

First record of *Ephemera separigata* Bae, 1995 (Insecta, Ephemeroptera) from Russia and Russian Far East

Первая находка *Ephemera separigata* Bae, 1995 (Insecta, Ephemeroptera) в России и на Дальнем Востоке

Т.М. Тиунова*, А.А. Семенченко**
Т.М. Тиунова*, А.А. Семенченко**

* Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, Prosp. 100-letiya Vladivostoka 159, Vladivostok 690022 Russia. E-mail: tiunova@biosoil.ru.

** Федеральный научный центр биоразнообразия наземной биоты Восточной Азии ДВО РАН, просп. 100-летия Владивостока 159, Владивосток 690022 Россия.

** Laboratory of Ecology and Evolutionary Biology of Aquatic Organisms, Far Eastern Federal University, Suhanova Str. 8, Vladivostok 690950 Russia. E-mail: semenchenko_alexander@mail.ru.

** Лаборатория экологии и эволюционной биологии водных организмов, Дальневосточный Федеральный университет, ул. Суханова 8, Владивосток 690950 Россия.

Key words: Ephemeroptera, mayfly, new record, Russia, Far East.

Ключевые слова: Ephemeroptera, поденки, новая находка, Россия, Дальний Восток.

Abstract. The first record of *Ephemera separigata* Bae, 1995 from Russia and Far East is given. The mitochondrial COI gene of *E. separigata* and *E. strigata* Eaton, 1892 occurring in Russian Far East were analyzed to confirm the species identification.

Резюме. Для фауны России и Дальнего Востока (Приморский край) приводится первая находка поденки *Ephemera separigata* Bae, 1995. Митохондриальный ген COI *Ephemera separigata* и *E. strigata* Eaton, 1892 встречающихся на Дальнем Востоке России, был проанализирован для подтверждения вида.

Introduction

At the present time, in the Far East of Russia, there are five species of mayflies belonging to the genus *Ephemera* Linnaeus 1758: East Asian Island *E. japonica* McLachlan 1875; East Palaearctic *E. sachalinensis* Matsumura 1911, *E. strigata* Eaton 1892, *E. transbaikalica* Tshernova 1973, and Palaearctic *E. orientalis* McLachlan 1875 [Tiunova, 2009].

The first record of *Ephemera separigata* Bae, 1995 is reported from Russian Far East on the basis of specimens collected in the Primorskiy Krai. We made a comparison between Far Eastern *E. separigata* and *E. strigata* and sequences from GeneBank and BOLD systems. To confirm monophyly of each species phylogenetic tree was also provided.

The material is deposited in the collection of the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch, Russian Academy of Sciences, Vladivostok, Russia.

Material and methods

Nine *Ephemera* specimens were used for molecular study (Table 1). Total DNA was extracted using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) from thorax or legs of larvae and imago and the resultant DNA was eluted in 100 µl. The COI fragment was amplified using LCO1490 (5'-GGTCAACAATCAT-AAAGATATTGG-3') and HCO2198 (5'-TAAACT-TCAGGGTGACCAAAAATCA-3') [Folmer et al., 1994]. The 10 µl PCR reaction mix contained 3 µl of ultrapure water, 1 µl of DNA additionally diluted 10x, 0.5 µl of each primer (10 µM), and 5 Go Taq Green Master Mix (Promega corp, Madison, WI, USA). The reaction was conducted with an initial step of 94 °C for 5 min, followed by a total of 30 cycles at 94 °C for 30 sec, 48 °C for 30 sec, 72 °C for 60 sec, and 72 °C for 5 min. Successful amplifications (checked by TBE Gel Electrophoresis on a 1.5 % agarose gel) were purified using Exonuclease I (ExoI) and Thermosensitive Alkaline Phosphatase (FastAP) (Thermo Fisher Scientific Inc., USA). Purification and bi-directional Sanger sequencing was performed by ABI 3130xl (Applied Biosystems, Carlsbad, CA) using Big Dye Terminator ver. 3.1 and BigDye 5x Sequencing Buffer.

