (36°58'N, 30°30'E), 13 vii 2002, coll. K.-G. .Heller; *Eupho- lidoptera annulipes* (Brunner v. Wattenwyl, 1882), CH5387, male, TURKEY: Mersin, below Güzeloluk (22 km to Erdemli) (36°44'N, 34°9'E), 1000 m, 6 vii 2002, coll. K.-G. Heller; CH5720, male, TURKEY: Mersin, below Güzeloluk, near Arslanlý (36°41'N, 34°11'E), 500 m, 6 vii 2002, coll. K.-G. Heller; *Eupholidoptera chabrieri garganica* (La Greca, 1959), CH6337-8, males, GREECE: Kerkyra, near Agios Spiridon (in the north of Kerkyra) (39°48'N, 19°50'E), 5 m, 15 vii 2004, coll. K.-G. Heller, M. Heller; CH3200, male, GREECE: Ioannina, Dodona (39°33'N, 20°45'E), 9 vi 1998, coll. Heller & Volleth; *Eupholidoptera epirotica* (Ramme, 1927), CH6141-6, males, CH6255, female, GREECE: Aitolia-Akarnania (Central Greece), Mt. Akarnania above Thirion (38°48'N, 20°58'E), 1400 m, 3 viii 2003, coll. K.-G. Heller, M. Heller, M. Volleth; *Eupholidoptera* sp., CH6151-2, males, CH6154-5, females, GREECE: Kikladhes: Andros, north of Vourkoti (37°52'N, 24°52'E), 30-31 vii 2003, coll. K.-G. Heller, M. Volleth; *Eupholidoptera icariensis* Willemse, 1980, CH3204, male, GREECE: Samos, Ikaria (37°36'N, 26°9'E), 23 v 1998, coll. Heller & Volleth; *Eupholidoptera karabagi* Salman, 1983, CH5381-2, CH5722, males, TURKEY: Antalya, Termessos (36°58'N, 30°30'E), 13 vii 2002, coll. K.-G. Heller; *Eupholidoptera megastyla* (Ramme, 1939), CH6148-50, CH6297, males, GREECE: Arta, Mt. Tsoumerka above Theodoriana (39°25'N, 21°10'E), 5-6 viii 2003, coll. K.-G. Heller, M. Heller, M. Volleth; *Eupholidoptera mersinensis* Salman, 1983, CH5391, male, TURKEY: Mersin, Fýndýkpýnarý (36°50'N, 34°20'E), 4 vii 2002, coll. K.-G. Heller; CH5390, male, TURKEY: Mersin, below Güzeloluk (near Köserlý) (36°45'N, 34°7'E), 1400 m, 6 vii 2002, coll. K.-G. Heller; *Eupholidoptera prasina* (Brunner v. Wattenwyl, 1882), CH5383-5, CH5388, CH5729, males, TURKEY: Mersin, Kasyayla (above Anamur) (36°15'N, 32°54'E), 9 vii 2002, coll. K.-G. Heller; *Eupholidoptera tauricola* (Ramme, 1930), CH5386, CH5734, males, TURKEY: Mersin, below Güzeloluk (22 km to Erdemli) (36°44'N, 34°9'E), 1000 m, 6 vii 2002, coll. K.-G. Heller; *Gampsocleis abbreviata* Herman, 1874, CH4784-6, males, GREECE: Fokis, 4 km north east of Gravia (38°40'N, 22°24'E), 21 vi 1997, coll. K.-G. Heller; *Metrioptera bicolor* (Philippi, 1830), 2 males, POLAND: Wieprzecka Mountain, (23°10'N, 50°35'E), 30-31 vii 1998, coll. E. Warchałowska-Śliwa, M. Jeleń; *Metrioptera brachyptera* (Linnaeus, 1761), 5 males, SLOVAKIA: Tatra Mountain, Tatrzańska Kotlina, near Lendak, (49°14'N, 20°2'E), 20 vii 2001, coll. E. Warchałowska-Śliwa, L. Śliwa; *Metrioptera* (*Roeseliana*) *roeselii ambitiosa* Uvarov, 1923, CH6344, male, GREECE: Ioannina, lake near Metsovon (39°49'N, 21°8'E), 27 vii 2004, coll. K.-G. & M. Heller; CH6345, female, GREECE: Thesprotia, lake of Kefalovriso, western shore (39°29'N, 20°27'E), 28 vii 2004, coll. K.