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Karyotypes of sawflies (Hymenoptera: Tenthredinidae) from the Moscow Province, Russia

Кариотипы пилильщиков (Hymenoptera: Tenthredinidae) из Московской области (Россия)

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КЛЮЧЕВЫЕ СЛОВА: Hymenoptera, Symphyta, Tenthredinidae, пилильщики, хромосомы, кариотипы.

ABSTRACT. Chromosomes of 13 species of the family Tenthredinidae from the Moscow Province were examined, including those 8 species studied for the first time*: *Cladius pectinicornis* (Geoffroy, 1785) (n = 6), **Euura clitellata* (Serville, 1823) (n = 9), **E. myosotidis* (Fabricius, 1804) (n = 8), **Eriocampa dorpatica* Konow, 1887 (n = 8, 9), *Nesoselandria morio* (Fabricius, 1781) (n = 6), *Pachyprotasis rapae* (Linnaeus, 1767) (n = 10), **Macrophya duodecimpunctata* (Linnaeus, 1758) (n = 10), **Sciapteryx consobrina* (Klug, 1816) (n = 12), **Empria pumila* (Konow, 1896) (n = 13), **Allantus calceatus* (Klug, 1818) (n = 9), *A. cinctus* (Linnaeus, 1758) (n = 9), **Claremontia puncticeps* (Konow, 1886) (n = 10) and *Eutomostethus luteiventris* (Klug, 1816) (n = 10). In a few cases, different chromosome numbers were detected within apparently the same sawfly species. Specifically, n = 6 was previously reported for *E. luteiventris* from Germany, although identification errors and/or presence of cryptic species cannot be completely ruled out. In almost all embryos derived from both studied females of *E. myosotidis* n = 8 was found, except for a particular specimen having a similar chromosome set with n = 9 due to the presence of an additional acrocentric. Implications of the detected chromosomal variation for taxonomy and phylogeny of the family Tenthredinidae are discussed.

РЕЗЮМЕ. Исследованы хромосомы 13 видов семейства Tenthredinidae из Московской области, включая 8 видов, изученных впервые*: *Cladius pectinicornis* (Geoffroy, 1785) (n = 6), **Euura clitellata* (Serville, 1823) (n = 9), **E. myosotidis* (Fabricius, 1804) (n = 8, 9), **Eriocampa dorpatica* Konow, 1887 (n = 8), *Nesoselandria morio* (Fabricius, 1781) (n = 6), *Pachyprotasis rapae* (Linnaeus, 1767) (n = 10), **Macrophya duodecimpunctata* (Linnaeus, 1758) (n = 10), **Sciapteryx consobrina* (Klug, 1816) (n = 12), **Empria pumila* (Konow, 1896) (n = 13), **Allantus calceatus* (Klug, 1818) (n = 9), *A. cinctus* (Linnaeus, 1758) (n = 9), **Claremontia puncticeps* (Konow, 1886) (n = 10) и *Eutomostethus luteiventris* (Klug, 1816) (n = 10). В нескольких случаях, разные хромосомные числа были обнаружены, возможно, внутри одних и тех же видов пилильщиков. В частности, для *E. luteiventris* в Германии было ранее указано n = 6, хотя в данном случае нельзя полностью исключить ошибки в определении или/и наличие криптических видов. Кроме того, почти у всех эмбрионов, полученных от обоих изученных самок *E. myosotidis* найдено n = 8, кроме одной особи, у которой был выявлен сходный хромосомный набор с n = 9 из-за наличия дополнительного акроцентрика. Обсуждается значение обнаруженной хромосомной изменчивости для таксономии и филогении семейства Tenthredinidae.

Introduction

The family Tenthredinidae s.l. currently harbors more than 5,500 sawfly species [Huber, 2017], although recent research supports the existence of separate smaller families within this group [Wutke *et al*., 2024]. Karyotypes of approximately 300 members of Tenthredinidae s.l. are studied up to now [Westendorff, 2006]. However, chromosome sets of many European species, especially those from Eastern Europe, remain totally unknown. At the same time, estimates of the fauna of this family in European Russia and adjacent territories suggest the presence of about 700 species of Tenthredinidae [Zhelokhovtsev, 1988]. I have recently studied about two dozen species from this family, mainly collected in the Moscow Province [Gokhman, 2023, 2024]. The present work therefore contains karyotype descriptions of another 13 species of Tenthredinidae from this region (Table 1).