PartitionFinder 2.1.1 [Lanfear et al., 2012] is used to select the best-fit partitioning scheme and models separately for each codon position of COI gene using the greedy algorithm with linked branch lengths for the corrected Bayesian Information Criterion as the optimality criterion for model selection. A Bayesian Inferences (BI) analysis was performed with MrBayes v.3.2.7 [Ronquist et al., 2012] under the following conditions:

Table 1. List of sequenced specimens *Ephemera separigata* Bae, 1995 and *E. strigata* Eaton, 1892
 Таблица 1. Список секвенированных образцов *Ephemera separigata* Бая, 1995 и *E. strigata* Этона, 1892

| Species | Isolate | Stage/sex | Country, collection locality | Geographic coordinate | GeneBank accession number |
|----------------------------|---------|-----------|---|--------------------------|---------------------------|
| <i>Ephemera strigata</i> | TMT170 | Larva | Primorsky Krai, Ryazanovka River | 42.823900, 131.234048 | ON505834 |
| <i>Ephemera strigata</i> | TMT342 | Imago, ♂ | Primorsky Krai, Krivoy Stream | 43.693182, 132.163682 | ON505835 |
| <i>Ephemera strigata</i> | TMT46 | Larva | Jewish Autonomous Oblast, Bira River | 49.007156, 131.884017 | ON505836 |
| <i>Ephemera strigata</i> | TMT540 | Larva | Primorsky Krai, Nezhinka River | 43.460720, 131.731141 | ON505837 |
| <i>Ephemera strigata</i> | TMT700 | Larva | Primorsky Krai, Tigrovaya River | 43.302552, 133.057668 | ON505838 |
| <i>Ephemera strigata</i> | TMT843 | Larva | Primorsky Krai, Avvakumovka River Basin | 43.825636, 134.905104 | ON505839 |
| <i>Ephemera strigata</i> | TMT851 | Larva | Primorsky Krai, Barabashevka River | 43.176817, 131.532742 | ON505840 |
| <i>Ephemera separigata</i> | TMT539 | Larva | Primorsky Krai, Nezhinka River | 43.460720, 131.731141 | ON505841 |
| <i>Ephemera separigata</i> | TMT593 | Larva | Primorsky Krai, Nezhinka River | 43.460720, 131.731141 | ON505842 |

5 million generations with sampling every 500 generations, four chains and a burn-in of 25 % trees. Sequences were deposited in GeneBank under accession numbers ON505834 - ON505842 (Table 1).

Description

Ephemera separigata Bae

Figs 1–3.

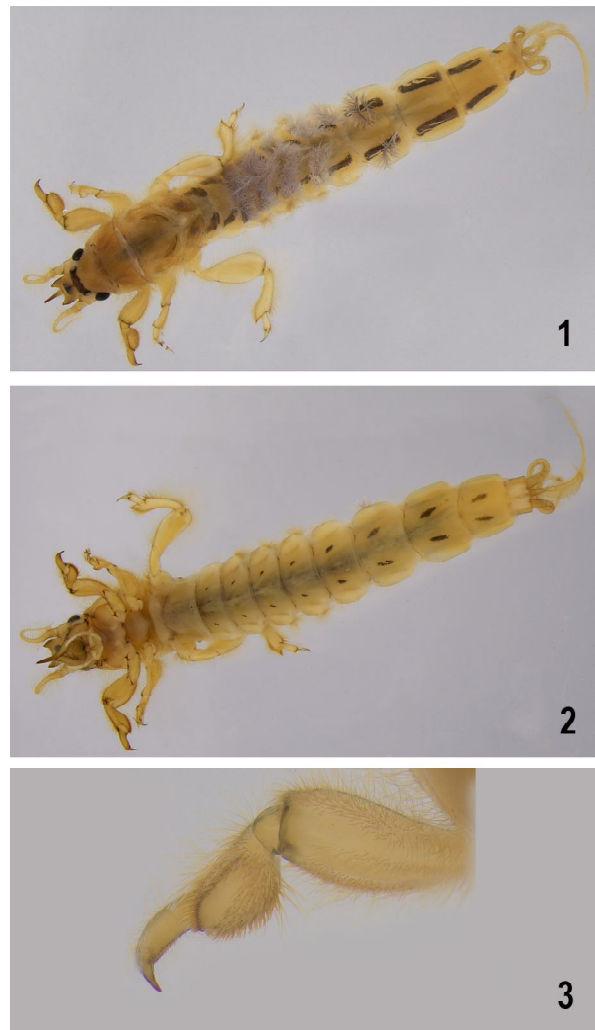
Ephemera separigata Bae, 1995: 160; Quan Y.T. et al., 2002: 250; Bae Y.J., Liu G.C. 1999:5.