-G. Heller; *Metrioptera* (*Roeseliana*) *roeselii roeselii* (Hagenbach, 1822), 6 males, POLAND: Ojców National Park, Sąspowska valley, (50°15'N, 19°5'E), 10 vii 1997, coll. E. Warchałowska-Śliwa, A. Maryańska-Nadachowska; *Metrioptera* (*Vichetia*) *oblongicollis* (Brunner v. Wattenwyl, 1882), CH6343, male, GREECE: Drama, 3 km south- southwest of Perithori (41°18'N, 23°46'E), 24 vii 2004, coll. K.-G. & M. Heller; CH6396, male, GREECE: Ioannina, lake near Metsovon (39°49'N, 21°8'E), 27 vii 2004, coll. K.-G. & M. Heller; *Parapholidoptera signata* (Brunner v. Wattenwyl, 1861), CH5394, male, TURKEY: Mersin, below Güzeloluk (22 km to Erdemli) (36°44'N, 34°9'E), 1000 m, 6 vii 2002, coll. K.-G. Heller; *Pholidoptera aptera aptera* (Fabricius, 1793), 4 males, POLAND, Pieniny Mountains, polana Walusiówka, alt. 724 m (49°25'N, 20°28'E), 17 viii 1998 coll. E. Warchałowska-Śliwa, L. Śliwa; *Pholidoptera frivaldskyi* (Herman, 1871), CH6341, male, GREECE: Drama, ca. 5 km north of Elatia (41°30'N, 24°18'E), 23 vii 2004, coll. K.-G. & M. Heller; *Pholidoptera griseoaptera* (De Geer, 1773), 6 males, POLAND, Ojców National Park, Sąspowska valley, (50°15'N, 19°50'E), 1-30 vii 1999, coll. E. Warchałowska-Śliwa, A. Maryańska-Nadachowska; *Pholidoptera macedonica* Ramme, 1928 (or a related species), CH6342, CH6399, males, GREECE: Drama, near Elatia (41°30'N, 24°18'E), 23 vii 2004, coll. K.-G. & M. Heller; *Platycleis* (*Parnassiana*) *tenuis* Heller et Willemse, 1989, CH6275, male, GREECE: Arta, Mt. Tsoumerka above Vourgareli, Grat (39°24'N, 21°9'E), 1850 m, 6-7 viii 2003, coll. K.-G. Heller, M. Heller, M. Volleth; *Psorodonotus fieberi macedonicus* Ramme, 1931, CH6349-52, males, GREECE: Ioannina, lake near Metsovon (39°49'N, 21°8'E), 27 vii 2004, coll. K.-G. & M. Heller; *Pterolepis edentata* (Willemse, 1982), CH6251, male, CH6174, male, CH6260, male, GREECE: Aitolia-Akarnania (Central Greece), Mt. Akarnania above Voustrio (38°48'N, 20°59'E), 1100 m, 3-4 viii 2003, coll. K.-G. Heller, M. Heller, M. Volleth; *Pterolepis ferdinandi* (L.Willemse & Tilmans 1986), CH6173, male, GREECE: Aitolia-Akarnania (Central Greece), near Plakoti (39°0'N, 21°9'E), 4 viii 2003, coll. K.-G. Heller, M. Heller, M. Volleth; *Pterolepis germanica* (Herrich-Schaffer, 1840), CH6172, male, GREECE: Arta, Mt. Gavrogo (39°13'N, 21°15'E), 1600-1800 m, 4-5 viii 2003, coll. K.-G. Heller, M. Heller, M. Volleth; CH6268, male, GREECE: Arta, Mt. Tsoumerka above Vourgareli, southern slope (39°23'N, 21°9'E), 1500 m, 6 viii 2003, coll. K.-G. Heller, M. Heller, M. Volleth; *Pterolepis insularis* (Ramme, 1928), CH6169-70, CH6234, males, GREECE: Kikladhes: Andros, above Apikia (37°52'N, 24°55'E), 30 vii 2003, coll. K.-G. Heller, M. Heller, M. Volleth; *Pterolepis* n. sp., CH6175-6, males, CH6178, female (paratypes), GREECE: Arta, Mt. Gavrogo (39°13'N, 21°15'E), 1600-1800 m, 4-5 viii 2003, coll. K.-G. Heller, M. Heller, M. Volleth.