

Morphometric analysis of chromosomes of sawflies of the genera *Tenthredo* Linnaeus, 1758 and *Athalia* Leach, 1817 (Hymenoptera: Tenthredinidae: Athaliidae)

Морфометрический анализ хромосом пилильщиков родов *Tenthredo* Linnaeus, 1758 и *Athalia* Leach, 1817 (Hymenoptera: Tenthredinidae: Athaliidae)

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КЛЮЧЕВЫЕ СЛОВА: Hymenoptera, Symphyta, Tenthredinidae, пилильщики, морфометрия хромосом.

ABSTRACT. Morphometric analysis of chromosomes of twelve sawfly species of the families Tenthredinidae and Athaliidae, namely, *Tenthredo arcuata* Forster, 1771, *T. campestris* Linnaeus, 1758, *T. mesomela* Linnaeus, 1758, *T. omissa* (Förster, 1844), *T. velox* Fabricius, 1798, *T. vespa* Retzius, 1783 (in all these species $n = 10$), *T. amoena* Gravenhorst, 1807 and *T. brevicornis* (Konow, 1886) ($n = 18$ in both), as well as of *Athalia bicolor* Serville, 1823, *A. circularis* (Klug, 1815) (in both $n = 6$), *A. scutellariae* Cameron, 1880 ($n = 7$) and *A. rosae* (Linnaeus, 1758) ($n = 8$) was performed. Karyotype differences between species with the same chromosome numbers have been revealed. Possible implications of chromosome morphometry for the sawfly taxonomy and phylogeny were discussed.

РЕЗЮМЕ. Проведен морфометрический анализ хромосом двенадцати видов пилильщиков семейств Tenthredinidae и Athaliidae: *Tenthredo arcuata* Forster, 1771, *T. campestris* Linnaeus, 1758, *T. mesomela* Linnaeus, 1758, *T. omissa* (Förster, 1844), *T. velox* Fabricius, 1798, *T. vespa* Retzius, 1783 (у всех этих видов $n = 10$), *T. amoena* Gravenhorst, 1807 и *T. brevicornis* (Konow, 1886) (у обоих $n = 18$), а также *Athalia bicolor* Serville, 1823, *A. circularis* (Klug, 1815) (у обоих $n = 6$), *A. scutellariae* Cameron, 1880 ($n = 7$) и *A. rosae* (Linnaeus, 1758) ($n = 8$). Выявлены различия между видами с одинаковыми хромосомными числами. Обсуждены возможности использования морфометрии хромосом в систематике и филогенетике пилильщиков.

Introduction

Tenthredinidae s.l. harbor more than 5,500 sawfly species [Huber, 2017]. However, recent research supports the existence of smaller subordinate families within this group [Wutke *et al.*, 2024]. Among them, Athaliidae, which include the genus *Athalia* Leach, 1817 and related taxa, can be named [Niu *et al.*, 2022]. Most sawflies can be more or less easily recognized, although few groups apparently contain cryptic species [Taeger, 1984, etc.]. To distinguish between closely related forms of the order Hymenoptera, genetic techniques are often used (see, e.g., [Gokhman [2018]]. Among these techniques, chromosomal analysis proved effective to detect cryptic species of sawflies [Westendorff, 2006; Gokhman, 2023]. Although karyotypes of approximately 300 members of Tenthredinidae s.l. are studied up to now, their chromosome sets are often described without giving much detail, scarcely going beyond the 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 [Naito, 1982; Westendorff, 2006]. However, Nishimoto *et al.* [2014] presented a thorough study of chromosomal polymorphism in *Dolerus genuicinctus* Zaddach, 1859 (Tenthredinidae s.str.). Moreover, Naito [1978a, b] conducted a detailed morphometric analysis of chromosomes of Japanese sawflies of the genera *Tenthredo*, 1758 and *Macrophya* Dahlbom, 1835 from the same family. The two latter studies defined several types of karyotype structure that were characteristic of certain species and species

groups. On the other hand, morphometric analysis allowed to trace specific pathways of karyotype evolution in other closely related Hymenoptera, i.e., parasitoids [Gokhman *et al.*, 2017; König *et al.*, 2019]. The present work therefore represents a further attempt to apply analysis of this kind to the karyotypes of other sawflies, i.e., eight and four species of the genera *Tenthredo* and *Athalia*, respectively (Table 1). The results of this study are given below.

