

Karyotypes of four species of the genus *Telenomus* Haliday, 1833 (Hymenoptera: Scelionidae)

Кариотипы четырех видов рода *Telenomus* Haliday, 1833 (Hymenoptera: Scelionidae)

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KEY WORDS. Telenominae, karyotype, chromosome morphometrics.

КЛЮЧЕВЫЕ СЛОВА. Telenominae, кариотип, морфометрия хромосом.

ABSTRACT. Karyotypes of four species of the genus *Telenomus* Haliday, 1833, namely, *T. acrobates* Giard, 1895, *T. angustatus* (Thomson, 1861), *T. heydeni* Mayr, 1879 and *T. turesis* Walker, 1836 were studied for the first time using chromosome morphometrics. Although all examined members of the genus appeared to have $n = 10$, substantial differences between karyotypes of certain species were revealed.

РЕЗЮМЕ. С использованием морфометрии хромосом впервые изучены кариотипы четырёх видов рода *Telenomus* Haliday, 1833, а именно, *T. acrobates* Giard, 1895, *T. angustatus* (Thomson, 1861), *T. heydeni* Mayr, 1879 и *T. turesis* Walker, 1836. Хотя у всех исследованных представителей рода обнаружено $n = 10$, выявлены существенные различия между кариотипами некоторых видов.

Introduction

Parasitoid Hymenoptera is one of the most speciose, taxonomically complicated and economically important insect groups, with its estimated species number in the world fauna far exceeding one million [Quicke, 1997; Bebbler et al., 2014; Forbes et al., 2018]. Despite rapid accumulation of karyotypic data, they are still available for just about 500 species of parasitic wasps [Gokhman, 2009]. The parasitoid superfamily Platygastroidea, which currently comprises about 6000 described species [Timokhov, 2019], is traditionally subdivided into two separate families, Platygastriidae and Scelionidae. In turn, the latter group contains more than

4000 species in the world fauna [Timokhov, 2019]. Among the Scelionidae, karyotypes of six members of the subfamily Telenominae that belong to the genera *Trissolcus* Ashmead, 1893 and *Telenomus* Haliday, 1833 were previously studied (see Gokhman, Timokhov, [2019] for review). Although all examined species appeared to have the same haploid chromosome number, $n = 10$, at least some members of the family can differ in certain details of their karyotype structure [Gokhman, Timokhov, 2019]. Moreover, for both studied *Telenomus* species, either the chromosome number remains the only known karyotypic feature, as in *T. turesis* Walker, 1836 [= *chloropus* (Thomson, 1861)] [Gokhman, 2009], or the existing description of the chromosome set contains apparent errors, e.g. presence of the putative “sex chromosomes”, as in *T. fariai* Costa Lima, 1927 [Dreyfus, Breuer, 1944]. Using chromosome morphometrics, we have recently studied karyotypes of four members of the genus *Telenomus* including *T. turesis*. The results of this work are given below.

Material and methods

Origin of parasitoids

Parasitic wasps used in the present study were reared from host egg clutches or collected by sweeping in their natural habitats in the Moscow Province (Russia) in 2016–2019 (Table 1). Specifically, individuals of *Telenomus acrobates* Giard, 1895 and *T. turesis* were reared from eggs of an unidentified lacewing (Neuroptera: Chrysopidae) and *Palomena prasina* (Linnaeus, 1761) (Hemiptera: Pentatomidae) respectively, whereas adult

Table 1. Species of the genus *Telenomus* used in the present study
 Таблица 1. Виды рода *Telenomus*, использованные в настоящей работе

Species	Locality	No. studied specimens	No. studied (haploid) and diploid metaphase plates
<i>T. acrobates</i> Giard, 1895	Zvenigorod Biological Station, Moscow State University, about 55 km W Moscow	1	(3)
<i>T. angustatus</i> (Thomson, 1861)	Prioksko-Terrasny Natural Reserve, about 100 km S Moscow	2	4
<i>T. heydeni</i> Mayr, 1879	Prioksko-Terrasny Natural Reserve	4	(7) 9
<i>T. turesis</i> Walker, 1836	Prioksko-Terrasny Natural Reserve	3	5