Material. Russia: Primorskiy krai, Khasanskiy district: 4 larvae, Nezhinka River, about 3 km above the Vladivostok–Khasan highway, 14.VII.2017, leg. T. Tiunova; 6 larvae, same place, 28.VII.2017, leg. T. Tiunova; 3 larvae, same place, 24.VII.2018, leg. T. Tiunova; 3 larvae, Khasanskiy district, Gryaznaya River, road bridge, Vladivostok–Khasan highway, 20.VI.2017, leg. T. Tiunova.

Ephemera separigata is morphologically very similar to *E. strigata* and *E. japonica*, but distinguished by a pair of relatively narrow, separated, and laterally oriented longitudinal stripes on the abdominal terga 7–9 in larval (Figs 1–2), subimaginal, and adult stages of both sexes. Forefemora with rows of hair like setae along anterior and posterior margins, and with longitudinal hair like setal field on dorsal surface (Fig. 3).

Overall, we have sequenced fragments of the cytochrome oxidase I (658 bp in length) of 9 specimens belonging to 2 species (Table 1). *E. strigata* includes 7 specimens, which made it possible to evaluate the intraspecific distances which were 0.13% in average.

We compared obtained sequences with GeneBank and BOLD systems data. For each species, we found conspecific samples in databases and calculate interspecific distances. The closest sequence to Far Eastern *E. strigata* were *E. strigata* collected from Japan (MN961290-MN961294, LC644376, BIN BOLD:AED3237). The average p-distances between them were 5.71 % which shows their conspecificity [Morinière et al., 2017]. Obtained sequences of *E. separigata* were close to *E. separigata* (HQ257232-HQ257234, MH823275-MH823279) from South Korea, the average p-distance was 0.6 %. Interspecific distances between *E. strigata* — *E. japonica*, *E. japonica* — *E. separigata* and



Figs 1–3. *Ephemera separigata* Bae, 1995. 1 — larva, dorsal view; 2 — larva, ventral view; 3 — foreleg, dorsal view.

Рис. 1–3. *Ephemera separigata* Бая, 1995. 1 — личинка, вид сверху; 2 — личинка, вид снизу; 3 — передняя нога.