Material and methods

Unless otherwise stated, adult sawflies collected by the author in 2022–2023 near Ozhigovo, Russia (about 60 km SW Moscow: 55°28'N; 36°52'E) were mainly used in this study, except for a single specimen of *Tenthredo amoena*, which was collected near Bicaz, Romania (about 280 km N Bucharest: 46°54'N; 26°05'E). All 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); a specimen of *T. amoena* was also identified by M. Prous (University of Tartu, Tartu, Estonia). Taxonomic positions of all species are generally given according to the Electronic World Catalog of Symphyta [Taeger *et al.*, 2018].

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. Mitotic chromosomes were measured on ten haploid metaphases of each species using KaryoType software version 2.0 [Altnordu *et al.*, 2016]. Chromosomes were classified according to the guidelines provided by Levan *et al.* [1964], i.e., as metacentrics (M), submetacentrics (SM), subtelocentrics (ST) and acrocentrics (A).

Results

Tenthredo campestris (n = 10). All chromosomes of this species are obviously biarmed (Fig. 1, Table 2). In the karyotype of *T. campestris*, the first chromosome is about 1.3 times longer than the second one, which is, in turn, also 1.3 times longer than the third one. Third to fifth, sixth to eighth and ninth to tenth chromosomes form three consecutive size groups.

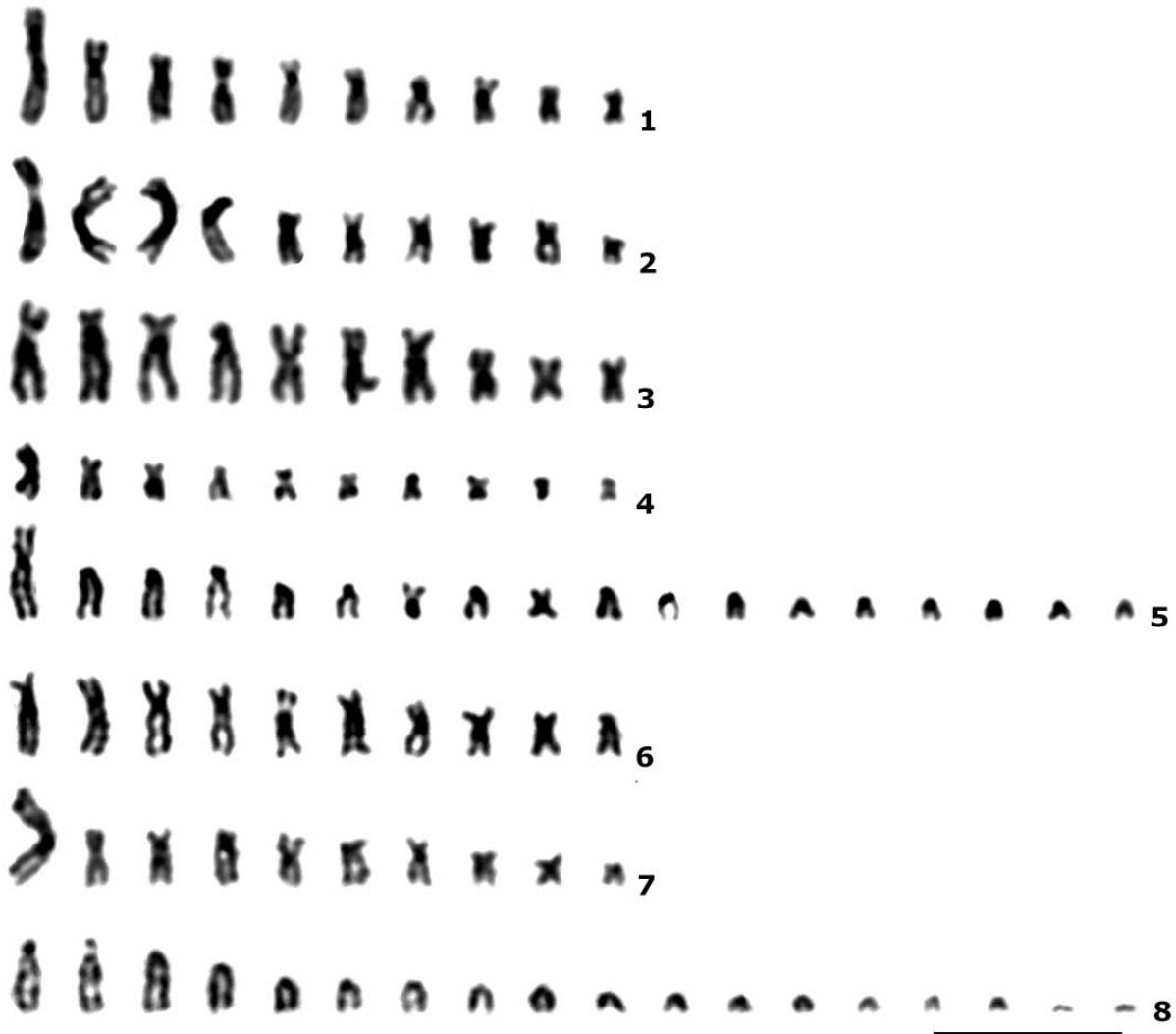
T. mesomela (n = 10). As in the previous species, all chromosomes are visibly biarmed (Fig. 2, Table 2). However, the first chromosome is only slightly longer than the second one. The remaining elements, i.e., third to fourth, fifth to ninth and the tenth chromosome represent three respective size groups.

