

## New registrations of some rare species of the genus *Gymnometriocnemus* Edwards, 1932 (Diptera: Chironomidae)

## Новые регистрации редких видов хирономид рода *Gymnometriocnemus* Edwards, 1932 (Diptera: Chironomidae)

M.G. Krivosheina<sup>1,2</sup>, D.D. Vinogradov<sup>1</sup>, S.M. Tsurikov<sup>1</sup>,  
A.G. Zuev<sup>1</sup>, I.V. Sotnikov<sup>1</sup>  
М.Г. Кривошеина<sup>1,2</sup>, Д.Д. Виноградов<sup>1</sup>, С.М. Цуриков<sup>1</sup>,  
А.Г. Зуев<sup>1</sup>, И.В. Сотников<sup>1</sup>

<sup>1</sup>A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Leninsky prospect, 33, Moscow 119071, Russia.  
E-mail: kriv2260@rambler.ru

<sup>1</sup>Институт проблем экологии и эволюции им. А.Н. Северцова РАН, Ленинский проспект, 33, Москва 119071, Россия.

<sup>2</sup>corresponding author

KEY WORDS: Diptera, Chironomidae, *Gymnometriocnemus*, Russia, new records.

КЛЮЧЕВЫЕ СЛОВА: Diptera, Chironomidae, *Gymnometriocnemus*, Россия, новые регистрации.

ABSTRACT. New data on the distribution of 2 species of the genus *Gymnometriocnemus* Edwards, 1932: *Gymnometriocnemus (Rhaphidocladius) brumalis* (Edwards, 1929) and *Gymnometriocnemus (Rhaphidocladius) kamimegavirgus* Sasa et Hirabayashi, 1993 in Russia are presented. The species were caught using an emergent trap installed on the territory of Moscow. The distribution of the species on the territory of the European part of Russia was proved using morphological and molecular methods.

РЕЗЮМЕ. Представлены новые данные о распространении 2 видов рода *Gymnometriocnemus* Edwards, 1932: *Gymnometriocnemus (Rhaphidocladius) brumalis* (Edwards, 1929) и *Gymnometriocnemus (Rhaphidocladius) kamimegavirgus* Sasa et Hirabayashi, 1993 на территории России. Виды были пойманы при помощи эмергент-ловушки, установленной на территории Москвы. Распространение видов на территории европейской части России было доказано с использованием морфологического и молекулярного методов.

### Introduction

The genus *Gymnometriocnemus* Edwards, 1932 includes about 20 species distributed mainly in the Holarctic region. The greatest number of species is known from the territory of the Palearctic (9 species), 3 species are recorded in the Nearctic region [Makarchenko, Makarchenko, 2019]. Larvae of most species have pre-

viously been regarded as terrestrial [Andersen et al., 2013], but there is evidence for at least semi-aquatic lifestyle in larvae of subgenus *Rhaphidocladius* from Norway [Stur, Ekrem, 2015]. The genus *Gymnometriocnemus* is divided in the 2 subgenera: *Gymnometriocnemus* s.str. and *Rhaphidocladius* Saether, 1983. The species of the subgenus *Rhaphidocladius* possess an extremely long virga with needle-like sclerotization in the adult males. The subgenus *Rhaphidocladius* included 4 valid species: *G. (R.) brumalis* (Edwards, 1929), *G. (R.) kamimegavirgus* Sasa et Hirabayashi, 1993, *G. (R.) tairaprimus* Sasa et Okazawa, 1994 and *G. (R.) volitans* (Goetghebuer, 1940). The study of the type specimens of *G. (R.) volitans* fulfilled by Stur & Ekrem [2015] showed that the species belongs to another genus — *Chaetocladius* Kieffer, 1911. As a result the three species only remained in *Rhaphidocladius*. A more detailed study of the material determined as *G. (R.) volitans*, carried out by the same authors, showed that some of the material on *G. (R.) volitans* was identified incorrectly and according to molecular genetic analysis, coincides with *G. (R.) kamimegavirgus*, described from Japan. Such specimens were discovered in Norway, the distribution of *G. (R.) kamimegavirgus* was precised. The species is quite rare and for a long time there was no information about its distribution in Russia until it was recently discovered in the Far East [Makarchenko, Makarchenko, 2019]. *G. (R.) tairaprimus*, described from Japan, was firstly registered in Russia in Jewish Autonomous Region by Makarchenko & Makarchenko [2019]. The third species, *G. (R.)*



Fig. 1. General view of the emergence trap.

Рис. 1. Общий вид эмергенц-ловушки.