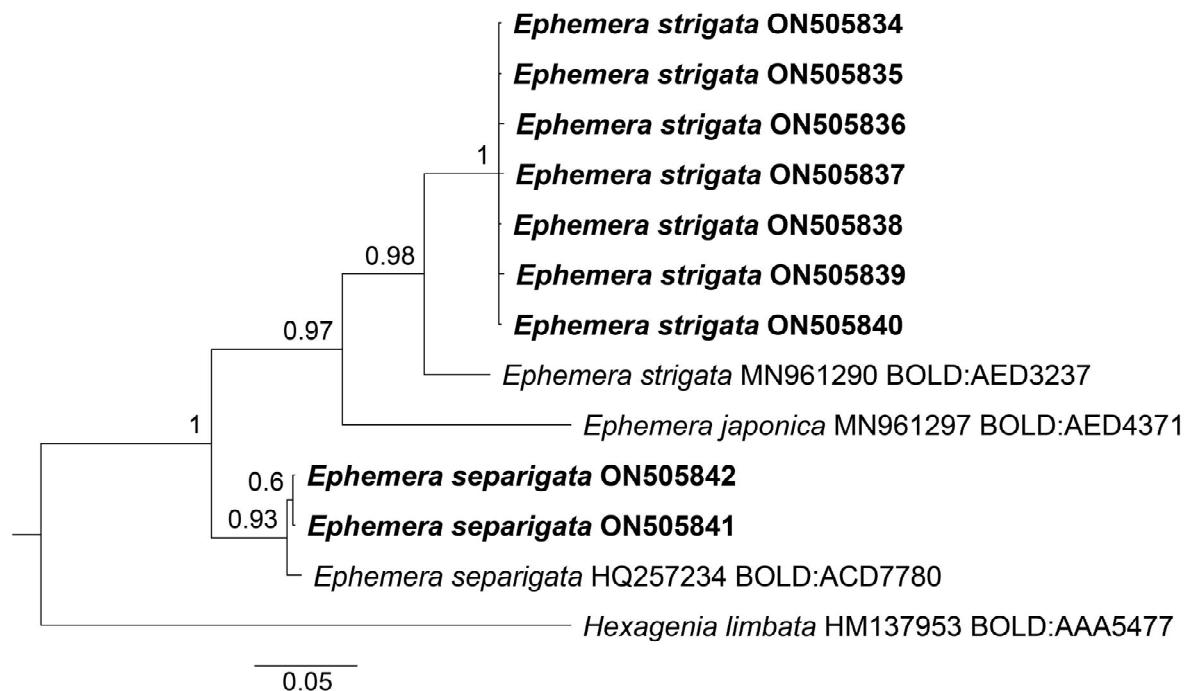


Fig. 4. Bayesian tree based on mitochondrion COI gene for obtained sequences and each available BIN number of three *Ephemera* species. *Hexagenia limbata* Servill was used as outgroup to root the tree. Bayesian posterior probabilities (PP) are given above tree nodes. Specimens obtained in this study are in bold.

Рис. 4. Байесовское дерево на основе митохондриального гена COI для полученных последовательностей и каждого доступного номера BIN трех видов *Ephemera*. *Hexagenia limbata* Servill использовалась как внешняя группа для укоренения дерева. Байесовские апостериорные вероятности (PP) даны над узлами дерева. Образцы, полученные в этом исследовании, выделены жирным шрифтом.

E. strigata — *E. separigata* were 10.51 %, 11.66 % and 11.14 % respectively which support the distinctiveness of each species [Morinière et al., 2017].

We used Bayesian Inference to reconstruct relationships of the three related *Ephemera* species (Fig. 4). To do this, we selected one sample from each BIN number from the BOLD systems and *Hexagenia limbata* Servill as outgroup. *E. strigata* was placed as sister to *E. japonica* (BIN BOLD: AED4371) with high support (Bayesian posterior probability, PP = 0.97). *E. separigata* was the earliest branching lineage and sister to two other species (PP = 1.00).

Distribution. Russian Far East (Primorye Territory), Korea, Northeast China.

Acknowledgments

The research was carried out within the state assignment of Ministry of Science and Higher Education of the Russian Federation (theme No. 121031000147-6).

References

- Bae Y.J. 1995. *Ephemera separigata*, a new species of Ephemeroidea (Insecta: Ephemeroptera) from Korea // Korean Journal of Systematic Zoology. Vol.11, No. 2. P.159–166.
- Bae Y.J., Liu G.C. 1999. Mayflies (Ephemeroptera) from Changbaishan area in Northeast Asia // Entomological Research Bulletin (Korean Entomological Institute). No.25. P.1–6.
- Folmer O., Black M., Hoeh W., Lutz R., Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates // Molecular Marine Biology and Biotechnology. No.3. P.294–299.
- Kumar S., Stecher G., Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets // Molecular Biology and Evolution. Vol.33, No.7. P.1870–1874.
- Lanfear R., Calcott B., Ho S.Y., Guindon S. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses // Molecular Biology and Evolution. Vol.29, No.6. P.1695–1701.
- Morinière J., Hendrich L., Balke M.T., Beermann A.J., Koenig T., Hess M., Koch S., Müller R., Leese F., Hebert P.D., Hausmann A., Schubart C.D., Haszprunar G. 2017. A DNA barcode library for Germany's mayflies, stoneflies and caddisflies (Ephemeroptera, Plecoptera and Trichoptera) // Molecular ecology resources. Vol.17, N. 6. P.1293–1307.
- Rambaut A., Drummond A.J., Xie D., Baele G., Suchard M.A. 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7 // Systematic Biology. Vol. 67, N. 5. P.901–904.
- Tiunova T.M. 2009. Biodiversity and distribution of mayflies (Ephemeroptera) in the Russian Far East (International Perspectives in Mayfly and Stonefly Research, Proceedings of the 12th International Conference on Ephemeroptera and the 16th International Symposium on Plecoptera). Stuttgart 2008 // Aquatic Insects. Vol. 31, Supplement 1. P. 671–691.
- Quan Y.T., Bae Y.J., Jung J.C., Lee J.W. 2002. Ephemeroptera (Insecta) fauna of Northeast China // Insecta Koreana. Vol.19, Nos 3–4 .P.241–269.