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Table 1. Chromosome numbers of sawflies studied in the present work.
Таблица 1. Хромосомные числа пилильщиков, изученные в настоящей работе.

Species	No. ind.	n
*Cladius pectinicorni*s (Geoffroy, 1785)	1	6
Euura clitellata (Serville, 1823)	1	9
E. myosotidis (Fabricius, 1804)	2	8, 9
Eriocampa dorpatica Konow, 1887	1	8
Nesoselandria morio (Fabricius, 1781)	1	6
Pachyprotasis rapae (Linnaeus, 1767)	1	10
Macrophya duodecimpunctata (Linnaeus, 1758)	1	10
Sciapteryx consobrina (Klug, 1816)	1	12
Empria pumila (Konow, 1896)	1	13
Allantus calceatus (Klug, 1818)	6	9
A. cinctus (Linnaeus, 1758)	1	9
Claremontia puncticeps (Konow, 1886)	1	10
Eutomostethus luteiventris (Klug, 1816)	1	10

Material and methods

Adult sawflies collected by the author in 2022-2023 near Ozhigovo, Russia (about 60 km SW Moscow: 55°28'N; 36°52'E) were used in this study. The specimens were preliminarily identified by the author; most identifications were then checked by S.A. Basov (Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia). Taxonomic positions of all species are generally given according to the Electronic World Catalog of Symphyta [Taeger *et al*., 2018]. Main clades of the family Tenthredinidae s.str., which are mostly given subfamily rank, are named according to the results of the recent cladistic analysis by Wutke *et al*. [2024]. Names of species which karyotypes were studied for the first time, are marked with an asterisk (*).

Chromosome preparations were obtained according to the guidelines provided by Naito [1982] and Imai *et al*. [1988] with a few modifications. The females were dissected in small Petri dishes in distilled water, unfertilized mature eggs were extracted from their bodies, placed into the dishes on a filter paper soaked with distilled water, and then incubated for 3–4 days at room temperature. Haploid embryos were extracted from the eggs and dissected in 0.5% hypotonic sodium citrate solution containing 0.005% colchicine. The embryos were then transferred to a fresh portion of hypotonic solution and incubated for about 30 min at room temperature. The material was transferred onto a pre-cleaned microscope slide using a Pasteur pipette and then gently flushed with Fixative I (glacial acetic acid: absolute ethanol: distilled water 3:3:4). The tissues were disrupted using dissecting needles in an additional drop of Fixative I. A drop of Fixative II (glacial acetic acid: absolute ethanol 1:1) was applied to the center of the area, and the more aqueous phase was blotted off the edges of the slide. The slides were then dried and stained with 3 per cent Giemsa solution for a few hours.

Mitotic divisions were studied and photographed using an optic microscope Zeiss Axioskop 40 FL fitted with a digital camera Axiocam 208 color (Carl Zeiss, Germany). To produce illustrations, the resulting images were handled with the image processing programs ZEN version 3.0 (blue edition) (Carl Zeiss) and GIMP version 2.10. Chromosomes were classified according to the guidelines provided by Levan *et al*. [1964], i.e., as metacentrics, submetacentrics, subtelocentrics and acrocentrics.

Results

Subfamily Nematinae

Cladius pectinicornis (n = 6). All chromosomes of this species are clearly biarmed, i.e., submetacentric and subtelocentrics (Fig. 1). The first two chromosomes are substantially longer than the remaining ones.

**Euura clitellata* (n = 9). As in the previous species, all chromosomes of *E. clitellata* are obviously biarmed, mostly being metacentric or submetacentric (Fig. 2). The first chromosome is about 1.5 times longer than the second one. Second to fourth, fifth to eighth and the ninth chromosomes represent three remaining size groups. On some metaphase plates, pericentromeric blocks of constitutive heterochromatin can be seen, with the largest one on the third submetacentric chromosome (Fig. 3).

**E. myosotidis* (n = 8, 9). Most metaphase plates obtained from two individuals of this species clearly contain eight metacentrics or submetacentrics. All chromosomes show more or less continuous gradation in size, with the fifth metacentric being somewhat shorter than the fourth one (Fig. 4). However, all studied mitotic divisions from a particular embryo consistently demonstrate n = 9 with an additional acrocentric, which is of approximately the same length as the smallest metacentric (Fig. 5). In the embryo with n = 9 all chromosomes except for the above-mentioned acrocentric look virtually identical to those of the basic karyotype having n = 8.