*brumalis* proved to have divergent genetic clusters [Stur, Ekrem, 2015] but the authors couldn't examine and barcode a sufficient number of specimens to confidently conclude if the divergent genetic clusters in *G. (R.) brumalis* can be regarded as separate species. They found this species in Norway. Makarchenko & Makarchenko [2019] registered *G. (R.) brumalis* from the Far East of Russia basing on the morphological characters of this species.

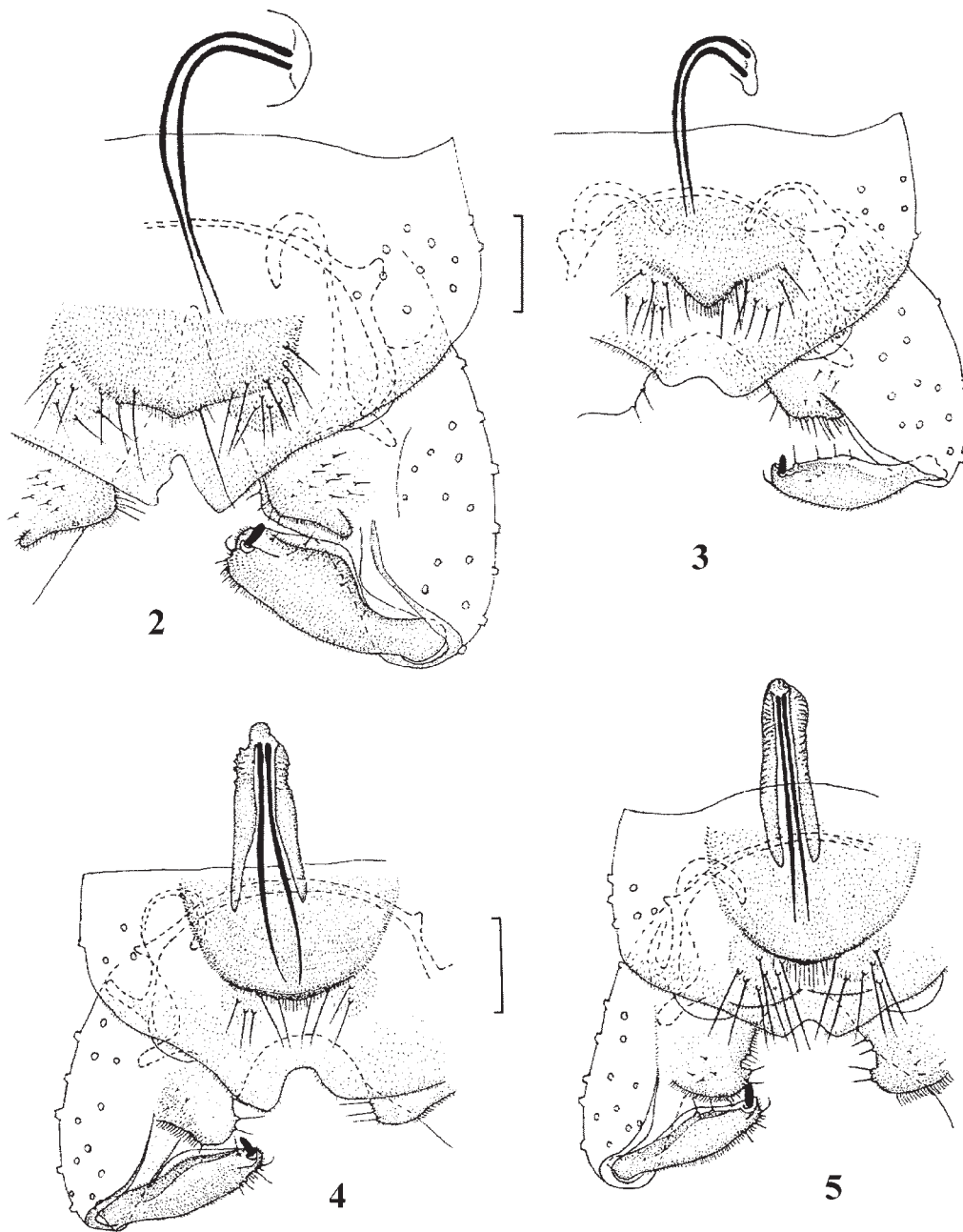
Our investigations on Diptera captured in emergence-traps in Moscow Region suddenly resulted in the registration of the following 2 species: *Gymnometriocnemus (Rhaphidocladius) brumalis* and *G. (R.) kamimega-virgus*. The species are quite rare. We register them in the European part of Russia and thus supplement the data on their real distribution.