T. velox (n = 10). Although all chromosomes of this species are biarmed, centromeric indices of the first four chromosomes are substantially lower than in *T. campestris* and *T. mesomela* (Fig. 3, Table 2). First to third, fourth to seventh, and eighth to tenth chromosomes also form three respective size groups.

Table 1. Data on sawfly karyotypes used in the present study.

Таблица 1. Данные о кариотипах пилильщиков, использованных в настоящей работе.

Species	No. ind.	n	Chromosomal formula
<i>Tenthredo (Endotethryx) campestris</i> Linnaeus, 1758	1	10	4M + 4M/SM + 2SM
<i>T. (Eurogaster) mesomela</i> Linnaeus, 1758	1	10	4M + 6M/SM
<i>T. (Tenthredella) velox</i> Fabricius, 1798	1	10	5M + 1M/SM + 2SM + 2SM/ST
<i>T. (Tenthredo) arcuata</i> Forster, 1771	1	10	6M + 4M/SM
<i>T. (T.) brevicornis</i> (Konow, 1886)	1	18	2M + 1M/SM + 1SM/ST + 14A
<i>T. (T.) omissa</i> (Förster, 1844)	1	10	1M + 6M/SM + 1SM + 2SM/ST
<i>T. (T.) vespa</i> Retzius, 1783	2	10	1M + 7M/SM + 2SM
<i>T. (Zonuledo) amoena</i> Gravenhorst, 1807	2	18	1ST + 17A
<i>Athalia bicolor</i> Serville, 1823	1	6	4M + 2M/SM
<i>A. circularis</i> (Klug, 1815)	2	6	2M + 2SM + 1SM/ST + 1A
<i>A. scutellariae</i> Cameron, 1880	1	7	4M + 3M/SM
<i>A. rosae</i> (Linnaeus, 1758)	3	8	7M + 1A



Figs 1–8. Haploid karyograms of the genus *Tenthredo*. 1 — *T. campestris*, 2 — *T. mesomela*, 3 — *T. velox*, 4 — *T. arcuata*, 5 — *T. brevicornis*, 6 — *T. omissa*, 7 — *T. vespa*, 8 — *T. amoena*. Bar = 10 μ m.

Рис. 1–8. Кариограммы гаплоидных наборов рода *Tenthredo*. 1 — *T. campestris*, 2 — *T. mesomela*, 3 — *T. velox*, 4 — *T. arcuata*, 5 — *T. brevicornis*, 6 — *T. omissa*, 7 — *T. vespa*, 8 — *T. amoena*. Масштаб 10 мкм.

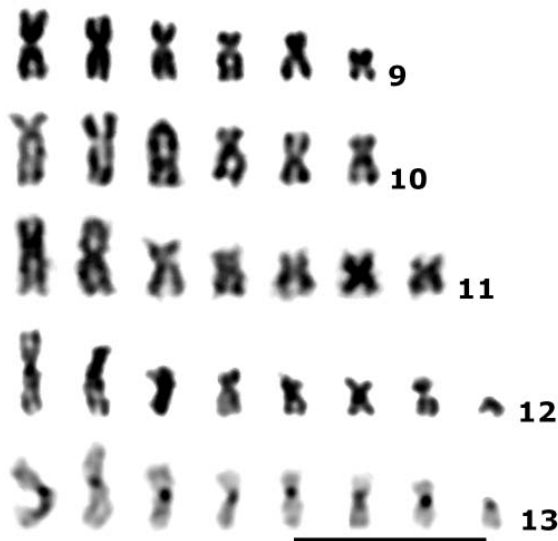
T. arcuata ($n = 10$). All chromosomes of *T. arcuata* are obviously biarmed, and the first element is about 1.3 times longer than the second one (Fig. 4, Table 2). The remaining chromosomes (third to fifth and sixth to tenth) represent two respective size groups.