Subfamily Selandriinae

**Eriocampa dorpatica* (n = 8). The chromosome set of this species generally resembles that of *E. myosotidis*, although the two last chromosomes are slightly shorter than the preceding ones (Fig. 6).

Nesoselandria morio (n = 6). As in most preceding species, all chromosomes of *N. morio* are obviously biarmed. The first chromosome clearly longer than the remaining ones, which form a continuous gradation in length (Fig. 7). On certain metaphase plates, pericentromeric blocks of constitutive heterochromatin are seen (Fig. 8).

Subfamily Tenthredininae

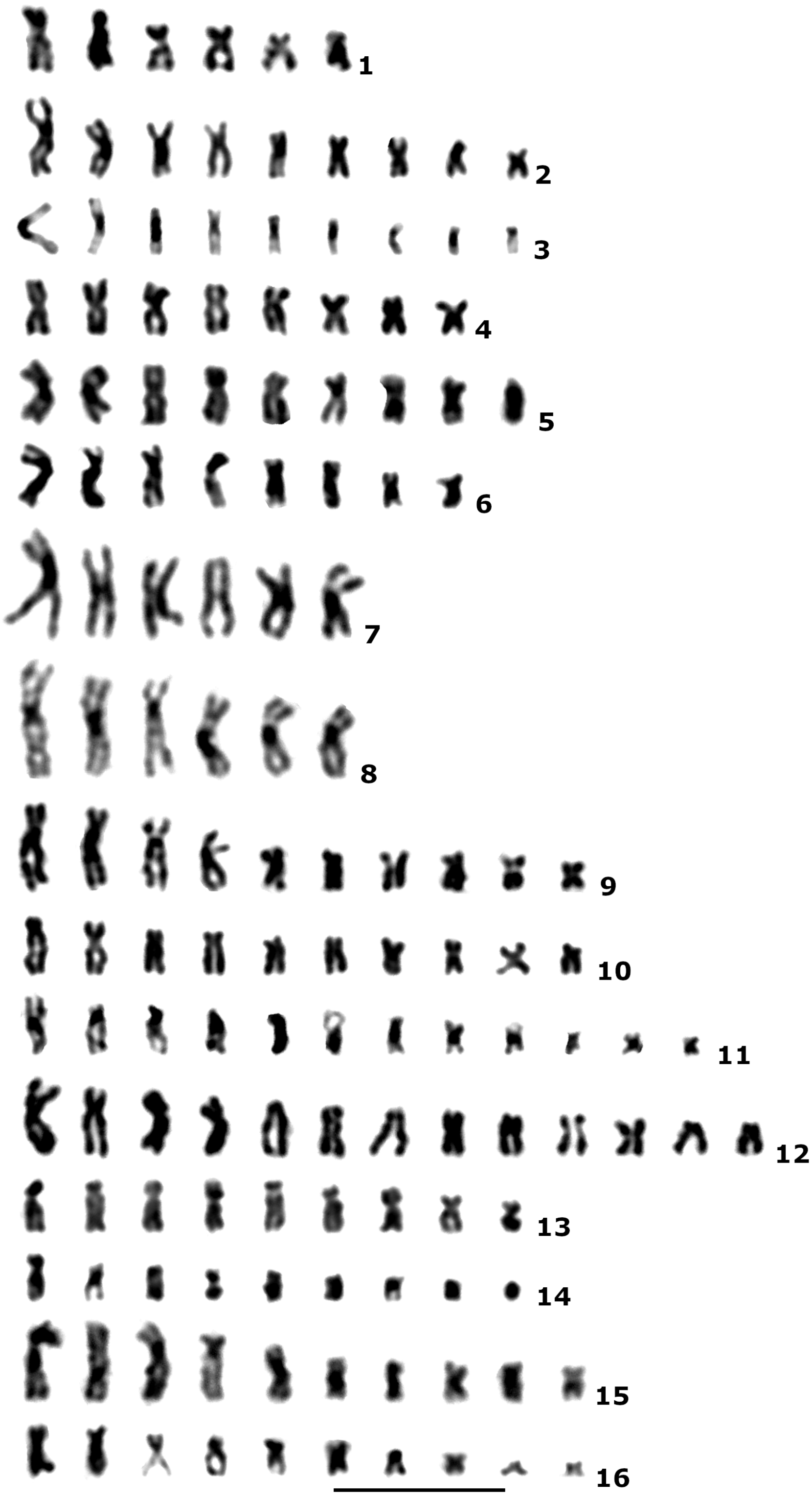
Pachyprotasis rapae (n = 10). All chromosomes of this species are visibly biarmed. The first submetacentric chromosome is somewhat longer than the second submetacentric and third subtelocentric. In turn, the fourth metacentric is obviously shorter than the preceding ones, and the remaining ones very gradually decrease in size, except for the last chromosome (Fig. 9).