specimens of *T. heydeni* were collected in the wild. Females of most parasitoid species were then transferred to egg clutches of lab hosts for oviposition, i.e. *Chrysoperla carnea* (Stephens, 1836) (Neuroptera: Chrysopidae) (for *T. acrobates*) and *Graphosoma lineatum* Linnaeus, 1758 (Hemiptera: Pentatomidae) (for both *T. heydeni* and *T. turesis*). To obtain wasp prepupae, parasitized host eggs were incubated in lab conditions for a few days. An ovipositing female of *T. angustatus* was observed on a single egg clutch of *Tabanus* sp. (Diptera: Tabanidae) in the field, and this clutch was taken to the lab and then used both for rearing adult wasps and obtaining immature stages for the cytogenetic study. All examined parasitoid species were identified by A.V. Timokhov. Voucher adult specimens of parasitic wasps are deposited in the collection of the Department of Entomology of Moscow State University (Moscow, Russia), except for several individuals of *T. heydeni* additionally deposited in the Florida State Collection of Arthropods (Gainesville, Florida, USA).

Preparation of chromosomes

Chromosome preparations were made from cerebral ganglia of parasitoid prepupae using a modified version of the technique described by Imai et al. [1988]. Wasps were dissected in 0.5% hypotonic sodium citrate solution containing 0.005% colchicine, and the tissues were incubated in fresh solution for 30 minutes at room temperature. The material was transferred to a pre-cleaned microscope slide using a Pasteur pipette and gently flushed with Fixative I (glacial acetic acid: absolute ethanol: distilled water 3:3:4). Tissues were disrupted in an additional drop of Fixative I using dissecting needles. Another drop of Fixative II (glacial acetic acid: absolute ethanol 1:1) was then applied to the center of the area and blotted off the edges of the slide. The slide was air dried at room temperature. Preparations were stained with freshly prepared 3% Giemsa solution in 0.05M Sorensen's phosphate buffer ($\text{Na}_2\text{HPO}_4 + \text{KH}_2\text{PO}_4$, pH 6.8).

Image acquisition and analysis

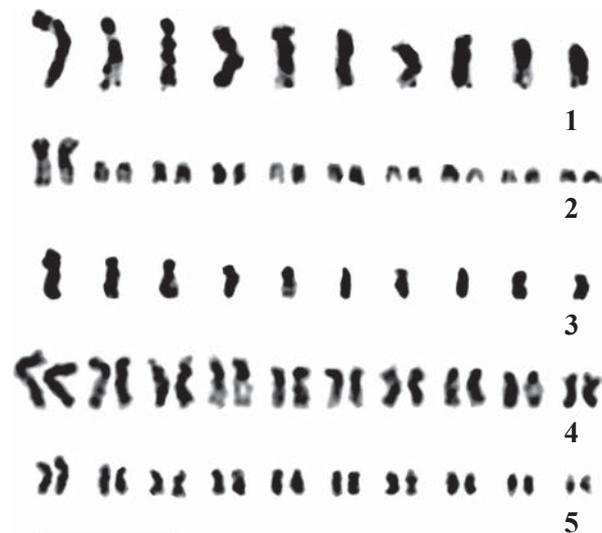
Mitotic divisions were studied and photographed using an optic microscope Zeiss Axioskop 40 FL fitted with a digital camera AxioCam 208 color (Carl Zeiss, Oberkochen, Germany). To obtain karyograms, the re-

sulting images were handled with image processing programs ZEN version 3.1 (blue edition) and GIMP version 2.10. Chromosomes from selected metaphase plates were measured using KaryoType software version 2.0 (Table 2). Since centromere positions could not be reliably identified for many chromosomes, only relative lengths of chromosomes (RLs) are given in the present paper. Nevertheless, certain chromosomes from selected metaphase plates were classified using criteria listed by Levan et al. [1964].

Results and discussion

Telenomus acrobates (Fig. 1). $n = 10$. Chromosomes more or less gradually decrease in size, but the first element of the karyotype is visibly longer than the remaining ones. At least some chromosomes, including the first one, are clearly bi-armed.