T. brevicornis ($n = 18$). The chromosome number of this species is substantially higher than in all previous ones (Fig. 5, Table 2). Moreover, most chromosomes of *T. brevicornis* are acrocentric. The first metacentric or submetacentric element is about 1.7 times longer than the second one. The second to fourth chromosomes (two acrocentrics and an apparent submetacentric or subtelocentric) are similar in size. The remaining chromosomes are mostly acrocentric (except for the seventh and ninth metacentrics); fifth to tenth and 11th to 18th elements form two respective size groups.

T. omissa ($n = 10$). All chromosomes of this species are biarmed, but the first element is only slightly longer than the second one (Fig. 6, Table 2). Second to third, fourth to sixth and seventh to tenth chromosomes form three consecutive size groups.

T. vespa ($n = 10$). As in most previous species, all chromosomes are obviously biarmed. The first metacentric is about 1.9 times longer than the second one (Fig. 7, Table 2). Second to fourth, fifth to seventh, and eighth to tenth chromosomes form three respective size groups.

T. amoena ($n = 18$). The chromosome number in this species is the same as in *T. brevicornis* (Fig. 8, Table 2). However, in *T. amoena* all chromosomes are acrocentric, except for the first subtelocentric element, which is only slightly longer than the second one. The second to



Figs 9–13. Haploid karyograms of the genus *Athalia*. 9 — *A. bicolor*, 10 — *A. circularis*, 11 — *A. scutellariae*, 12 — *A. rosae*, 13 — ditto, visualized heterochromatic segments. Bar = 10 μ m.

Рис. 9–13. Кариограммы гаплоидных наборов рода *Athalia*. 9 — *A. bicolor*, 10 — *A. circularis*, 11 — *A. scutellariae*, 12 — *A. rosae*, 13 — то же, показаны гетерохроматиновые блоки. Масштаб 10 мкм.

fourth, fifth to ninth and 10th to 18th chromosomes form three respective size groups. On most metaphase plates, the second chromosome bears a characteristic secondary constriction on its shorter arm near the centromere.

Athalia bicolor ($n = 6$). All chromosomes of this species are obviously biarmed (Fig. 9, Table 3), which is also confirmed by Westendorff *et al.* [1999]. They are either metacentric or submetacentric, although Westendorff [2006] also reports a single subtelocentric within the karyotype of *A. bicolor*. First to second, third, fourth to fifth, and the sixth chromosome form four respective size groups.

A. circularis ($n = 6$). Chromosomes of this species are mostly biarmed, except for the third acrocentric element (Fig. 10, Table 3). Size groups of different chromosomes

resemble those of the previous species, although the sixth element is only slightly shorter than the fifth one.

A. scutellariae ($n = 7$). As in *A. bicolor*, all chromosomes of this species are obviously biarmed (Fig. 11, Table 3). This observation is in contrast with the karyotype description provided by Westendorff [2006], who identified a particular subtelocentric / acrocentric within this chromosome set. First to second, third, and fourth to seventh chromosomes form three respective size groups.

A. rosae ($n = 8$). All chromosomes, except for the smallest acrocentric, are metacentric (Fig. 12, Table 3). First to second, third, fourth to seventh, and the eighth chromosome form four consecutive size groups. On some metaphase plates, medium-sized blocks of pericentromeric heterochromatin can be seen (Fig. 13).

Discussion

For all species studied in the present work, except for *T. vespa*, chromosome numbers, often accompanied by brief descriptions of the karyotypes, were previously known [Westendorff, 2006; Gokhman, 2023]. Nevertheless, morphometric analysis of chromosomes revealed certain previously unknown features of sawfly karyotypes. This is clearly seen, for example, in the genus *Athalia*. Specifically, all four studied species have generally similar chromosome sets, especially in terms of size groups of the chromosomes. In addition, a separate subgenus, *Dentathalia* Benson, 1931, was erected by Benson [1931] to accommodate *A. scutellariae*. Recent phylogenetic analysis [Niu *et al.*, 2022] suggests this is a valid genus, and, moreover, it probably belongs to the most basal clade within *Athalia* s.l. If this is true, then the karyotype of *A. scutellariae* with $n = 7$ can be considered ancestral. Consequently, chromosome sets of *Athalia* species having either $n = 6$ or 8 are derived by either chromosomal fusion or fission respectively. However, at present it is difficult to trace most chromosomal rearrangements in this genus, although, for instance, the smallest chromosome of *A. rosae* has apparently originated through some kind of fission. Moreover, karyotypes of *A. bicolor* and *A. circularis* with $n = 6$ are similar, but the smallest chromosome of the latter species is slightly longer than that of *A. bicolor*, and centromeric

Table 3. Relative lengths (RLs) and centromeric indices (CIs) of *Athalia* chromosomes.