## Material and methods

The flies were captured with the help of emergence trap. The emergent trap was designed to catch insects leaving the soil. It is a pyramid made of dense black cotton fabric with metal ribs, covering an area of 1 m<sup>2</sup>, with a container fixed to the top, transparent to the sun's rays, with a fixing liquid — 96% ethyl alcohol. The trap was installed from 27.04. to 5.07.2022 on soil in a pine forest (Fig. 1). The specimens were determined using the key by Makarchenko & Makar-

chenko [2019]. The final determination was carried out using molecular methods.

DNA extraction was carried out from several legs or head of insects in 50 µl of lysis buffer (Protease K, mercaptoethanol, Tris-HCl, EDTA) for 1.5 hours at 65°C with shaking. PCR was performed using the Encyclo Plus PCR kit (Evrogen, Russia) according to the manufacturer's protocol. For PCR, universal primers for the standard fragment of the mitochondrial COI gene were used: LepF1 (5'-AT-TCAACCAATCATAAAGATAT-3') and LepR1 (5'-TAAACTTCTGGATCCAAAAA-3') [Hebert *et al.*, 2004]. The obtained products were visualized by electrophoresis in 1% agarose gel. If the product was available, the target fragment was separated from nonspecific amplification products using preparative electrophoresis in 2% agarose gel and purified using the CleanUp Mini kit (Evrogen, Russia). Sequencing was carried out at the "Center for Collective Use Genome". The analysis of the obtained chromatograms was carried out using the BioEdit program [Hall, 1999]. The GenBank identification results [Altschul *et al.*, 1990] for *Gymnometriocnemus brumalis* matched 100% similarity and 100% coverage with the sequence of the same species numbered KP050314.1 from Norway. The sequence obtained for the species *Gymnometriocnemus kamimegavirgus* matched with 100% similarity and 98% coverage with the sequence of the species of the same name numbered HM421376 from Norway and *Gymnometriocnemus volitans* numbered MZ633347.1 from Finland. The sequences obtained have been submitted to GenBank with numbers OQ947086 for



Figs 2–5. General view of hypopygium of *Gymnometriocnemus* (*Rhaphidocladius*) species: 2, 3 — *G. (R.) brumalis*; 4, 5 — *G. (R.) kamimegavirgus*. Scale bar 50  $\mu\text{m}$  (after Makarchenko & Makarchenko, 2019, Figs 4, 5, 8, 9).

Рис. 2–5. Общий вид гипопигия самцов *Gymnometriocnemus* (*Rhaphidocladius*): 2, 3 — *G. (R.) brumalis*; 4, 5 — *G. (R.) kamimegavirgus*. Масштабная линейка 50 мкм (по Makarchenko & Makarchenko, 2019, Figs 4, 5, 8, 9).

*Gymnometriocnemus brumalis* and OQ947087 for *Gymnometriocnemus kamimegavirgus*.

The taxonomy of the genus, subgenera and species follows data from Stur & Ekrem [2015] and Makarchenko & Makarchenko [2019]. The examined specimens are kept in the Institute of Ecology and Evolution, Russian Academy of Sciences.

*Gymnometriocnemus* (*Rhaphidocladius*) *brumalis*  
(Edwards, 1929)

Figs 2, 3.

MATERIAL. **Russia**: Moscow Region, village Pervomayskoe, left bank of Zhiletovka river, pine forest with admixture of deciduous species 55.46164°N, 037.18284°E, 27.04–7.05.2022, S.M. Tsurikov, A.G. Zuev (19 males).

**DIAGNOSIS.** The species, like other members of the subgenus *Rhaphidocladius*, is characterized by a strongly developed virga beginning in tergite 8 and the absence of a process on tergite 9, which has only a medial fold. The species differs from *G. (R.) tairaprimus* by simple, not double, lower appendage of gonocoxite. The species differs from *G. (R.) kamimegavirgus* by wing membrane which has setae on at least half of wing, weakly sclerotized and often hardly visible lamella of virga and angulate lower appendage of gonocoxite (Figs 2, 3) [Makarchenko, Makarchenko, 2019].