T. angustatus (Fig. 2). $2n = 20$. The first pair of chromosomes is more than twice longer than the others,



Figs 1–5. Karyograms of *Telenomus* species: 1 — *T. acrobates*, haploid set, 2 — *T. angustatus*, diploid set, 3 — *T. heydeni*, haploid set, 4 — ditto, diploid set, 5 — *T. turesis*, diploid set. Bar — 5 μm .

Рис. 1–5. Кариограммы видов *Telenomus*: 1 — *T. acrobates*, гаплоидный набор, 2 — *T. angustatus*, диплоидный набор, 3 — *T. heydeni*, гаплоидный набор, 4 — то же, диплоидный набор, 5 — *T. turesis*, диплоидный набор. Масштаб — 5 мкм.

Table 2. Relative lengths of chromosomes of four species of the genus *Telenomus* (mean \pm SD)
Таблица 2. Относительная длина хромосом четырёх видов рода *Telenomus* (среднее значение \pm стандартное отклонение)

Chromosome no.	<i>T. acrobates</i>	<i>T. angustatus</i>	<i>T. heydeni</i>	<i>T. turesis</i>
1	15.21 \pm 1.49	23.24 \pm 1.03	15.59 \pm 0.91	14.07 \pm 0.59
2	12.52 \pm 0.33	10.28 \pm 0.44	12.27 \pm 0.63	11.98 \pm 0.69
3	11.42 \pm 0.49	9.84 \pm 0.15	11.00 \pm 0.67	11.41 \pm 0.59
4	11.05 \pm 0.60	9.51 \pm 0.18	10.13 \pm 0.33	10.22 \pm 0.45
5	10.19 \pm 1.05	9.20 \pm 0.29	9.53 \pm 0.33	9.80 \pm 0.02
6	9.63 \pm 1.27	8.76 \pm 0.25	9.04 \pm 0.21	9.64 \pm 0.22
7	8.88 \pm 0.41	8.07 \pm 0.06	8.77 \pm 0.23	9.28 \pm 0.11
8	8.30 \pm 0.17	7.65 \pm 0.11	8.41 \pm 0.28	8.83 \pm 0.25
9	7.12 \pm 1.53	6.95 \pm 0.21	8.00 \pm 0.44	7.75 \pm 0.31
10	5.68 \pm 0.93	6.50 \pm 0.25	7.26 \pm 0.73	7.02 \pm 0.96

which form a continuous gradation in size. Moreover, the largest chromosome pair is obviously bi-armed (metacentric/submetacentric), whereas the remaining ones are either subtelocentric or acrocentric.

T. heydeni (Figs 3–4). $n = 10$ and $2n = 20$. As in *T. acrobates*, chromosomes more or less gradually decrease in size. The first chromosome is slightly longer than the remaining elements. Again, at least some chromosomes (e.g. the first one) are clearly bi-armed.

T. turesis (Fig. 5). $2n = 20$. The karyotype of this species is generally similar to those of *T. acrobates* and *T. heydeni*.

Nowadays, chromosomes of five *Telenomus* species are known, and this work therefore represents the first comparative karyotypic study of the genus. Our research confirms that all members of the genus *Telenomus*, together with other Scelionidae, have the same chromosome number, $n = 10$ [Dreyfus, Breuer, 1944; Gokhman, 2009; Fusu et al., 2013; Gokhman, Timokhov, 2019]. Nevertheless, certain *Telenomus* species can differ in some details of their karyotype structure. For example, chromosomes of most members of this genus, as well as of other Scelionidae studied in this respect, more or less gradually decrease in size, except for *T. angustatus*, in which the first chromosome is more than twice longer than the remaining ones (Table 2). The latter member of the genus belongs to a separate species group, *tabanivorus*, whereas both *T. turesis* (formerly *T. chloropus*) and *T. heydeni* are currently placed into the *podisi* group [Johnson, 1984]. On the other hand, *Telenomus* species that develop inside neuropteran eggs, including *T. acrobates*, belong to the enormous and quite heterogeneous *californicus* species complex [Johnson, Bin, 1982] which is apparently related to the *podisi* group [Taekul et al., 2014]. Nevertheless, the karyotype structure is generally similar in all these species except *T. angustatus*.