Таблица 3. Относительная длина (RL) и центромерный индекс (CI) хромосом *Athalia*.

Sp. no.	<i>A. bicolor</i>		<i>A. circularis</i>		<i>A. rosae</i>		<i>A. scutellariae</i>	
	RL	CI	RL	CI	RL	CI	RL	CI
1	21.84 ± 0.64	47.63 ± 2.18	20.54 ± 0.74	44.21 ± 2.29	19.48 ± 1.67	45.42 ± 3.75	20.29 ± 1.33	44.84 ± 3.82
2	20.58 ± 0.75	46.75 ± 3.94	19.57 ± 0.72	27.22 ± 2.60	17.44 ± 0.74	44.27 ± 2.34	18.61 ± 0.88	39.36 ± 3.49
3	18.12 ± 0.75	42.38 ± 3.80	17.68 ± 0.92	0	14.42 ± 1.30	44.22 ± 2.38	14.93 ± 0.98	36.27 ± 3.95
4	15.78 ± 0.88	37.33 ± 3.45	15.35 ± 0.64	33.47 ± 3.62	11.82 ± 0.46	43.99 ± 3.16	12.65 ± 0.34	38.66 ± 2.99
5	13.65 ± 0.61	38.85 ± 3.64	13.96 ± 0.65	47.32 ± 2.27	11.23 ± 0.40	43.53 ± 2.76	11.80 ± 0.44	41.14 ± 3.47
6	10.03 ± 0.65	44.06 ± 3.38	12.90 ± 0.83	34.93 ± 2.34	10.35 ± 0.34	46.37 ± 1.93	11.10 ± 0.49	43.51 ± 3.99
7	–	–	–	–	9.73 ± 0.52	43.23 ± 3.64	10.62 ± 0.40	41.92 ± 3.58
8	–	–	–	–	5.53 ± 0.57	0	–	–

indices of some elements also differ. For example, the third chromosome is metacentric in most studied members of the genus *Athalia*, including *A. bicolor* with $n = 6$, but acrocentric in *A. circularis* with the same chromosome number, suggesting a pericentric inversion in the latter species. In addition, a similar acrocentric is also characteristic of *A. cordata* Serville, 1823 which has $n = 6$ as well [Westendorff *et al.*, 1999]. Anyway, advanced cytogenetic studies of this genus, e.g., C-banding and/or FISH experiments, would certainly provide further information about possible chromosomal rearrangements in different *Athalia* species.

The results described in the present work also show that chromosome sets of certain species of the genus *Tenthredo*, which belong to different subgroups, are more or less similar, and, at the same time, karyotypes of some related sawflies can have different chromosome numbers. For instance, $n = 10$ was found in the members of the subgenera *Endotethryx* Lacourt, 1997, *Eurogaster* Zirngiebl, 1953, *Tenthredella* Rohwer, 1910 and *Tenthredo* s.str. Karyotypes of most these species, however, can be distinguished by different relative lengths and/or centromeric indices of particular chromosomes, even if these sawflies belong to the same subgenus (e.g., *T. omissa* and *T. vespa* from *Tenthredo* s.str.). On the other hand, the chromosome set of *T. mesomela* does resemble karyotypes of some other members of its species subgroup [Naito, 1978b]. In addition, morphologically similar *T. arcuata* and *T. brevicornis*, which both also belong to *Tenthredo* s.str., have strongly divergent chromosome sets with $n = 10$ and 18 respectively. Similarly, substantial differences can be found between *T. brevicornis* and *T. amoena*, which both have $n = 18$. Since the putative ancestral karyotype of the genus *Tenthredo* apparently contained ten bivalents chromosomes with continuous gradation in length, possible rearrangements responsible for karyotype transformations in this group might include translocations, inversions as well as chromosomal fissions and fusions (see Gokhman [2023]).

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