

## Chromosomes of *Glyphomerus stigma* (Fabricius, 1793) and a review of karyotypes of the family Torymidae (Hymenoptera, Chalcidoidea)

### Хромосомы *Glyphomerus stigma* (Fabricius, 1793) и обзор кариотипов семейства Torymidae (Hymenoptera, Chalcidoidea)

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**Key words:** Chalcidoidea, chromosomes, Glyphomerinae, *Glyphomerus stigma*, Hymenoptera, karyotypes, parasitoids, Torymidae.

**Ключевые слова:** Кариотипы, паразитоиды, хромосомы, Chalcidoidea, Glyphomerinae, *Glyphomerus stigma*, Hymenoptera, Torymidae.

**Abstract.** Chromosomes of *Glyphomerus stigma* (Fabricius, 1793), the first studied member of the subfamily Glyphomerinae (Torymidae), are examined. The diploid karyotype of this species contains five pairs of metacentric and submetacentric chromosomes ( $2n = 10$ ). A brief overview of chromosome sets of the family Torymidae is given. For most clades of Torymidae, initial haploid karyotypes apparently had  $n = 6$ , comprising five large banded chromosomes and a smaller subtelocentric / acrocentric. However, the ancestral karyotype for the whole family might have had  $n = 10$  and predominantly contained subtelocentrics and/or acrocentrics.

**Резюме.** Исследованы хромосомы *Glyphomerus stigma* (Fabricius, 1793), первого изученного представителя подсемейства Glyphomerinae (Torymidae). Диплоидный кариотип этого вида включает пять пар метацентрических и субметацентрических хромосом ( $2n = 10$ ). Приведен краткий обзор хромосомных наборов семейства Torymidae. Исходные для большинства ветвей Torymidae гаплоидные кариотипы, очевидно, имели  $n = 6$ , и при этом включали пять крупных двуплечих хромосом и небольшой субтелоцентрик / акроцентрик. Тем не менее, предковый кариотип для всего семейства, возможно, имел  $n = 10$  и в основном содержал субтелоцентрики и/или акроцентрики.

## Introduction

Chalcidoidea, or chalcid wasps, is an extremely species-rich and biologically diverse superfamily of insects, which includes about 500,000 potentially recognized species [Cruaud et al., 2024]. Among these parasitoids, Torymidae represent a middle-sized family, which harbors more than 1100 species [Janšta et al., 2018]. Nevertheless, chromosomes of only 23 species of Torymidae, excluding two members of the former Megastigminae, which is currently considered a family of its own [Janšta et al., 2018], are studied up to now [Gokhman, 2009, 2020]. These parasitoids belong to the subfamilies

Toryminae, Monodontomerinae and Podagrioninae. A few years ago, I managed to examine chromosomes of *Glyphomerus stigma* (Fabricius, 1793), which is now placed in a separate subfamily, Glyphomerinae [Janšta et al., 2018]. The description of its karyotype, together with a brief overview of chromosome sets of the family Torymidae, is given below.

This paper is dedicated to the 80th anniversary of Prof. Arkady S. Lelej, a world-class expert on systematics, phylogeny and ecology of the order Hymenoptera.

## Material and methods

An adult female of *G. stigma* was reared from galls of *Diplolepis mayri* (Schlechtendal, 1877) (Hymenoptera, Cynipidae) on *Rosa* sp. (Rosaceae) collected by the author in August 2020 in the Dubovsky District of the Volgograd Province of Russia (49°01'N, 44°43'E). The chromosome preparation was obtained from ovaries according to the technique developed by Gokhman [2009] with certain modifications. Specifically, the female was dissected in 0.5 % hypotonic sodium citrate solution containing 0.005 % colchicine. The extracted ovaries were incubated in a fresh portion of the hypotonic solution for 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 tissue was 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 slide was dried for approximately 30 min and stored at room temperature.

The chromosome preparation was stained overnight using a freshly prepared 3 % Giemsa solution (Merck,

Germany). Eight diploid metaphase plates of *G. stigma* were examined and photographed with an optical microscope Zeiss Axioskop 40 FL fitted with a digital color camera Axiocam 208 (Carl Zeiss, Germany). To produce illustrations, the resulting images were processed with the image processing programs ZEN version 3.0 (blue edition) and GIMP version 3.2. KaryoType version 2.0 software [Altınordu et al., 2016] was also used for taking measurements from five metaphase plates with the best morphology of chromosomes. The chromosomes were classified following guidelines provided by Levan et al. [1964].