Although a detailed phylogenetic study has not yet been performed for most members of the genus *Telenomus*, a preliminary analysis suggests that less advanced *Telenomus* species mainly attack true bugs (Hemiptera: Heteroptera) [Taekul et al., 2014]. If this is true, then the

karyotype structure found in *T. heydeni* and *T. turesis*, as well as in *T. acrobates*, may represent the plesiomorphous character state, and that characteristic of *T. angustatus* is thus apomorphous. The present work therefore demonstrates that closely related species of the genus *Telenomus* and of the family Scelionidae in general can have substantially different chromosome sets, and we believe that further karyotypic research will confirm this assumption.

Acknowledgements. The present study is partly supported by a research grant from the Russian Foundation for Basic Research no. 18-04-00611. The work of AVT was carried out within the framework of the Russian State Research Project no. AAAA-A16-116021660101-5.

References

- Bebber D.P., Polaszek A., Wood J.R.I., Barker C., Scotland R.W. 2014. Taxonomic capacity and author inflation // *New Phytologist*. Vol.202. P.741–742. doi: 10.1111/nph.12745.
- Dreyfus A., Breuer M.E. 1944. Chromosomes and sex determination in the parasitic hymenopteron *Telenomus fariai* (Lima) // *Genetics*. Vol.29. P.75–82.
- Forbes A.A., Bagley R.K., Beer M.A., Hippee A.C., Widmayer H.A. 2018. Quantifying the unquantifiable: Why Hymenoptera, not Coleoptera, is the most speciose animal order // *BMC Ecology*. Vol.18. Paper 21. doi: 10.1186/s12898-018-0176-x.
- Fusu L., Bin F., Popovici O.A. 2013. First report of chromosomes of the parasitoid wasp *Trissolcus basalis* (Wollaston) (Hymenoptera: Platygastridae: Telenominae) // *Entomological Science*. Vol.16. P.263–265. doi: 10.1111/ens.12011.
- Gokhman V.E. 2009. Karyotypes of parasitic Hymenoptera. Dordrecht: Springer. XIII + 183p p. doi: 10.1007/978-1-4020-9807-9.
- Gokhman V.E., Timokhov A.V. 2019. Karyotypes of three species of the genus *Trissolcus* Ashmead, 1893 (Hymenoptera: Scelionidae) // *Russian Entomological Journal*. Vol.28. No.1. P.69–71.
- Imai H.T., Taylor R.W., Crosland M.W.J., Crozier R.H. 1988. Modes of spontaneous chromosomal mutation and karyotype evolution in ants with reference to the minimum interaction hypothesis // *Japanese Journal of Genetics*. Vol.63. P.159–185. doi: 10.1266/jjg.63.159.
- Johnson N.F. 1984. Systematics of Nearctic *Telenomus*: classification and revisions of the *podisi* and *phymatae* species groups (Hymenoptera: Scelionidae) // *Bulletin of the Ohio Biological Survey*. New Series. Vol.6. No.3. P.1–113.

- Johnson N.F., Bin F. 1982. Species of *Telenomus* (Hym., Scelionidae), parasitoids of stalked eggs of Neuroptera (Chrysopidae and Berothidae) // *Redia*. Vol.65. P.189–206.
- Levan A., Fredga K., Sandberg A.A. 1964. Nomenclature for centromeric position on chromosomes // *Hereditas*. Vol.52. P.201–220. doi: 10.1111/j.1601-5223.1964.tb01953.x.
- Quicke D.L.J. 1997. Parasitic wasps. London: Chapman & Hall. XVII + 470 pp.
- Taekul C., Valerio A.A., Austin A.D., Klompen H., Johnson N.F. 2014. Molecular phylogeny of telenomine egg parasitoids (Hymenoptera: Platygasteridae s.l.: Telenominae): evolution of host shifts and implications for classification // *Systematic Entomology*. Vol.39. P.24–35. doi: 10.1111/syen.12032.
- Timokhov A.V. 2019. Superfamily Platygastroidea // Belokobylskij S.A., Samartsev K.G., Il'inskaya A.S. (eds.). Annotated catalogue of the Hymenoptera of Russia. Vol.2. Apocrita: Parasitica. Proceedings of the Zoological Institute Russian Academy of Sciences. Suppl.8. St Petersburg: Zoological Institute RAS. P.42–57.