**Macrophya duodecimpunctata* (n = 10). The karyotype of this species is very similar to that of *P. rapae* (Fig. 10).

**Sciapteryx consobrina* (n = 12). Most chromosomes of this species are apparently biarmed, although their morphology sometimes cannot be clearly seen (Fig. 11). The first submetacentric is substantially longer than the remaining chromosomes, which, in turn, form a continuous gradation in length (except for the three last ones).

Subfamily Allantinae (core)

**Empria pumila* (n = 13). Although clearly biarmed chromosomes prevail in the karyotype of this species, it also includes a few subtelocentrics and/or acrocentrics (Fig. 12). All chromosomes continuously decrease in size.



Figs 1–16. Haploid karyograms of Tenthredinidae. 1 — *Cladius pectinicornis*, 2 — *Euura clitellata*, 3 — ditto, visualized heterochromatic segments, 4 — *E. myosotidis*, karyotype with n = 8, 5 — ditto, n = 9, 6 — *Eriocampa dorpatica*, 7 — *Nesoselandria morio*, 8 — ditto, visualized heterochromatic segments, 9 — *Pachyprotasis rapae*, 10 — *Macrophya duodecimpunctata*, 11 — *Sciapteryx consobrina*, 12 — *Empria pumila*, 13 — *Allantus calceatus*, 14 — *A. cinctus*, 15 — *Claremontia puncticeps*, 16 — *Eutomostethus luteiventris*. Bar = 10 µm.

Рис. 1–16. Кариограммы гаплоидных наборов Tenthredinidae. 1 — *Cladius pectinicornis*, 2 — *Euura clitellata*, 3 — то же, показаны гетерохроматиновые блоки, 4 — *E. myosotidis*, кариотип с n = 8, 5 — то же, n = 9, 6 — *Eriocampa dorpatica*, 7 — *Nesoselandria morio*, 8 — то же, показаны гетерохроматиновые блоки, 9 — *Pachyprotasis rapae*, 10 — *Macrophya duodecimpunctata*, 11 — *Sciapteryx consobrina*, 12 — *Empria pumila*, 13 — *Allantus calceatus*, 14 — *A. cinctus*, 15 — *Claremontia puncticeps*, 16 — *Eutomostethus luteiventris*. Масштаб 10 мкм.

**Allantus calceatus* (n = 9). All chromosomes of this species are obviously biarmed. First to fifth chromosome form a very slow gradation in size, the remaining ones more visibly decrease in length (Fig. 13).

A. cinctus (n = 9). Overall karyotype structure is presumably similar to that of the previous species, although morphology of certain chromosomes cannot be clearly seen (Fig. 14).

Clade "Allantinae II / Blennocampinae II"

**Claremontia puncticeps* (n = 10). All chromosomes of this species are biarmed. The first chromosome is somewhat longer than the others. Second to fifth chromosome from a visible gradation in length, the remaining ones slowly decrease in size (Fig. 15).

Subfamily Blennocampinae (core)

Eutomostethus luteiventris (n = 10). All chromosomes of *E. luteiventris* are apparently biarmed. First to second, third to sixth, seventh to eighth and ninth to tenth chromosomes form four size groups (Fig. 16).

Discussion

Among the sawflies examined in the present study, most species, i.e., 8 out of 13, were studied for the first time (see above). However, different chromosome numbers are sometimes recorded even for the same members of the family. Specifically, the karyotype with n = 6, mostly containing biarmed chromosomes, was found in *Eutomostethus luteiventris* in Germany [Westendorff, 2006], whereas in the present paper n = 10 is reported for apparently the same species from the Moscow Province. Since other studied members of the same genus have both above-mentioned chromosome numbers [Westendorff, 2006], this discrepancy could be explained either by identification errors or by the presence of cryptic species. For some other genera of Tenthredinidae, new chromosome numbers were also revealed. Specifically, only n = 9 was previously known for both *Eriocampa* Hartig, 1837 and *Claremontia* Rohwer, 1909 [Naito, 1982; Westendorff, 2006], whereas newly studied members of these genera turned to have n = 8 and 10 respectively (see above).