The present work is registered in ZooBank (www.zoobank.org) under urn:lsid:zoobank.org:pub:6D0F7385-6DEB-4D0C-AE91-DD5785FEA424

## Results and discussion

The diploid karyotype of *G. stigma* contains five pairs of biarmed chromosomes of similar size ( $2n = 10$ ; Fig. 1). Chromosomes of the first, third and fifth pairs are clearly metacentric, those of the second pair are submetacentric, and chromosomes of the fourth pair are intermediate between these classes (Table 1). This species is the first member of the subfamily Glyphomerinae, which chromosome set has been recently studied. However, karyotypes that contain five similar metacentric and/or submetacentric chromosomes, were previously reported for two other subfamilies of Torymidae, i.e., Toryminae and Monodontomerinae [Gokhman, 2009; Gokhman, Gumovsky, 2013]. Moreover, the haploid chromosome set, which comprises five large metacentrics / submetacentrics and a small subtelocentric / acrocentric chromosome ( $n = 6$ ), predominates among Torymidae (Table 2, Fig. 2). This karyotype therefore considered ancestral at least for all the above-mentioned subfamilies [Gokhman, 2013, 2020]. Furthermore, it can also be found in a number of other more or less distantly related clades of the superfamily Chalcidoidea, e.g., in many Eulophidae, Agaonidae and some Ormyridae ([Gokhman, 2013], also see [Cruaud et al., 2024]). This karyotype structure apparently originated via parallel pairwise chromosomal fusions from an ancestral karyotype of Chalcidoidea with  $n = 11$ , which might contain eleven subtelocentrics and/or acrocentrics [Gokhman, 2009, 2020]. In all these families, as well as in Megastigmidae, chromosome sets,



Fig. 1. Diploid karyogram of *G. stigma*. Scale bar: 5  $\mu$ m.

Рис. 1. Диплоидная кариограмма *G. stigma*. Масштаб: 5 мкм.

which comprise five large metacentrics or submetacentrics, were also found ([Gokhman, 2009], see Table 2). Among other parasitoids, this karyotype is now detected in *G. stigma* (Fig. 1, Table 1). In turn, these chromosome sets likely resulted from independent fusions of the smaller subtelocentric / acrocentric to one of the larger biarmed chromosomes [Gokhman, 2013].

Nevertheless, two karyotypes of the genus *Podagrion* Spinola, 1811 with  $n = 10$ , which belongs to the subfamily Podagrioninae (Torymidae), represent an obvious exception to this pattern (Table 2). Chromosome sets of both *Podagrion gibbum* Bernard, 1938 and *P. pachymerum* (Walker, 1833) mostly contain subtelocentrics and acrocentrics of gradually decreasing length, except for a large submetacentric and another medium-sized one [Fusu, 2008]. It is tempting to suppose that these karyotypes could directly originate from the ancestral chalcid karyotype with  $n = 11$  (see above) via a single chromosomal fusion and a few additional rearrangements, such as pericentric inversions and/or translocations. However, members of other main clades of Torymidae with known karyotypes, i.e., Toryminae (including the tribes Torymoidini and Torymini), Glyphomerinae and Monodontomerinae, have  $n = 4$  to 6, with the latter chromosome number obviously prevailing among these species (Table 2). This effectively requires parallel series of chromosomal fusions in all these subfamilies, which would lead to similar chromosome sets (Fig. 2). On the other hand, both higher and lower chromosome numbers,

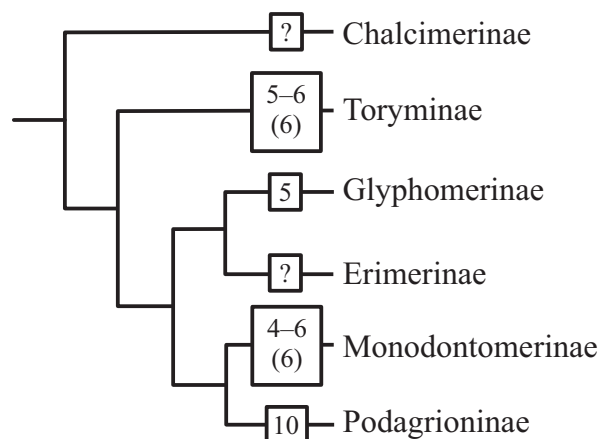


Table 2. Chromosome numbers of the family Torymidae  
Таблица 2. Хромосомные числа семейства Torymidae