**DISTRIBUTION.** The species was described for Great Britain [Edwards, 1929]. Registered in Norway [Stur, Ekrem, 2015]. According to the data in Fauna Europaea the species is also known from Sweden, Finland, Ireland, France, Germany, Hungary, Luxemburg, Poland, Switzerland, Netherlands and Spain [De Jong *et al.*, 2014]. According to the World database of Diptera the species is also widely distributed in Canada and USA [Evenhuis, Pape, 2023]. The species is not rare but for a long time there was no information about its distribution in Russia. It was recently registered in Primorsky Krai (Lazovsky District), Jewish Autonomous Region and Khabarovsk Krai (Khabarovsk District) [Makarchenko, Makarchenko, 2019]. We prove the distribution of the species in European Part of Russia.

*Gymnometriocnemus (Rhaphidocladius)*  
*kamimegavirgus* Sasa et Hirabayashi, 1993  
Figs 4, 5.

**MATERIAL. Russia:** Moscow Region, village Pervomayskoe, left bank of Zhiletovka river, pine forest with admixture of deciduous species 55.46164°N, 037.18284°E, 27.04–7.05.2022, S.M. Tsurikov, A.G. Zuev (2 males, 7 females).

**DIAGNOSIS.** Males of this species seem to be much darker in comparison with specimens of *G. (R.) brumalis*: thorax and abdominal tergites dark brown, almost black. Wing membrane has setae on the most apex only. The species differs from *G. (R.) tairaprimus* by simple, not double, lower appendage of gonocoxite. The species differs from *G. (R.) brumalis* by strongly sclerotized and well visible lamella of virga and rounded, not angulate lower appendage of gonocoxite (Figs 4, 5) [Makarchenko, Makarchenko, 2019].

**DISTRIBUTION.** The species was described from the territory of Japan (Nagano, Honshu Island) [Sasa, Hirabayashi, 1993]. Registered in Norway [Stur, Ekrem, 2015]. In Russia it was found in the Far East (Magadan Region) only

[Makarchenko, Makarchenko, 2019]. Our registration is the first for the European part of Russia.

**Competing interests.** The authors declare no competing interests.

**Acknowledgements.** The investigation was supported by the Russian Science Foundation No 22-14-00363.

## References

- Altschul S.F., Gish W., Miller W., Myers E.W., Lipman D.J. 1990. Basic local alignment search tool // *Journal of Molecular Biology*. Vol.215. P.403–410. doi:10.1016/S0022-2836(05)80360-2.
- Andersen T., Sæther O.A., Cranston P.S., Epler J.H. 2013. The larvae of the Orthoclaadiinae (Diptera: Chironomidae) of the Holarctic Region — Keys and diagnoses // Andersen T., Cranston P.S., Epler J.H. (eds.). *The larvae of Chironomidae (Diptera) of the Holarctic Region — Keys and diagnoses*. Insect Systematics and Evolution Supplement. No.66. P.189–386.
- De Jong Y., Verbeek M., Michelsen V. *et al.* 2014. Fauna Europaea — all European animal species on the web // *Biodiversity Data Journal*. Vol.2. No.e4034. doi: 10.3897/BDJ.2.e4034.
- Edwards F.W. 1929. British non-biting midges (Diptera, Chironomidae) // *Transactions of the Entomological Society of London*. Vol.77. P.279–430. doi: 10.1111/j.1365-2311.1929.tb00692.x
- Evenhuis N.L., Pape T. (editors). 2023. *Systema Dipteroformum*, Version 4.1, last updated 15 March 2023. <http://diptera.org/>, accessed on 3 May 2023.
- Hall T.A. 1999. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT // *Nucleic Acids Symposium Series*. No.41. P.95–98.
- Hebert P.D.N., Penton E.H., Burns J.M., Janzen D.H., Hallwachs W. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator* // *Proceedings of the National Academy of Sciences USA*. Vol.101. P.14812–14817. doi:10.1073/pnas.0406166101
- Makarchenko E.A., Makarchenko M.A. 2019. Review of the genus *Gymnometriocnemus* Edwards (Diptera, Chironomidae, Orthoclaadiinae) from the Russian Far East // *Eurasian entomological Journal*. Vol.18. No.5. P.333–340. doi: 10.15298/euroasentj.18.5.5.
- Sasa M., Hirabayashi K. 1993. Studies on the additional chironomids (Diptera, Chironomidae) collected at Kamikochi and Asama-Onsen, Nagano, Japan // *Japanese Journal of Sanitary Zoology*. Vol.44. P.361–393.
- Stur E., Ekrem T. 2015. A review of Norwegian *Gymnometriocnemus* (Diptera, Chironomidae) including the description of two new species and a new name for *Gymnometriocnemus volitans* (Goetghebuer) sensu Brundin // *ZooKeys*. Vol.508. P.127–142. doi: 10.3897/zookeys.508.9874