It should be noted that only chromosome numbers and small-sized images of metaphase plates, often accompanied by general descriptions in terms of the proportion of metacentrics and submetacentrics vs. subtelocentrics and acrocentrics, are available for most Tenthredinidae [Naito, 1982; Westendorff, 2006]. Under these circumstances, detailed studies of sawfly karyotypes [Naito, 1978a, b; Nishimoto *et al*., 2014; Gokhman, 2024] can be very helpful. For example, Naito [1978a] conducted morphometric analysis of chromosomes of twelve Japanese species of the genus *Macrophya* Dahlbom, 1835. In addition, chromosomes of a few other members of this group were studied by Westendorff *et al*. [1999]. Naito [1982] also examined the karyotype of *Pachyprotasis rapae*, together with about 30 other *Pachyprotasis* Hartig, 1837 species. The chromosome set of *P. rapae* was also studied by Westendorff [2006]. According to these authors, the karyotype of the latter species, as well as many other members of this genus, contains ten biarmed chromosomes, and this is confirmed in the present work. Moreover, this chromosome set is similar to karyotypes of certain *Macrophya* species, including *M. duodecimpunctata* [Naito, 1978; this study]. Nevertheless, this does not seem surprising, since closely related *Pachyprotasis* Hartig, 1837 and *Macrophya* both belong to the tribe Macrophyini [Naito, 1978; Westendorff *et al*., 1999]. The karyotype structure shared by *M. duodecimpunctata* and *P. rapae* may therefore represent a symplesiomorphy of these genera.

The genus *Empria* Lepeletier et Serville, 1828 is also of considerable interest in terms of the karyotype study. Specifically, five members of this group, including recently examined *E. pumila*, turned to have four different chromosome numbers, i.e., n = 10, 11, 13 and 15 [Westendorff, 2006; Gokhman, 2023]. Karyotypes of these species also follow the general trait found in other Symphyta, according to which the proportion of subtelocentrics and acrocentrics in a given chromosome set grows together with an increase in the chromosome number [Gokhman, 2023]. Indeed, all chromosomes of *E. pallimacula* (Serville, 1823) with n = 10 are biarmed, whereas karyotypes of *E. pumila* and *E. sexpunctata* (Serville, 1823) as well as *Empria* sp. with n = 13 and 15 respectively contain considerable proportions of subtelocentric / acrocentric chromosomes [Naito, 1982; Gokhman, 2023; present paper].

Interesting results were also obtained for the two members of the taxonomically challenging genus *Euura* Newman, 1837 (= *Nematus* auct.). While all analyzed mitotic divisions of *E. clitellata* uniformly demonstrated n = 9, two different chromosome sets were discovered in *E. myosotidis*. Specifically, both karyotypes found in the two studied individuals of the latter species contained eight metacentric and submetacentric chromosomes, but all cell divisions from a particular embryo included an additional acrocentric. Since the latter chromosome was present on all metaphase plates from a certain developing egg, I presume that this phenomenon should be classified as aneuploidy, rather than presence of a B chromosome (see, e.g., [Gokhman, 2009]).

Additionally, distribution of the constitutive heterochromatin was examined in *Euura clitellata* and *Neoselandria morio* (see above). In both species, heterochromatic segments are exclusively or predominantly pericentromeric, which is also characteristic of a few other studied members of the superfamily Tenthredinoidea [Rousselet *et al*., 1998; Kuznetsova *et al*., 2001; Gokhman, 2024].

As mentioned above, a new comprehensive phylogeny of Symphyta has recently been published [Wutke *et al*., 2024]. This phylogenetic reconstruction would allow to consider all accumulated information on sawfly karyotypes in a new context. However, at first glance, it seems that in most clades of the resulting tree of Tenthredinidae the chromosome number fluctuates around n = 10, probably except for slightly lower n values in the subfamily Nematinae (see [Westendorff, 2006]). Nevertheless, the above-mentioned reconstruction confirms the previous conclusion on the apparent decrease in the chromosome number in Tenthredinoidea if compared to other Symphyta [Gokhman, 2023].

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