| Species                                                                | n(2n)  | References                        |
|------------------------------------------------------------------------|--------|-----------------------------------|
| <b>Subfamily Toryminae</b>                                             |        |                                   |
| <b>Tribe Torymoidini</b>                                               |        |                                   |
| <i>Pseudotorymus</i> sp.                                               | (12)   | Gokhman, Gumovsky, 2013           |
| <b>Tribe Torymini</b>                                                  |        |                                   |
| <i>Diomorus aiolomorphi</i> Kamijo, 1964                               | 5*     | Yuan et al., 2024                 |
| <i>Torymus baccharidis</i> (Huber, 1927)                               | 6(12)  | Goodpasture, Grissell, 1975       |
| <i>T. bedeguaris</i> (Linnaeus, 1758)                                  | (12)** | Gokhman, Mikhailenko, 2007        |
| <i>T. californicus</i> (Ashmead, 1886)                                 | 6(12)  | Goodpasture, Grissell, 1975       |
| <i>T. capillaceus</i> (Huber, 1927)                                    | 6(12)  | Goodpasture, Grissell, 1975       |
| <i>T. chloromerus</i> (Walker, 1833)                                   | (12)   | Gokhman, Mikhailenko, 2007        |
| <i>T. koebelei</i> (Huber, 1927)                                       | 5(10)  | Goodpasture, Grissell, 1975       |
| <i>T. microstigma</i> (Walker, 1833)                                   | (12)   | Gokhman, Gumovsky, 2013           |
| <i>T. occidentalis</i> (Huber, 1927)                                   | 6(12)  | Goodpasture, Grissell, 1975       |
| <i>T. rubi</i> (Schränk, 1781)                                         | (12)   | Gokhman, Gumovsky, 2013           |
| <i>T. tubicola</i> (Osten Sacken, 1870)                                | 6(12)  | Goodpasture, Grissell, 1975       |
| <i>T. umbilicatus</i> (Gahan, 1919)                                    | 5(10)  | Goodpasture, Grissell, 1975       |
| <i>T. vesiculi</i> Moser, 1956                                         | (12)   | Goodpasture, Grissell, 1975       |
| <i>T. warreni</i> (Cockerell, 1911)                                    | 6(12)  | Goodpasture, Grissell, 1975       |
| <i>Torymus</i> sp.                                                     | (12)   | Gokhman, Gumovsky, 2013           |
| <b>Subfamily Glyphomerinae</b>                                         |        |                                   |
| <i>Glyphomerus stigma</i> (Fabricius, 1793)                            | (10)   | Present paper                     |
| <b>Subfamily Monodontomerinae</b>                                      |        |                                   |
| <i>Monodontomerus aeneus</i> (Fonscolombe, 1832)                       | 4(8)   | Goodpasture, 1975                 |
| <i>M. clementi</i> Grissell, 1973                                      | 6(12)  | Grissell, 1973; Goodpasture, 1975 |
| <i>M. montivagus</i> Ashmead, 1890                                     | 6      | Goodpasture, 1975                 |
| <i>M. obscurus</i> Westwood, 1833                                      | 6(12)  | MacDonald, Krunic, 1971           |
| <i>M. viridiscapus</i> Gahan, 1941 (= <i>saltuosus</i> Grissell, 1973) | 5(10)  | Grissell, 1973; Goodpasture, 1975 |
| <b>Subfamily Podagrioninae</b>                                         |        |                                   |
| <i>Podagrion gibbum</i> Bernard, 1938                                  | (20)   | Fusu, 2008                        |
| <i>P. pachymerum</i> (Walker, 1833)                                    | 10(20) | Fusu, 2008                        |

Note. \* — estimate resulting from the chromosome-level genome assembly; \*\* — Gokhman et al. [2014] also reported a single trisomic with  $2n = 13$  in this species, which karyotype carried three copies of the smaller acrocentric

Примечание. \* — оценка по результатам сборки генома на хромосомном уровне; \*\* — в работе Gokhman et al. [2014] сообщается о трисомии данного вида с  $2n = 13$ , кариотип которого содержал три копии небольшого акроцентрика

with  $n$  values decreasing from  $n = 10$  to five, are also found in a few other higher taxa of Chalcidoidea, e.g., in the genera *Metaphycus* Mercet, 1917 (Encyrtidae) and *Eurytoma* Illiger, 1807 (Eurytomidae) [Gokhman, 2020]. In these genera, the ancestral character of karyotypes with  $n = 10$  is already proven, and parallel chromosomal fusions in several clades of Torymidae therefore seem plausible as well. Furthermore, an alternative evolutionary pathway, i.e., a series of chromosomal fissions in the Podagrioninae clade from the putative ancestral karyotype of Torymidae with  $n = 6$  is even less likely, since these fissions are rarely found among other Chalcidoidea [Gokhman, 2020]. Nevertheless, this scenario cannot be fully excluded (see [Fusu, 2008]).

Historically, *Torymus* and *Monodontomerus* became the first studied parasitoid genera which included species with different chromosome numbers [Goodpasture, 1975; Goodpasture, Grissell, 1975]. Although cryptic species of Torymidae with different karyotypes have not been detected up to now (see [Gokhman, 2018]),  $n = 4$  and  $6$ , were previously reported for the same Holarctic parasitoid, *Monodontomerus obscurus* Westwood, 1833 [MacDonald, Krunic, 1971; Goodpasture, 1975]. However, Grissell [2007] found out that at least the specimens with  $n = 4$  and  $2n = 8$  studied by Goodpasture [1975] actually belonged to *M. aeneus* (Fonscolombe, 1832).

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